Chapter 2

Structure and Functional Heterogeneity of Fucoxanthin-Chlorophyll Proteins in Diatoms

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Summary

Fucoxanthin-chlorophyll proteins (FCPs) of diatoms are divided into three groups, the main light harvesting antennas Lhcf, the photosystem I-specific Lhcr, and Lhcx involved in photoprotection. All are closely related to higher plant light harvesting complexes (LHCs) when comparing sequences, albeit smaller and more hydrophobic. However, pigmentation differs from higher plant LHCs with around eight chlorophyll *a*, two chlorophyll *c* and six fucoxanthin per monomer. Fucoxanthin, with a carbonyl moiety conjugated to the polyene backbone, undergoes extreme bathochromic shifts upon protein binding, dividing the different fucoxanthins into more red, green and blue absorbing ones. Excitation energy transfer is extremely efficient, either directly from chlorophyll *c* to chlorophyll *a* or from fucoxanthin to chlorophyll *a* involving the S_1/ICT state of fucoxanthin. Most Lhcf assemble into trimers, whereby only in centric diatoms Lhcx was found in trimers as well, and specific oligomeric FCP complexes are present. Whereas the arrangement of FCPs around the photosystems is largely unknown, spectroscopic measurements together with homology considerations allow for a first rough model of the pigment arrangement in trimeric and oligomeric FCP complexes. Blue fucoxanthin is bound analogously to lutein in LHCII, surrounded by the same four chlorophyll *a*, since binding sites are conserved. Additionally, chlorophyll *a* can be found in

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a604, a614, b605 and a611, although binding of the latter has to be different due to the lack of long wavelength absorption in FCPs. Chlorophyll *c* is most probably bound in b609 and a613. The red fucoxanthin cluster around helix 2, which has less sequence homology to LHCII. The green fucoxanthins are most probably located around the violaxanthin and b601 binding sites of LHCII, whereby the former is probably a mixed site for fucoxanthins and diadinoxanthin/diatoxanthin.

I. Introduction

Membrane-intrinsic light-harvesting proteins belonging to the same family as higher plant LHC are wide-spread amongst other eukaryotic photoautotrophic organism like e.g. stramenopiles, including brown algae and diatoms. This review will focus on diatom light- harvesting proteins, which, due to their main carotenoid, are also called fucoxanthin-chlorophyll proteins (FCPs).

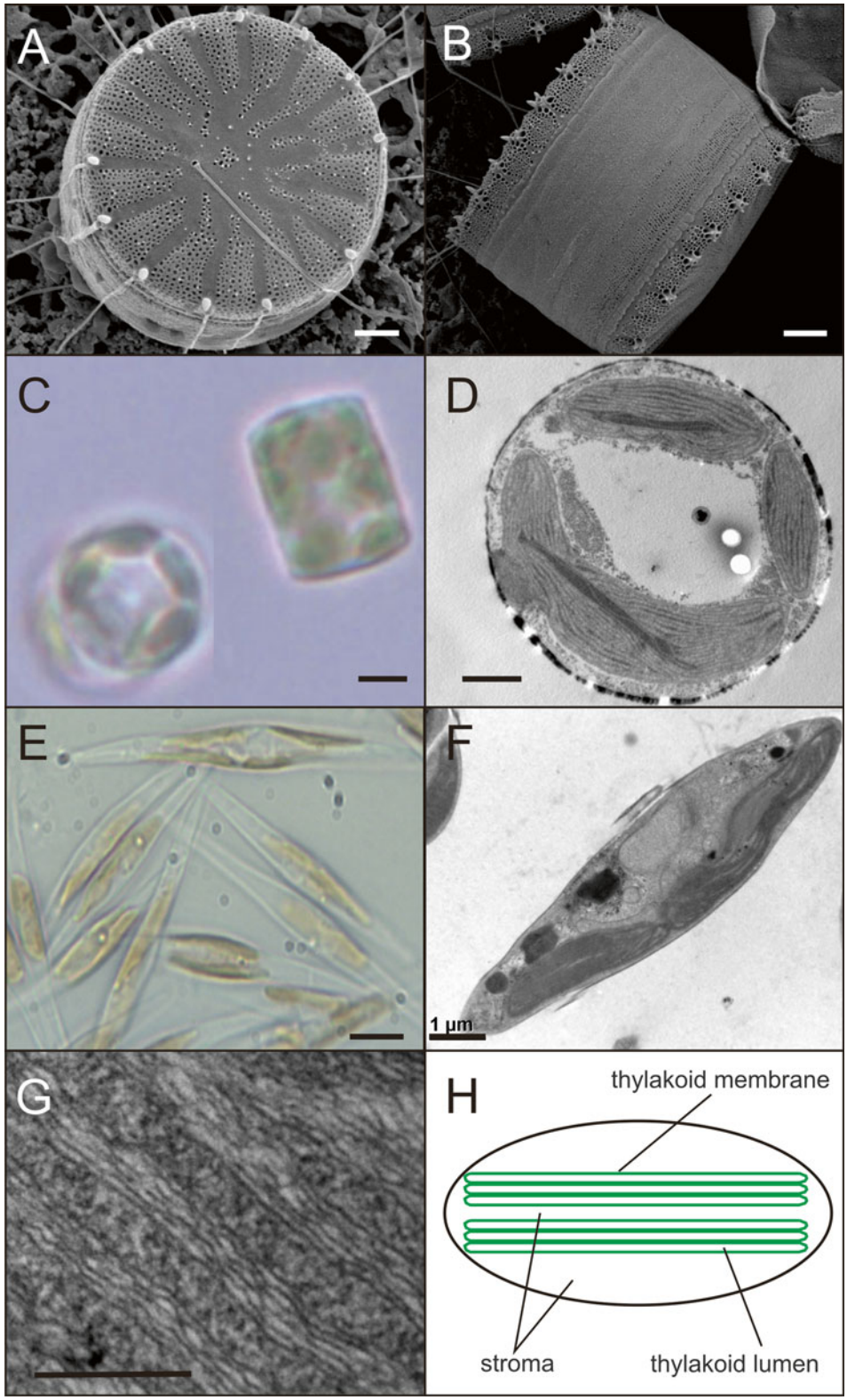
Diatoms are unicellular photosynthetic organisms characterized by an ornamental cell wall made of silica (Raven and Waite 2004) (Fig. 2.1a, b). They fall into two main groups, the so-called pennate diatoms and centric diatoms (Medlin et al. 1996). Whereas the former are longish in shape and usually contain one or two plastids per cell, the centrics have a rotational symmetry and are characterized by more than two plastids (Medlin et al. 1996) (Fig. 2.1c–f). In the public data bases, two analyzed genomes are available at the moment, one of the pennate diatom *Phaeodactylum tricornutum* and one from the centric *Thalassiosira pseudonana* (Armbrust et al. 2004; Bowler et al. 2008). See also Chap. 18 for information on carbon fixation in Diatoms.

Stramenopiles plastids are derived from a secondary endosymbiosis (for recent review see Green (2011)). Ancient red algae are postulated to be the endosymbionts, i.e. the ancestors of the plastids (Archibald and Keeling 2002; Wolfe et al. 1994), but genes typical of green algae and plants can be found as well (Moustafa et al. 2009). Due to the secondary endosymbiosis, chloroplasts are enveloped by four membranes instead of two membranes indicating a primary endosymbiotic event. The outer chloroplast membrane is in connection with the nuclear endoplasmatic reticulum that possesses ribosomes (Gibbs 1970).

The thylakoid structure in brown algae and diatoms is different as well: no grana – stroma differentiation (Gibbs 1970) can be found and thus no lateral heterogeneity concerning the distribution of photosystem (PS) I and PS II was detected so far (Pyszniak and Gibbs 1992). Thylakoids are instead organized in long bands of three thylakoids (i.e. six membranes) each, running along the whole length of the plastids (Pyszniak and Gibbs 1992) (Fig. 2.1g, h). The thylakoid membranes contain the multi-protein

Abbreviations: Chl – Chlorophyll; Dd – Diadinoxanthin; Dt – Diatoxanthin; FCP – Fucoxanthin-chlorophyll protein; Fx – Fucoxanthin; LHC – Light-harvesting complex; NPQ – Non-photochemical quenching; PS – Photosystem

Fig. 2.1. Diatoms are unicellular organisms characterized by an ornamental silica cell wall as seen by scanning electron microscopy for the centric diatom *C. meneghiniana* (**a**, **b**). These diatoms contain more than two chloroplasts per cell as shown in the light micrograph (**c**) and the transmission electron microscopy picture (**d**) of *C. meneghiniana*. The morphology of pennate diatoms, here *P. tricornutum*, is demonstrated in (**e**, light microscopy) and (**f**, transmission electron microscopy). The arrangement of thylakoid membranes in bands of three thylakoids each are shown in thin sectioning in (**g**) and in a schematic overview of the plastid in (**h**). *Bars* represent 1 µm in (**a**), (**b**), (**d**) and (**f**), 2 µm in (**c**) and (**e**), and 0.2 µm in (**g**).


A
B
C
D
E
F
1 µm
G
H
thylakoid membrane
stroma
thylakoid lumen

complexes usually found in eukaryotic thylakoids, i.e. photosystem I and II, cytochrome b_6/f complexes (see Chap. 8), ATP synthases (see Chap. 6) and antenna systems. Photosystems are homologous to those of higher plants except for some minor subunits (Ikeda et al. 2008; Nagao et al. 2007, 2010; Veith et al. 2009; Veith and Büchel 2007), and in general light capture and electron transport thus resemble those of spermatophytes. However, the lack of grana poses the questions of how energy is distributed between the photosystems and how regulation is achieved. Some indications exist for a domain structure, e.g. results of circular dichroism (CD) measurements of intact diatom cells (Szabó et al. 2008) were interpreted to indicate domains of different lipid composition (Lepetit et al. 2010).

II. The Light Harvesting Proteins of Diatoms

A. The Fucoxanthin-Chlorophyll Proteins

All FCPs are rather similar at the protein level. Prediction from gene sequences show three membrane spanning helices, whereby helix 1 and 3 are homologous to those in higher plant LHC and therefore assumed to form a similar cross-like superhelical structure (Green and Kühlbrandt 1995; Green and Pichersky 1994) (Fig. 2.2b, according to Eppard and Rhiel (1998)). In contrast, helix 2 shows significant deviations. In addition, FCPs are generally smaller than LHC proteins due to smaller loops and termini. Thus, molecular weights are in the range of 18–21 kDa (Eppard and Rhiel 1998). As a consequence, the proteins are even more hydrophobic than higher plant LHCs.

FCPs can be divided into three groups (Fig. 2.2a): the main light harvesting proteins, called Lhcf nowadays, are homologous between diatoms and their relatives, e.g. brown algae. These proteins are very abundant and were the first to be identified at the gene-level (Bhaya and Grossman 1993; Eppard et al. 2000; Eppard and Rhiel 1998, 2000). A second group is called Lhcr, due to their similarity to red algal LHCI genes (Durnford et al. 1996). The third group is related to LhcSR (former LI818) proteins of *Chlamydomonas reinhardtii*, which are involved in light protection (Eppard and Rhiel 1998; Peers et al. 2009; Richard et al. 2000; Zhu and Green 2010), and are called Lhcx in diatoms. Whereas the Lhcr and Lhcx proteins are very similar in centric and pennate diatoms, the Lhcf proteins fall into three different groups (Fig. 2.2, adapted from Gundermann et al. 2013), whereby two groups are more specific for pennates or centrics, respectively, and the third group contains members from both.

As can already be seen from the list of members of the Lhcf group (Fig. 2.2a), diatoms possess quite a number of FCP genes, e.g. for *P. tricornutum* 17 Lhcf genes, 14 Lhcr and 4 Lhcx are annotated and for *T. pseudonana* 11 Lhcf, 14 Lhcr and 7 Lhcx genes are described. All are expressed according to EST data or their expression was proven otherwise (Nymark et al. 2009). Whereas a few of those are identical and thus probably the result of gene duplications, most of them show very small sequence differences. The high number of almost identical proteins might be related to the huge adaptability of diatoms. The even larger number of FCPs encoded by the genome of *Fragilariopsis cylindrus* (http://genome.jgi-psf.org/), a psychrophilic alga living in the Antarctic ice, argues for this.

B. Supramolecular Organization of Fucoxanthin-Chlorophyll Proteins

Biochemical work on FCPs dates back to the 80s, when mainly sucrose density centrifugation was used to separate photosystems from FCPs as a whole (Alberte et al. 1981; Brown 1988; Caron and Brown 1987; Fawley et al. 1986; Friedman and Alberte 1984, 1986; Gugliemelli 1984; Owens 1986, 1988; Owens and Wold 1986). Most FCPs can be isolated as ‘free’ FCPs, i.e. separate from the photosystems, by many biochemical methods

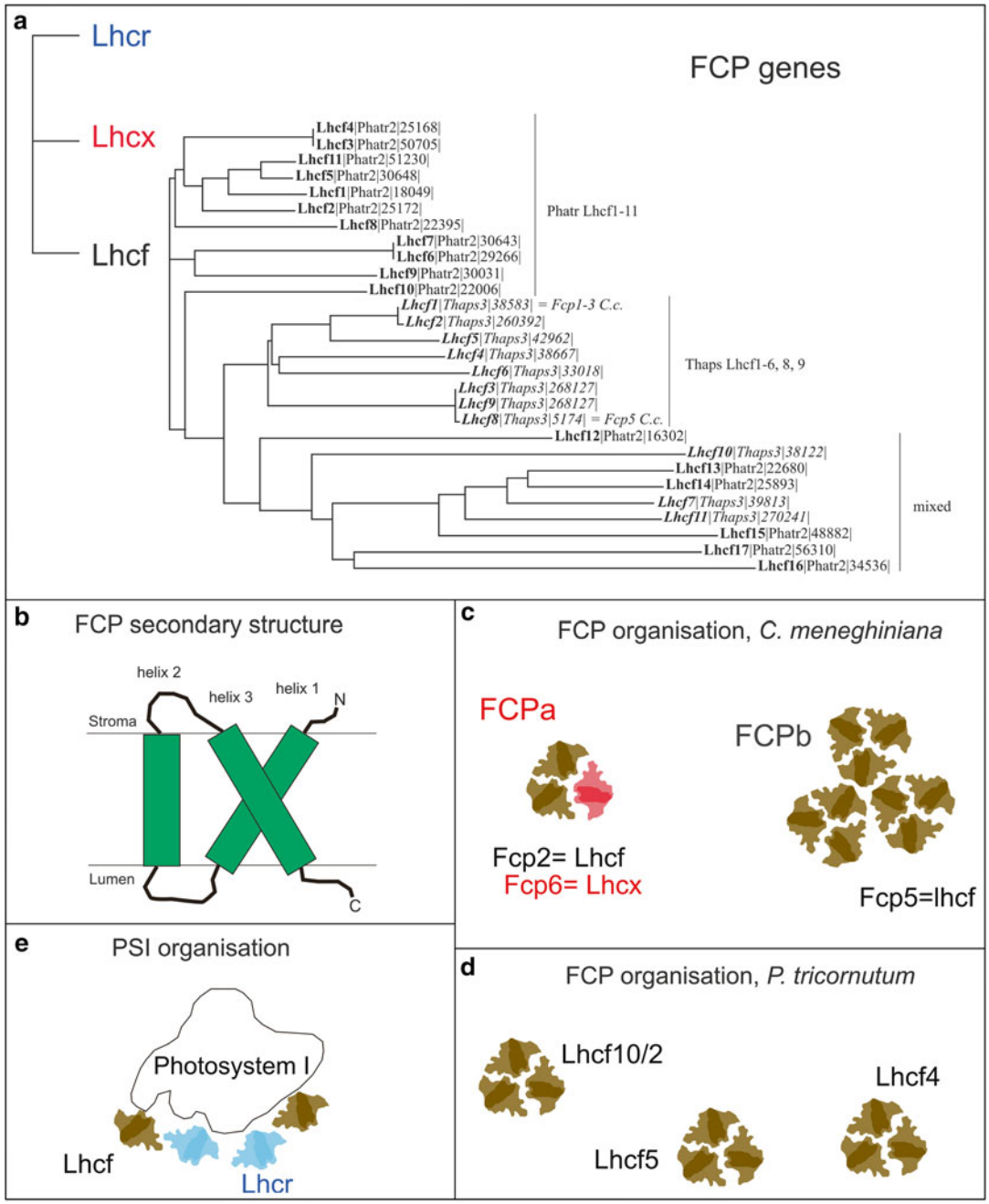


Fig. 2.2. Diatom light-harvesting polypeptides can be differentiated into three groups, Lhcr, Lhcf and Lhcx proteins. Concerning pennate and centric diatoms, most of the Lhcf genes are different between the two groups as demonstrated by the phylogenetic tree (Adapted from Gundermann et al. 2013) (**a**, Phatr = *P. tricornutum*, Thaps = *T. pseudonana*). All of them share the same topology with three membrane-spanning helices predicted to show the same arrangement as in LHCII, as depicted in the cartoon as side view into the membrane (**b**). However, the oligomeric state and polypeptide composition of the FCP complexes is different between centrics and pennates: whereas in the centric diatom *C. meneghiniana* (**c**) trimers (FCPa) and higher oligomers (FCPb) make up the main antenna complexes, only trimers can be found in the pennate *P. tricornutum* (**d**), whereby the trimer population is quite inhomogeneous. Labels refer to the main polypeptides only. In the trimers found in *C. meneghiniana*, also Lhcx polypeptides are present, which have so far not been reported for *P. tricornutum*. On the other hand the composition of the photosystem I antenna (**e**) seems to be similar with mainly Lhcr and Lhcf proteins bound.

(sucrose density centrifugation, ion exchange chromatography, blue native polyacrylamide electrophoresis) (Beer et al. 2006, 2011; Berkaloff et al. 1990; Brakemann et al. 2006; Büchel 2003; Grouneva et al. 2011; Guglielmi et al. 2005; Gundermann and Büchel 2008; Lavaud et al. 2003). This 'pool' of FCPs consists of trimeric FCP complexes (Büchel 2003; Lepetit et al. 2007), whereby in centrics specific complexes of higher oligomeric state were found as well (Beer et al. 2006, 2011; Büchel 2003; Grouneva et al. 2011; Gundermann and Büchel 2008) (Fig. 2.2c). In *P. tricornutum*, a pennate diatom, three major trimers were isolated lately, composed of Lhcf5, Lhcf10/2 and Lhcf4 with different interaction partners, respectively (Gundermann et al. 2013) (Fig. 2.2d). No members of the other Lhc families (Lhcr or Lhcx) could be found in these trimers. This is in contrast to *Cyclotella meneghiniana*, a centric diatom closely related to *T. pseudonana*, where Lhcx (Fcp6) proteins were found in the major trimeric complex, named FCPa, accompanied by Lhcf proteins, mainly by Fcp2 (Fig. 2.2c). The oligomeric complex found in these organisms, named FCPb, was composed solely of Lhcf polypeptides, most probably Fcp5 (Beer et al. 2006; Büchel 2003). Since diatoms contain many more Lhc genes than higher plants a larger hetereogeneity in trimer and oligomer composition than elucidated so far may be revealed using more sophisticated biochemical separation methods.

In higher plants there is excellent knowledge about the supramolecular structure of photosystem I as well as photosystem II (for review see e.g. Dekker and Boekema (2005)). In diatoms, in contrast, the attribution of the different FCPs to the two photosystems and/or their supramolecular structure remains ill-defined. Photosystem I supercomplexes were isolated early (Berkaloff et al. 1990) and from several organisms (Brakemann et al. 2006; Grouneva et al. 2011; Ikeda et al. 2008; Veith et al. 2009; Veith and Büchel 2007). Since gene sequences became available, Lhcr proteins were always supposed to serve as PSI antennas, which could indeed be shown at the protein level as well (Grouneva et al. 2011; Veith et al. 2009) (Fig. 2.2e). There is still some controversy, as to whether this PSI antenna is exclusively composed of Lhcr proteins (Lepetit et al. 2010). Results by blue native polyacrylamide electrophoresis (Grouneva et al. 2011), a method more stringent than e.g. sucrose density centrifugation, supported earlier data about the presence of Lhcf proteins as part of the PSI antenna (Brakemann et al. 2006; Juhas and Büchel 2012; Veith et al. 2009; Veith and Büchel 2007). In addition, Lhcx polypeptides were found, but solely in pennates (Grouneva et al. 2011). Like in higher plants, PSI of diatoms is a monomer (Veith and Büchel 2007), but no data about the arrangement of the antenna proteins are currently available.

Very little is known about PSII-specific interactions of FCPs so far, since supercomplexes as can be isolated from higher plants (Boekema et al. 1995) have not been obtained yet. Nagao et al. (2007) and Nagao et al. (2010) were able to isolate PSII complexes from *Chaetoceros gracilis*, which still contained FCP proteins, but unfortunately these FCPs were too loosely bound to allow for a more detailed analysis. Thus, no proof for minor Lhcs like CP24, CP26, or CP29 found in plant systems, is available so far, despite immunological similarities (Rhiel et al. 1997).

In summary, Lhcf polypeptides are the main constituents of FCP trimers, whereas Lhcx proteins were found only in trimers from centric diatoms so far. In those organisms also higher oligomers built from specific Lhcf polypeptides are present as well. PSI is associated with Lhcr proteins, but also with Lhcf (and Lhcx) polypeptides. The structural association of FCPs with PSII remains unresolved. Only some data obtained in *C. meneghiniana*, points to FCPb complexes being more closely associated with PSI, and FCPa serving PSII (Szabó et al. 2010; Veith and Büchel 2007). This severe lack of precise knowledge concerning protein associations also extends to the overall arrangement of the complexes in the thylakoid membranes.

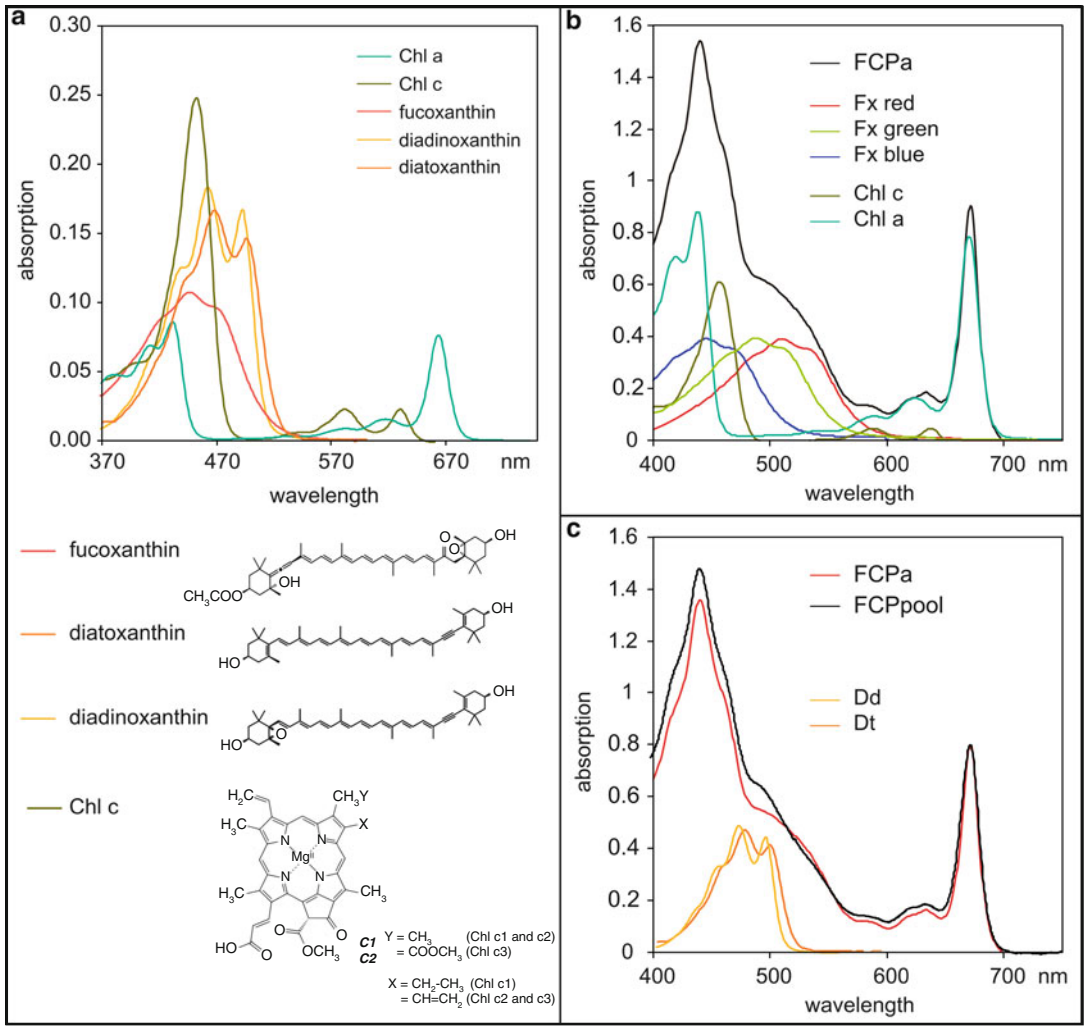


Fig. 2.3. Chl *a*, Chl *c* and Fx are bound to FCP complexes besides the xanthophyll cycle pigments Dd and Dt. In (**a**) the absorbance spectra of all pigments (1 mM in 80 % acetone) are shown. Upon binding to the protein, Fx undergoes extreme bathochromic shifts, whereby several populations can be distinguished, i.e. '*red*', '*green*' and '*blue*' Fx (**b**) (Adopted from Premvardhan et al. 2008). Depending on the isolation method (sucrose gradient centrifugation, FCP pool, or ion exchange chromatography, FCPa) different amounts of Dd and Dt can be found in the preparations as demonstrated by the absorbance spectra in (**c**).

C. Pigmentation of Fucoxanthin-Chlorophyll Proteins

When comparing plant LHC with FCPs the most obvious difference is the pigmentation. Diatoms exhibit a brownish color, which is due to the carotenoid fucoxanthin (Fx) bound to FCPs. This carotenoid is found in much higher amounts in FCPs than carotenoids in LHCII: the molar Chl/carotenoid ratio is almost 1:1 in FCPs compared to the 14:4 in LHCII (Beer et al. 2006; Papagiannakis et al. 2005). Fucoxanthin is a rather peculiar carotenoid with a carbonyl moiety in conjugation with the polyene backbone that is also found in peridinin (Damjanović et al. 2000; Frank et al. 2000; Katoh et al. 1991; Zigmantas et al. 2004) (Fig. 2.3a). Fx displays an extreme bathochromic shift upon protein binding, extending the absorption from

390 nm up to 580 nm (Premvardhan et al. 2009). This light-absorbing capability in the blue-green range is used for successful photosynthesis in the aquatic environments. Since the bathochromic shift depends strongly on the polarity of the protein environment (Koyama et al. 1996), different shifts are exhibited by the different Fx found in a FCP monomer. Using stark and resonance Raman spectroscopy, more 'blue', 'green' and 'red' absorbing Fx molecules could be detected (Fig. 2.3b) in FCPa as well as in FCPb (Premvardhan et al. 2008, 2009, 2010). The existence of differently absorbing Fx was also demonstrated in whole cells using electrochromic shift measurements (Szabó et al. 2010). In carotenoids in general, due to their symmetry, absorption from the ground state to the lowest-energy singlet S_1 state (2^1A_g) is symmetry-forbidden. Instead, excitation of the ground state (1^1A_g) results in the S_2 state (1^1B_u). In carbonyl-containing carotenoids it was shown that after excitation into S_2 the so-called ICT (intramolecular charge transfer) state is populated (Zigmantas et al. 2004). This state is strongly coupled to the S_1 state and more generally referred to as S_1/ICT. Indeed, the excitation energy transfer from Fx to Chl *a* was shown to proceed mainly via the S_1/ICT state in FCPs (Gildenhoff et al. 2010a; Papagiannakis et al. 2005). Stark spectroscopy reveals that upon photon absorption by Fx in solvent, a huge change in the static dipole moment of 17 D takes place, indicating photo-induced charge transfer of the Fx molecules. When Fx in FCPs is examined, two population with changes of 17 D and up to 40 D can be distinguished, again underlining the different properties of the different Fx molecules bound (Premvardhan et al. 2008).

As accessory chlorophyll Chl *c* is found instead of Chl *b*, but only 1 Chl *c* per 4 Chl *a* is bound. In contrast to Chl *b*, Chl *c* is characterized by a huge Soret-band absorption and little Q_Y or Q_X absorption (Fig. 2.3a, b). The absorption in the Soret is shifted to the red in comparison to Chl *a*. Three different Chl *c* exist, distinguished by their residues at the porphyrin ring as shown in Fig. 2.3a. Since this residue contains a double bond, which is part of the conjugated system in Chl c_2 and c_3, these Chl *c*'s absorb at slightly longer wavelengths as compared to Chl c_1. In prymnesiophytes Chl c_3 and in dinoflagellates Chl c_2 was described as the major Chl *c*, whereas diatoms usually contain Chl c_1 and some Chl c_2 as well (Fawley 1989; Jeffrey and Humphrey 1975; Kraay et al. 1992). In all Chl *c*'s, the lack of a phytol chain makes the molecule much more polar and, from a structural point of view, much smaller than Chl *a* or Chl *b*.

In addition to these pigments diatoms contain diadinoxanthin (Dd) and diatoxanthin (Dt). Dd is de-epoxidised to Dt under conditions of increased light intensities in the so-called xanthophyll cycle (Lavaud et al. 2002; Lohr and Wilhelm 1999). The amount of Dd or Dt found in the various FCP preparations differs tremendously depending on the isolation method (Fig. 2.3c). Usually the more xanthophyll cycle pigments are found, the more lipids are still contained in the samples (Beer et al. 2006; Büchel 2003; Lepetit et al. 2010). However, this does not necessarily argue for a localization of xanthophyll pigments in the lipid phase, i.e. not bound to the protein. Using more rigid methods, which get rid of almost all lipid, Dd or Dt is still found in e.g. FCPa preparations, and the amount depends on the presence of Fcp6, a Lhcx protein (Beer et al. 2006, 2011). However, most probably part of the Dd is found in the lipid shell around the FCP complexes (Gundermann and Büchel 2012; Lepetit et al. 2010), serving as a pool of xanthophylls pigment. Dt is thought to play an important role in the protection against an surplus of light, and the Dt (and Dd) that is newly synthesized in response to high-light was recently demonstrated to be protein-bound (Alexandre et al. 2014).

A long ongoing debate is the actual pigment to protein stoichiometry inside FCPs. Most isolated complexes exhibit rather similar absorption spectra, whereby only the amount of xanthophyll cycle pigments differs, visible as a shoulder around 485 nm (Fig. 2.3c). In LHCII, 8 Chl *a*, 6 Chl *b* and

4 carotenoids are bound per monomer (Liu et al. 2004; Standfuss et al. 2005). The pigment ratio of FCPs is around 3–4 Chl *a* : 1 Chl *c* : 3–4 Fx, depending on isolation procedure and species, whereby the FCPs of *P. tricornutum* are characterized by a higher amount of Fx (Beer et al. 2006; Gundermann et al. 2013; Joshi-Deo et al. 2010; Lepetit et al. 2007; Papagiannakis et al. 2005). Because the Q_Y absorption of FCPs is at a relatively short wavelength (671 nm, see Fig. 2.3a, b) it was argued that especially Chl *a* molecules have to be further apart than in LHCII in order to avoid excitonic interactions, which result in longer wavelength absorbing Chls. This argument was strengthened by CD spectra, where no excitonic interactions are visible in the Q_Y band (Büchel 2003; Joshi-Deo et al. 2010; Szabó et al. 2008). Using resonance Raman spectroscopy, two differently bound Chl *c* molecules were identified in FCPa, as well as in FCPb, by their signature 'ring-breathing modes' at ~1,360 cm^{-1} (Premvardhan et al. 2010). Thus, most likely around 8 Chl *a*, 2 Chl *c* and up to 8 Fx are bound per FCP monomer.

D. Excitation Energy Transfer Between Pigments in Fucoxanthin-Chlorophyll Proteins

Light-harvesting systems are first of all characterized by their efficiency to absorb and transfer light energy. Early on the S_1/ICT state was identified as one major state upon absorption of solar energy by fucoxanthin, being responsible for up to 60 % of the energy transfer to Chl *a* (Papagiannakis et al. 2005). No transfer into Chl *c* could be observed within the limit of the instrumentation (<100 fs), giving rise to the assumption that Fx transfers its absorbed energy directly (or via other Fx) to Chl *a* molecules. On the other hand this means that Chl *c* absorption will lead to direct transfer to Chl *a* (Gildenhoff et al. 2010a; Papagiannakis et al. 2005; Premvardhan et al. 2009).

In intact systems the transfer to Chl *a* from fucoxanthin is extremely fast. Gildenhoff et al. (2010a) determined lifetimes for FCPa of <150 fs for the fucoxanthin S_2 state (transferring directly into the Q_X state of Chl *a*), and 0.6/0.9 ps for the unrelaxed and 2.6/4.2 ps for the relaxed S_1/ICT state (transferring into Q_Y), respectively, whereby the higher values represent the lifetimes of the 'red' Fx molecules and the lower ones those of the 'blue' Fx. Thus, when exciting the 'blue/green' and the 'red' Fx molecules to different extents, the observed dynamics change. For FCPa an additional time constant at around 25 ps was found after excitation at 500 nm and assigned to the intrinsic lifetime of the blue absorbing fucoxanthins engaged in Fx – Fx excitation energy transfer (Gildenhoff et al. 2010a). Using anisotropy measurements it was concluded that one Fx'red' and two of the Fx'blue/green' transfer their energy directly to Chl *a*, whereas a further Fx'blue/green' is depending on another Fx molecule for excitation energy transfer to Chl *a* (Gildenhoff et al. 2010b).

When comparing the different oligomeric states of FCPa and FCPb, the trimeric FCPa has the more efficient energy transfer, which is also reflected in its higher Chl a fluorescence quantum yield. On the other hand the oligomeric FCPb is intrinsically less fluorescent (Gundermann and Büchel 2008), and thus some of the lifetimes mentioned above are even shorter (Gildenhoff et al. 2010a).

E. Lhcx and Photoprotection

Diatoms, like higher plants, protect themselves against fast changing levels of light intensities. Energy fluxes that exceed the conversion capacity of the photosynthetic machinery can cause damage. To avoid impairment the surplus of energy is very efficiently dissipated as heat, a mechanism which is called non-photochemical quenching (NPQ) due to its simultaneous reduction in fluorescence emission. In diatoms NPQ is more pronounced than in higher plants (Ruban et al. 2004), correlates with the de-epoxidation of Dd to Dt (Lavaud et al. 2002, 2003), is pH-dependent as in higher plants but no PsbS protein or homologues exists in

diatoms. Here we will focus mainly on the contribution of Lhcx proteins to NPQ.

Lhcx proteins, whether from pennates or centrics, are up-regulated during prolonged high light (Bailleul et al. 2010; Becker and Rhiel 2006; Beer et al. 2006; Janssen et al. 2001; Lepetit et al. 2010; Nymark et al. 2009; Oeltjen et al. 2002, 2004; Zhu and Green 2010). A direct relationship between the amount of Lhcx proteins expressed and the capability for NPQ was also demonstrated (Bailleul et al. 2010; Zhu and Green 2010). However, when comparing Lhcx proteins in pennate and centric diatoms, a crucial difference becomes obvious. In centrics, a Lhcx protein (Fcp6) was found as a constituent of a trimeric complex (FCPa). The amount of Fcp6 depended on the light intensity during growth, as did the diatoxanthin content (Beer et al. 2006). In contrast, in the pennate *P. tricornutum*, no Lhcx proteins could be found in trimeric complexes so far (Grouneva et al. 2011; Gundermann et al. 2013). Lepetit et al. (2012) were the first to propose that it is unlikely that Lhcx1 of *P. tricornutum* binds Dd or Dt, based on their interpretation of the work of Bailleul et al. (2010). Lepetit and coworkers hypothesized that Lhcx1 might play a role in NPQ through the induction of conformational changes, which in turn influence the energy transfer to the photosystems, in analogy to what is hypothesized about psbS in higher plants (for review see Szabò et al. 2005; Ruban et al. 2012). The same role was attributed to Lhcx1 from a centric diatom, *T. pseudonana*, because its amount did not change dramatically upon high-light stress, in contrast to Lhcx6 (Zhu and Green 2010). No protein comparable to Lhcx6 is described so far in *C. meneghiniana*, since Fcp6 closely resembles Lhcx1. Gundermann and Büchel (2008) were able to show that the Fcp6 (Lhcx1)-containing FCPa of *C. meneghiniana* changes its fluorescence yield in dependence on Dt and Fcp6 content, implying a function of Lhcx1 in the regulation of fluorescence emission in centrics.

Since antenna aggregation was proposed as a mechanism to reduce fluorescence yield *in vivo* (Miloslavina et al. 2009), influence of protein distance, pH and content of xanthophyll cycle pigments on the Lhcx containing FCPa in proteoliposomes was analysed (Gundermann and Büchel 2012) as well. Recently it had already been demonstrated that FCPb is changing fluorescence yield depending on aggregation, but not according to Dt content or pH (Gundermann and Büchel 2008). Indeed, FCPa protein aggregation led to reduced fluorescence yields, which was in addition strongly influenced by the pH. On the other hand Dt reduced fluorescence emission in addition, but independently of pH and protein aggregation. Thus it seems that in centric diatoms a constituent of the trimeric complexes is an active player in NPQ, whereas in pennates at least Lhcx1 is working in a more independent manner. This is rather surprising, since, when comparing Lhcx proteins, Lhcx1 of *P. tricornutum* is most closely related to Fcp6 of *C. meneghiniana*. Since differences mainly concern helix 2 of those proteins (data not shown) they might directly relate to the pigment binding capacities of Lhcx1 of *P. tricornutum* (see below).

F. Structural Model Based on Spectroscopic Data

No structural data are available on other membrane-intrinsic light-harvesting proteins besides LHCII (Liu et al. 2004; Standfuss et al. 2005) and the quite similar CP29 from higher plants (Pan et al. 2011). Since FCPa and FCPb complexes from the centric diatom *C. meneghiniana* are by far the most studied diatom antenna systems using several spectrometric methods, our model here (Fig. 2.4) is based on results obtained from this organism.

Eppard and Rhiel (1998) were the first to model sequences of FCP using the LHCII structure as template. They identified five conserved Chl *a* binding sites (a602, a603, a610, a612 and a613, nomenclature according to Liu et al. (2004)). With more sequences available, Premvardhan et al. (2010) were able to identify two further conserved binding sites, a614 and b609. Premvardhan and

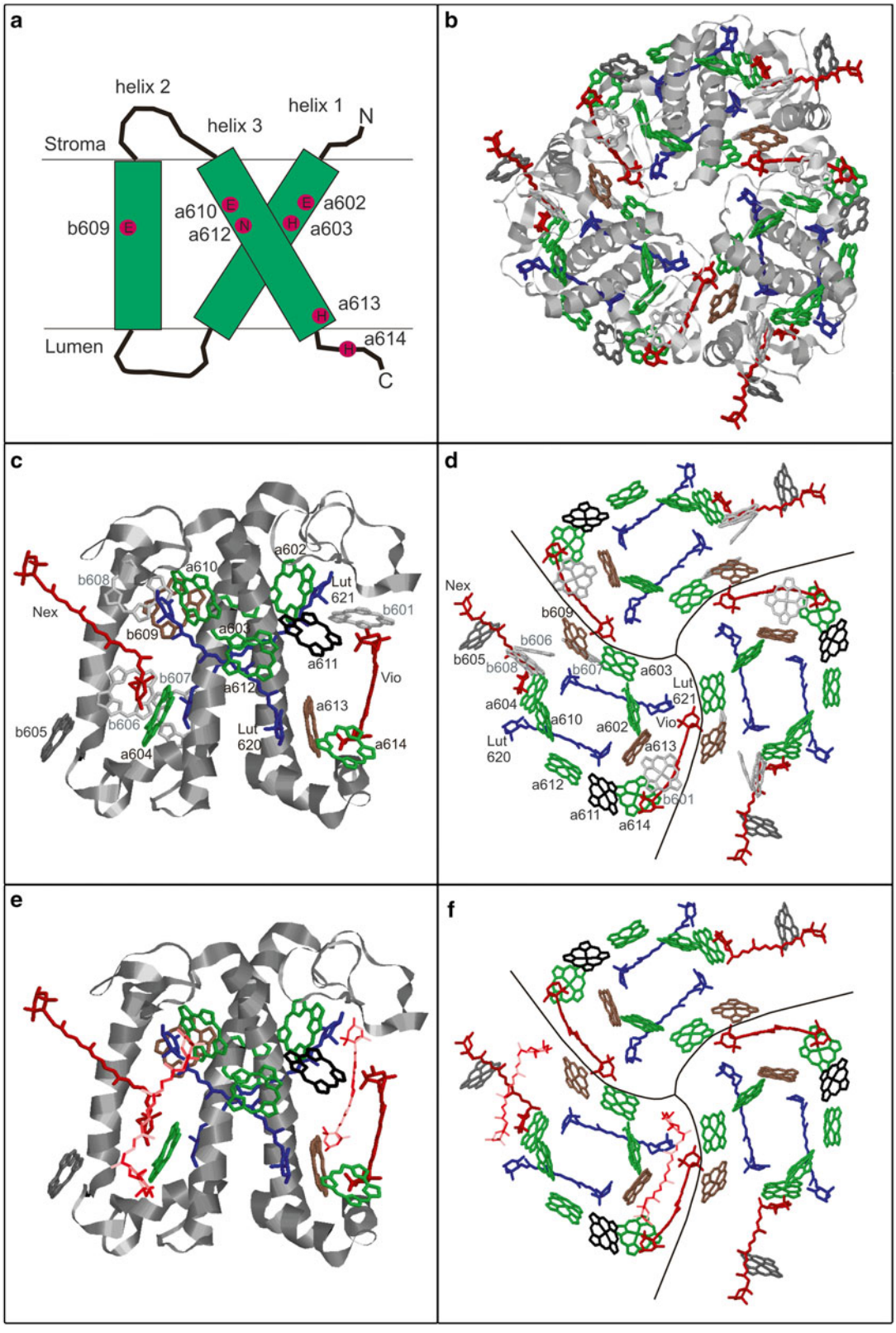
coworkers built a model, which had to fit some major requirements: (i) the pigment content of FCPa and FCPb is based on 2 Chl *c* per monomer, i.e. the FCP contain 6–8 Chl *a* : 2 Chl *c* : 5–6 Fx in total; (ii) the Chl *a* molecules are not allowed to be arranged in a way to favor excitonic interactions; (iii) Fx is not transferring energy to Chl *c* and Fx and Chl *c* should thus be in an appropriate distance and/or orientation; (iv) Chl *c* to Chl *a* transfer is extremely fast, i.e. Chl *c* has to be in close proximity to Chl *a* molecule(s).

The two 'blue'Fx were proposed to be located homologous to the central luteins of LHCII (Premvardhan et al. 2009) clustering around helix 1 and 3. In close distance four symmetrically related Chl-binding sites can be found, which are conserved in FCPs (a610, a612, a602, a603). The frequently observed fast energy transfer from Fx to Chl *a* supports the occupation of these chlorophyll-binding sites by Chl *a*.

Further away two additional Chl *a*'s are found in LHCII, namely a613 (binding site conserved) and a604, the latter being ligated by a water molecule in LHCII. Thus, no prove for binding or non-binding is available from sequence comparison only. However, the quite similar structure prediction around those central helices makes it very likely that this symmetry-related site is occupied by Chl in FCP, as well. This also holds for the binding sites a614 and b609, where the amino acids responsible for Chl binding are conserved.

When comparing FCPa and FCPb, spectral differences are quite low. However, the precise stoichiometry of pigments is slightly different. 6 Fx : 6–7 Chl *a*: 2 Chl *c* can be found in the trimeric FCPa, whereas 5 Fx : 8 Chl *a* : 2 Chl *c* are bound to the oligomeric FCPb. Thus, FCPa has a slightly reduced Chl *a* content as compared to FCPb, which in turn is reduced in Fx, but is characterized by 'red' Fx absorbing at even longer wavelength than those in FCPa (Premvardhan et al. 2009). This difference in Chl *a* content can either be due to missing binding sites, which is unlikely due to the high similarity of the protein sequences, or to loss of pigments during the isolation procedures. If the difference in Chl *a* content is due to pigment loss, this may indicate that one to two of the Chl *a* molecules are more loosely, i.e. peripherally, bound. The most peripheral Chls in the LHCII structure are b605 and a611, the latter being slightly more central but only bound by a lipid. Those Chls might thus be present in FCPb, but missing in FCPa preparations. The Chl *a* molecule of LHCII bound at a611 participates in forming a dimer with Chl *a*612, which can be detected by its red shifted absorption (Georgakopoulou et al. 2007), which is missing in all FCP complexes. Thus, if the a611 binding site is present and occupied, the Chl *a* will have to adopt a different orientation in order to avoid excitonic dimer formation with Chl *a*612.

Thus, the ten Chl sites in FCPs could be identified with some probability, whereby only six (a602, a603, a610, a611, a612 and b605) are likely to be occupied by Chl *a*. In LHCII, the Chl *b* in b609 is strongly coupled to Chl *a* (a603) and both binding sites are predicted to exist in FCPs as well. In contrast, no direct interaction partner to a604 is conserved in FCPs. This renders b609 a probable candidate for Chl *c* binding in FCPs, whereas a604 is then probably occupied by Chl *a*. The other two sites left, a614 and a613, are rather close together, but further away from other Chls. One of these Chls is most probably a Chl *c* in FCPs, since in LHCII the two Chl *a* molecules interact exitonically (Georgakopoulou et al. 2007), but nothing like that can be seen in FCPs. Thus, to break this excitonic couple, one site is most probably occupied by Chl *c*. The Chl *c* molecules in FCPs can also be distinguished as more 'red' and 'blue'-absorbing. It has been argued that if one Chl *c* occupies b609, the lowest energy site in LHCII, the remaining Chl *c* should absorb at higher energies, which would make a613, the lower energy site in LHCII, less probable (Premvardhan et al. 2010). However, this argument implies almost identical protein environments in LHCII and FCP complexes, which is rather unlikely. On the other hand the porphyrin in a614 is in closer contact to the violaxanthin binding site, a scenario to be avoided, because of the lack of Fx – Chl *c* excitation energy transfer. Thus, in Fig. 2.4 Chl *c* is presented in a613.


a
helix 2
helix 3
helix 1
N
Stroma
b609
a610
a612
a602
a603
a613
a614
Lumen
C
b
c
b608
a610
a602
Lut
621
Nex
b609
b601
a603
a611
Vio
b607
a612
a613
b605
b606
Lut
620
a604
a614
d
Nex
b609
b606
b605
b608
b607
a603
Lut
621
a604
a610
a602
Vio
Lut
620
a613
a612
b601
a611
a614
e
f

Since only ten Chls are bound to the FCPs, the space occupied by the remaining 4 Chls and the two additional phytol chains in LHCII can be filled by carotenoids in FCPs. The minimal number of Fx is five (in case of FCPb) or six (in case of FCPa), whereby the absorption properties differentiate them into blue Fx (2 molecules), green Fx (1–2 molecules) and red Fx (2 molecules). The blue Fx should be located in the lutein binding sites as outlined above. Using Stark and resonance Raman spectroscopy (Premvardhan et al. 2008, 2009), a model for the location of the different Fx molecules was developed. In this model, red Fx molecules are located near helix 2, whereby the structural differences between neoxanthin and fucoxanthin make it unlikely that one Fx is exactly bound like neoxanthin. One of the green Fx most probably occupies the violaxanthin binding site, and the other green Fx is located in the b601 site (Premvardhan et al. 2009). These data were supported by Gildenhoff et al. (2010b) demonstrating fast energy transfer from two of the blue/green Fx, as well as from one Fx'red' to Chl *a*, whereas a further Fx'blue/green' was depending on another Fx molecule. The other 2–3 Fx found in FCPs isolated using more gentle preparations, or in FCPs from *P. tricornutum*, have then to be more loosely bound and are not shown in Fig. 2.4 The model holds for FCPb, as well as FCPa, whereby in the latter the Fcp6 (Lhcx1) polypeptide carries Dd as well. This Dd was assumed to be located in the violaxanthin pocket as a mixed binding site with the green Fx (Premvardhan et al. 2010).

One implication of the relatively short wavelength absorption (671 nm) and the lack of excitonic Chl *a*/Chl *a* interactions is striking: a610/a611/a612 cannot work together as final emitters as shown for higher plants (Novoderezhkin et al. 2004). Thus the energy landscape of pigments in FCPs must also differ from that of LHCII, not only because of the replacement of Chl *b* by Chl *c*, but also concerning the Chl *a* molecules, despite some of them being bound in comparable sites.

Although the models based on spectroscopic data are quite elaborate by now, many questions remain, which only a proper structure determination would solve. One of the unresolved questions it the arrangement of the Fx molecules, which - in contrast to the symmetric lutein - is highly asymmetric. This implies that always two orientations exist, that are not accounted for by the current model, but are important in excitation energy transfer. Another important open question is the precise localization and function of Dd and Dt.

III. Conclusions

Despite all homologies, FCP complexes of diatoms are quite different from higher plant LHCs. Obvious deviations concern pigmentation, and thus the overall energy landscape. Not so obvious differences slowly emerge with our increasing knowledge about the structure of FCP complexes. The three different groups of FCP polypeptides, Lhcf, Lhcr and Lhcx, have no direct counterparts and plant Lhc proteins, except for the main light harvester Lhcf. Even Lhcr polypeptides, which might be compared to LHCI, are not the sole PSI antenna components. Lhcx proteins have a counterpart only in green algae and functional similarities to psbS, but are 3-helix proteins and, at least in centric

Fig. 2.4. Homology model based on the LHCII structure (pdb 1RWT) and spectroscopic analyses (Modified after Premvardhan et al. 2010). Graphics were made using RasMol and pigments are labeled according to Liu et al. (2004). In (**a**) potential Chl *a*-binding sites are shown, identified by sequence alignment. (**b**) Gives the *top view* of a trimer with pigments and protein backbone, the latter is removed in (**d**) and (**f**) for clarity. In (**c**) and (**e**) *side views* of one monomer are shown. Pigments are colored according to their attribution in FCPs: *green* = Chl *a*, *brown* = Chl *c*, *white* = Chls not present in FCPs, *gray* and *black* = Chl *a* missing in FCPa but present in FCPb, *blue* = 'blue' Fx and *red* = all other Fx molecules. In the *lower panel* two carotenoids per monomer are added as compared to LHCII in the volumes left by the missing Chl molecules. Note that the precise arrangement of each of these pigments is not known, especially in the case of the carotenoids and pigments around helix 2.

diatoms, contain the full complement of pigments. Thus, obtaining a molecular structure of FCPs to further elucidate pigment-binding and excitation energy transfer, as well as data about supercomplex formation are needed. This additional information will be extremely useful in the future to understand two major features of diatoms: their extreme ecological success, being responsible for up to 25 % of the biomass production on earth (Falkowski et al. 1998), and the related ability to regulate light harvesting versus photoprotection very efficiently.

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