Light Harvesting Strategies of Cryptophyte Algae

by

Scott McKay

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Graduate Department of Chemistry
University of Toronto

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University of Toronto

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Abstract

Cryptophyte algae employ a unique system of free floating phycobiliproteins that shuttle photosynthetic excitations to membrane bound chlorophyll a/c complexes which in turn are connected to the photosystem reaction center (Larkum, 2010). Using absorption, emission and excitation spectroscopy, we attempt to determine, firstly, the manner in which the algae first absorb incoming photons and then shuttle the excitation from the outlying peripheral proteins to the core complex reaction centers and secondly, how efficient this process is within the organism.

The complex relationship between light harvesting efficiency and the antenna proteins was examined by performing experiments on organisms that varied in their antenna structure both energetically and structurally. The adaptability to varying environmental conditions was also studied by exposing organisms to varying ambient light intensity, a factor which is well known to influence photosynthetic efficiency (Falkowski & Owens, 1980). Contributions of alternate dissipative pathways were determined by chemically decoupling key systems in the electron transport chain.
Acknowledgments

There is one Dilbert comic that I am particularly fond of, in it, the Pointy Haired Boss is tasked with finding a new CEO for the company. During the welcoming speech for the new CEO, the Pointy Haired Boss reveals to the company that the new hire has no relevant experience or knowledge in their field. The Pointy Haired Boss informs the company that this was done on purpose as they wanted someone who “Didn’t know what can’t be done”. I often think how well this sums up my career in academia.

With that I would like to thank my supervisor, Dr. Greg Scholes, for taking me on as a graduate student. I am very grateful for being given the opportunity to work under such a passionate and knowledgeable person.

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Gēð ā Wyrd swā hīo scel!

-Beowulf
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Abbreviations and Acronyms

269  Chroomonas Mesostigmatica
344  Rhodomonas Minutae
705  Proteomonas Sulcata
705h Proteomonas Sulcata grown under high light conditions
706  Hemiselmis Pacifica
abs  Absorbance
APC  Allophycocyanin
ADP  adenosine diphosphate
ATP  adenosine triphosphate
CAC complex chlorophyll a/c complex
ChlA  chlorophyll a
ChlC  chlorophyll c2
DCMU  3-(3,4-dichlorophenyl)-1,1-dimethylurea
em  Fluorescence emission
exc  Fluorescence excitation
GA  billions of years ago
LHC  light harvesting complex
NADP  nicotenamide-adenine dinucleotide phosphate
NPQ  non photochemical quenching
PC577 phycocyanin 577 isolated from Hemiselmis Pacifica
PC645 phycocyanin 645 isolated from Chroomonas Mesostigmatica
PE545 phycoerythrin 545 isolated from Rhodomonas Minutae or Proteomonas Sulcata
PSI  photosystem I
PSII  photosystem II
PBP  phycobiliprotein
Pheo  pheophytin
PIC  plastocyanin
PIQ  plastoquinone
PQ  photochemical quenching
QA/B terminal electron acceptors in PSII
qH  vibrational quenching
qR  radiative quenching (fluorescence)
RC  reaction center
WT  algae without DCMU
Chapter 1

Introduction

1.1 Photosynthesis

Every second, a tremendous amount of energy in the form of solar radiation is deposited on the surface of the Earth (Larkum, 2010). Less than a billion years (Hohmann-Marriott & Blankenship, 2011) after our planet coalesced out of the primordial debris surrounding the infant sun, the first organisms capable of anaerobic photosynthesis (Xiong & Bauer, 2002) made their eternal mark on the biosphere. Now, in the present day, these early ancestors have sired an impressive lineage of light harvesting organisms that form the foundation of all life on Earth. Three billion years of evolutionary tinkering have produced a diverse set of methods for making use of solar energy (Koziol, et al., 2007) (Ruban, 2014). From the capture of photons, to ensuring their safe and rapid journey to the photosynthetic reaction centers, Nature has provided us with many models to investigate and motivate the design of our own solar devices (Scholes, Fleming, Olaya-Castro, & van Grondelle, 2011).

Aerobic photosynthesis is the process by which an organism sequesters carbon dioxide from the atmosphere and fixes it into sugar (Arnon, 1971). This is driven by electrons produced through the splitting of water molecules by captured photons (Åkerlund, Jansson, & Andersson, 1982). For phototrophs, such as plants, algae and some bacteria, this is the primary mode for energy production. Heterotrophs consume these so called primary producers as their source of energy. The waste product of photosynthesis, molecular oxygen, is necessary for sustaining aerobic life on earth (Dismukes, et al., 2001). Photosynthesis is a global phenomenon, active in terrestrial and aquatic environments (Antoine, André, & Morel, 1996). Annually, photosynthesis produces an equivalent of 450 (Pisciotta, Zou, & Baskakov, 2010) TW of energy and over 100 billion tons of biomass.

2.5 billion years ago, ancestral cyanobacteria evolved (Drews, 2011) to utilize water splitting to drive their biochemistry, after a few millions of years, the waste oxygen produced reached toxic levels in the atmosphere, leading to a major extinction event, the “Oxygen
Catastrophe” (Hsia, Schmitz, Lambertz, Perry, & Maina, 2013), which was responsible for the death of most anaerobic life on earth.

Nature rapidly learned to utilize the more energetic oxygen gas and more complex organisms rapidly spread across the planet (Larkum, 2010). Eukaryotic photosynthesis was the next major evolutionary milestone, it is widely accepted that an early eukaryotic heterotroph consumed an ancestral cyanobacteria (Yoon, Hackett, Ciniglia, Pinto, & Bhattacharya, 2004) and instead of metabolizing it, incorporated it into its own physiology. This so called primary endosymbiosis occurred around 1.5 billion years ago and was the ancestor of modern plants and algae (McFadden, 2001). Following this, numerous secondary endosymbiotic events occurred, giving rise to the many different types of photosynthetic organisms. Of interest is the secondary endosymbiotic event in which an ancestral red algae was engulfed by some early protozoan, giving rise to the cryptophyceae lineage (Petersem, Teich, Brinkmann, & Cerff, 2006) (Burki, Okamoto, Pombert, & Keeling, 2012). These cryptophyceae (commonly, and hereto referred to Cryptophytes) possess a novel method (Ingram & Hiller, 1983) (Spear-Bernstein & Miller, 1985) for capturing and controlling photons which will be further explored and elaborated on in the following chapters.

Figure 1.) Simplified timeline of the evolution of photosynthetic eukaryotes. a) A heterotrophic eukaryotic cell engulfs an ancestral cyanobacterium b) The eukaryote does not digest the cyanobacterium and instead incorporates it into its physiology c) The early progenitor of the photosynthetic eukaryotes gives rise the major classes we see today, including the Red Algae d) During a secondary endosymbiotic event, an ancestral Red Algae is engulfed by an early eukaryote e) The Red Algae is incorporated into the eukaryote, losing its plastid and giving rise to the Cryptophyte lineage. Adapted from Reference
Cryptophytes are found in nearly all aquatic environments, both fresh and salt water (Javornický & Hindák, 1970). They range in size from 10-50 µm and can be readily seen with an optical microscope. For mobility they rely on flagella and specialized organelles called ejectosomes which propel them away from environmental stressors. Cryptophytes possess a chloroplast which contains chlorophyll a and c (Fawley, 1989), as well as phycobiliproteins and carotenoids. It is the phycobiliproteins (Apt, Collier, & Grossman, 1995), as well as chlorophyll (French, Brown, & Lawrence, 1972), that give the algae their colour (Scholes, Mirkovic, Turner, Fassioli, & Buchleitner, 2012), which ranges from red to blue as well as functioning as the major unit for capturing incoming photons.
1.2 Light Harvesting Architecture

1.2.1 Chromophores and Proteins

The simplest organisms which utilize light energy are the photosynthetic bacteria (Jensen, Aasmundred, & Eimhjellen, 1964). The nature of the prokaryotic cell does not allow for complex, specialized organisms in the same way that the eukaryotic cell does. The photosynthetic proteins are simply embedded in the cell membrane which may or may not be folded in to the cytosol in sheets, or as free floating vesicles, as a means of increasing the effective surface area (Bryant & Frigaard, 2006).

Higher plants and algae have a specialized organelle called the chloroplast where photosynthesis occurs (Cooper, 2000). The chloroplast is a double membrane structure which is filled with an aqueous fluid known as the stroma. Inside the stroma is where the major machinery of photosynthesis sits, it contains stacks of flattened disks individually called thylakoids (Nielsen, Smillie, Hennigsen, & von Wettstein, 1979). The proteins and structure responsible for photosynthesis are contained within the thylakoid membrane. This includes the pigments that absorb the light energy, as well as the enzymes and proteins that form the electron transport chain (Rochaix, 2010), and the pumps that drive the formation of a pH gradient across the thylakoid membrane which ultimately leads to the formation of ATP (Allen, 2002). The pigments in the thylakoid (eukaryotes) are often arranged in to large complex structures called a light harvesting complex (LHC) (McDermott, et al., 1995) (Kiihlbrandt, Wang, & Fujiyoshi, 1994). The LHC acts as an antenna (Horton & Ruban, 2005) and greatly increases the absorption cross section of the pigments and allows for the efficient collection of photons. The LHC is spatially and energetically arranged to ensure that the excitations caused by photon capture make their way rapidly and efficiently to the reaction center (RC). This phenomenon is known as the antenna effect and will be examined in this study.
Photosynthetic organisms utilize a wide variety of pigments not only to collect energy, but also as a means of photoprotection (Bilger & Björkman, 1990). The specific arrangement and identity of pigments contained by the organism is largely determined at the species level in response to the environmental conditions in its habitat. The ratios of these pigments can however change in response to local ambient conditions.

The predominant pigment found in photosynthetic organisms is chlorophyll. Several different subtypes of chlorophyll exist in nature but all absorb strongly in the red and blue regions of the solar spectrum (Krause, 1991). In order to take advantage of the window in the yellow and green part of the solar spectrum, photosynthetic organisms also employ such pigments as phycobiliproteins (PBPs) in algae and the carotenoids.

In higher plants and red and green algae, the LHC is a highly organized and complex structure, bound in the thylakoid membrane. In addition to this, the red algae use a special arrangement of PBPs called the phycobilisome to supplement their light harvesting (Grossman, Schaefer, Chiang, & Collier, 1993) (Kursar & Alberte, 1983). Interestingly, the Cryptophyte lineage seems to have taken a step backwards in complexity, opting to forego the complex phycobilisome of its red algae ancestors and instead transporting the PBPs to the lumen of the thylakoid where they are randomly oriented and free floating (Kana, Prásil, & Mullineaux, 2009).

Figure 2. Schematic of the antenna effect, chromophores far from the reaction center are higher in energy, chromophores nearer the RC are lower in energy with the RC being the lowest. This ensures that excitations are funneled into the RC.
The Cryptophytes, in addition to their unique spatial arrangement of pigments, have a combination of pigments not seen in any other photosynthetic lineage (van der Weij-De Wit, et al., How Energy Funnels from the Phycoerythrin Antenna Complex to Photosystem I and Photosystem II in Cryptophyte Rhodomonas CS24 Cells, 2006). Phycoerythrin (PE) and Phycocyanin (PC) are the main light harvesting pigments residing in the thylakoid lumen, they absorb yellow and green light and range from red to green, and purple to blue in colour (Scholes, Mirkovic, Turner, Fassioli, & Buchleitner, 2012). A particular species of Cryptophyte will have either PE or PC but not both. The membrane bound portion of the LHC in Cryptophyte contain Chlorophyll a/c (CAC) complexes with chlorophyll c₂ as the main component. The CAC complex receives energy as fluorescence emission from the PBP. Also contained within the CAC complex is the carotenoid alloxanthin. The exact function of alloxanthin is not precisely known but it is likely that it acts as a photo protective pigment much like the carotenes of higher plants. The CAC complex then passes excitations on to Photosystem I and II (PSI/PSII) where the excitation is converted into a charge separated pair which is passed off through the ETC in a series of oxidation-reduction reactions.

1.2.2 Photochemical Quenching

In the context of this manuscript, Photochemical Quenching (PQ) broadly refers to the fate of the photo induced excitation once it reaches one of the photosystem RCs. This process is
outlined in the so called ‘Z-Scheme”, where an electron starting at PSII is shuttled through a
series of proteins and charge carriers including Plastoquinone (PlQ), cytochrome b₆f,
Plastocyanin (PlC), PSI and terminating at ferredoxin where it drives the formation of NADPH
from NADP (Stirbet & Govindjee, 2011).

In addition to producing ATP and NADPH, oxygenic photosynthesis produces protons
which establish a chemiosmotic gradient which is used to power the conversion of ADP to ATP
by ATP Synthase. Maximal production of ATP is achieved when PQ is the main sink for
excitations, however there are two other major pathways that compete with PQ which do not
result in the formation of ATP. It is worthwhile to note that while producing as much ATP as
possible is crucial to the growth and reproduction of the organism, it is often advantageous to the
organism to operate at a submaximal rate as this provides it with the capability to buffer changes
in the environment that might otherwise prove fatal.

The net production from the Z-Scheme or oxygenic photosynthesis is

$$2H_2O + 2NADP^+ + 3ADP + 3P_l \rightarrow O_2 + 2NADPH + 3ATP + 2H^+$$
1.2.3 Other Dissipative Pathways

In addition to PQ we introduce the idea of non-photochemical quenching (NPQ) which manifests itself in two basic ways. NPQ broadly refers to any quenching of a photo induced excitation which does not lead to inclusion in the ETC. Here NPQ will be considered to be either radiative quenching ($q_R$) or dissipative quenching ($q_H$) (Govindjee, 1995).

Radiative quenching is any fluorescence event after absorption of a photon. There are a number of steps in excitation transfer where this can occur. In Cryptophytes, fluorescence is mostly observed from the PBP and from PSII with some contribution from PSI.

Dissipative quenching is the conversion of an excitation to heat energy, in the context of a photo protective mechanism (Muller, Li, & Niyogi, 2001). The exact nature of this in Cryptophytes is not currently known. In higher plants, $q_H$ is mediated by the carotenoids.
1.3 Spectroscopy

In this study of the Cryptophytes, basic steady-state spectroscopy will be used to determine the parameters used for quantifying the energy transfer efficiency and excitation pathways in the Cryptophyte LHC. While many sophisticated methods have been used to study in detail, elements of the Cryptophyte LHC (Doust, et al., 2004) (Curutchet, et al., 2013) (van der Weij-De Wit, et al., How Energy Funnels from the Phycoerythrin Antenna Complex to Photosystem I and Photosystem II in Cryptophyte Rhodomonas CS24 Cells, 2006), these more straightforward methods will be more useful to a global investigation.

1.3.1 Linear Absorption

Linear absorption is a measure the degree that a material absorbs an incident beam of light. The absorption of a chemical species in solution can be calculated using the Beer-Lambert law

\[ A = -\log_{10} \left( \frac{I_0}{I} \right) \varepsilon lc \]

Where \( I_0 \) and \( I \) are the intensity of the incident and transmitted light respectively. \( \varepsilon \) is the molar absorptivity, \( l \) is the path length of the sample and \( c \) is the concentration of the sample. The molar absorptivity is wavelength dependent and unique to each chemical present. Thus the total absorption of a material at a given wavelength is, to first order, a linear combination of the absorption of each chemical species contained within the sample. Absorption spectroscopy will be used to map out the energy landscape of the Cryptophyte chromophores as a first step to determining how they function as a cohesive unit. It will also be used to estimate the relative amount of each chromophore in a sample of algae.

1.3.2 Fluorescence

When a molecule becomes excited, it can relax back to its ground state in a number of ways. It often accomplishes this by emission of a photon some short time after excitation. Fluorescence occurs from the first singlet excited state. If a molecule is excited to the second excited state, it will typically vibrationally relax to the first excited state before fluorescing. The emitted photon is red shifted relative to where it was absorbed, thus the fluorescence emission
spectrum will be reminiscent of the absorption spectrum. Fluorescence can be measured in two ways, we can measure the emission spectrum by exciting the sample at a specific wavelength and then monitor the intensity of the emissions at longer wavelengths. Fluorescence emission can be used to infer the presence of energy transfer between two chromophores. Fluorescence from a second chromophore after excitation of an initial chromophore indicates that energy transfer has occurred between the two.

The excitation spectrum can also be measured. This involves monitoring the fluorescence intensity at a particular wavelength and exciting a range of shorter wavelengths. The signal indicates where energy came from and when paired with the emission spectrum can be used to determine which chromophores are spectrally connected. Additionally, by comparing the normalized excitation and absorption spectrum, the efficiency of energy transfer between two chromophores can be calculated (Blankenship, 2002).

Figure 5.) The efficiency of energy transfer between two chromophores can be determined with the normalized absorption and fluorescence excitation spectrum. In this example we consider a simple system of two chromophores, called A and D, where A is the acceptor with an absorption maximum $\lambda=510$ nm and D is the donor with absorption maximum $\lambda=490$ nm. Both spectra are normalized at 510 nm and the ratio at 490 nm is determined. In this case the transfer efficiency is found to be 70%.

This efficiency parameter will be the sought after quantity in this study. While calculation of the efficiency is straightforward in a two chromophore system with no other competing
dissipation pathways, the analysis rapidly becomes intractable in a system with multiple chromophores with significant spectral overlap and with numerous transfer and dissipation pathways.
Chapter 2

Energy Transfer under Standard Conditions

2.1 Methods and Introduction

The light harvesting structures employed by the Cryptophytes shows a novel design with a compliment of chromophores found nowhere else in nature. We are interested in knowing how efficient the Cryptophyte light harvesting complex is and how robust it is to changes in environmental conditions. Originally, four species of Cryptophyte, varying in phycobiliproteins compositions were investigated for periods of two to three weeks in order to determine the effect that aging of the algal colony had on the composition and efficiency of the Cryptophyte LHC.

The four species investigated were *Proteomonas Sulcata* (PE545), *Rhodomonas Minutae* (PE545), *Hemiselmis Pacifica* (PC577), and *Chroomonas Mesostigmatic* (PC645). Only *P. Sulcata* will be reported here as it was the only species that was investigated in all three experiments. The spectra of the other species were considered to be too convoluted due to overlap of multiple chromophores to be of use to this study.

Algal colonies were grown in Erlenmeyer flasks and kept on a 12 hour day-night cycle. The closed nature of the flask meant that the colony would eventually perish due to nutrient deficiency and exposure to toxic metabolic and life cycle byproducts. The flasks were kept at a constant distance from their light source to ensure consistent illumination. An additional colony of *P. Sulcata* was grown at a much closer distance to the light source to simulate a high light stress environment. Measurements were performed every 2 to 3 days during the lifetime of the colony in order to follow changes in photosynthetic behavior through exponential growth and eventually death. Measurement consisted of linear, steady state absorption in the visible region, and fluorescence emission and excitation. Fluorescence targets were identified by matching peaks in the absorption spectrum to chromophores previous identified in the Cryptophyte. Absorbance measurements were performed on a Varian Cary 6000i UV-Vis-NIRspectrophotometer and fluorescence measurements were performed on Cary Eclipse Fluorometer. This method was devised by Dr. Tihana Mirkovic and Laura Campitelli in an
earlier initial study of the Cryptophytes. Of the five species outlined above *P. Sulcata* and *R. Minutae* were selected for initial study because of their lesser spectral overlap between their PBP and photosystems. Dr. Mirkovic also provided a great service in maintaining the algae colonies for this experiment as well as assisting in collecting the spectra shown in this chapter.

### 2.2 Spectroscopic Investigation under Normal Conditions

The first part of the experiment sought to determine the light harvesting and transfer efficiency of the algal species under ‘normal’ conditions to set a baseline for the future experiments. This was meant intended as a breadth study to see what common trends could be identified in all species. Due to the congested absorbance profile of the PC containing species, attention was initially given to the two PE containing species as well as the high light *P. Sulcata*.

**Figure 6.** Normalized absorption spectra of *H. Pacifica* and *P. Sulcata*, containing PC and PE respectively. The major contributors to the absorbance profile are: chlorophyll a S2 (440 nm), chlorophyll c1:S2 (470 nm), allophan (490 nm), phycoerythrin (545-555 nm), phycoerythrin (575-625 nm), chlorophyll c2:S1 (630 nm), and chlorophyll a S1 (680 nm). Overlap of PC and ChlA /C complicates the energy transfer efficiency calculations.
2.2.1 Absorbance Spectroscopy

*P. Sulcata* Absorbance Spectrum Normalized at 677 nm

![Absorbance Spectrum Normalized at 677 nm](image1)

*P. Sulcata* High Light Absorbance Spectrum Normalized at 677 nm

![High Light Absorbance Spectrum Normalized at 677 nm](image2)

*R. Minutae* Absorbance Spectrum Normalized at 677 nm

![Absorbance Spectrum Normalized at 677 nm](image3)

Figure 7.) The three graphs above show how the absorbance profile of each algal species changes over the lifetime of the colony. In all three, there is a large variation in the absorbance between 400 and 600 nm and a large decrease in PE545. The relative amounts of ChlA to ChlC remain more or less constant.
PE545 acts as an antenna for incoming photons and so it would seem that it is in the organisms’ best interest to have as much as possible in order to maximize photon capture. It is clear from the above graphs that *P. Sulcata*, after a short amount of time, opts to produce less antenna per reaction center. The effect that this has on the transfer efficiency will be investigated in the next sections.

**2.2.2 Fluorescence Spectroscopy**

With the major chromophore targets identified, we can now, with fluorescence measurements, begin to determine how the chromophores are spectrally linked. If excitation of one chromophore leads to fluorescence emission from one or more other chromophores then we can deduce that there is some degree of energy transfer between the two. Energy transfer requires an overlap between the emission spectrum of the first chromophore and the absorption spectrum of the next. 705, 705h and 344 were excited at 440 nm (ChlA), 490 nm (carotenoids) and 530 nm (PE).

![Comparison of PE545-ChlA Ratio in *P. Sulcata* and *R. Minutae* During Colony Lifetime](image)

Figure 8.) By plotting the relative amount of PE545 (545 nm) to ChlA (677 nm) we can see how the pigment composition changes over time. 705 maintains a consistent ratio until around Day 15 where it then begins to drop. 344 appears to not lose PE545 over the duration of the study. 705h declines rapidly with a significant drop after Day 9. The loss of PE545 is visible to the eye, as the colony ages, the colour fades from deep red to a pale yellow.
The spectra were taken using different excitation and emission slit widths each day so direct comparison of the absolute changes in spectra is not possible here, instead we are comparing the changes in peak height with the spectra normalized at 685 nm. This will be Figure 9.) Fluorescence emission spectra from 705, 705h and 344 (top to bottom). Excitation wavelengths at 440, 490 and 530 nm (left to right).
addressed in Chapter 3. Each of the PE545 containing species show similar trends in their emission spectrum. Deviations from the general trends on D2, D4, and D21, D23 are attributed to low algae concentration and presence of cellular debris respectively.

Excitation at 440 nm corresponds to exciting the s2 band of ChlA. The excited ChlA rapidly converts to the s1 excited state (677 nm) where it is then fluoresced by PSII (685 nm) and PSI (740 nm). The ratio of the fluorescence emission from PSII and PSI is very consistent over the lifetime of the algal colony and between the three species at approximately 16-18%. This suggests that the relative quantities and spatial relationships between PSII and PSI are fixed over the lifetime of the colony. The small emission peaks at 530 and 590 nm are likely artefacts of exciting the blue edge of the carotenoids and PE545.

Excitation at 490 nm corresponds, broadly, to the carotenoids present in that region, particularly alloxanthin. Despite the three major emission bands that appear, it is thought that the fluorescence contribution from the carotenoids is minimal as they are expected to vibrationally relax to the ground state, thus emitting no photon. The large fluorescence emission at 590 nm is from PE545, which has a broad absorption peak which overlaps with carotenoids. Emission at PSII and PSI arises from the excited PE545 transferring energy to the Chlorophyll a/c complex. The difference in the maximum peak height and rate of decay between the three species at 590 nm correlates with the relative amount of PE545 as measured by absorption spectroscopy.

Direct excitation of the PE545 at 530 nm produces a much larger emission band at 590 nm as well the expected emission at 685 and 740 nm. Despite having a similar PE545/ChlA ratio, 705 and 705h show a considerable difference in the maximum and minimum height of the 590 nm band, with 705h having both a greater maximum and a smaller minimum. If the relative amount of PE545 is the same, then increased emission at 590 nm may be interpreted as a greater fluorescence leakage from the PE545, that is, a greater proportion of the photons absorbed by the PE545 do not make it to the CAC complex. The rapid decrease in emission by 705h again can be rationalized by the loss of PE545 as per absorption measurements.
Figure 10.) Fluorescence emission spectra from 705, 705h and 344 (top to bottom). Emission wavelengths at 685 and 730 nm (left to right)
By measuring the excitation spectrum at the emission peaks of PSII (685 nm) and PSI (730 nm), we can read off where each photosystem receives its energy from, however, this does not tell us the exact nature of the path the excitation takes. Because the excitation spectra are normalized at 677 nm, there are some cases where the 685 nm excitation spectra are unreliable due to the excitation beam being convoluted with the signal at 677 nm. This was remedied in future experiments by monitoring the PSII excitation spectrum at 695 nm.

For both PSII and PSI, 705 shows a constant excitation signal from the S2 chlorophyll region (440 nm) as well as from the CAC complex over the colony lifetime. We expect these regions to have very little variation since they arise from the same chlorophylls that make up the photosystems and CAC complexes. The signal from the PE545 region starts at a maximum and slowly decreases from D7. Measurements from D2 and D4 were compromised due to signal overlap from the excitation beam.

705h shows a more interesting trend, in both photosystems, the PE545 signal starts at an intermediate intensity, rises and peaks and then rapidly drops. Again the regions dominated by the S2 chlorophyll bands and the CAC complex remain relatively consistent.

344 shows a much slower decline in the PE545 region which only begins to manifest after about 3 weeks. 344 grows at a considerably slower rate than the 705 species and so changes over time are much less drastic. This will be addressed in the second iteration of this experiment.

In all cases, the excitation signal from the PE545 is slightly higher in the PSI spectrum than the PSII spectrum. This may be a results of the structure of the thylakoid membrane and organization of the photosystems. PSII is blocked on the luminal side of the membrane by the OEC and accessory proteins which screens the RC from direct energy transfer from the PBP pool. PSI on the other hand is not blocked to the same degree as PSII and thus has more direct access to energy transfer. An excitation must first be transferred to a CAC complex in the thylakoid membrane before it can be transferred to PSII.

\[ \varepsilon = \frac{I_{\lambda_{\text{max}}D_{\text{exc}}}}{I_{\lambda_{\text{max}}A_{\text{abs}}}} \]

*Figure 11.* Calculation of energy transfer efficiency. \( I_{\lambda_{\text{max}}D_{\text{exc}}} \) is the Donor fluorescence excitation at the absorption maxima of the donor. \( I_{\lambda_{\text{max}}A_{\text{abs}}} \) is the acceptor absorption spectrum normalized at the Acceptor absorption maxima.
Figure 12.) Transfer efficiency from the PBP antenna to PSII and PSI for P. Sulcata. Efficiencies above 100% are physically unrealistic and arise from the simple two chromophore, single transfer step model.
Figure 13.) Transfer efficiency from the PBP antenna to PSII and PSI for High Light P. Sulcata. Efficiencies above 100% are physically unrealistic and arise from the simple two chromophore, single transfer step model.
Figure 14.) Transfer efficiency from the PBP antenna to PSII and PSI for R. Minutae. Efficiencies above 100% are physically unrealistic and arise from the simple two chromophore, single transfer step model.
Determining the efficiency of energy transfer from the excited PE545 pool to PSII and PSI as originally outlined in chapter 1.3.2 yields the above results. The energy transfer efficiency we are discussing is the amount of photons that have been absorbed by the phycobiliproteins and then fluoresced and absorbed by the photosystems. Of immediate notice is that the calculated transfer efficiency is greater than 100% in all species and for both photosystems. Since this is unphysical, we conclude that this method of analysis is inappropriate for this system. It is important to reiterate that this method is effective when contributions from other transfer pathways or dissipation mechanisms are negligible compared to the pathway of interest. Given the convoluted nature of the light harvesting complex of the Cryptophyte, it is not surprising that this simplistic treatment fails to capture the transfer efficiency. This will be addressed in the preceding chapters.

While this treatment does not allow comparison of absolute numbers, comparison of relative values is still possible within a species. 705 and 705h both show a very constant efficiency despite a large variation in the relative amount of PE545 to ChlA. The transfer efficiency in 344 appears to increasing slowly over time. From this we can conclude that all three species, despite a large variation in the relative amount of PE545 to ChlA are able to provide a consistent amount of energy to both photosystems, thus, a portion of the luminal PE545 is redundant in transferring energy to the photosystems. PSI apparently enjoys a slightly more efficient transfer from PE545 than PSII. Commenting on which photosystem receives more energy from the PE545 is not possible at this stage as it depends on many biological and physical factors although we can note that PSII is shielded from direct irradiation from the lumen by the optically inactive proteins of the Oxygen Evolving Complex and so it must receive energy through a CAC complex intermediate. PSI has direct line of sight to the thylakoid lumen and so can receive incoming radiation directly.
Chapter 3

Contributions of Dissipative Pathways

In order to gain some insight into how to better calculate the energy transfer efficiency, we make reference back to Chapter 1.2.3 in order to determine how alternate transfer pathways and dissipation mechanisms affect the transfer of energy from the PBP pool in the thylakoid lumen to the RC.

Initially we considered a highly simplified model where we only took into account transfer from an antenna (PBP) to the reaction center of either photosystem. The reality is of course more complicated and requires acknowledgment that the antenna complex and the reaction center have an internal structure that alter the simple donor and acceptor model. The full complexity model is based on the measurements performed in chapter 2 (Strasser, Tsimilli-Michael, & Srivastava, 2004).

Figure 15.) Full complexity model of energy transfer in the Cryptophyte. $J_{545}$ and $J_{677}$ are the 545 and 677 nm photon flux. $PE_L$ and $PE_M$ are the luminal and membrane associated PE. $J_{LF}$ is fluorescence from luminal PE. $E_{LM}$ is transfer between $PE_L$ and $PE_M$. $E_{M(I)}$ is energy transfer from the $PE_M$ to PSI. NPQ$_{CH}$ and $J_{CF}$ are NPQ and fluorescence from the CAC complex. $E_{C(I)}$ and $E_{C(II)}$ are energy transfer from the CAC complex to PSI and PSII. $J_{(II)F}$ and $J_{(I)F}$ are fluorescence from PSII and PSI. $E_{(II)PQ}$ and $E_{(I)PQ}$ are energy transfer via charge separation from PSII and PSI.
In coming up with the above map we begin by considering first the thylakoid lumen, the location of the antenna, the PBPs can be subdivided into two groups. The first is what will be called the membrane associated PBPs, these are the ones in close proximity to the thylakoid membrane and hence, close to the CAC complex and photosystems. The second group is luminal PBPs, these are farther out from the membrane and have no line of sight with it. If we consider a membrane associated PBP, fluorescence can be readily absorbed by the adjacent CAC complex, a LPBP however is screened on all sides by other PBPs and so fluorescence from a luminal PBP is less likely to contribute to energy transfer ending in a PS. From these results we hypothesize that while every phycobiliprotein in the thylakoid lumen is capable of absorbing an incoming photon, they do not have equal probabilities of transferring that excitation to a photosystem. The excitation may hop from PBP to PBP until it reaches its destination but generally the probability decreases as distance, and therefore number of intermediate hops, from the thylakoid membrane increases.

Examining the absorption and emission spectra of the PBPs, there is non negligible overlap in some regions, this leads to the possibility of a PBP emitting a photon, only to be absorbed by a second PBP. The emission spectra of the PBPs are also of interest. Taking PE545 as an example, the major emission peak is at 590 nm, there is a minor peak centered around 630 nm. The absorption maximum of ChlC is at 630 nm.

![Overlap of PE545 and 705 Absorption Spectrum](image)

**Figure 16.** Showing the overlap between PE545 and the absorption spectrum of 705, we can see that despite the high fluorescence yield of PE545, only a small portion of it is of the appropriate wavelength to transfer energy to the CAC complex or the photosystems.
The CAC complex (Kereîche, et al., 2008) as its name suggests is composed of ChlA and ChlC, with a larger amount of the latter (Larkum, 2010). ChlA is red shifted compared to ChlC so in following the energy gradient towards the RC, it will encounter ChlC first. There is very little fluorescence emission from ChlC and so the transfer from ChlC to ChlA is considered lossless. The CAC is also thought to be the major site of NPQ in the Cryptophyte (Kaña, Kotabová, Sobotka, & Prášil, 2012). It is thought to be pH dependent and likely involves some participation from carotenoids within the CAC complex. While the exact process is not known, it is known that it does not involve cycling of carotenoids as in higher plants and other photosynthetic algae, indicating a novel NPQ scheme. ChlA has an emission maximum around 680 which is overlaps strongly with PSII, there is also a broad shoulder extending out to about 740 nm which overlaps with PSI. The only fluorescence seen on excitation of chlorophyll is at 685 and 740 nm, PSII and PSI respectively, indicating a very efficient transfer of an excitation from the CAC complex to the photosystems.

Once in the photosystem reaction center, the excitation will either undergo charge separation and photochemistry will occur, or the excitation will be emitted as fluorescence. The excitation may also be thermally dissipated.

This chapter will deal with the attempts at shutting down the pathways that provide alternate fates for an absorbed photon in order to maximize the fluorescence emission from the photosystems. By doing this we can begin to address the failings of our simple transfer model by allowing for other dissipative pathways and begin to quantify their effect. Firstly, an attempt will be made to minimize the effects of NPQ, secondly, an attempt will be made to shut down primary photochemistry at PSII and PSI.
3.1 Non Photochemical Quenching

Non Photochemical Quenching occurs as a response to high light conditions as a protective mechanism to dissipate excess excitations that might otherwise damage the organism. There are several timescales that NPQ works on depending on the organism. In general there is a rapid quenching that activates within minutes. There is also a long term response that operates on the timescale of hours. There may also exist a number of intermediate time scale NPQ effects (Müller, Li, & Niyogi, 2001).

In order to determine the effect of NPQ, the algae were exposed to either high, low, regular or no light for two hours prior to measurement at the beginning of their day. Two hours was deemed sufficient time for any NPQ effects to manifest, the measurements were performed at the beginning of the day in order to minimize normal physiological changes that occur over the course of the day.

D corresponds to no light, R corresponds to regular light (10 μmol m⁻² s⁻¹) and serves as the control. L and H are low (2 μmol m⁻² s⁻¹) and high light (30 μmol m⁻² s⁻¹), corresponding to about a one order of magnitude decrease and increase in ambient light intensity. There is some additional complication in determining the transfer efficiency in *H. Pacifica* and *C. Mesostigma*ica (not shown) due to the high overlap of their phycobiliproteins and the CAC complex. More sophisticated methods will be required to determine the absolute transfer efficiency in the PC containing species.

![Figure 17](image.png)

**Figure 17.** Transfer efficiency from the PBP antenna to PSII and PSI.
The dark adapted sample generally showed a slightly higher transfer efficiency to both photosystems over the course of the experiment. The low light sample also appeared to be slightly more efficient for the first two weeks at which point it was similar to the regular and high light samples. The regular and high light samples were more or less equal over the course of the four weeks. It is likely that the high light conditions were not intense enough to elicit the full NPQ response that was sought after. Káňa et al. suggest that full recruitment of the NPQ mechanisms (Káňa, Kotabová, Sobotka, & Prášil, 2012) occurs around 600 μmol m⁻² s⁻¹ whereas our high light sample was around 30 μmol m⁻² s⁻¹. If NPQ had been fully recruited by the algae, we would expect to measure decreased fluorescence from the PBP peak in the photosystem excitation spectra since photons that would have otherwise made it to the reaction center are dissipated as heat and thus, a decreased transfer efficiency.

Despite not being able to achieve a high enough photon flux with our setup to estimate the contributions of NPQ it is still worthwhile to comment on the capability of these organisms to buffer large changes in ambient light intensity with little loss in their ability to transfer energy absorbed by their light harvesting antenna to their reaction centers.

### 3.2 Photochemical Quenching

Chlorophyll fluorescence has been widely used as a tool to study photosynthesis and plant health via the Kautsky transient for a number of decades. By measuring the minimal and maximal fluorescence from PSII in a dark adapted plant, as well as the time it takes to reach this maximum and the fluorescence intensity at a few key points, dozens of parameters can be calculated that report on many aspects of the workings of the photosystems. Use of certain pesticides in these type of measurements is also common in order to shut down specific parts of the ETC in order to gain information about the pathways that compete with photosynthesis.

Use of the Chlorophyll Fluorescence Transient (CFT) was developed for the study of higher plants and relies on a number of assumptions in the calculation of its parameters that make it unreliable in analyzing anything other than higher plants. One of these assumptions is that the organism does not contain PBPs (Stirbet & Govindjee, 2011). This immediately disqualifies the Cryptophytes from dependable analysis with this method. Even though the LHC and antenna system show great variability across all photosynthetic organisms, the photosystems...
are largely identical. Thus we can use the same pesticides used in the analysis of the CFT to roughly the same effect in this study since we are probing fluorescence from the photosystems.

Photochemical quenching is the dissipation of an absorbed photon by charge separation at both photosystems and its subsequent transfer to the ETC. Any excitation that follows this path will not be measurable by our instruments and thus will make the transfer efficiency appear greater than it actually is. In PSII, directly after charge separation, the electron is transferred via pheophytin to quinone pool. Once a quinone has accumulated two electrons, it is oxidized by plastoquinone thereby regenerating the quinone.

3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) is a pesticide which blocks the binding of PIQ to PSII (Ridley, 1977) thus preventing the flow of electrons into the ETC. DCMU is highly selective and doesn’t interfere with any other photosynthetic reactions, it is widely used in CFT studies to reduce the complexity of PSII. The next section will investigate the effect of DCMU on Cryptophyte algae.

Few studies have been done investigating the effect of DCMU on the Cryptophytes and so its reliability as a PSII probe in this system is unknown but for the sake of simplicity it will be assumed to have the same effect. A colony of 705 will be grown under normal conditions and a sample will have the same fluorescence and absorbance measurements performed on it. The sample will then be allowed to dark adapt for 20 minutes at which point it will be poisoned with 6 µL of 10mM DCMU (Kaňa, Kotabová, Sobotka, & Prášil, 2012) in ethanol and then allowed to dark adapt for another 20 minutes. The sample will then have the same measurements.
performed on it in order to determine how DCMU affects PSII and to get a better estimate of the transfer efficiency. Two other fluorescence targets will be investigated, the emission spectrum from 460 nm and the excitation spectrum monitored at 645 nm. 5 nm slits will be used in all measurements so direct comparison of absolute fluorescence intensity is possible.

Figure 19.) Absolute (top) and normalized (bottom) fluorescence emission spectra of *P. Sulcata*, before and after addition of DCMU. WT refers to measurements performed without DCMU
Addition of DCMU to *P. Sulcata* has a significant effect on the emission spectrum, in all measured spectra, the corresponding fluorescence from PE545 at 590 nm remains the same but the fluorescence intensity from PSII and PSI are greatly increased. Addition of DCMU also maintains the ratio between PSII and PSI fluorescence as seen in the normalized spectra. The DCMU has therefore blocked a significant portion of photochemical quenching from PSII and the excitations that normally would have entered the ETC from PSII are being emitted as fluorescence. At this point we acknowledge that the existence of other dissipation mechanisms is

**Figure 20.** Absolute (top) and normalized (bottom) fluorescence excitation spectra of *P. Sulcata*, before and after addition of DCMU. WT refers to measurements performed without DCMU.
are likely. The adjacent CAC complexes may provide a route for the excitation to dissipate to where it would then undergo NPQ.

Looking first at the excitation spectra at 645 nm, corresponding to the CAC complex, we see that the signal arises from PE545 with little contribution elsewhere. In both the absolute and normalized spectra, there is no change in the fluorescence intensity or relative shape of the spectra. This assures us that the DCMU has had no noticeable effect on the energy transfer from the thylakoid lumen to the CAC complex. Both PSII and PSI show a greatly increased signal in the absolute fluorescence spectrum with PSII showing the greatest change as expected. The normalized spectrum reveals that the shape of the excitation spectrum has changed slightly, with less contribution coming from the PE545 region. We can immediately note that this will lower the apparent energy transfer efficiency when compared to the absorption spectrum.

\[ \varepsilon = \frac{J_{685} + PQ + qH2}{J_{590} + J_{685} + qH1 + qH2 + PQ} \]

Figure 21.) Schematic for efficiency calculation. J545 is the absorbed photon flux at 545 nm. J590 is the fluorescence from PE545. ET1 is energy transfer from PE545 to the CAC complex, qH1 is thermal dissipation from the CAC complex. ET2 is energy transfer from the CAC complex to a photosystem (PSII). J685 is fluorescence from PSII. qH2 is thermal dissipation from PSII. PQ is photochemical quenching at PSII. If NPQ is deactivated (dark adapted) then qH1 = qH2 = 0. With DCMU, PQ = 0.
Figure 22.) ChlA (top), ChlC (middle) s2 band, and carotenoid (bottom) absolute emission spectra before and after addition of DCMU
The emissions spectra from 440, 460 and 490 nm, which correspond to exciting the s2 band of ChlA, ChlC carotenoids, show a massive increase in fluorescence from PSII and PSI after addition of DCMU. Over the course of the three weeks, the PSII-PSI emission ratio stays approximately the same. The small emission signal at 590 nm arises from exciting the far blue end of the absorption spectrum of PE545 which becomes much more significant in the 490 nm excitation. The PE545 emission spectra show that PE545 emission is not changed with the addition of DCMU however the fluorescence from PSII and PSI are greatly increased. The photosystem fluorescence intensity from excitation of PE545 is greater than the intensity when chlorophyll is directly excited evidencing the utility of the PE545 as an antenna.

Figure 23.) PE545 (top and bottom) absolute and normalized emission spectra before and after addition of DCMU.
The emission spectra of the control colony all show the same gradual increase in both the PE545 and PSII emission intensity which then decreases as the colony ages. However the PE545 intensity peaks around Day 11 whereas the PSII intensity peaks around Day 16 before leveling off. The DCMU samples all show a significantly increases emission from PSII and PSI with almost identical emission from PE545 as the control group.

Figure 24.) Comparison of PE545 (top) and PSII (bottom) fluorescence with and without DCMU over colony lifetime.
**P. Sulcata** Normalized Absorption and PSII Excitation Spectrum

|----------------|----------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|

**P. Sulcata** Normalized Absorption and PSII Excitation Spectrum with DCMU

|----------------|-----------------|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|

**P. Sulcata** PE545 - PSII Energy Transfer Efficiency

- PSII-WT
- PSII_DCMU

Figure 25.) *P. Sulcata* Energy Transfer from PE545 to PSII, taking into account PSII photochemical quenching by addition of DCMU
Figure 26.) *P. Sulcata* Energy Transfer from PE545 to PSI, taking in to account PSII photochemical quenching by addition of DCMU
Examining the excitation spectra and corresponding energy transfer efficiencies we see that in the control, PSII at PSI start at a high efficiency, decreases over the next week only to peak again on Day 18 where is finally drops a second time. The DCMU samples show a gradual increases peaking around Day 20 when it starts to drop off again. Having an excess of PBP in the thylakoid may be disadvantageous to the organism if we consider only the transfer efficiency. If we consider our hypothetical partitioning of the PE545 in the thylakoid into membrane associated and luminal PBP, the membrane associated PE545 makes up a smaller portion of the total PBP. Increasing the production of PBP would then increase the path length of an excitation captured in the lumen by adding more PBP in this region, thus a greater chance of fluorescence quenching before reaching the membrane.

Comparing the emissions spectra trends we see that the maximum fluorescence from PSII and PE545 correspond to the lowest transfer efficiency. This is consistent with our hypothetical model as more photons are fluorescing from the thylakoid lumen without subsequent reabsorption in the membrane.

DCMU is effective in blocking the transport of excitations into the ETC from PSII, thus lowering the apparent energy transfer efficiency in both photosystems. This can be used to estimate the contribution of photochemical quenching from PSII through the quinone pool but provides no information about alternate routes out of PSII. More study is required to determine if DCMU is in fact completely shutting off PQ at PSII or if the dark adapted quinone pool is able to buffer a significant number of excitations before shutting down. The contribution of PQ from PSI also requires investigation to more completely shut down all routes to the ETC.
Chapter 4

Conclusions

This thesis studied the light harvesting complex of the Cryptophyte algae and the efficiency and robustness of its light harvesting complex. The Cryptophytes are unique in their photosynthetic architecture, consisting of free floating phycobiliproteins in the thylakoid lumen, membrane bound CAC complexes, and PSI and PSII. Four species of algae were investigated in this study, *Proteomonas Sulcata* (PE545), *Rhodomonas Minutae* (PE545), *Hemiselmis Pacifica* (PC577), and *Chroomonas Mesostigmatica* (PC645). Basic spectroscopic techniques such as linear absorption, and fluorescence emission and excitation were used to calculate the energy transfer efficiency from the phycobiliproteins in the luminal space to the photosystems. Techniques from chlorophyll fluorescence transient studies were later used to gain further insight in to the many different fates a photon may succumb to once inside the light harvesting complex.

Chapter 1 introduced photosynthesis and the variety of different ways in which nature has devised a means to harness solar light energy and how the rules that guide biological development differ significantly from how we might engineer a device in the lab. An overview of spectroscopic techniques and how they would be used to determine energy transfer efficiency was also introduced.

Chapter 2 investigated the normal growth conditions of *P. Sulcata* and *R. Minutae* to determine a reference point to compare efficiencies under nonstandard conditions and to determine the best method for measuring the necessary spectroscopic parameters. Simple consideration of donor-acceptor energy transfer within the Cryptophyte leads to transfer efficiencies greater than 100%. Since this is not physical, we are forced to consider a more complex model that includes alternate pathways for an excitation between the thylakoid lumen and photosystems.

Chapter 3 sought to determine the degree that non-photochemical and photochemical quenching played in energy transfer and whether or not it was a significant contributor the apparent greater than 100% energy transfer. *H. Pacafica* and *C. Mesostigmata* were studied here
as well to compare phycocyanin containing species to phycoerythrin containing species. The two containing phycocyanin have an additional complication in determining energy transfer efficiency as there is significant spectral overlap between the phycocyanin and CAC complex. High light exposed samples only showed a marginal decrease in fluorescence intensity compared to the dark, low and regular light samples in all four species. It is likely that the high light samples would not have shown significant effects due to non-photochemical quenching until a photon flux of 600 μmol m⁻² s⁻¹. When exposed to DCMU, P. Sulcata showed a significant change in its fluorescence emission and excitation spectrum. PSII and PSI both showed increased emission intensity while emission from PE545 remained the same. The excitation spectra from PSII and PSI also show a decreased signal from the PE545 region which corresponds to a significantly decreased energy transfer efficiency. However this was still not enough to extract a physically realistic number for the energy transfer efficiency.
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