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The light-harvesting antenna of the diatom *Phaeodactylum tricornutum*: Evidence for a diadinoxanthin binding sub-complex

Gérard Guglielmi, Johann Lavaud[∇], Bernard Rousseau, Anne-Lise Etienne, Jean Houmard and Alexander V. Ruban[⊥]

From the Organismes Photosynthétiques et Environnement, FRE 2433 CNRS, Département de Biologie, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05, France

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Present addresses:

Corresponding author: Gérard Guglielmi, Organismes Photosynthétiques et Environnement, UMR 8541 CNRS, Département de Biologie, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris cedex 05, France. Tel. +33 1 44 32 35 30; fax +33 1 44 32 39 41 E-mail:

ggugliel@biologie.ens.fr, <http://www.biologie.ens.fr/opeaec/>

(∇) Pflanzliche Ökophysiologie, Fachbereich Biologie, Universität Konstanz, Postfach M611, Universitätsstr. 10, 78457 Konstanz, Germany

(⊥) The Robert Hill Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, UK

Abbreviations: XC, xanthophyll cycle; DD, diadinoxanthin; DT, diatoxanthin; NPQ, non-photochemical chlorophyll fluorescence quenching; PSI and PSII, Photosystem I and II; LHC, light harvesting complex; α -DM, n-dodecyl- α ,D-maltoside; β -DM, n-dodecyl- β ,D-maltoside; PAGE, polyacrylamide gel electrophoresis.

Keywords: Diatom, light-harvesting complex, photoprotection, xanthophyll cycle, fucoxanthin

Summary

Diatoms differ from higher plants by their antenna system, in terms of both polypeptide and pigment contents. A rapid isolation procedure was designed for the membrane-intrinsic light harvesting complexes (LHC) of the diatom *Phaeodactylum tricornutum* to show whether different LHC subcomplexes may exist, as well as an uneven distribution between them of pigments and polypeptides. Two distinct fractions were separated that contain functional oligomeric complexes. A major and more stable complex (about 75% of total polypeptides) carries most of the Chl a , and almost only one type of carotenoid, fucoxanthin. The minor component, carrying approximately 10-15% of the total antenna chlorophyll and only a little chlorophyll c , is highly enriched in diadinoxanthin, the main xanthophyll cycle carotenoid. The two complexes also differ in their polypeptide composition suggesting specialized functions within the antenna. The diadinoxanthin-enriched complex could be where de-epoxidation of diadinoxanthin into diatoxanthin mostly occur.

Introduction

Diatoms constitute a dominant group of phytoplanktonic algae, playing an important role in the carbon, silica and nitrogen biogeochemical cycles [1-3]. Their photosynthetic efficiency and subsequent productivity depend upon the light environment which can vary greatly due to water motions [4,5]. Fluctuating irradiances and especially excess light exposure can be harmful for photosynthesis, in particular photosystem II (PS II), causing a decrease in productivity and fitness [6,7]. One of the photoprotective mechanisms used by diatoms is the dissipation of excess energy in the light-harvesting complex (LHC) of PS II to prevent over excitation of the photosystems (so-called NPQ for non-photochemical chlorophyll fluorescence quenching). NPQ

is triggered by a transthylakoidal proton gradient (ΔpH) and results from a modulation in the xanthophyll content [8-12]. In diatoms, the xanthophyll cycle (XC) is made up of diadinoxanthin (DD), which is converted under an excess of light into its de-epoxidised form, diathoxanthin (DT) [13]. The presence of DT is mandatory for NPQ [11,14-16]. Additionally, a reverse de-epoxidation of violaxanthin into zeaxanthin via antheraxanthin, similar to that which occurs in plants, has been demonstrated in diatoms submitted to a prolonged exposure to excess light [17]. The diatom photosynthetic apparatus differs in many aspects from that of green plants and algae. There are no grana stacking and no segregation of the photosystems [18]. The main components of the antenna are the Fucoxanthin Chlorophyll (Chl) Proteins (FCP) encoded by a multigene family [19]. FCP share common features with plants CAB (Chl *a* binding) proteins [20], and in the diatom *Cyclotella meneghiniana*, two 18 and 19 kDa subunits were recently shown to form trimers and higher oligomers [21]. However, no obvious orthologues of some of the plant LHC minor components (e. g. PsbS, CP26 and 29) have been found in the fully sequenced diatom genome [22].

In diatoms, the accessory pigments are also different. Chl *c* is the secondary chlorophyll, fucoxanthin is the main xanthophyll, and the XC pigments are DD and DT. The xanthophylls / Chl ratio can be 2-4 times higher than in plants [23]. In higher plants, the XC pigments are bound to both major (LHC II trimers) and minor (PsbS, CP 24, 26 and 29) components of the LHC [24,25]. In diatoms, DD and DT are mainly associated with the FCP antenna (12) but their exact localization in the different sub-fractions of the antenna has not yet been determined.

The goal of the present report was to address this question. Different isolation procedures were applied to obtain purified LHC fractions. The apparent molecular size, polypeptide and pigment compositions, as well as the spectroscopic properties of the various fractions were compared.

The data show that oligomeric FCP subcomplexes exist which have different polypeptides and pigment contents.

Results

Sucrose gradient preparations of pigment-protein complexes from Phaeodactylum tricornutum. The mild detergent α -DM was used for the solubilization of the pigment-protein complexes from the thylakoid membrane [21]. Freshly solubilized *P. tricornutum* pigment-protein complexes were loaded onto a sucrose density gradient under either low salt (LS) or high salt (HS) conditions. The two lower bands at densities of approximately 0.6 and 0.75 M sucrose (Fig. 1) correspond to PSII and PSI, respectively, as deduced from comparison with the sucrose gradient separation of the PSII-enriched particles from spinach chloroplasts (Fig. 1B), and already published data [12,26]. The upper bands correspond to the fucoxanthin containing light-harvesting protein complexes (FCP or LHCF) fraction. The major brown colored band designated F ran where FCP's were reported to be localized [12,21] and at a density similar to that of the spinach LHCIIB monomers (compare Fig. 1A and 1B). This fraction contained approximately 80-85% of the total LHC chlorophyll (Chl) *a*. A lighter yellow band (termed D) ran at ~ 0.3 M sucrose and contained about 10-15% of the total LHC Chl *a*. With the high salt buffer, conditions known to better keep the integrity of the oligomeric states of protein complexes, two F bands were resolved: F1, similar to F, and F2. F2 ran at a higher sucrose concentration (0.45 M, Fig. 1C), *i.e.* in between the position for the spinach LHCIIB monomers and trimers.

Further purification of the F and D fractions by gel filtration. The LHC fractions D and F from the sucrose gradients were buffer-exchanged and immediately applied onto a FPLC column. Figure 2 shows typical elution profiles recorded by absorption at 280 nm, except for the free pigment fraction (fp) which did not contain any polypeptide and was recorded at 436 nm. Isolated LHCII trimers, monomers and the free pigment fraction obtained from spinach chloroplasts by the sucrose gradient procedure (Fig. 1B) were used for size calibration. Fraction F eluted like the LHCII monomers (at 47 min) with a shoulder around 49-50 min (Fig. 2, trace 1), and fraction D at 50 min, with a minor-shoulder at 47-48 min (Fig. 2, trace 3). The shoulders are likely to be due to a cross-contamination of F by D, and *vice versa*. All the elution times were reproducible with at most a 8% variation, and co-chromatographies of *P. tricornutum* F and D with monomeric spinach LHC fractions were performed to validate the comparison of the elution times (data not shown). Regardless of the salt conditions used for the sucrose gradients, the apparent molecular size of the F and D complexes was always the same. For F, it corresponded to that observed for the spinach LHCII monomer while D eluted at about 50 min well ahead of the spinach free pigment fraction. Absorption at 280 nm showed that both fractions do contain polypeptides. No significant differences in terms of pigment composition, spectroscopic properties or chromatographic behaviour on gel filtration columns could be detected between the D fractions, whether they were isolated from LS or HS conditions, nor between the F, F1 and F2 fractions. A higher salt concentration allowed fractioning of the F fraction into F1 and F2, the latter likely representing a higher aggregation state (dimers?) of the same subcomplexes which is not stable enough to be kept during the gel chromatography.

In another set of experiments, fractions F and D were additionally treated with β -DM before gel filtration. These stronger detergent conditions led to more loosening of the subcomplex

interactions. Following this treatment, the retention time increased for the D fraction (Fig. 2, trace 4), and fraction F (Fig. 2, trace 2) appeared as two peaks, the second one corresponding to that obtained with the β -DM-treated D fraction. Table 1 shows the pigment composition of the different fractions. Fucoxanthin is the major pigment in all the fractions. Its concentration is higher than that of Chl *a*, in contrast to what is observed for lutein, the main xanthophyll of the higher plant LHCs [27]. DD, as well as Chl *c*, are unevenly distributed. Compared to F, D fractions are highly enriched in diadinoxanthin (DD) and contain less Chl *c*.

Absorption (Fig. 3) and 77K fluorescence spectra (Fig. 4) were recorded for fractions F and D. Fraction F exhibited an absorption spectrum (Fig. 3B) which reflected its pigment content: Chl *c* peaks at 463 nm and 636 nm, and a large fucoxanthin 500-550 nm absorbance band was visible with two distinct peaks at 505 and 536 nm. This is characteristic of the absorption properties of the LHC bound fucoxanthin observed with whole cells [12,28]. In agreement with the low DD content of the F fractions, no peak corresponding to the DD absorption (around 490 nm) is observed. The 77 K emission (Fig. 4A) and excitation (Fig. 4B) fluorescence spectra confirmed that energy couplings between Chl *c*, as well as fucoxanthin and Chl *a*, are preserved in the F fraction. The lack of a 635 nm peak in the emission spectrum and the peak at 463 nm in the excitation spectrum were indicative of a coupled Chl *c*. The two shoulders at 505 and 536 nm in the excitation spectrum were indicative of a coupled fucoxanthin. We therefore concluded that fraction F was obtained in a form very close to the *in vivo* state. The fraction D showed different absorption and fluorescence spectra. According to its low Chl *c* content, no peak corresponding to the Chl *c* absorption (at 463 and 636 nm) was visible in the absorption (Fig. 3A) and the excitation (at 463 nm) spectra (Fig. 4B). Although the amount of fucoxanthin was higher in fraction D, no 500-550 nm LHC bound fucoxanthin band could be observed on the absorption

spectrum (Fig. 3A). The uncoupling of fucoxanthin was confirmed by the excitation spectrum (Fig. 4B). It resulted in an increased absorption in the Soret region (Fig. 3A) with a specific 486 nm peak characteristic of the blue shifted absorbance of decoupled fucoxanthin [28]. Since the ratio DD/fucoxanthin was small, the absorption peak corresponding to DD was not detectable (Fig. 3A). Hence, in fraction D, only the Chl *a* molecules (Fig. 4) appear to be still energetically coupled. Finally, the D fraction showed a broader Chl *a* band in the red absorption region than did the F fraction (compare the respective 670 nm peaks in Fig. 3A and B). This indicated a somewhat different environment for the chlorophyll molecules in the two fractions, which was confirmed by a 2 nm shift of the Chl *a* fluorescence peak in the F fraction (Fig. 4A).

The polypeptide composition of the two fractions was analyzed by SDS-PAGE electrophoresis (Fig. 5). Diatom FCPs have molecular sizes ranging from 17 to 23 kDa [19,21-22]. The D and F fractions share a common band at ~18.5 kDa that is a doublet at least in D. A second polypeptide of ~18 kDa is present only in F. Additional polypeptides in the 10-17 and 20-66 kDa range are present in the D fraction. The F fraction is particularly rich in FCP polypeptides.

Direct gel filtration of the solubilized pigment-protein complexes. To obtain LHC fractions that have kept their *in vivo* oligomeric state as far as possible, a new procedure was devised. Following plastid isolation, the detergent treatment was reduced to a minimum and the sucrose gradient step avoided. Plastids were solubilized by a 5 min α -DM treatment in 600 mM NaKPO₄, and immediately loaded on a gel filtration column. Three fractions were obtained, with the first two that elute corresponding to the photosystems, and the third one to a large FCP oligomer termed LHCo (Fig. 6). This LHCo started to elute at 40 min, with a peak at 43 min, and presented a tail which extended up to about 50 min. Spinach LHCI trimers used for size calibration eluted between 41 and 45 min, peaking at 43 min (see Fig. 2, dashed line). The

majority of the soluble proteins were not embedded into micelles and eluted as a very broad peak centered at around 80 min (not shown). These new conditions thus allow the isolation in a stable form of a LHC of higher apparent molecular size, suggesting that it corresponds to an oligomeric complex.

The LHCo elution peak was asymmetric, indicative of heterogeneity. This fraction further treated with β -DM and rechromatographed gave a two-peak profile (Fig. 6, dashed line). From the OD₂₈₀ profile, the first peak (F) would contain about 75% of the LHCo polypeptides. Pigment composition and absorption spectra showed that these peaks corresponded to the above described F and D fractions (data not shown). We thus decided to separately collect and analyze, from this LHCo, the fractions that eluted between 40 and 44 min (LHCo-1, first fraction) and between 45 and 50 min (LHCo-2, second fraction). Pigment compositions are presented in Table 2. Compared to the whole LHCo, LHCo-1 contained two times less DD, whereas LHCo-2 was enriched in DD (3 fold) and fucoxanthin (1.7 fold), and contained about two times less Chl *c*. After treatment with β -DM and a new gel filtration, the LHCo-1 gave a major peak with an elution time corresponding to that of the F fraction (47 min), and a minor one eluting at 52 min that resembled D fraction (retention times similar to traces 2 and 4 of Fig. 2). The opposite was observed for LHCo-2 which gave a major peak corresponding to a D fraction. The pigment composition of each of the major peaks is close to that of the F and D fractions obtained from sucrose gradients, once treated with β -DM (Table 1). The oligomeric LHCo isolated with the new procedure thus corresponds to the association of F and D sub-complexes which likely reflects the *in vivo* spatial state of the LHC. This statement is well supported by the spectral properties of the LHCo-1 and -2 fractions which both showed energy coupling between Chl *c*, fucoxanthin and Chl *a* (Fig. 7).

Figure 8 presents the SDS-PAGE polypeptide profiles of the LHCo fractions. Subfractions LHCo-1 and -2 (Figure 8A, lanes 2 and 3, respectively) showed a different polypeptide composition, especially in the range 15-22 kDa. Two polypeptides (15 and 17 kDa, solid arrows) visible in the LHCo fraction were only present in the LHCo-2 subfraction (D analogue) and one at 22 kDa (dashed arrows) almost only found in the LHCo-1 (F analogue). This observation was confirmed by a further purification of both LHCo-1 and -2 subfractions with β -DM (Fig. 8B). The lowest band of the 15 kDa doublet and the 17 kDa polypeptide are clearly specific to LHCo-2, and the 22 kDa polypeptide specific of the LHCo-1. Compared to the F and D fractions obtained after sucrose gradients (Fig. 5), the polypeptide patterns of the latter two β -DM treated fractions show that they almost only contain polypeptides in the 12-20 kDa range. The lower contaminations by high molecular mass polypeptides suggest that with the new isolation procedure, all the macromolecular complexes, including PSI and PSII, have kept a more "native" aggregation state.

Discussion

In contrast to the plant light-harvesting complexes, LHCI and LHCII, the diatom LHC is presently poorly characterized even in terms of polypeptide and pigment composition. Concerning the FCPs, six genes have been described for *P. tricornutum* whose products share 86-99% similarity [19], but up to 20 or even more would exist in *Cyclotella cryptica* and *Thalassiosira pseudonana* [22,29]. On the other hand, the diatom xanthophyll cycle required for the establishment of the photoprotective non-photochemical fluorescence quenching (NPQ) also differs. This cycle mainly occurs between two forms, diadinoxanthin (DD) and its de-epoxidized form, diatoxanthin (DT), while three different ones are required in higher plants [13]. Our goal

was to better characterize the *P. tricornutum* LHC, looking for the existence of putative subcomplexes that would contain the xanthophyll pigments. Because it is known from studies on higher plant LHC antennae that pigment-protein complexes can have different stabilities and that their binding affinity for pigment can vary greatly [30,31], we designed a new fractionation procedure, and compared it with previously used isolation techniques.

The organization of the light-harvesting antenna in diatoms. Omitting the sucrose gradient step and using a mild and short detergent treatment under high salt conditions, immediately followed by gel filtration chromatography, we could separate from PSI and PSII a diatom LHC as an oligomer, LHCo, whose molecular size resembles that of the spinach trimers. We further showed that this LHCo is made up of two different subcomplexes. The first part of the LHCo peak essentially corresponds to the F fraction that was previously isolated from sucrose gradient preparations [12], and the second to the D fraction obtained by the same procedure. The isolation of the LHCo as an asymmetric peak (see Fig. 6) strongly suggests that interactions between the two subcomplexes do exist *in vivo*. Compared to the total LHCo, the LHCo-1 (F analogue) is depleted in diadinoxanthin while LHCo-2 (D analogue) is depleted in Chl *c* and highly enriched in diadinoxanthin. Our analyses also demonstrated that the two oligomeric subcomplexes that were isolated have a different polypeptide composition (Fig. 8). Moreover, both fractions can efficiently transfer energy from fucoxanthin to Chl *a*. Thus none of them correspond to free pigments. This means that two subcomplexes do exist and that, by using the newly designed procedure, they have kept a more "native" state than the F and D fractions obtained from the sucrose gradients. A recent study was conducted on the *Cyclotella meneghiniana* LHC, in which two FCP fractions were separated from sucrose gradients, A and B, the latter having a higher apparent molecular size than A [21]. Fraction A is mainly composed of 18 kDa polypeptides and exhibits a 486 nm absorption shoulder; fraction B does not have this shoulder and is made up of

18 and 19 kDa polypeptides. The pigment content of each of these fractions was however not provided. In the present study it is shown that only the D fraction and its analogue (LHCo-2) from the LHCo have a 486 nm absorption peak, and they contain polypeptides of lower molecular masses than the F and LHCo-1 fractions. Fractions D and LHCo-2 thus resemble the fraction A, whereas F and LHCo-1 correspond to the fraction B of *C. meneghiniana*. Büchel [21] also reported that the B fraction is more stable than the A, and our results show that the F fraction (LHCo-1) is similarly more stable than the D (LHCo-2) one. Indeed, a β -DM treatment modifies the molecular size of D (Fig. 2) and LHCo-2 but not that of F, and a loss of energy coupling between fucoxanthin and Chl *a* was observed for the D but not for the F fractions. All of the presently available data confirm that, though sharing a common ancestor, diatoms exhibit an organization and pigment composition for their LHC that clearly differ from that of extant higher plants, in terms of both polypeptide and pigment content.

Consequences of the LHC organization on the mechanism of the excess energy dissipation (NPQ). The spatial organization of the LHC in diatoms is very likely at the origin of the huge NPQs that diatoms can exhibit [16]. Different minor LHC polypeptides (in particular CP26, CP29), as well as PSII small subunits (PsbS = CP22 and PsbZ = Ycf9) have been implicated in the NPQ formation in plant and green algae, underlying that it is a rather complex phenomenon not yet totally understood [32-34]. In plants and green algae, the PsbS protein binds zeaxanthin (the DT analogue) and is required for the NPQ to develop [33]. No CP26, CP29 and PsbS orthologues have been recognized in the fully sequenced diatom genome [22]. In the green microalga *Chlamydomonas reinhardtii* a CAB polypeptide, PsbZ, involved in the oligomeric organization of the LHC was found to affect: i) the deepoxidation of xanthophylls; and ii) the kinetics and amplitude of nonphotochemical quenching [34]. PsbZ (Ycf9) genes are also present

in red algae, diatoms and cyanobacteria. Our working hypothesis is that the functional diatom orthologues of such polypeptides are present in the D and LHCo-2 minor sub-fractions that we purified from *P. tricornutum*. One of the two polypeptides (15 or 17 kDa) specifically found in this LHC subcomplex might play a functional role in DD binding and NPQ formation. When grown under an intermittent light regime, *P. tricornutum* cells show a very high NPQ that was correlated with a specific up to 3-fold enrichment of the LHC in DD and DT [11,12,16]. In this context, the intermittent-light grown *P. tricornutum* cells could constitute a unique model to elucidate the exact role played by the organization of the LHC in the photoprotective energy dissipation. Compared to plants and green algae, the different organization of the diatom LHC, as well as the distribution of the xanthophyll pigments between the two subcomplexes, might ensure more flexibility and thus quicker responses to the important light intensity fluctuations that diatoms encounter in their natural habitat.

Materials and Methods

Culture conditions - *Phaeodactylum tricornutum* Böhlin cells (Laboratoire Arago algal collection, Banyuls-sur Mer, France) were grown photoautotrophically in sterile seawater as described in [35]. Briefly, cultures were incubated at 18 °C in airlifts continuously bubbled with air to maintain the cells in suspension, and under a 16 h light/8 h dark cycle. They were grown under a white light of 40 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by fluorescent tubes (Claude, Blanc Industry, France). Under this light intensity there is no DT formed during the light periods and therefore after purification, the antenna only contains DD. When needed, DT is formed by exposure of the cells to a strong illumination [12,16].

Plastid preparation and membrane solubilization - Diatoms were collected after 4 to 5 days in their exponential growth phase by centrifugation at 3,000 g for 10 min and resuspended in

medium containing 600 mM NaKPO₄ buffer, pH7.5, 5 mM EDTA, 1/100 (v/v) of the Sigma protease inhibitor cocktail. Cells were broken by two 15 s cycles of sonication and centrifuged for 5 min at 400 g. The pellet was sonicated for a second time and centrifuged as above. Chloroplasts from the two supernatants were pelleted by centrifugation at 12,000 g for 10 min and resuspended in the same high salt buffer, at a chlorophyll concentration of 2 mg.ml⁻¹.

P. tricornutum chloroplasts were solubilized with α -DM at a 1/15 chlorophyll detergent ratio for 15-30 min and centrifuged in Eppendorf tubes at 12,000 g for 10 min to remove insoluble material. All these steps were performed at 4°C.

Sucrose gradients - Exponential 7-step sucrose gradients were prepared in either a low-salt buffer containing 50 mM HEPES, pH 7.5, 5 mM EDTA, 0.03 % of α -DM and 1mM PMSF or a high-salt buffer containing 100 mM NaKPO₄, pH 7.5, 5 mM EDTA, 0.03% of α -DM and 1 mM PMSF. Detergent-solubilized membrane fractions in 600 mM NaKPO₄ were buffer exchanged on PD-10 column (Amersham Pharmacia) against low-salt or high-salt buffers before loading on appropriate gradients. Centrifugation was performed using a SW41 rotor in a Beckman XL-90 ultracentrifuge at 250,000 g for 17-20 hrs at 4°C. Monomeric and trimeric forms of LHCII from market spinach were used as standards for the sucrose gradient and gel filtration procedures. The preparation of PSII membranes from spinach thylakoids was performed as described by Burke *et al.* [26].

Gel filtration - Gel filtration chromatographies were performed using the Biologic Duo flow system (Biorad). Fractions from the gradients were collected and further purified by gel filtration on a Superdex TM 200 10/300GL Tricorn column (Amersham) with a flow rate of 0.3 ml.min⁻¹. The running buffer was 20 mM NaKPO₄, pH 7.5 supplemented with 10 mM EDTA, 1mM PMSF and 0.03% α -DM. Elution profiles were recorded at 280 nm to detect proteins or 436 nm

to detect chlorophylls, and 0.3 ml fractions were collected. A further purification using β -DM was sometimes used as indicated in the text and figure legends.

A direct gel filtration, without any previous sucrose gradient step, was performed following a shorter detergent treatment (5 min α -DM solubilization of thylakoids on ice, using a 1/15 chlorophyll/detergent ratio). This new procedure was devised in an attempt to get better-preserved fractions. Solubilized membrane fractions were centrifuged at 12,000 g in Eppendorf tubes to remove insoluble material before applying the samples onto the column.

Spectroscopic analyses - Absorption measurements were performed using a DW-2 Aminco spectrophotometer. 77K fluorescence emission and excitation spectra were measured on a Hitachi F-4500 spectrophotometer with 2.5 nm spectral resolution for both types of measurements.

Pigment analysis - Pigment contents of cells, plastids and isolated fractions were determined by the HPLC method as described earlier [12]. Extraction was performed using the phase separation procedure first with one volume of a methanol/acetone (50:50, v/v) solution followed by one volume of ether and two volumes of a 10% NaCl solution.

Gel electrophoresis - Polyacrylamide gel electrophoresis was performed using 10-15 % gels according to Laemmli and stained with silver nitrate (Amersham Biosciences kit).

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Table 1. Pigment composition of *P. tricornutum* plastids and antenna fractions obtained after solubilization of the plastids by n-dodecyl- α ,D-maltoside followed by separation on the sucrose gradient (see Fig. 1). Whenever indicated (+) the fractions have been treated with n-dodecyl- β ,D-maltoside before gel filtration. Pigment composition is given in mol per 100 mol of Chl*a*.

β -DM treatment	Plastids	F fraction		D fraction	
	-	-	+	-	+
Chl <i>a</i>	100	100	100	100	100
Chl <i>c</i>	15.5 \pm 0.8	30.9 \pm 1.5	21.8 \pm 1.1	10.8 \pm 0.5	3.1 \pm 0.2
Fucoxanthin	63.3 \pm 3.2	122 \pm 6.1	106.6 \pm 5.3	163.7 \pm 8.2	145.1 \pm 7.2
Diadinoxanthin	9.06 \pm 0.5	6.6 \pm 0.3	2.1 \pm 0.1	41.7 \pm 2.1	60 \pm 3.0

Table 2. Pigment composition of LHC*o* fractions prepared from isolated *P. tricornutum* plastids solubilized with n-dodecyl- α ,D-maltoside and separated on the gel filtration column. Whenever indicated (+) the fractions have been treated with n-dodecyl- β ,D-maltoside before gel filtration. Pigment composition is given in mol per 100 mol of Chl*a*.

β -DM treatment	Total LHC <i>o</i>	LHC <i>o</i> -1		LHC <i>o</i> -2	
	-	-	+	-	+
Chl <i>a</i>	100	100	100	100	100
Chl <i>c</i>	24.9 \pm 1.2	23.5 \pm 1.2	26.7 \pm 1.3	13.4 \pm 0.7	8 \pm 0.4
Fucoxanthin	108.5 \pm 5.4	111.9 \pm 5.6	110.9 \pm 5.5	171.2 \pm 8.6	179.2 \pm 9.0
Diadinoxanthin	9.6 \pm 0.5	4.6 \pm 0.2	1.4 \pm 0.1	26.6 \pm 1.3	51 \pm 2.6

Figure legends

Figure 1. Schematic representation of the pigment-protein complexes separated by sucrose gradients. *P. tricornutum* isolated plastids and spinach membranes were solubilized by n-dodecyl- α ,D-maltoside. A) *P. tricornutum* plastids in the low-salt buffer; B) Enriched PSII membranes from spinach in the low salt buffer (see Materials and Methods); C) *P. tricornutum* plastids in the high-salt buffer. Labelings on the left: D, F, F1 and F2 correspond to LHC fractions of the diatom antenna; for the spinach chloroplasts, Fp corresponds to the free pigment fraction, PSI and PSII to photosystems I and II, respectively.

Figure 2. Elution profiles after gel filtration of LHC fractions obtained from sucrose gradients. Trace 1, F fraction; trace 2, F fraction pretreated with 1 % n-dodecyl- β ,D-maltoside for 10 min; trace 3, D fraction; and trace 4, D fraction pretreated with n-dodecyl- β ,D-maltoside. Trimer (t), monomer (m) and free pigment (fp) correspond to the gel filtration traces of the fractions obtained from spinach particles after sucrose gradient procedure as shown on Fig. 1B. Absorption was monitored at 280 nm, except for the free pigment (fp) monitored at 436 nm.

Figure 3. Absorption spectra of purified D and F fractions obtained by the gel filtration procedure after sucrose gradients. The dashed lines represent the second derivatives of the spectra; for clarity, a multiplying factor of 4 was applied to draw the trace from 400 to 570 nm. The x4 label indicates that the multiplying factor used to draw the trace.

Figure 4. 77K chlorophyll fluorescence spectra of purified D (solid line) and F (dashed line) fractions obtained by the gel filtration procedure after sucrose gradients. A) Spectra were

normalized to the ~670 nm peak, and B) excitation spectra of the fluorescence emission at 672 nm.

Figure 5. SDS-PAGE analysis of LHC fractions prepared by gel filtration: D and F originate from sucrose gradients. Th, proteins from whole plastids; MM, molecular mass markers.

Figure 6. Gel filtration profiles of *P. tricornutum* plastids solubilized with α -D-maltoside (solid line), and of the LHCo thus obtained and further treated with β -D-maltoside (dashed line).

Figure 7. 77K excitation spectra of chlorophyll fluorescence emission at 672 nm for the two LHCo fractions.

Figure 8. SDS-PAGE analysis of the fractions obtained after direct gel filtration of α -D-maltoside solubilized plastids (see Fig 6). A) LHCo; LHCo-1 (1°) and LHCo-2 (2°), MM corresponds to the molecular mass markers. Solid arrows point to polypeptides present in LHCo and LHCo-2 but absent from the LHCo-1 fraction; dashed arrows to those specific to LHCo-1. B) Lane 1 corresponds to the LHCo-1 and lane 2 to that fraction after β -D-maltoside treatment and a second gel filtration, lane 3 to the LHCo-2 and lane 4 to the β -D-maltoside treated LHCo-2 fraction; MM shows the molecular mass markers. Loadings were based on Chl *a* contents: 0.5 μ g for LHCo (lane 1 of part A) and for LHCo-2 (lanes 3 and 4 of part B); 0.1 μ g for LHCo-1 and LHCo-2 (lanes 2 and 3 of part A) and lanes 1 and 2 of part B.

Figure 1. Guglielmi et al.

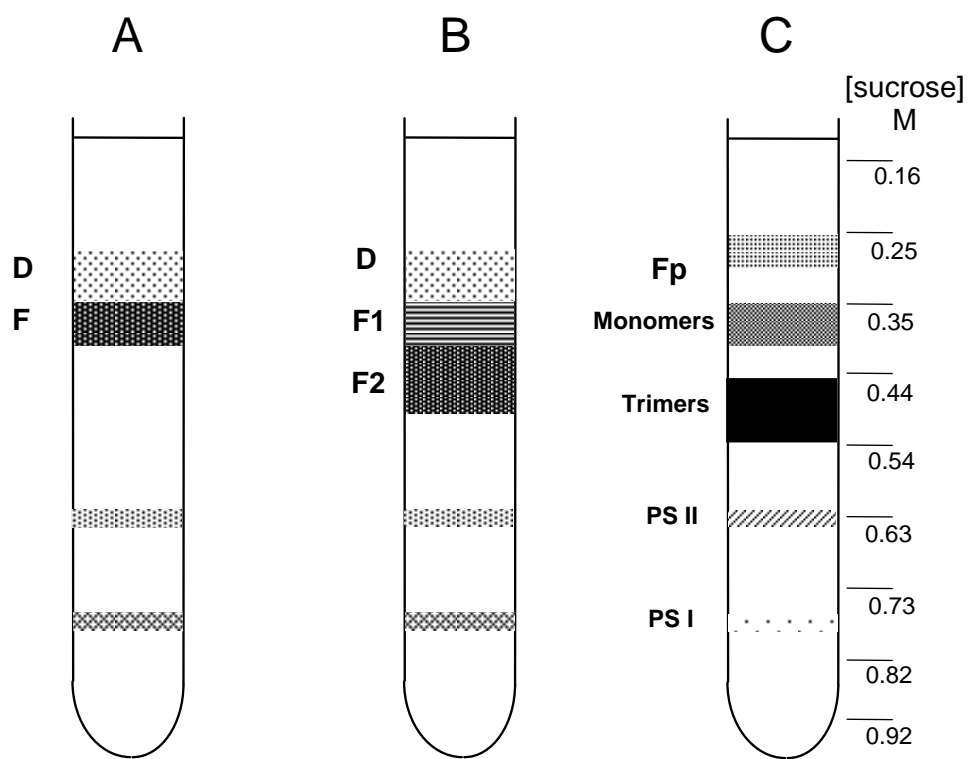


Figure 2. Guglielmi et al.

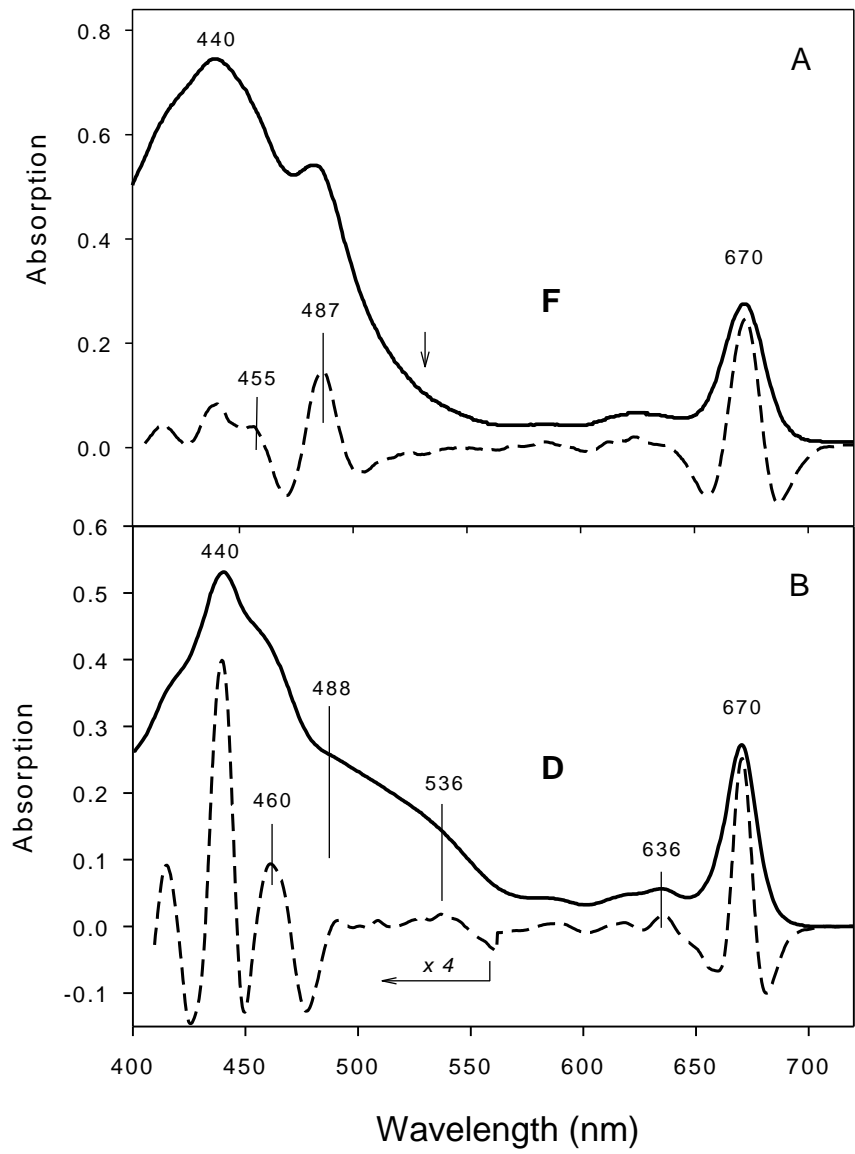


Figure 3. Guglielmi et al.

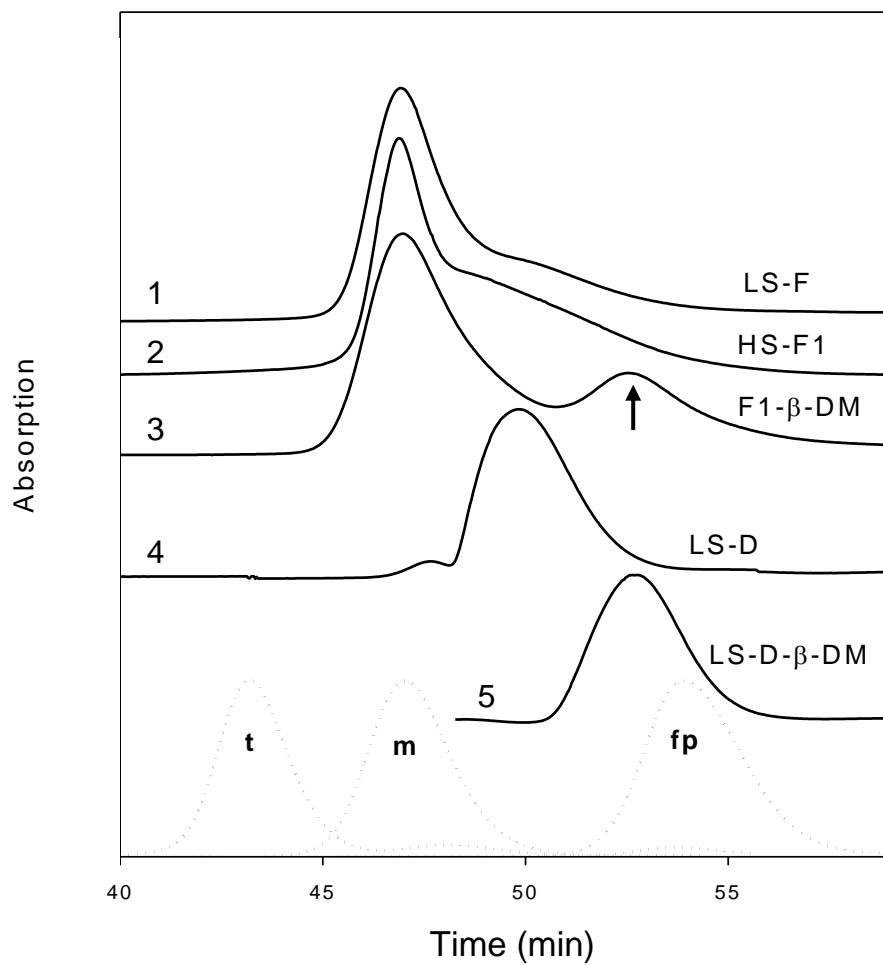


Figure 4. Guglielmi et al.

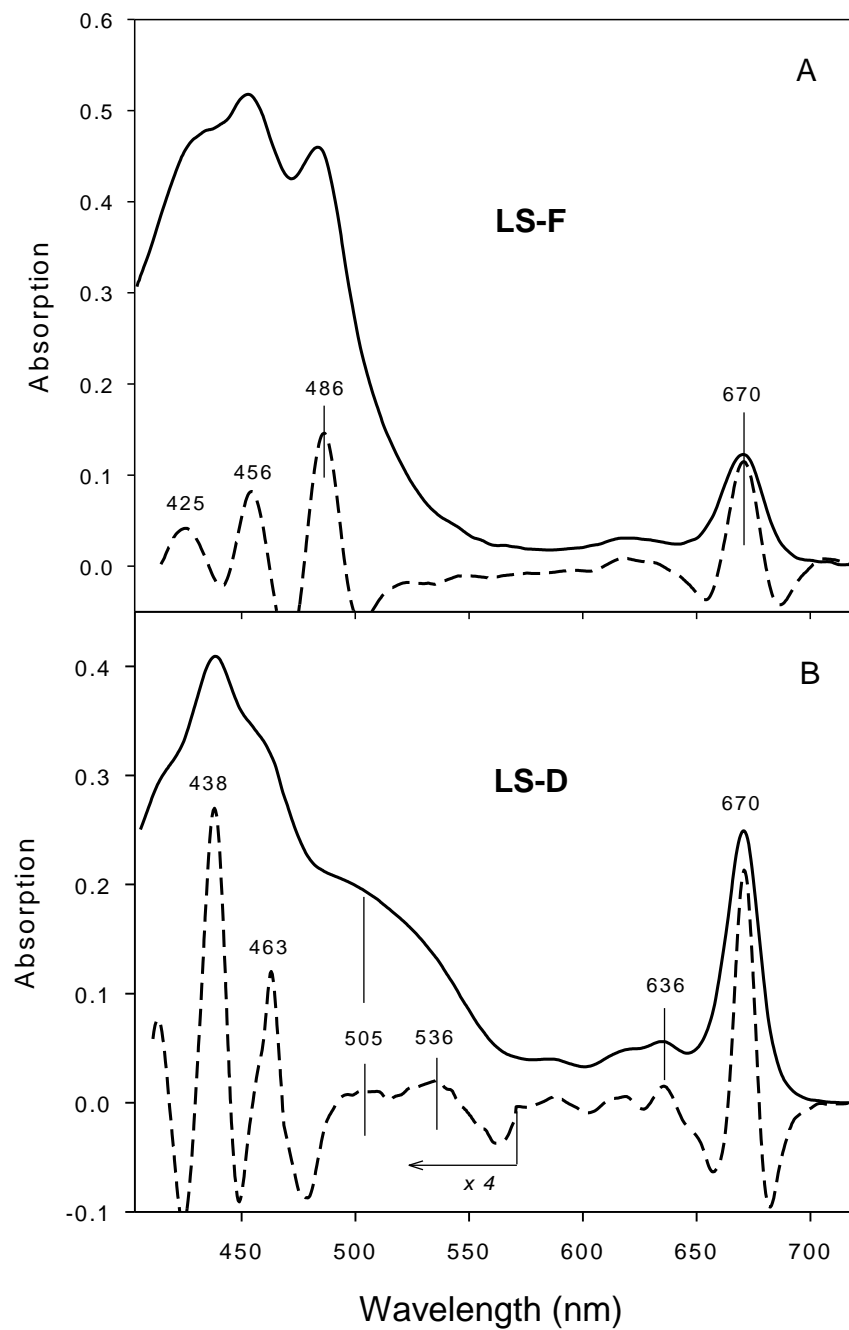


Figure 5. Guglielmi et al.

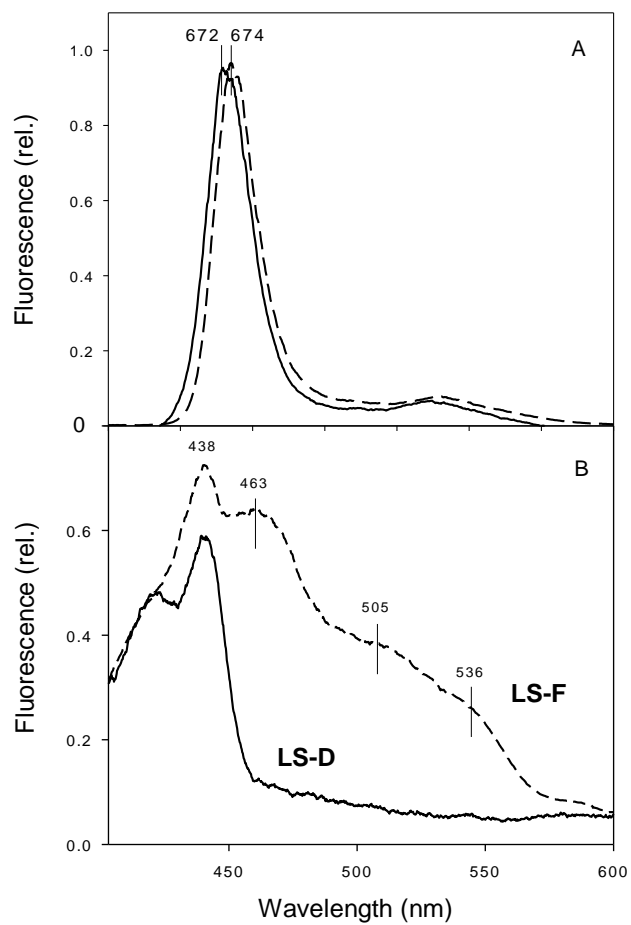


Figure 6. Guglielmi et al.

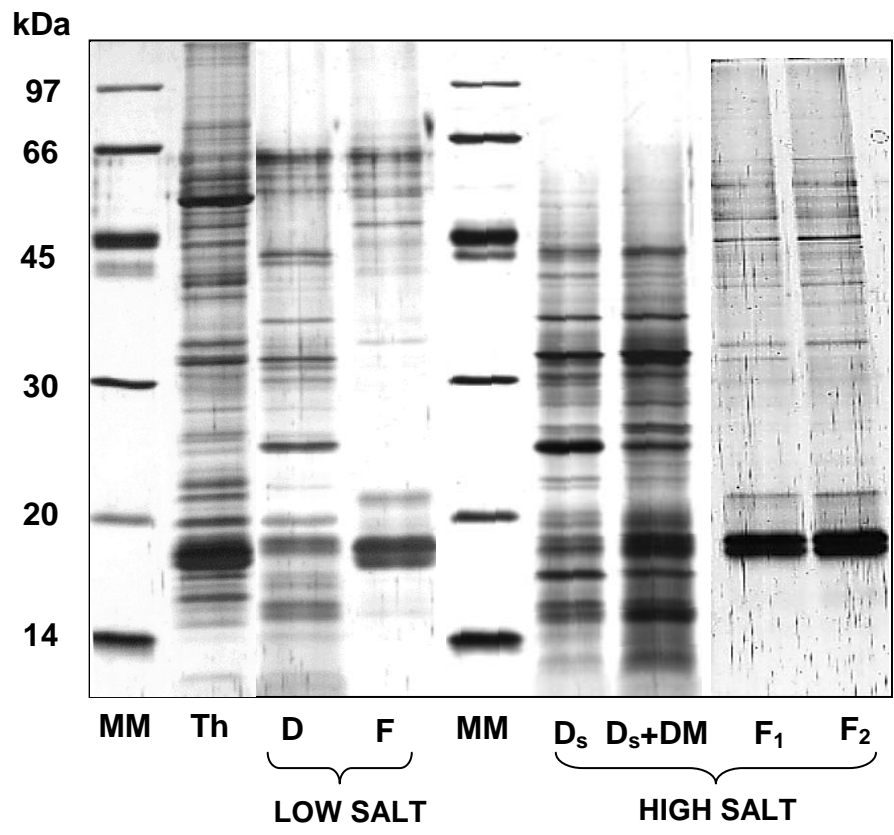


Figure 7. Guglielmi et al.

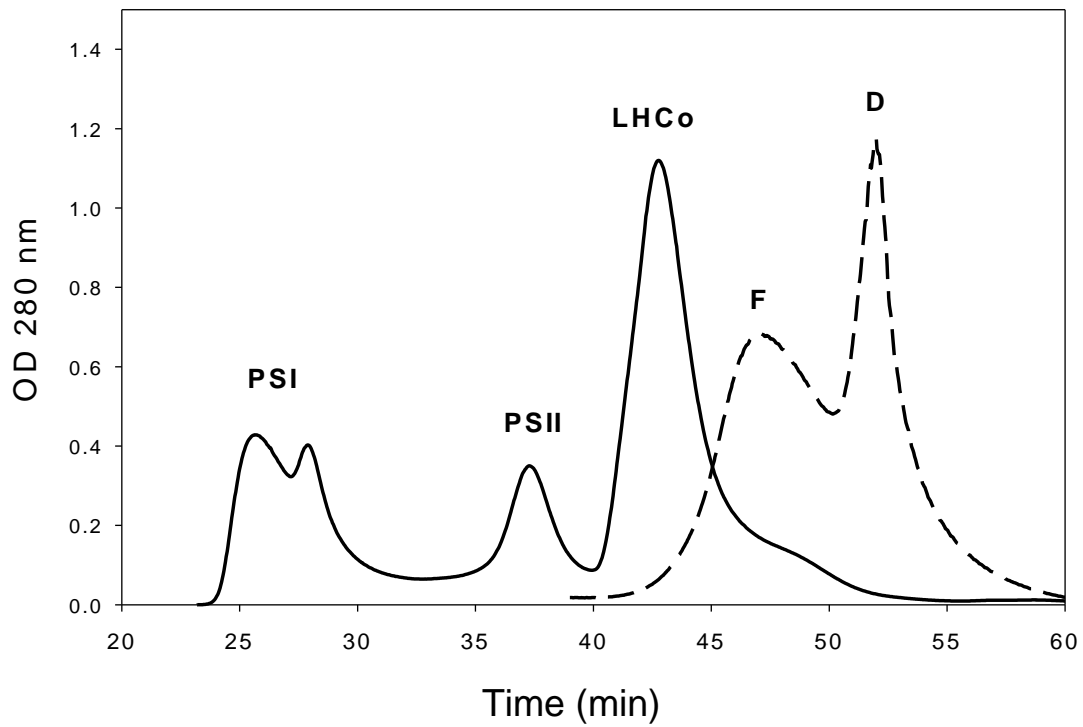


Figure 8. Guglielmi et al.

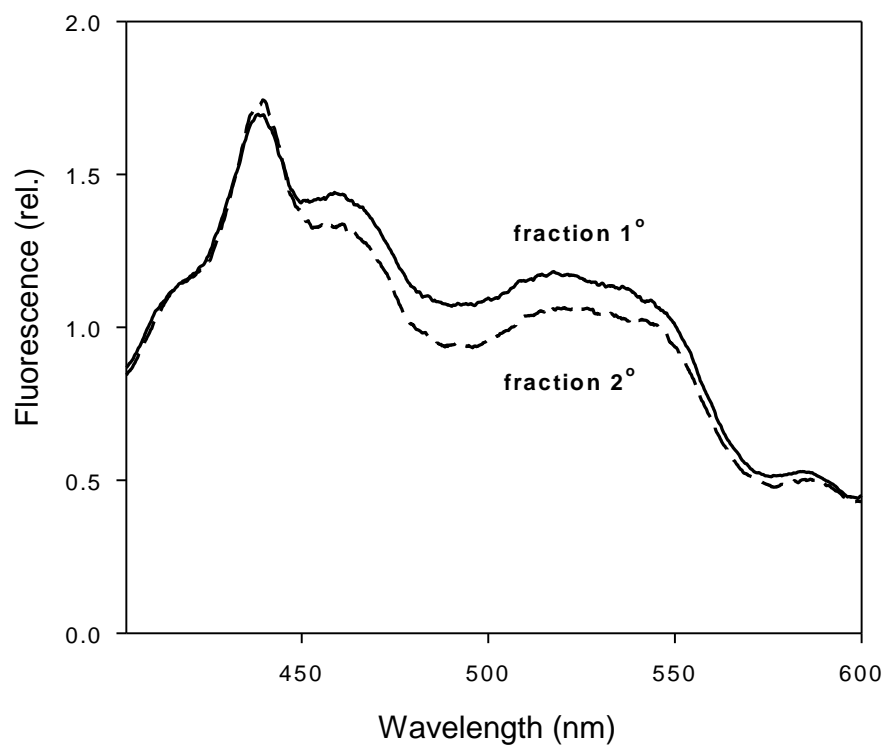


Figure 9. Guglielmi et al.

