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Investigation of the Effect of Xanthophyll Composition on Energy Transfer and Quenching in Light-Harvesting Complexes from the Diatom, Phaeodactylum tricornutum

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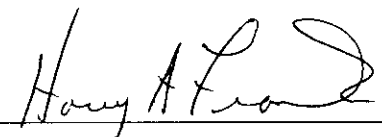
Major Advisor 

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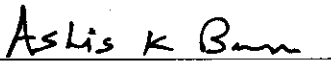
Associate Advisor 

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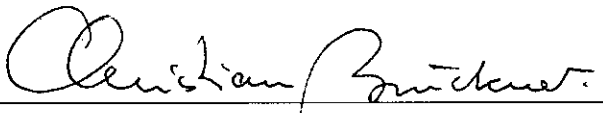
Associate Advisor 

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Introduction

Photosynthetic organisms contain light-harvesting, also called antenna, pigment-protein complexes that capture light energy and channel it to the reaction center to be converted into electrical potential.[1-7] Excess energy beyond that required for photosynthesis must be deactivated because energy not trapped by the reaction center may result in the formation of chlorophyll (Chl) triplet states which can sensitize the formation of singlet oxygen, a powerful oxidizing agent of Chl.[8-12] The dissipation of excess light energy can be measured by monitoring Chl fluorescence originating from the antenna and examining its extent of quenching under different physical and chemical conditions. The dissipation of excess energy is referred to as nonphotochemical quenching (NPQ), and xanthophyll pigments are involved in the process.[13-19]

There are several different components of NPQ, however the major part of it is denoted qE.[19-2122-26] qE is induced by high intensity illumination which results in acidification of the thylakoid lumen, the formation of a trans-membrane ΔpH,[27] and the enzymatic de-epoxidation of violaxanthin to zeaxanthin via the so-called "xanthophyll-cycle" also known as the "violaxanthin cycle" (Figure 1).[20,28-35] qE is also correlated with the presence of a light-harvesting protein denoted, PsbS[19,36-38] and recent studies have sought to understand its precise role in NPQ.[38-49] Thus, sSeveral correlations exist between qE and various molecular parameters, but the site of qE activity has not yet been identified, nor has the molecular mechanism of nonradiative energy dissipation been elucidated.

In higher plants, the focus of the investigation into the molecular mechanism of qE quenching is on the light-harvesting pigment-protein complexes associated with Photosystem II (PSII)[50-53] where the majority of the xanthophyll cycle carotenoids are found[54,55] This suggests that the site of qE quenching is located within these proteins. In many species of microalgae, of which the diatom *Phaeodactylum (P.) tricornutum* is a good example, the xanthophyll, diadinoxanthin, is converted into diatoxanthin via a one-step de-epoxidation reaction known as the "diadinoxanthin cycle" (Figure 2) and this may provide the same function in terms of dissipation of excess excitation energy in algae as the two step de-epoxidation of violaxanthin into zeaxanthin does in higher plants.[56-60] Some algae exhibiting the diadinoxanthin cycle may also possess the violaxanthin cycle, which is only visible when the algae are exposed to prolonged periods of light stress.[61] The violaxanthin cycle pigments are biosynthetic precursors of the diadinoxanthin cycle pigments, diadinoxanthin and diatoxanthin, and fucoxanthin.[61,62] The de-epoxidation rate of diadioxanthin is four times faster than violaxanthin, whereas the re-epoxidation rate is about the same for both xanthophyll cycles.[61]

In order to evaluate the molecular factors controlling light-harvesting and NPQ, it is important to understand the electronic nature of the excited states of xanthophylls. Based on years of optical spectroscopic studies of polyenes, carotenes, and xanthophylls, it is known that many of the spectroscopic properties of these molecules can be described using the energy level diagram shown in Figure 3.[63-68] The first excited singlet state is denoted S_1 or $2^1A_g^-$. Direct excitation into S_1 from the ground state, S_0 or $1^1A_g^-$, by one-photon absorption is forbidden by symmetry. The second excited singlet state is denoted

S_2 or $1^1B_u^+$ and is responsible for the major visible absorption and strong coloration characteristic of all long-chain conjugated polyenes.[69-74]

There have been two hypotheses proposed to explain the molecular basis of qE: Direct and indirect quenching. In the direct quenching model, the S_1 state of longer, more conjugated xanthophylls (zeaxanthin or diatoxanthin, Figures 1 and 2) formed upon de-epoxidation under high-light stress, is low enough to accept energy from Chl thereby acting as a trap for excess excited state energy of Chl. This would provide photoprotection as well as a means of regulating the flow of energy in the antenna.[73-75] This model was first suggested by Demmig-Adams[33] and Owens[76] and gained support from determinations of the energies of the low-lying S_1 states of xanthophylls, polyenes, and carotenoids.[75,77-81] This scheme, termed the "molecular gear shift mechanism",[77] requires that it be energetically feasible for the S_1 state of zeaxanthin or diatoxanthin to deactivate the Chl excited singlet state. In contrast, the higher S_1 energies of violaxanthin and diadinoxanthin might render it incapable of quenching Chl fluorescence, but perhaps still effective at light-harvesting (Figure 4).

Some reports indicate direct quenching of Chl excited states by xanthophylls may not occur by energy transfer at all but rather by electron transfer.[82-85] This evidence has emerged from femtosecond time-resolved spectroscopic investigations carried out on thylakoid membranes from spinach and *Arabidopsis thaliana* by Holt *et al*.[86,87] These authors observed a xanthophyll radical cation signal in the near infrared (NIR) region and correlated it with the presence of the PsbS protein. Then they proposed that excess excitation is quenched via excitation transfer from bulk antenna-bound Chl to a special zeaxanthin-Chl (ZeaChl) heterodimer which undergoes ultrafast (0.1 to 1 ps) electronic

charge separation to form a $Zea^{+}Chl^{-}$ radical pair.[87] Xanthophyll radical cations are thought to be efficient quenchers of Chl fluorescence suggesting they may be involved in qE in both major and minor antenna complexes.[87-89]

The indirect quenching model has been advanced largely by Horton and co-workers[22-24,90-92] who have argued that xanthophylls exert control over the aggregation state of the antenna complexes which leads to quenching. Experiments carried out on isolated complexes have supported the hypothesis that zeaxanthin promotes aggregation of antenna complexes whereas violaxanthin has an opposite effect. In this model, either the xanthophylls or Chl *a* aggregates (or both) may be the quenchers, and the role of the xanthophylls is thought of as allosteric, affecting regulation of energy flow via modulation of the architecture of the overall protein complex.[92-97]

In fact, neither the direct nor indirect quenching mechanisms have been proven unequivocally. Thus, further studies of the energy transfer properties of pigment-protein complexes containing different compositions of xanthophyll cycle carotenoids are needed.

Many of the antenna proteins found in green plants and algal systems are similar by virtue of their being integral to the membrane, but their pigment composition can be completely different.[98] Many algal systems, including diatoms, bind Chl *a*, but instead of Chl *b*, they have Chl *c* as a secondary accessory pigment.[98] Also their xanthophyll composition may be different. Instead of lutein, neoxanthin and violaxanthin which are the major xanthophylls in the light-harvesting complexes (LHCs) of green plants, diatoms synthesize fucoxanthin and diadnoxanthin.[99] Fucoxanthin is used by diatoms to improve the efficiency of light capture in the visible wavelength region between 460 nm to 570

nm in the marine environment. The high stoichiometric amounts of fucoxanthin are the reason some antenna proteins from diatoms are termed fucoxanthin-chlorophyll proteins (FCPs). In addition, the Chl-to-xanthophyll stoichiometric ratios of LHCs and FCPs differs. Whereas green plant LHCs bind more Chls than xanthophylls, FCPs exhibit an approximate 1:1 ratio of these pigments.[99-101] Also, the relative amount of Chl *c* in FCPs is usually smaller than that of Chl *b* in LHCs.[102]

For the work described in this thesis, whole cells of the diatom, *P. tricornutum* (Figure 5) were subjected to either low-light (LL) or high-light (HL) illumination conditions to affect the xanthophyll cycle pigment composition. Then, using a protein isolation procedure that employed β-dodecyl-maltoside detergent to fractionate the complexes, followed by sucrose density gradient ultracentrifugation purification,[57] two major protein bands denoted D and F were collected. The pigment compositions of the bands were analyzed using high-performance liquid chromatography (HPLC). The protein composition was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fucoxanthin was found to be the major carotenoid in all the pigment-protein complexes. The xanthophyll cycle carotenoids, diadinoxanthin and diatoxanthin, were also present, but in lower amounts than fucoxanthin. Diadinoxanthin and diatoxanthin were found in higher amounts in the D band compared to the F band as previously reported.[57] Also, the amount of diatoxanthin was over an order of magnitude larger in the complex prepared from the high-light stressed cells. Steady-state and fast transient absorption and fluorescence spectroscopic experiments were carried out on the isolated pigment-protein complexes to evaluate the role of the protein-bound xanthophylls in light-harvesting and fluorescence quenching. The overall objectives of

the work are to: (1) Examine the differences in the steady-state and transient spectroscopic features of pigment-protein complexes isolated from LL and HL cells of *P. tricornutum*; and (2) Explore the effect of xanthophyll composition on the dynamics and efficiency of light-harvesting and excited state quenching in the pigment-protein complexes.

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Figure 1. The xanthophyll cycle in higher plants whereby violaxanthin and zeaxanthin are enzymatically interconverted under different conditions of illumination.

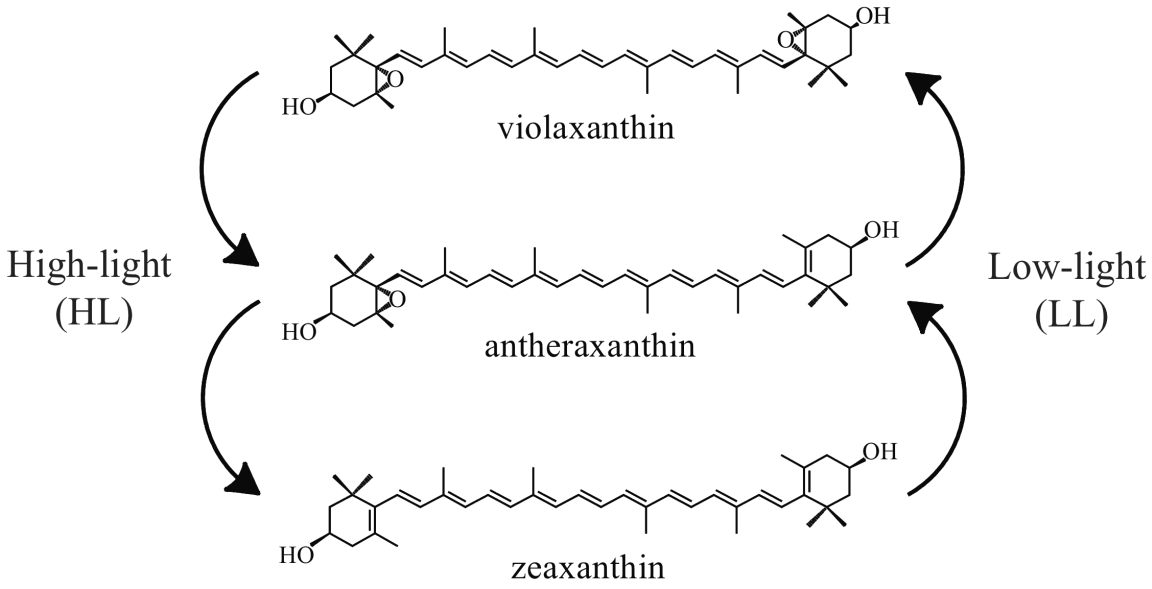

High-light
(HL)
violaxanthin
antheraxanthin
zeaxanthin
Low-light
(LL)
HO
OH
O

Figure 2. The xanthophyll cycle in algal systems whereby diadinoxanthin and diatoxanthin are enzymatically interconverted under different conditions of illumination.

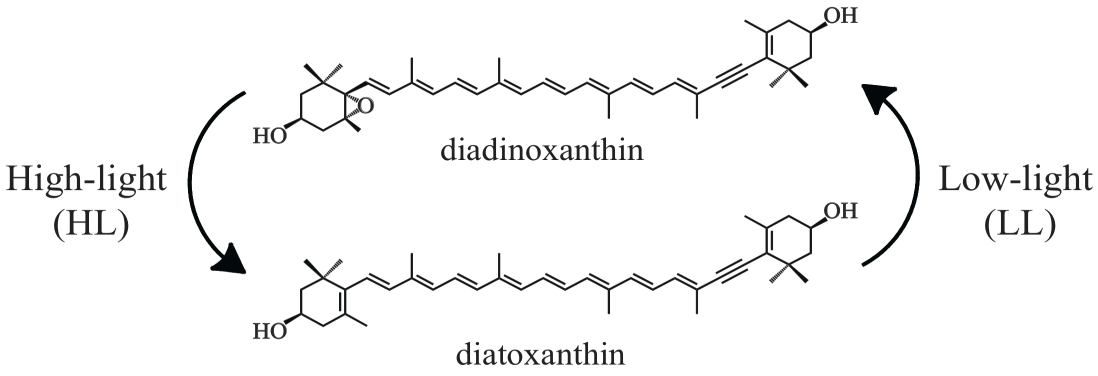

High-light
(HL)
Low-light
(LL)
HO
O
OH
diadinoxanthin
HO
OH
diatoxanthin

Figure 3. The state designations and symmetry assignments of the low-lying singlet states of polyenes, carotenes, and xanthophylls. a, absorption; f, fluorescence; ic, internal conversion.

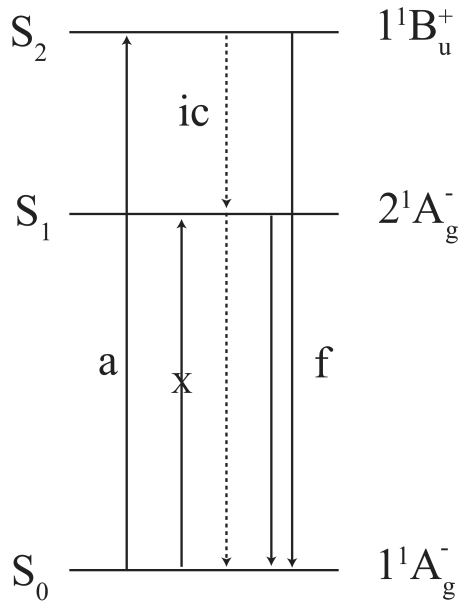

S2
1¹B+u
ic
S1
2¹A-g
a
x
f
S0
1¹A-g

Figure 4. The energy states of zeaxanthin and violaxanthin relative to Chl *a*. The figure represents a potential mechanism for zeaxanthin-mediated Chl fluorescence quenching and violaxanthin-mediated light-harvesting.

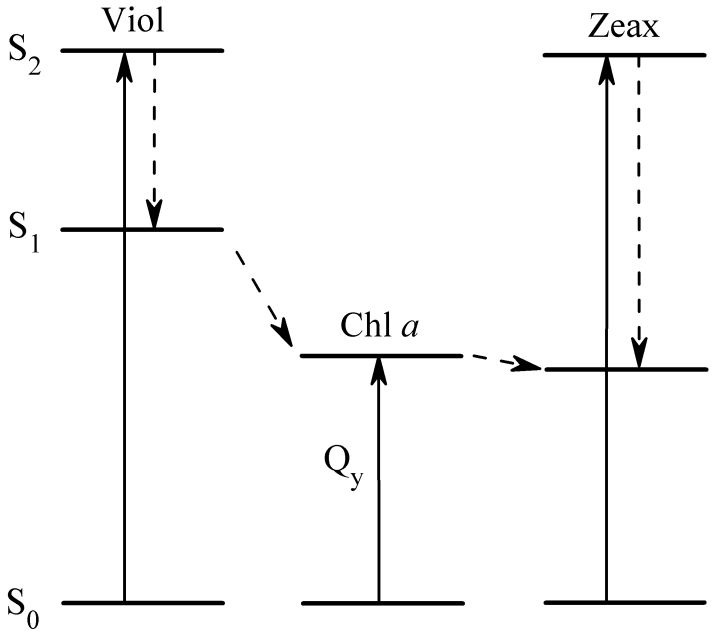
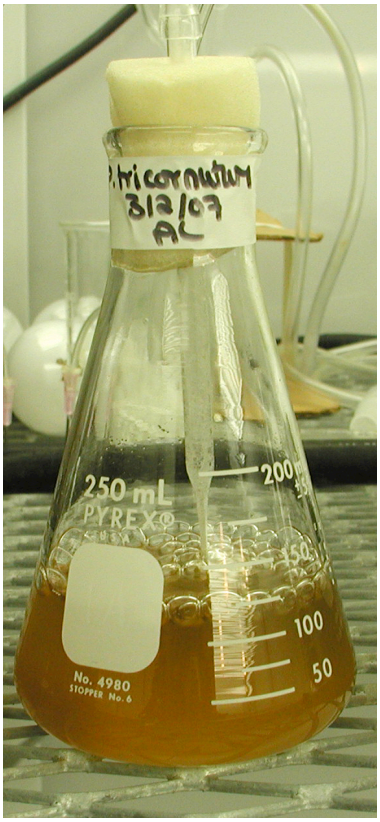

Viol
Zeax
S2
S1
Chl a
Qy
S0

Figure 5. Culture of *P. tricornutum* grown in our laboratory.


P.tricornutum
312/07
AC
250 mL
PYREX
200
150
100
50
No. 4980
STOPPER No. 6

Chapter II

Materials and Methods

2.1 Cell culture growth

Cells of the marine diatom, *Phaeodactylum tricornutum* (Bohlin, CCMP 1327), were grown in f/2 media as previously described.[1] Briefly, the cells were grown in 250 mL Erlenmeyer flasks under low-light (LL) conditions (12 μmol photons m^{-2} s^{-1}) and harvested during the exponential growth phase. In order to induce formation of diatoxanthin, a portion of the cells were transferred to a shallow Pyrex dish and subjected to high-light (HL) exposure (430 μmol photons m^{-2} s^{-1}) for a period of 3 hr using a Cary Model 1471200 tungsten-halogen light source positioned 30 cm above the surface of the liquid culture which was gently and continuously stirred.

The treatment with HL initiated the enzymatic conversion of diadinoxanthin to diatoxanthin.[2] Twenty-four hours prior to HL treatment, 20 mg/L of $CdCl_2$ was added to inhibit diatoxanthin epoxidase activity and prevent the back-conversion of diatoxanthin to diadinoxanthin after the high-light stress period.[3] During HL exposure, aliquots of the culture were taken every hour to monitor the pigment composition and assess the conversion of diadinoxanthin to diatoxanthin via high-performance liquid chromatography (HPLC, see below). The temperature of the growth chamber was kept between 30-34ºC. The temperature of the media was monitored to ensure it did not exceed 35ºC.

2.2 Preparation of thylakoid membranes

The whole cells were pelleted by centrifugation at 3,000 x *g* for 5 min at 4ºC using an SS34 rotor in a Sorvall RC-5B Superspeed centrifuge. Subsequently, the cells were resuspended in a small amount of 15 mM Hepes, 5 mM EDTA, pH 7.5, buffer. Thylakoids were isolated using a procedure similar to that previously described[4] in which the resuspended cells were passed 3 times through a French press at 3,000 psi and spun at 800 x *g* in the SS34 rotor for 5 minutes at 4ºC to remove debris and unbroken cells. The supernatant was then centrifuged for 1 hr at 180,000 x *g* at 4ºC using a 55.2 Ti rotor in a Beckman L8-55M ultracentrifuge. The pelleted thylakoid membranes were then washed twice using the suspension buffer and concentrated.

2.3 Isolation of the pigment-protein complexes

Freshly prepared thylakoids were adjusted to a chlorophyll (Chl) *a* concentration of 0.100 mg/mL using the suspension buffer and solubilized using 10 mM β-dodecyl-maltoside (β-dodecyl-maltoside/Chl *a* 51:1, w/w) by stirring the sample for 20 minutes on ice. The Chl concentration of the thylakoids was determined spectrophotometrically using a previously reported protocol in a 1 cm path length quartz cuvette in acetone 90% (v/v).[5] Subsequently, the sample was loaded onto centrifuge tubes containing continuous sucrose density gradients prepared by freezing (to -20ºC) and thawing (to 4ºC) solutions containing 0.5 M sucrose,[6] in 50 mM Hepes, 5 mM EDTA, 0.3% (w/v) β-dodecyl-maltoside, pH 7.5.[7] Prior to freezing and thawing, the tubes containing the sucrose density gradients were placed in a vacuum dessicator for 1 hr to eliminate dissolved gases that could cause bubbles to form during centrifugation. Ultracentrifugation of the

samples on the sucrose density gradients was carried out for 20 hr at 285,000 x *g* at 4ºC using an SW 40 Ti rotor in the ultracentrifuge.[7]

Two major bands were resolved on the sucrose density gradients for both the LL- and HL-treated cells. These bands were harvested using a syringe and were labeled D for the less dense band and F for the more dense band as denoted in the literature.[7]

2.4 Pigment Analysis

The pigment composition of the whole cells before and after high-light treatment was analyzed using aliquots of both the LL and HL cultures spun at 3,000 x *g* for 5 minutes at 4°C in the SS34 rotor to pellet the cells. The pigments were extracted from the cells using 3 mL of methanol:acetone (1:1, v/v). The solvent was removed from the extract using a gentle stream of nitrogen gas, and the dried pigments were re-dissolved in acetonitrile for injection into a Waters 600E multi-solvent delivery system HPLC equipped with a 2996 Waters photodiode array detector. The HPLC protocol used a Nova-Pak C_{18} column (3.9 x 300 mm, 4 µm particle size) with a mobile phase consisting of 99% solvent A (acetonitrile:methanol:water, 87:10:3, v/v/v) with 1% solvent B (100% ethyl acetate) run in isocratic mode for 10 minutes. Then, from 10 min to 40 min the mobile phase was run in linear gradient mode whereby solvent A was decreased to 60% and solvent B was increased to 40%. From 40 to 50 min the mobile phase was held at 60% solvent A and 40% solvent B.

Aliquots of broken cells obtained from the French pressure disruption were analyzed in a similar fashion to verify the pigment composition. After disruption, 3 mL of the supernatant from the slow speed spin were extracted using 6 mL of

acetone:methanol:methyl-*tert*-butyl ether (MTBE) 1:1:2 (v/v/v) and dried using a gentle stream of nitrogen gas. Once again, the dried pigments were re-dissolved in the HPLC-injection solvent, acetonitrile, for analysis using the HPLC protocol describe above. Also, to assess the carotenoid composition of isolated thylakoids, the same extraction and HPLC analysis procedures were carried out after ultracentrifugation before the detergent treatment.

The pigment composition of the D and F bands isolated from the sucrose density gradient ultracentrifugation were analyzed using 1 mL aliquots dialyzed for 18 hours against 3 L of a buffer consisting of 10 mM Hepes, 5 mM EDTA, 0.3% (w/v) Triton X-100, pH 7.5, to remove the sucrose. The dialyzed samples were lyophilized for 24 hr and extracted using acetone. The solvent was then removed using a gentle stream of nitrogen gas and the residue was re-dissolved in acetonitrile for injection into the HPLC.

2.5 Gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a Hoefer SE 600 vertical electrophoresis unit (Amersham Pharmacia Biotech). The procedure used 30 μL samples from protein bands D and F obtained after sucrose density centrifugation of fractionated LL-treated cells. The proteins contained 0.3 μg of Chl *a* and were denatured in a buffer containing 2% SDS, 60 mM Tris, 10% glycerol, 2% mercaptoethanol, and 0.05% (w/v) bromophenol blue at pH 6.8 by heating the sample in boiling water for 2 min and loading the material on the gel as previously described.[8] In addition the same procedure was carried out using 15 μL of protein samples containing 0.2 μg of Chl *a* from detergent-solubilized thylakoid membranes and proteins from band

D and F obtained after sucrose density centrifugation of fractionated LL and HL treated cells, and stained with silver nitrate.

2.6 Spectroscopic analyses

The pigment-protein complexes associated with the D and F bands isolated using sucrose density gradient ultracentrifugation were concentrated using Amicon filters having a protein molecular weight cutoff of 30 kDa,[6] to an optical density (OD) of ~0.3 in a 2 mm path length quartz cuvette at 500 nm for use in the ultrafast time-resolved spectroscopic experiments. For the fluorescence experiments the samples were diluted to an OD of ~0.05 in a 4 mm path length quartz cuvette at 630 nm and then further diluted by a factor of 25 to avoid saturation of the fluorescence detector.

Steady-state absorption spectra were recorded using a Varian Cary 50 UV/Vis spectrometer. Ultrafast time-resolved transient absorption spectroscopic measurements were carried out at room temperature using the laser spectrometer system previously described.[9] Band D isolated from the LL cells was excited at 493 nm, whereas band D from the HL cells was excited at 502 nm. Also, band D from both LL and HL material was excited at 676 nm. Band F from both LL and HL material was excited at 502 nm, 550 nm and 676 nm.[10] The pump laser beam had an energy of 0.5 μJ/pulse corresponding to an intensity of ~1.3 x 10^{14} photons cm^{-2} $pulse^{-1}$ at 500 nm and intensity of ~1.7 x 10^{14} photons cm^{-2} $pulse^{-1}$ at 676 nm for the experiments carried out on band D prepared from both LL and HL cells. For the experiments on the F band from both LL and HL cells, the pump laser beam had an energy of ~0.6 μJ/pulse corresponding to an intensity of ~1.5 x 10^{14} photons cm^{-2} $pulse^{-1}$ at 502 nm, an intensity of ~1.7 x 10^{14} photons cm^{-2} $pulse^{-1}$ at

550 nm, and an intensity of ~2.0 x 10^{14} photons cm^{-2} $pulse^{-1}$ at 676 nm. Steady-state absorption spectra were recorded before and after each transient absorption measurement to ensure sample integrity. Surface Xplorer Pro (v.1.0.6) software was used for the chirp correction, and ASUfit version 3.0 software was used for the global analysis of the transient absorption spectroscopic data.

Fluorescence spectra were recorded at room temperature using a Jobin-Yvon Horiba Fluorolog-3 Model FL3-22 equipped with excitation and emission double monocromators having 1200 grooves/mm gratings. Excitation was provided by a 450 W ozone-free Osram XBO xenon arc lamp. A Hamamatsu R928 photomultiplier was used for detection. Fluorescence spectra were detected at a right angle relative to the excitation beam using an excitation monochromator slit width corresponding to a 5 nm bandpass and an emission monochromator slit width corresponding to a 2 nm bandpass. Excitation spectra were recorded using an excitation monochromator slit width corresponding to a 2 nm bandpass and an emission monochromator slit width corresponding to a 5 nm bandpass. Time-resolved fluorescence decays were recorded using a Jobin-Yvon Horiba NanoLED-670L pulsed diode light source having a peak wavelength of 665 nm, a repetition rate of 1 MHz, a pulse duration of < 200 ps. Decay analysis software version 6 (DAS6) was used to fit the transient data to a sum of exponentials function. The spectra of the various fluorescence components were obtained from plots of the pre-exponential factors of the transient responses detected from 675 to 700 nm in increments of 5 nm.

2.7 References

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Chapter III

Results and Discussion

3.1 Results

3.1.1 Pigment composition

3.1.1.1 Whole cells

High performance liquid chromatography (HPLC) of the pigment extracts from LL- and HL-treated *Phaeodactylum* (*P.*) *tricornutum* cells were carried out to assess the relative concentrations of the xanthophylls, fucoxanthin, diadinoxanthin, and diatoxanthin, whose structures are shown in Figure 1. Chls *a*, *c* and β-carotene were also found in the extracts from the cell samples.[1,2] No diatoxanthin was found in the cultures grown under LL conditions, but the mole ratio of diatoxanthin to diadinoxanthin was found to be ~1:1 in cells that were subjected to HL, as shown in Figure 2 and reported in Table 1.

3.1.1.2 Thylakoids

The pigment composition of the HL-treated thylakoids was determined after French pressing the cells and again after ultracentrifugation to ensure that the HL-induced conversion of diadinoxanthin into diatoxanthin was maintained. The pigment composition of HL-treated thylakoids was 61 ± 8% fucoxanthin, 3 ± 1% diadinoxanthin, 5 ± 1% diatoxanthin, 30 ± 8% Chl *a*, and 2 ± 1% β-carotene. No Chl c was detected.

These values are within experimental error of those obtained from HL-treated whole cells given in Table 1. Moreover, the extract of thylakoids prepared from HL-treated cells was found to have a fucoxanthin to Chl *a* ratio of ~2:1 which is not too far from the value of 1.5:1 found from the extract of the HL treated whole cells (Table 1). This indicates that the pigment composition was not significantly altered during French pressure disruption or ultracentrifugation.

During initial attempts to prepare thylakoids, it became apparent that the diatoxanthin produced in the HL-treated cells was rapidly converting back into diadinoxanthin during the preparation. To inhibit this back conversion, 20 mg/L of $CdCl_2$ was added as a solid directly to the culture of *P. tricornutum* 24 hr prior to HL treatment.[3] The efficacy of $CdCl_2$ treatment was confirmed by extracting and analyzing aliquots of $CdCl_2$-treated, HL-illuminated whole cells hourly for a 5 hr period after illumination. It was found that the addition of $CdCl_2$ in combination with keeping the cells at 4ºC delayed the back conversion for a period of ~2 hr which was sufficient time for the preparation of the thylakoids without significantly reducing the diatoxanthin content.

3.1.1.3 Pigment-protein complexes

Figure 3 shows the results from ultracentrifugation of detergent-solubilized thylakoid membranes. Two bands were observed and labeled D and F as in previous work.[2] Band F appeared dark brown in the centrifuge tube, while band D appeared green. HPLC chromatograms of the pigments extracted from the isolated bands are shown in Figure 4, and their pigment compositions are given in Table 1. Fucoxanthin was the major pigment in all cases. (See Table 1.) Diatoxanthin was present in a larger amount

in the pigment-protein complexes isolated from cells exposed to HL conditions. Chl *a* was present in all bands, but was more abundant in band F than in band D.

3.1.2 Polypeptide composition

The results of SDS-PAGE of the isolated pigment-protein complexes in bands D and F obtained from cells grown under LL conditions are presented in Figure 5. The gels were stained with either coomassie blue (Fig. 5A) or silver nitrate (Fig. 5B) and show that a single prominent protein band having an effective molecular weight of ~18.5 kDa is common to both bands D and F from the ultracentrifugation. As reported previously,[2] band D was resolved on the gel as a doublet. This could be attributed to different conformers of the protein which is not entirely denatured during SDS treatment, or to a heterogeneous protein mixture.

3.1.3 Steady-state absorption

Figure 6 shows the steady-state absorption spectra of the isolated pigment-protein complexes normalized to the Chl *a* Soret band at ~440 nm. Spectral features that were observed for band D include the Soret and Q_y band of Chl *a* which appeared at 442 nm and 673 nm, respectively. For band F the Soret and Q_y band of Chl *a* were found at 441 nm and 672 nm, respectively. These differences are seen more clearly in the top panel of Figure 7 and can be attributed to distinct protein environments of Chl *a* in the two different complexes that results in a slight shift of their Q_y bands. Although Chl *c* was barely evident from the HPLC analysis, a small amount of the absorption near 630 nm may be attributed to this pigment. Carotenoid absorption bands appear between 480 and 570 nm.[4-6] As seen clearly in the insert in Figure 6, the absorption spectra of band F taken

from both LL and HL samples show significantly more intensity between 500 and 570 nm compared to band D from both LL and HL samples. This additional absorption in this wavelength region explains why band F appears brown and much darker than band D in the ultracentrifuge tube (Figure 3).

3.1.4 Steady-state fluorescence

The bottom panel of Figure 7 shows the Chl *a* emission spectra of bands D and F from complexes prepared from LL and HL cells. The maxima in the fluorescence spectra were found to be 676 nm for band F and red-shifted to 679 nm for band D. This is consistent with the differences in their absorption bands (Figures 6 and 7) that can be attributed to distinct protein environments of Chl *a* in the two different complexes. Also, a low-energy vibrational band was observed at 740 nm in the spectra of all of the isolated pigment-protein complexes.[4] There was no observable difference in the position of these bands between the LL and HL samples.[6,7]

Figure 8 shows fluorescence excitation spectra of the samples monitored at 730 nm overlaid with their respective 1-minus-transmission (1-T) spectra of the isolated pigment-protein complexes. The excitation feature between 480 and 560 nm in the carotenoid absorption region for band F (bottom figures) indicates energy transfer from the carotenoids to Chl *a*.[2] This feature is absent for band D (upper figures) indicating no energy transfer from the carotenoids to Chl *a* is taking place.

3.1.5 Time-resolved fluorescence

Decay-associated spectra generated from the time-resolved fluorescence data of the pigment-protein complexes associated with bands D and F prepared from LL and HL cells are given in Figure 9. Two kinetic components having lifetimes of 5.5 ± 0.2 ns to 6.1 ± 0.2 ns and 1.4 ± 0.7 ns to 2.9 ± 0.5 ns were found. The kinetic component with the longer lifetime has a maximum at 676 nm for band F and at 679 nm for band D. These are the same wavelengths observed for the maxima in the steady-state spectra (Figure 7) indicating that this longer lifetime component is attributable to Chl *a*. The similarity in the values of the lifetimes and amplitudes between the LL and HL samples suggests that the rate of Chl *a* excited state population decay is not dependent on the xanthophyll composition. The kinetic component with the shorter lifetime has its maximum at a shorter wavelength than 675 nm in all cases, and has a slightly higher amplitude in band F compared to band D. This component may be attributable to fluorescence from Chl *c*.

3.1.6 Transient absorption spectra

Figure 10 shows the transient absorption (TA) spectra recorded at various time delays after excitation at 493 nm for band D (LL), at 502 nm for band D (HL) and band F (LL and HL), and at 550 nm for band F (LL and HL). The excitation wavelength was chosen to excite the carotenoids diadinoxanthin, diatoxanthin, and a short wavelength-absorbing fucoxanthin. The pump laser tuned to 550 nm results in the excitation of a longer wavelength-absorbing fucoxanthin exclusively.[4,8-10] Spectra obtained by exciting at 493 nm or 502 nm show band structure between 530 and 580 nm typical of $S_1 \rightarrow S_n$ transitions of xanthophylls.[11-13] The spectra in this region from the HL samples from both

bands D and F were significantly broader and had prominent intensity at longer wavelength compared to the spectra from the LL samples. This broadening is undoubtedly due to the presence of a substantial amount of diatoxanthin in the HL samples (Table 2) which has a longer π-electron chain length than diadinoxanthin (Figure 1) and therefore, upon excitation, is expected to display a red-shifted $S_1 \rightarrow S_n$ absorption band compared to that seen in the LL samples. Also, all of the spectra show bleaching of the $S_0 \rightarrow S_2$ absorption of the xanthophylls and, at early times, stimulated emission from S_2. Spectra from band F (LL and HL) taken after excitation at either 502 or 550 nm show bleaching of the Q_y band of Chl *a* at 676 nm which was not observed in spectra taken from band D (LL and HL). This clearly shows that carotenoid-to-Chl energy transfer does not occur in the pigment-protein complex associated with band D, consistent with the results of the steady-state fluorescence excitation measurements (Figure 8). Also, TA spectra from band D taken after excitation into the carotenoid region at 493 nm (LL) or 502 nm (HL) (top panels in Figure 10), and TA spectra taken from band F after excitation into the carotenoid region at 502 (LL and HL) (middle panels in Figure 10) show a strong positive feature in the 550 to 600 nm region associated with the $S_1 \rightarrow S_n$ transition of carotenoids. However, TA spectra from band F taken after excitation into the lowest energy absorbing fucoxanthin at 550 nm (lower panels in Figure 10) lack this feature. The fact that an $S_1 \rightarrow S_n$ absorption band is not observed after excitation of the long wavelength-absorbing fucoxanthin in band F, but bleaching of the Chl Q_y band is observed, indicates that energy transfer from the S_1 state of fucoxanthin to Chl in band F is nearly 100% efficient. This same conclusion is evident from the results of the steady-state fluorescence excitation experiments shown in Figure 8 where good agreement is

seen between the 1-T and the fluorescence excitation spectra near 550 nm for band F, but the agreement diminishes substantially at shorter wavelength. The fact that carotenoid $S_1 \rightarrow S_n$ absorption bands are seen in the TA spectra upon excitation of the short wavelength-absorbing xanthophylls in bands D and F indicates that energy transfer to Chl from the S_1 states of these carotenoids is less than 100% efficient.

Figure 11 shows TA spectra taken from bands D and F (LL and HL) after excitation of the Q_y band of Chl *a* at 676 nm. All TA spectra taken with this excitation show an immediate onset of Q_y band bleaching that persists for more than hundreds of picoseconds. Besides this bleaching, spectra of both band D samples (LL and HL, top panels in Figure 11) show a weak, positive signal between 500 and 600 nm that rises slightly to shorter wavelengths. This feature is not observed in the TA spectra of band F (bottom panels in Figure 11). The fact that the signal is relatively long-lived and occurs in this wavelength region suggests that it may be attributed to xanthophyll triplet states formed by triplet energy transfer from Chl *a*. Xanthophylls have been reported to have triplet-triplet absorption bands in this wavelength region.[14,15]

Global analysis of the TA datasets based on a sequential decay model yielded evolution associated difference spectra (EADS) for the spectra taken using different excitation wavelengths. (See Figures 12 and 13.) The TA datasets taken from band D prepared from LL- and HL-treated cells excited at 493 and 502 nm, respectively, required five kinetics components for a good fit (Figure 12 top panels). When these samples were excited at 676 nm, two components were sufficient for a good fit (Figure 12 bottom panels). Figure 13 shows the EADS fits to the TA spectra for band F prepared from LL- and HL-treated cells and excited at 502 nm, 550 nm and 676 nm. For the TA datasets

using 502 nm excitation (top panels in Figure 13), six components were required for a good fit, whereas for the TA datasets generated using 550 nm excitation, three components were required for a good fit (middle panels in Figure 13). For the TA datasets generated using excitation at 676 nm, only two components were needed. All the kinetic components obtained from the global fitting analysis are summarized in Table 2.

Previous experiments characterizing the pigment-protein complexes from the diatom *P. tricornutum* were conducted by Owens and Wold.[1] These authors used 1% Triton X-100 to solubilize thylakoid membranes obtained from whole cells grown under 120 μE m^{-2} s^{-1} of light and reported three bands from sucrose density gradient ultracentrifugation. The less dense band was labeled as a Chl *a*/*c* complex because it contained Chls *a*, c_1 and c_2 in a mole ratio of 1.0:0.23:0.26. No fucoxanthin was detected in this band. The middle band was termed the Chl *a*/*c* fucoxanthin light-harvesting complex and was found to have Chls *a*, c_1, c_2 and fucoxanthin in a mole ratio of 1.0:0.09:0.28:2.22. The most dense band was the P700-Chl *a* protein complex which was enriched in Photosystem I.[1] Previous studies by Guglielmi et al.[2] on *P. tricornutum* plastids obtained from LL treated cells solubilized with α-dodecyl-maltoside reported a ratio of fucoxanthin to Chl *a* of ~1:1.5. In the present work the ratio of fucoxanthin to Chl *a* in LL-grown whole cells was found to be ~1:1 in the LL sample which is reasonably consistent with the findings on plastids, but different from the 1:2.22 ratio reported by Owens and Wold[1] in their work using Triton X-100 to solubilize the thylakoid membranes. This emphasizes the need for careful biochemical characterization of the complexes prior to carrying out spectroscopic investigations.

The diadinoxanthin composition of bands D and F reported here (Table 1) are consistent with previous studies that reported a larger amount of diadinoxanthin in band D isolated from cells grown under LL conditions than in band F from the same cells.[2] Also, it has been reported that band F isolated from cells grown under LL conditions showed a higher Chl *a* content than band D. This is marginally true in the current preparations (Table 1), however, the relative amount of Chl *a* increased substantially in band F from cells exposed to HL conditions. These results and the protein analysis using SDS-PAGE indicate that the pigment-protein complexes prepared here for use in the spectroscopic studies are of high integrity.

No energy transfer was observed from any carotenoids to Chl *a* in band D isolated from cells subjected to either LL or HL conditions. However, energy transfer from carotenoids to Chl *a* was observed in both LL and HL band F preparations. As described by Guglielmi et al.,[2] despite a substantial amount of fucoxanthin being present in band D, the lack of energy transfer in this complex may be attributed to the loss of energy coupling between fucoxanthin and Chl *a*.[2] In a different study carried out by Gildenhoff et al.,[4] a trimer of 18 kDa proteins labeled FCPa and an aggregate of 18 kDa and 19 kDa polypeptides and labeled FCPb were prepared from the diatom, *Cyclotella meneghiniana*. In their work, detergent treatment with β-dodecyl-maltoside was followed by ion exchange chromatography and sucrose density gradient ultracentrifugation. FCPa and FCPb preparations were obtained from cells subjected to LL and HL conditions, and energy transfer from bound carotenoids to Chl *a* was reported in all cases.[4] However, no significant differences in the dynamics of energy transfer or excited state decay were evident between the proteins obtained from the LL or HL material.[4]

The steady-state spectrum of fucoxanthin depends on the protein environment and results in both long wavelength-absorbing (red) and short wavelength-absorbing (blue) fucoxanthin molecules in the pigment-protein complexes.[8,16,17] When 500 nm light is used for excitation, the blue fucoxanthin and the xanthophyll cycle carotenoids, diadinoxanthin and diatoxanthin, are excited. Excitation at 550 nm selectively excites the red fucoxanthin molecule.[8-10]

Global fitting of band D (LL) excited at 493 nm and band D (HL) excited at 502 nm are presented in Figure 12 (top panel). The kinetic components resulting from the fit are summarized in Table 2. The first component has a lifetime of 130 fs for band D (LL) and 170 fs for band D (HL) and contains a number of negative bands ranging from 500 to 600 nm. These are due to combination of bleaching of the ground state absorption bands and stimulated emission from the S_2 state of carotenoid. The second EADS component has a lifetime of 520 fs for band D (LL) and 580 fs for band D (HL) and due to the broadness on the long wavelength side of the band that narrows upon relaxation to the next component can be assigned to vibrationally nonequilibrated (or "hot") S_1 states of the xanthophylls. The next two components from band D (LL) can be attributed to the S_1 states of xanthophylls, one of which has a lifetime of 12.6 ps and can assigned to the S_1 state of the blue fucoxanthin. The other has a lifetime of 34 ps and can be assigned to the S_1 state of diadinoxanthin. The same two components for band D (HL) have lifetimes of 5.2 ps which can be attributed to a mixture of the S_1 state lifetime of diatoxanthin and the blue fucoxanthin. As for the band D (LL) sample, the 34 ps lifetime is associated with the S_1 state of diadinoxanthin which has a similar long-lifetime in solution.[13] The non-

decaying (infinite) component for band D (LL and HL) corresponds to the long-lived bleaching of the Q_y band of Chl *a*.

Both global analyses of LL and HL band D excited at 676 nm required two components for the fit (Figure 12 bottom panel and Table 2). The short-lived component, in band D (LL) 5.1 ps and in band D (HL) 18.7 ps are most likely due to exciton annihilation that is commonly observed upon Chl excitation.[18] The other non-decaying (infinite) component corresponds to the long-lived bleaching of the Q_y band of Chl *a*.

Figure 13 presents the results of global fits to the datasets recorded from band F (LL) (top panel, left) excited at 502 nm. The kinetic components are summarized in Table 2. The first component has a lifetime of 150 fs and shows negative bands between 500 and 600 nm attributable to the carotenoid absorption. The 450 fs component exhibits a broad $S_1 \rightarrow S_n$ excited state absorption of a carotenoid as well as bleaching of the Q_y absorption band at 680 nm. The excited state absorption can be assigned to a transition from vibronically excited S_1 state of xanthophylls, and the Q_y bleaching is indicative of energy transfer to Chl *a* in this same time domain, most likely originating from the S_2 state of a carotenoid. The next component has a 2.2 ps lifetime and can be assigned to the red fucoxanthin which undergoes efficient energy transfer to Chl *a*. The 6.8 ps component may be attributed to the blue fucoxanthin which is less efficient at energy transfer to Chl *a*. The 41 ps component can be assigned to diadinoxanthin, which by virtue of its long lifetime,[13] suggests negligible energy transfer to Chl *a*. The infinite component is the bleaching of Q_y band of Chl *a*.

Figure 13 (top panel, right) and Table 2 also show the results of the global analysis of band F prepared from HL treated cells. Negative bands between 500-600 nm can be observed for the 170 ps component, and these can be assigned to bleaching of the $S_0 \rightarrow S_2$ transition of carotenoids. The next component has a 450 fs lifetime which can be attributed to an excited state transition from a vibronically excited S_1 state. Bleaching of the Q_y band of Chl *a* was also observed in this component indicating that energy transfer to Chl *a* is occurring in this time domain. The next component has a lifetime of 2.0 ps and, as in the LL material, can be attributed to a red absorbing fucoxanthin which undergoes efficient energy transfer to Chl *a*. The 11 ps, which is not present in the LL material, can be assigned to either the blue fucoxanthin or diatoxanthin. The 41 ps, as in the LL material, can be assigned to diadinoxanthin. Also an infinite component was required and it can be attributed to the long-lived bleaching of the Q_y band of Chl *a*.

The TA datasets measured after 550 nm excitation of band F (bottom panels in Figure 10) were fit in the range 575 to 760 nm to avoid the strong negative signal associated with the scattered laser light at 550 nm in the experiment. The global fitting of band F (LL and HL) required three components (Figure 13, middle panel). The shortest component had a lifetime of 2.7 ps in the LL sample and 2.4 ps in the HL. These correspond well to energy transfer to Chl *a* from the S_1 state of the red fucoxanthin. The origin of the 58 ps in LL band F remains unclear, whereas the 14 ps component in HL band F may be attributed to energy transfer originating from the S_1 state of the blue fucoxanthin. The infinite components are associated with the long-lived bleaching of the Q_y band of Chl *a*.

Global analysis of the datasets from band F (LL and HL) that were excited at 676 nm is shown in Figures 13 (bottom panels). The first component, 5.7 ps for LL and 13.3 ps for HL can be assigned to exciton annihilation that occurs upon direct Chl excitation.[18] In both samples a non-decaying (infinite) component was required and represents the long-lived bleaching of the Q_y band of Chl *a*.

Previous work by Gildenhoff, et al.[4] on pigment-protein complexes prepared from LL and HL-treated *Cyclotella memeghiniana* used single wavelength analyses to fit the TA data. In that work five kinetic components were required for a satisfactory fit. In the present analysis of the band F pigment-protein complex prepared from LL and HL-treated *P. tricornutum*, global analysis required six components for a satisfactory fit. Whereas Gildenhoff et al.,[4] reported a 25 ps component assigned to the S_1 state of a blue fucoxanthin or diadinoxanthin or diatoxanthin,[4] the present work is more precise in distinguishing 6.8 ps and 41 ps components for LL material, and 11 ps and 41 ps components for HL material. The 6.8 ps and 11 ps component can be assigned specifically to the decay of the S_1 states of the blue fucoxanthin or diatoxanthin, and the 41 ps can be attributed to the decay of the S_1 state of diadinoxanthin.

Taking together the results from the global analysis with the steady-state fluorescence excitation spectra, it is clear that xanthophyll to Chl energy transfer occurs in band F prepared from both LL and HL treated cells. However, only very minor differences exist between the kinetic components obtained from the LL and HL datasets of band F regardless of whether 502 nm, 550 nm and 676 nm was used.

3.2 Conclusions

Varying the xanthophyll composition of the light-harvesting pigment-protein complexes of *P. tricornutum* by exposing cells to HL conditions has little effect on the efficiency of energy transfer to Chl *a* in this system. This is consistent with results published on *Cyclotella meneghiniana*.[4] Also, the additional amount of diatoxanthin that accumulates in the pigment-protein complexes prepared from HL cells does not affect the Chl excited state quenching characteristics compared to complexes prepared from LL cells that have a lesser amount of the xanthophyll. Nevertheless, the present work on band F from both LL and HL preparations shows clearly that the blue fucoxanthin, perhaps augmented by diadinoxanthin or diatoxanthin, transfers energy to Chl *a*. However, the red fucoxanthin was more efficient in this process consistent with previous results on a different organism.[4,5] The pigment-protein complex associated with band D, despite having a substantial amount of bound fucoxanthin, was found here to be incapable of carotenoid-to-Chl energy transfer. A schematic model summarizing the energy transfer pathways in the pigment-protein complex associated with band F is depicted in Figure 14. The shortest lifetime reported in the literature for fucoxanthin is 23 ps in methanol.[11] Thus, the 2.2 ps lifetime for the red fucoxanthin reported here suggests an energy transfer efficiency to Chl *a* of greater than 90%.

3.3 References

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Table 1. Pigment composition derived from HPLC analyses of the extracts of whole cells and isolated pigment-protein complexes prepared from cells subjected to low-light (LL) and high-light (HL) conditions. Chl *a* content in band F includes the presence of the Chl epimers. The values are expressed as a percent of the total pigment composition. The uncertainties in the percentages for the band D (LL) sample were based on the results of three separate HPLC determinations. All others represent the average of two determinations, the first being done using freshly extracted whole cells and pigment-protein complexes whereas the other injection was done with material that had been stored at -20ºC.

Sample	Chl *c*	fucoxanthin	diadinoxanthin	diatoxanthin	Chl *a*	β-carotene
Whole cells (LL)	2 ± 2	45 ±10	8 ± 1	0	43 ± 13	2 ± 1
Whole cells (HL)	2 ± 2	54 ± 2	4 ± 1	4 ± 1	35 ± 1	1 ± 1
Thylakoids (HL)	0	61 ± 8	3 ± 1	5 ± 1	30 ± 8	2 ± 1
Band D (LL)	0	70 ± 11	7 ± 1	0	23 ± 13	0
Band D (HL)	0	70 ± 20	6 ± 2	6 ± 1	26 ± 5	3 ± 1
Band F (LL)	0	69 ± 1	3 ± 2	0	28 ± 1	0
Band F (HL)	0	55 ± 12	3 ± 1	2 ± 1	40 ± 6	0

Table 2. Excited singlet states lifetimes, τ, of the pigment-protein complexes obtained from LL and HL treated cells. The top part of the table presents the kinetics components obtained from global analysis of datasets taken using 493 nm, 502 nm and 550 nm excitation. The bottom part of the table is from data using 676 nm excitation. In both sets of data an infinitely long-lived component (not listed) was also required for a good fit.

pigment protein complex	excitation wavelength/nm	τ_1/fs	τ_2/fs	τ_3/ps	τ_4/ps	τ_5/ps
Band D (LL)	493	130 ± 50	520 ± 50		12.6 ± 0.4	34.0 ± 0.5
Band F (LL)	502	150 ± 50	450 ± 50	2.2 ± 0.1	6.8 ± 0.2	41 ± 1
	550			2.7 ± 0.6		58 ± 3
Band D (HL)	502	170 ± 50	580 ± 50		5.2 ± 0.1	34.0 ± 0.3
Band F (HL)	502	170 ± 50	450 ± 50	2.0 ± 0.2	11.0 ± 0.4	41 ± 1
	550			2.4 ± 0.1	14.7 ± 0.5	
Band D (LL)	676					5.1 ± 0.1
Band F (LL)	676					5.7 ± 0.4
Band D (HL)	676					18.7 ± 2
Band F (HL)	676					13.3 ± 0.5

Figure 1. Structures of fucoxanthin and the xanthophyll cycle carotenoids, diadinoxanthin and diatoxanthin.

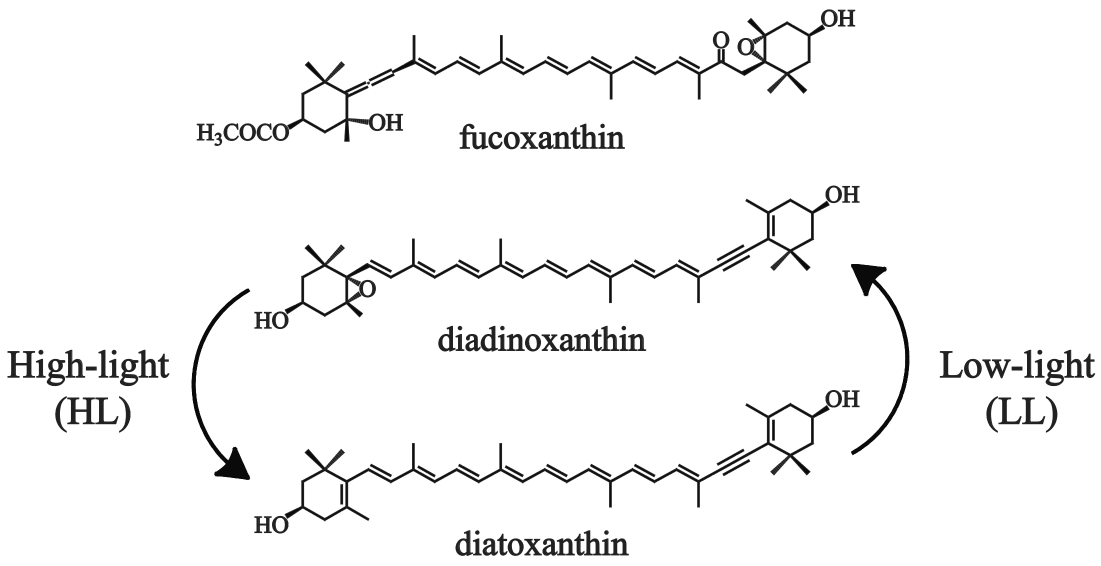

OH
O
O
H3COCO
OH
fucoxanthin
OH
O
HO
diadinoxanthin
High-light
(HL)
Low-light
(LL)
OH
HO
diatoxanthin

Figure 2. HPLC chromatogram monitored at 450 nm of the pigments extracted from LL- and HL-treated *P. tricornutum* whole cells. The pigments are denoted Fx for fucoxanthin, Dd for diadinoxanthin, and Dt for diatoxanthin.

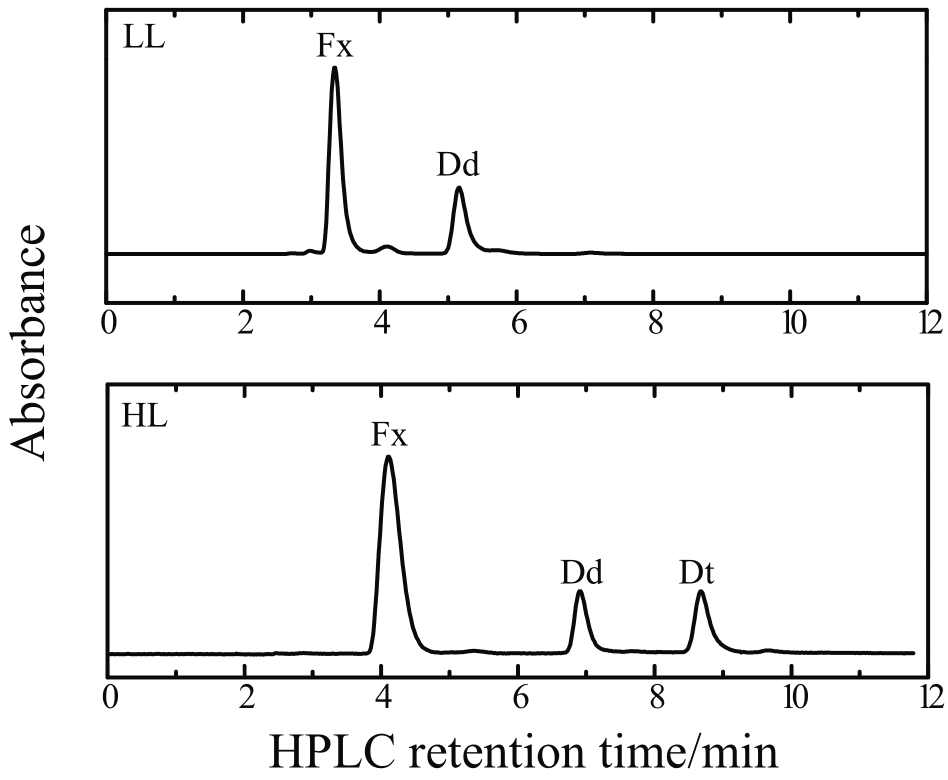

LL
Fx
Dd
HL
Fx
Dd
Dt
0
2
4
6
8
10
12
Absorbance
HPLC retention time/min

Figure 3. Sucrose density gradient ultracentrifugation tube containing two bands labeled D and F obtained after detergent solubilization of thylakoids prepared from *P. tricornutum*.

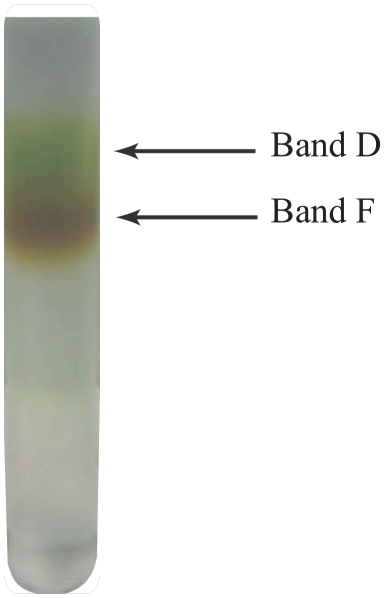

Band D
Band F

Figure 4. HPLC chromatograms monitored at 440 nm of the D and F bands isolated using sucrose density gradient ultracentrifugation. Left panels are from samples prepared from LL cells, and right panels are from samples prepared from HL cells.

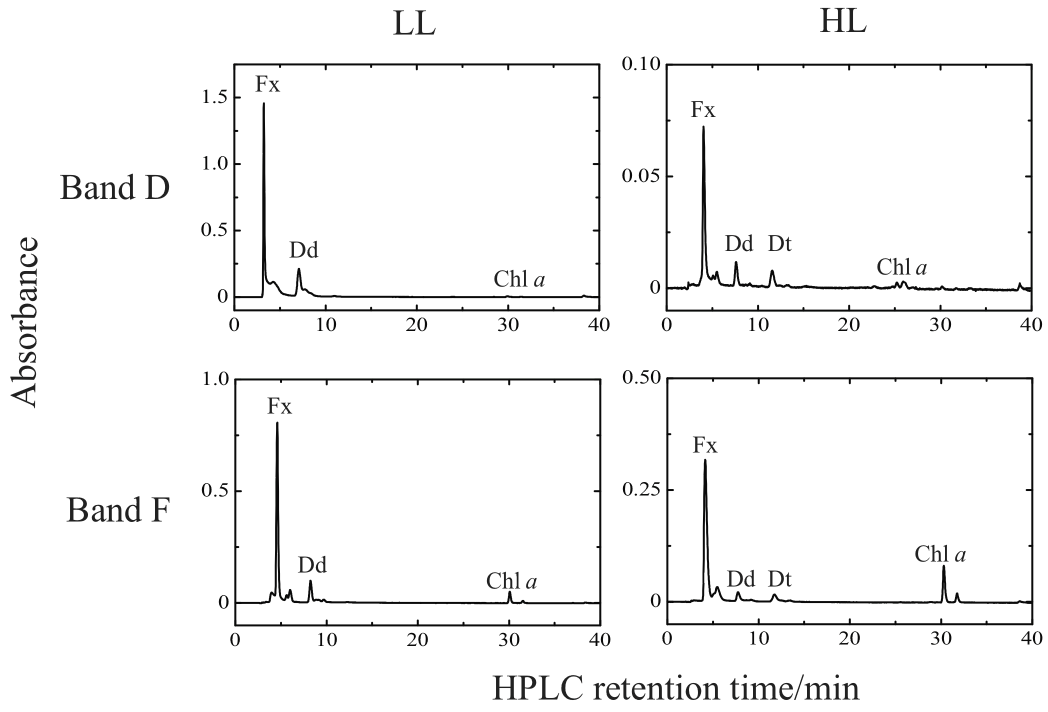
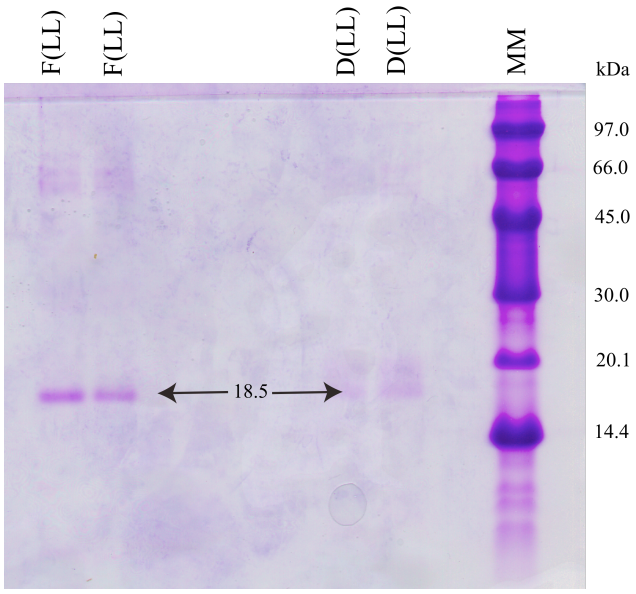

LL
HL
Band D
Band F
Absorbance
HPLC retention time/min
Fx
Dd
Dt
Chl a

Figure 5. SDS-PAGE of the pigment-protein complexes associated with bands D and F resulting from fractionation and ultracentrifugation of LL- (D (LL) and F (LL)) and HL- (D (HL) and F (HL)) treated whole cells of *P. tricornutum*. . Also shown are the protein extracts from thylakoids obtained from LL- (T (LL)) and HL- (T (HL)) treated cells. (A) Gel stained with coomassi blue. (B) Gel stained with silver nitrate. The lanes containing molecular mass standards are labeled MM.

A


F(LL)
F(LL)
D(LL)
D(LL)
MM
kDa
97.0
66.0
45.0
30.0
20.1
14.4
18.5

B

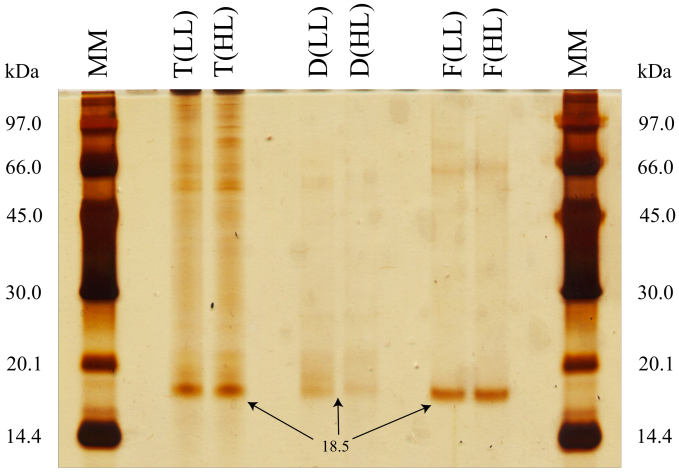

kDa
MM
T(LL)
T(HL)
D(LL)
D(HL)
F(LL)
F(HL)
MM
kDa
97.0
66.0
45.0
30.0
20.1
14.4
18.5

Figure 6. Steady-state absorption spectra of the isolated pigment-protein complexes. The spectra were normalized to the Soret band near 440 nm.

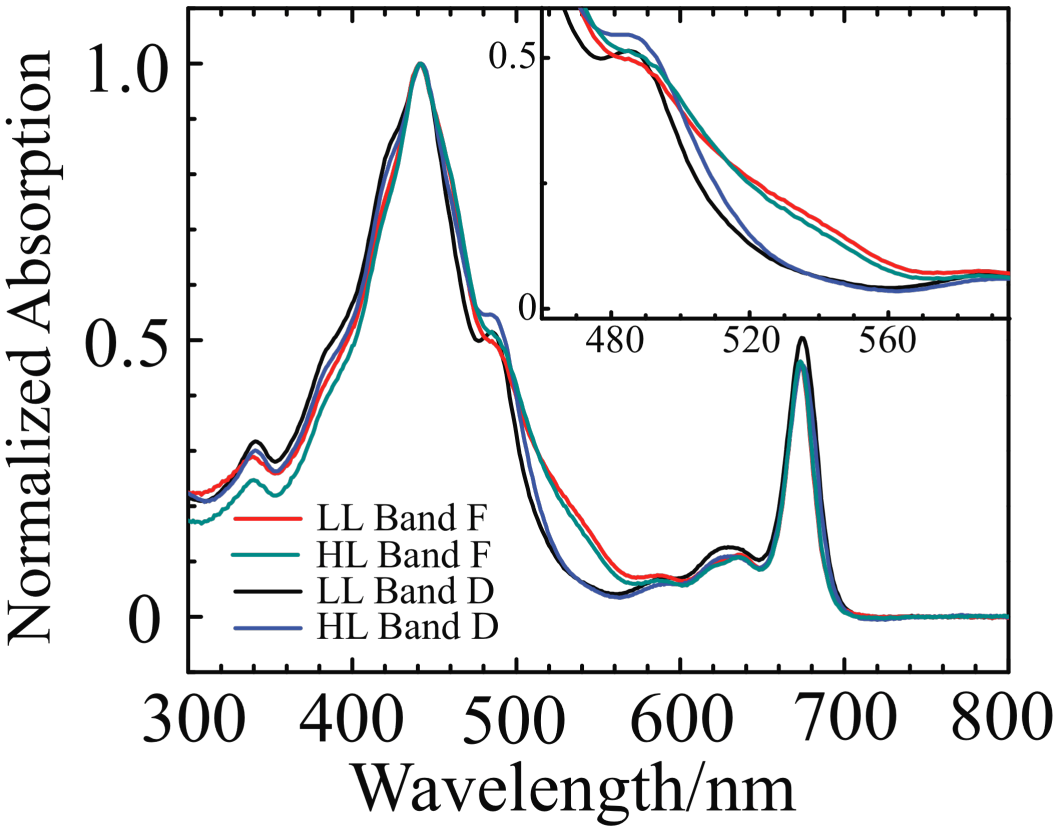
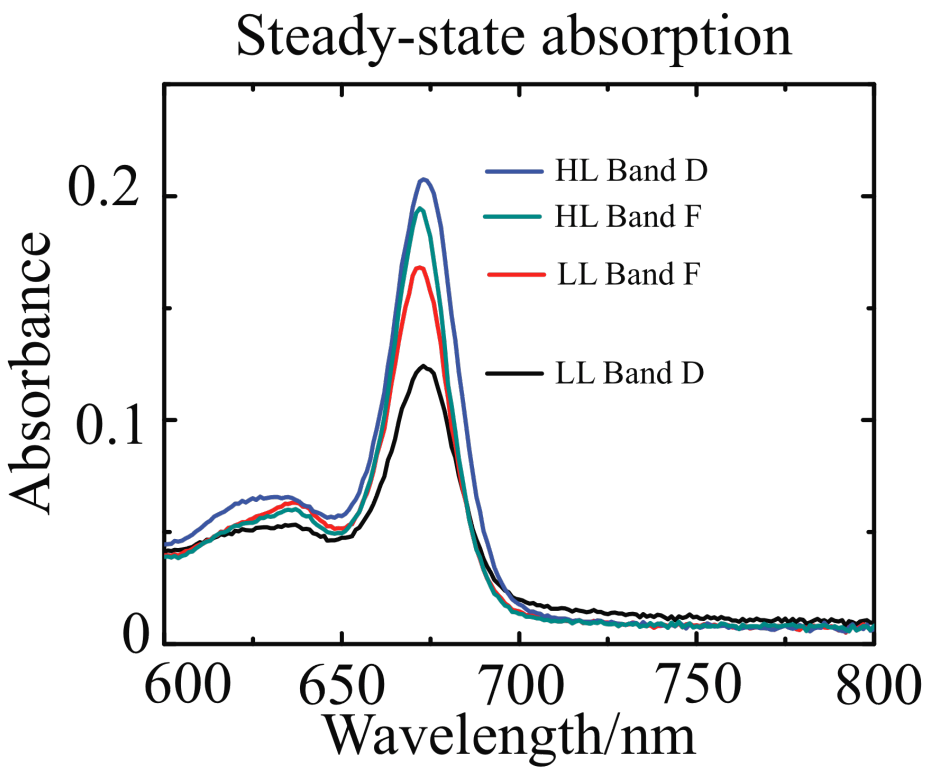

Normalized Absorption
1.0
0.5
0
300
400
500
600
700
800
Wavelength/nm
LL Band F
HL Band F
LL Band D
HL Band D
0.5
0
480
520
560

Figure 7. Steady-state absorption spectra (top) of the isolated pigment-protein complexes in the Q_y spectral region, and fluorescence spectra (bottom) taken using 630 nm excitation.


Steady-state absorption
Absorbance
0.2
0.1
0
600
650
700
750
800
Wavelength/nm
HL Band D
HL Band F
LL Band F
LL Band D

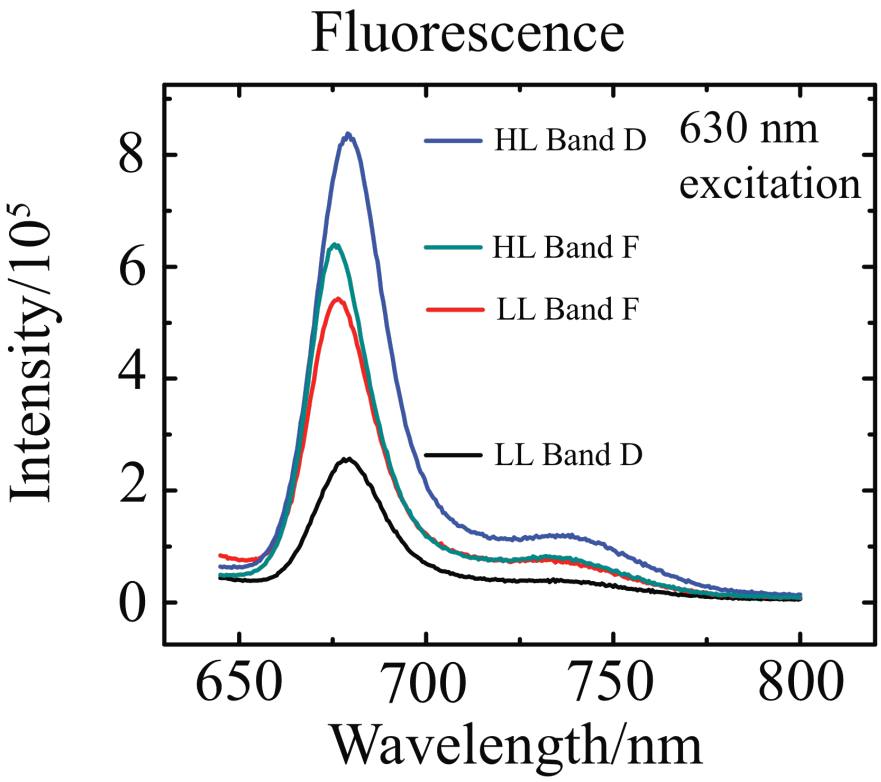

Fluorescence
Intensity/10^5
8
6
4
2
0
650
700
750
800
Wavelength/nm
HL Band D
HL Band F
LL Band F
LL Band D
630 nm
excitation

Figure 8. Overlay of 1-minus-transmission (1-T) spectra (black lines) and fluorescence excitation spectra (red lines) monitored at 730 nm of the pigment-protein complexes isolated using sucrose density ultracentrifugation.

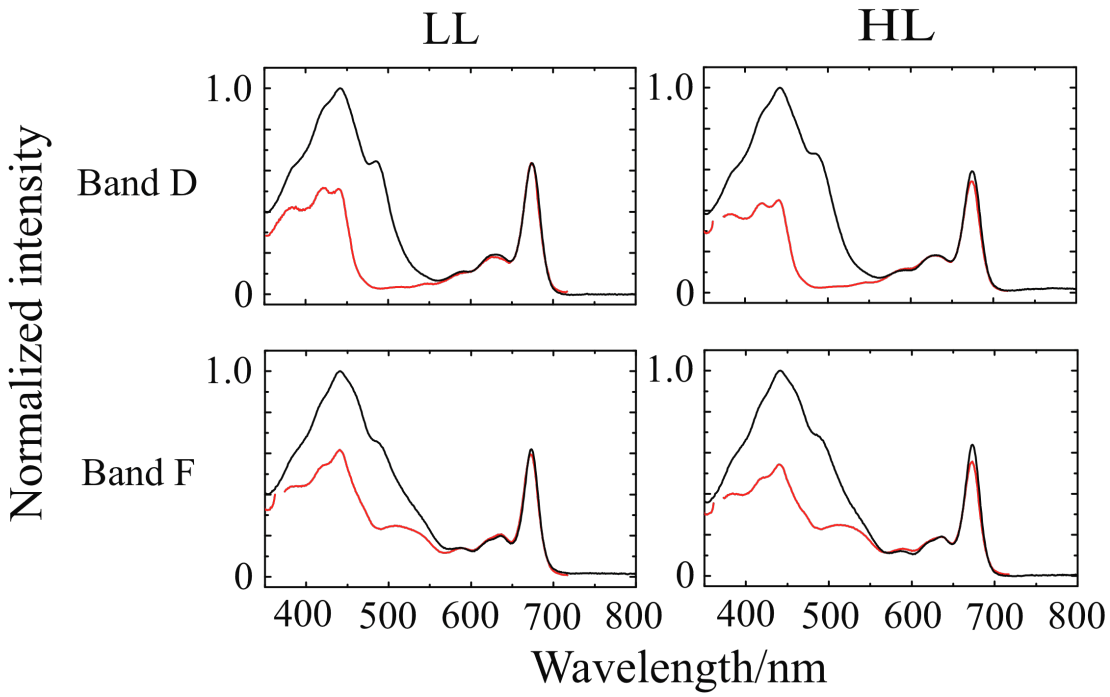

LL
HL
Normalized intensity
Band D
Band F
1.0
0
400
500
600
700
800
Wavelength/nm

Figure 9. Decay-associated spectra of time-resolved fluorescence of the isolated pigment-protein complexes taken using 665 nm excitation and recorded 5 nm intervals. Left panel LL and right panel HL.

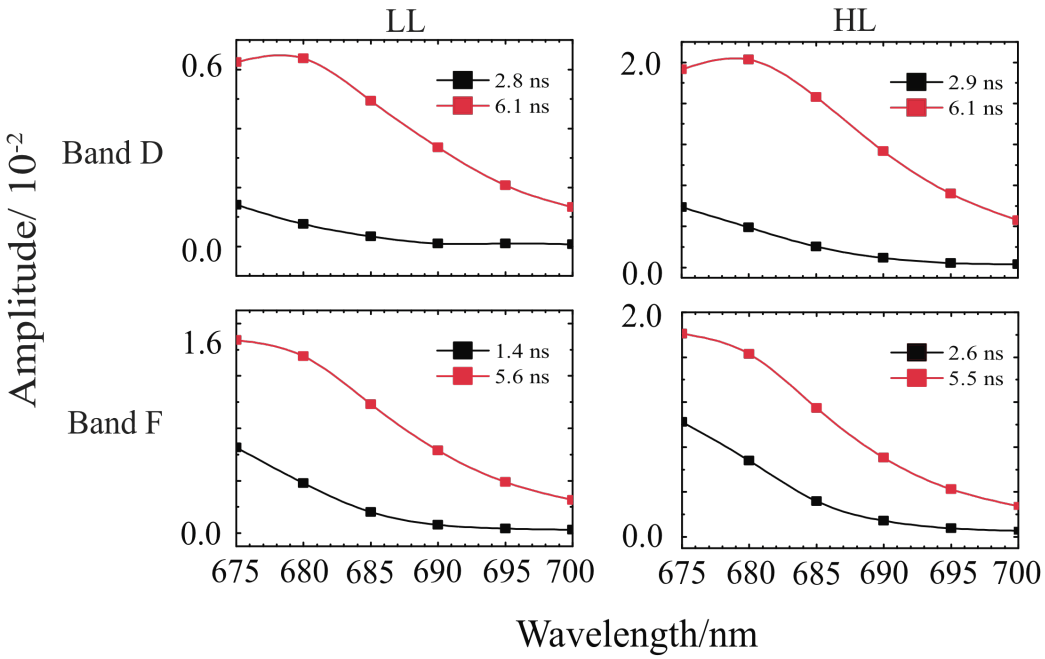

LL
HL
Band D
Band F
Amplitude/ 10-2
0.6
0.0
2.0
0.0
1.6
0.0
2.0
0.0
2.8 ns
6.1 ns
2.9 ns
6.1 ns
1.4 ns
5.6 ns
2.6 ns
5.5 ns
675 680 685 690 695 700
675 680 685 690 695 700
Wavelength/nm

Figure 10. Ultrafast time-resolved absorption spectra of band D and F isolated from LL and HL cells. The spectra were taken at room temperature at various delay times after excitation at 493 nm, 502 nm and 550 nm as indicated.

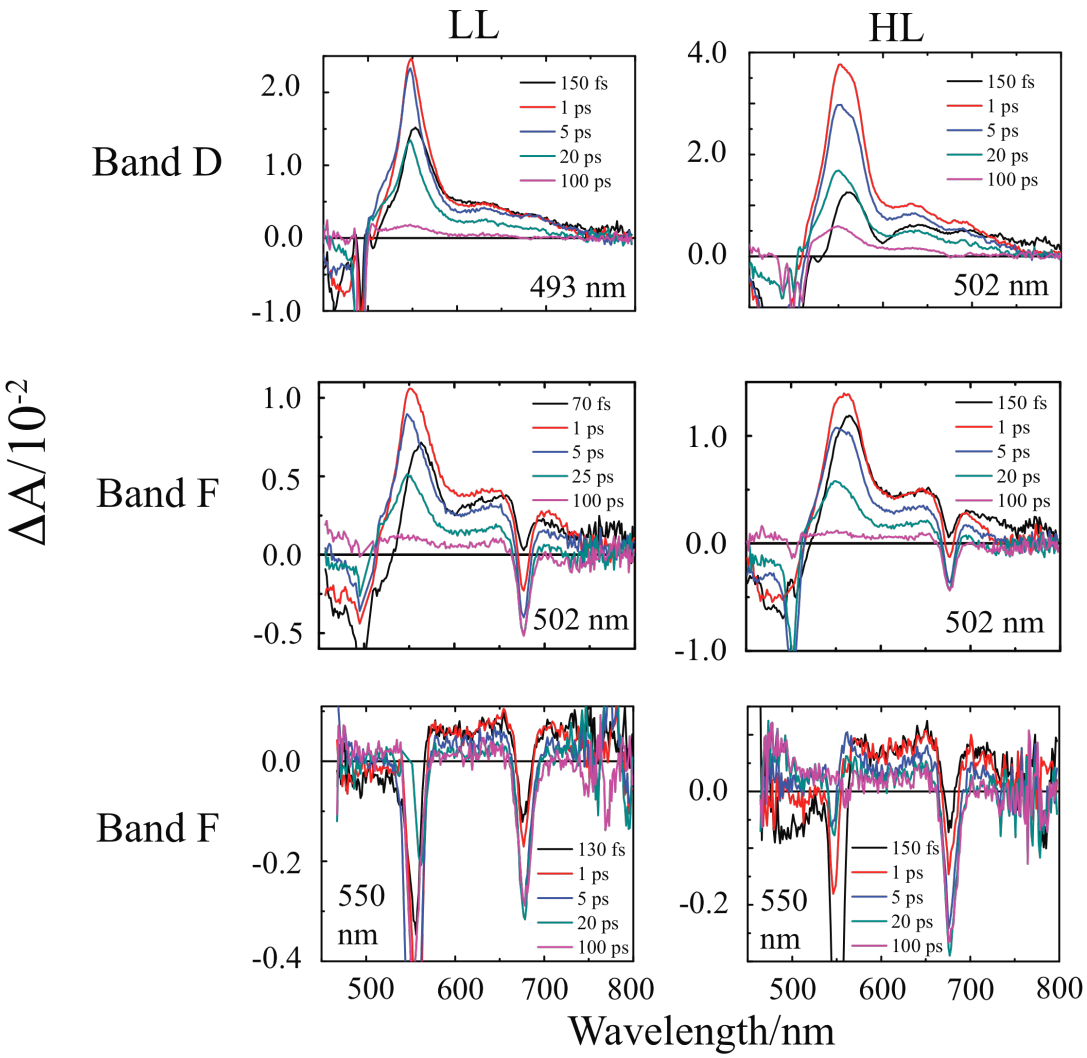

LL
HL
Band D
Band F
Band F
ΔA/10-2
150 fs
1 ps
5 ps
20 ps
100 ps
493 nm
502 nm
70 fs
25 ps
130 fs
550 nm
Wavelength/nm

Figure 11. Ultrafast time-resolved absorption spectra of band D and F isolated from LL and HL cells. The spectra were taken at room temperature at various delay times after excitation at 676 nm.

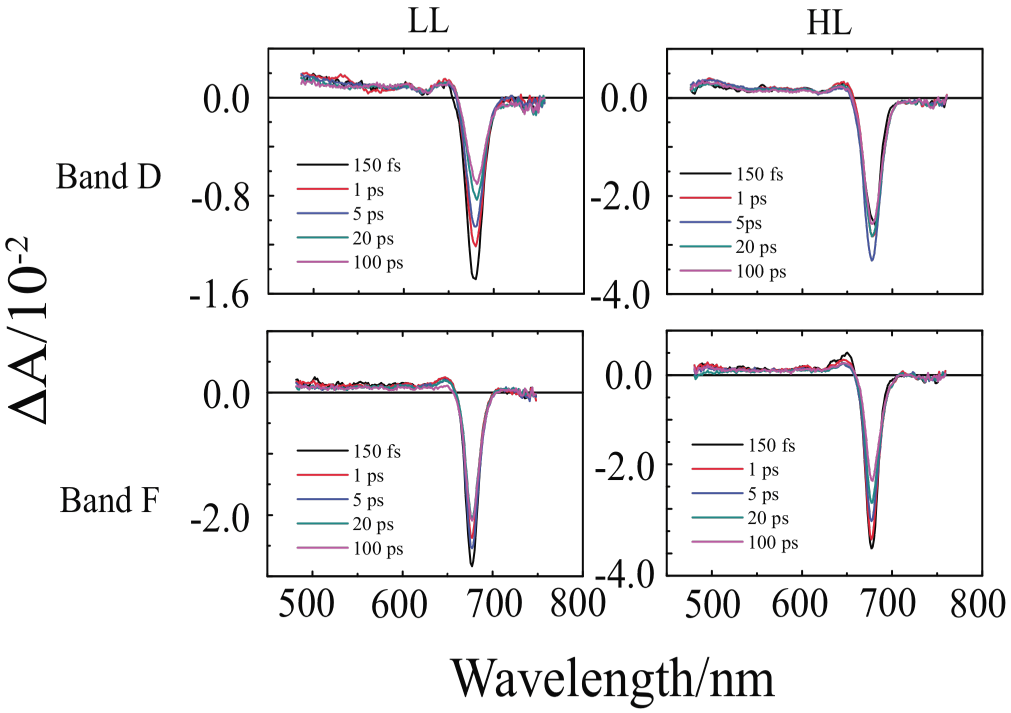

LL
HL
Band D
Band F
ΔA/10-2
0.0
-0.8
-1.6
-2.0
-4.0
150 fs
1 ps
5 ps
5ps
20 ps
100 ps
500
600
700
800
Wavelength/nm

Figure 12. Evolution associated difference spectra (EADS) obtained from global fitting of the TA datasets of band D isolated from LL and HL cells.

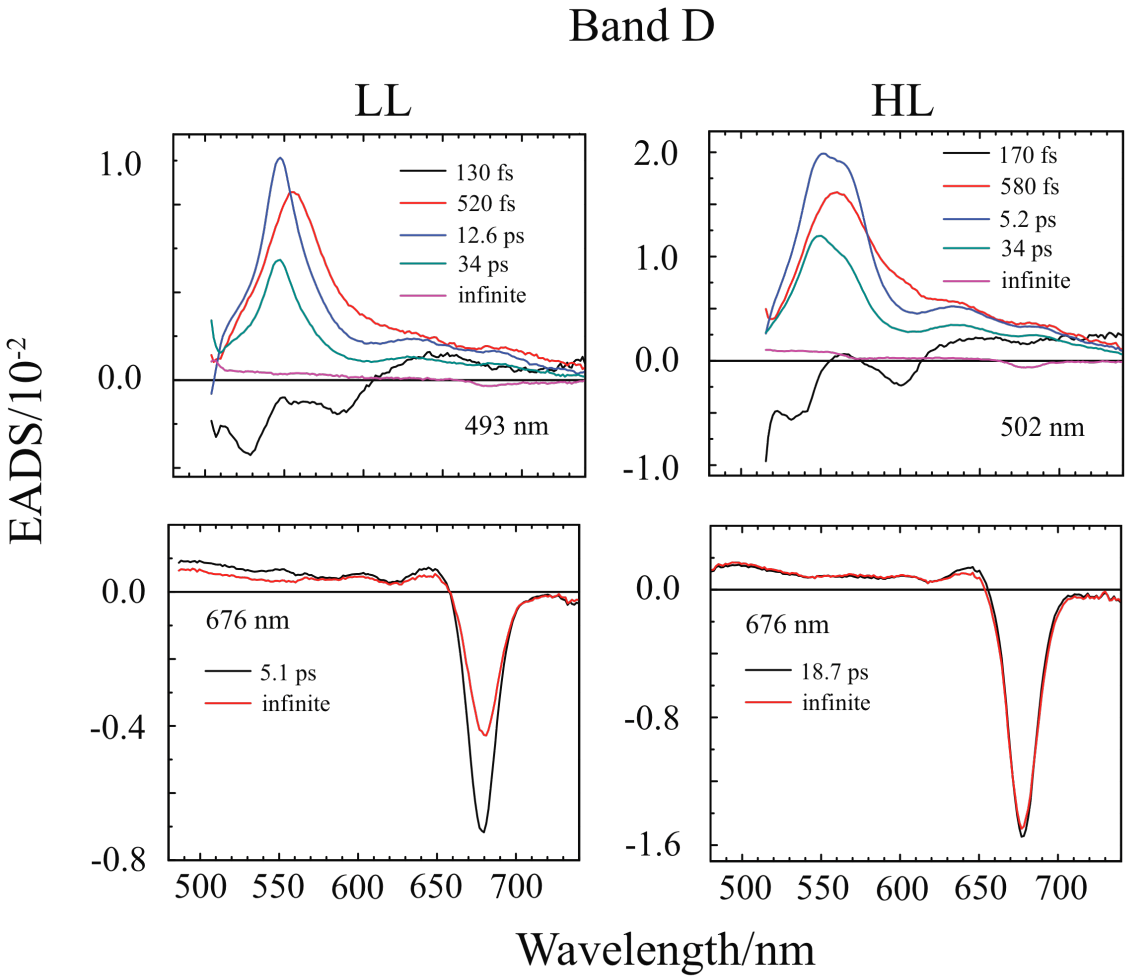

Band D
LL
HL
EADS/10-2
1.0
0.0
130 fs
520 fs
12.6 ps
34 ps
infinite
493 nm
2.0
1.0
0.0
-1.0
170 fs
580 fs
5.2 ps
34 ps
infinite
502 nm
0.0
-0.4
-0.8
676 nm
5.1 ps
infinite
0.0
-0.8
-1.6
676 nm
18.7 ps
infinite
500 550 600 650 700
500 550 600 650 700
Wavelength/nm

Figure 13. Evolution associated difference spectra (EADS) obtained from global fitting of the TA datasets of band F isolated from LL and HL cells.

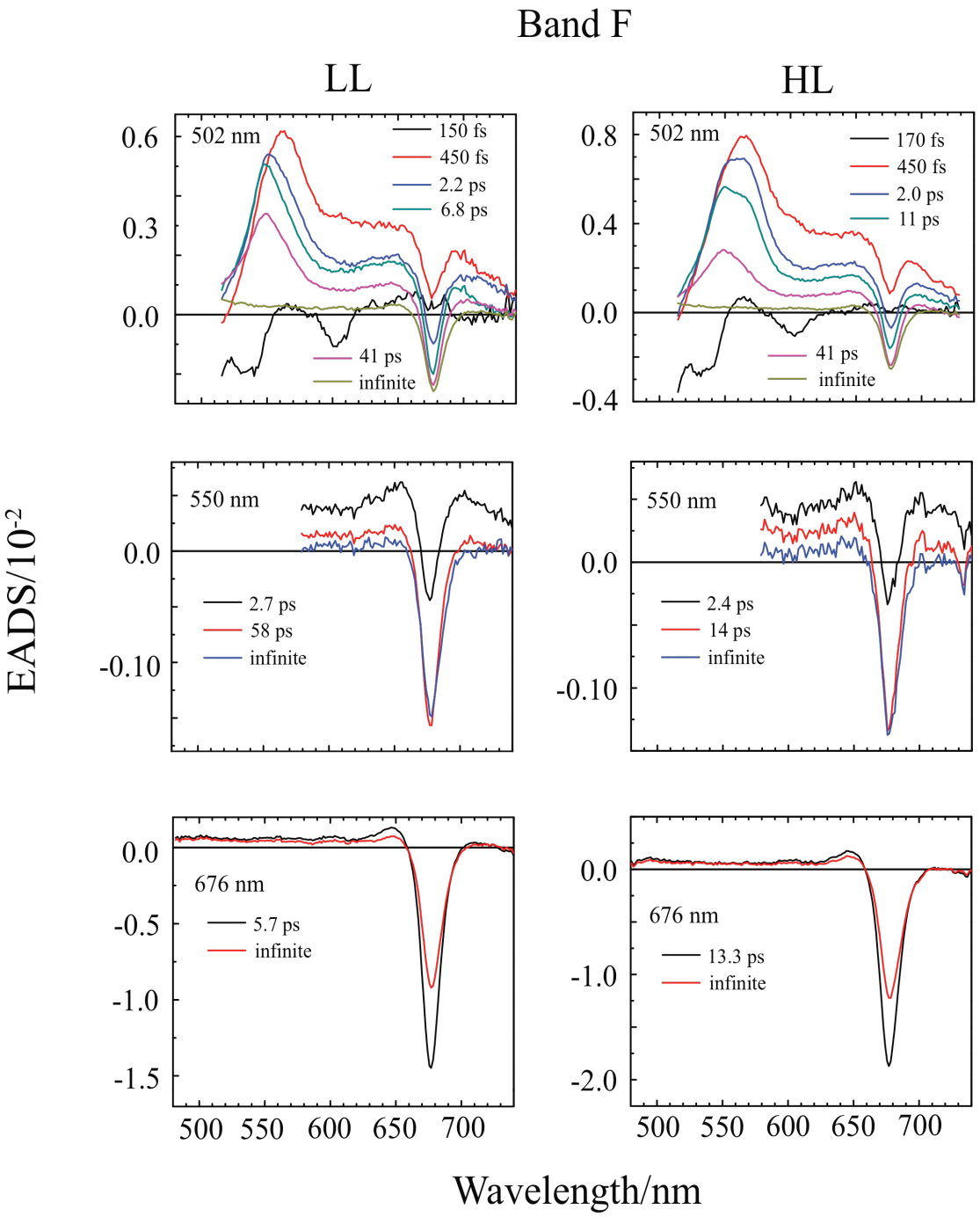
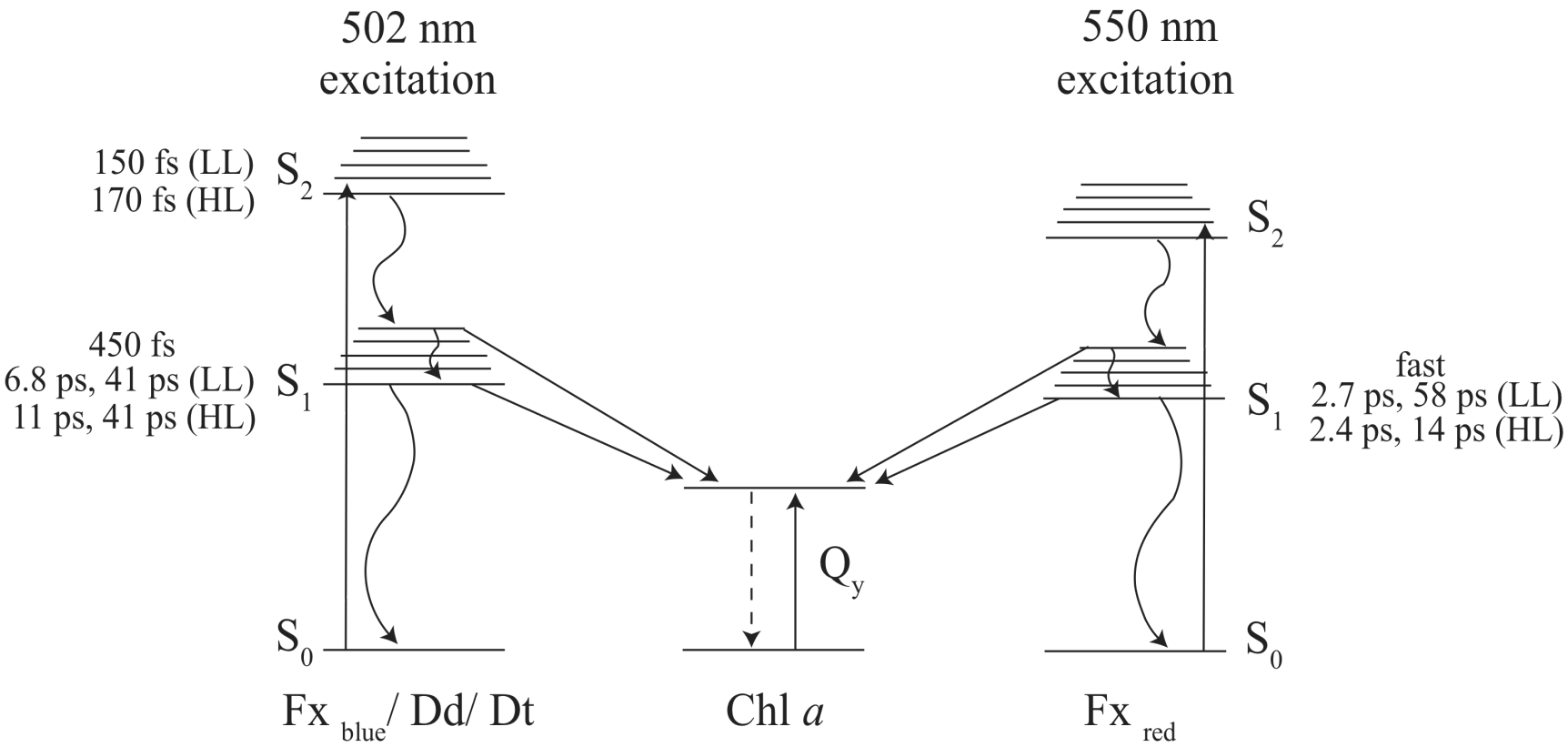

Band F
LL
HL
502 nm
150 fs
450 fs
2.2 ps
6.8 ps
41 ps
infinite
170 fs
2.0 ps
11 ps
550 nm
2.7 ps
58 ps
2.4 ps
14 ps
676 nm
5.7 ps
13.3 ps
EADS/10-2
Wavelength/nm

Figure 14. Schematic model describing the energy transfer in band F involving the blue absorbning fucoxanthin and/or diadinoxanthin/diatoxanthin and the red absorbing fucoxanthin. The solid arrows show the main energy transfer channels, wave arrows the nonradiative relaxation processes and the dashed arrows the fluorescence of Chl *a*.


502 nm
excitation
550 nm
excitation
150 fs (LL)
170 fs (HL)
S2
450 fs
6.8 ps, 41 ps (LL)
11 ps, 41 ps (HL)
S1
S0
Qy
S2
S1
fast
2.7 ps, 58 ps (LL)
2.4 ps, 14 ps (HL)
S0
Fx blue/ Dd/ Dt
Chl a
Fx red