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## **Photosynthetic Light Reactions in Diatoms. I. The Lipids and Light-Harvesting Complexes of the Thylakoid Membrane**

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### ► **To cite this version:**

Claudia Büchel, Reimund Goss, Benjamin Bailleul, Douglas Campbell, Johann Lavaud, et al.. Photosynthetic Light Reactions in Diatoms. I. The Lipids and Light-Harvesting Complexes of the Thylakoid Membrane. *The Molecular Life of Diatoms*, Springer International Publishing, pp.397-422, 2022, <10.1007/978-3-030-92499-7\_15>. <hal-03672196>

**HAL Id: hal-03672196**

**<https://hal.science/hal-03672196v1>**

Submitted on 19 May 2022

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1 Chapter 1: Photosynthetic light reactions in diatoms. I. The  
2 lipids and light-harvesting complexes of the thylakoid  
3 membrane

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18

19

20 **Abstract**

21 Light harvesting and photochemistry is performed by photosystems coupled to specific  
22 antennae embedded in the thylakoid membrane, a common principle across diatoms, plants and  
23 green algae. Still, unique features of diatoms within this common principle have been unraveled  
24 in recent decades, likely resulting from the complex evolutionary history of diatoms. These  
25 unique features are found in (i) the lipid composition of the thylakoid membrane, ii) the spatial  
26 organization of the light harvesting complexes, and iii) their protein and pigment composition.  
27 This chapter summarizes current knowledge of these three specific features, with a focus on  
28 structural and functional properties.

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33 *Key words: diatoms, FCP, LHC, light harvesting, lipids, thylakoids, xanthophyll cycle*

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52 **Abbreviations**

- 53 *C. meneghiniana*: *Cyclotella meneghiniana*  
54 *Ch. gracilis*: *Chaetoceros gracilis*  
55 Chl: chlorophyll  
56 Dd: diadinoxanthin  
57 DGDG: digalactosyldiacylglycerol  
58 DGGC: diacylglycerylcarboxyhydroxymethylcholine  
59 DGTA: diacylglycerylhydroxymethyl-N,N,N-trimethyl- $\beta$ -alanine  
60 DGTS: diacylglyceryl-N-trimethylhomoserine  
61 DHA: docosahexaenoic acid  
62 Dt: diatoxanthin  
63 EPA: eicosapentaenoic acid  
64 FCP: fucoxanthin-chlorophyll-protein complex  
65 Fx: fucoxanthin  
66 H<sub>II</sub>: inverted hexagonal phase  
67 *H. ostrearia*: *Haslea ostrearia*  
68 ICT: intramolecular charge transfer  
69 LHC: light-harvesting complex  
70 MGDG: monogalactosyldiacylglycerol  
71 *P. tricorutum*: *Phaeodactylum tricorutum*  
72 PG: phosphatidylglycerol  
73 PSI: photosystem I  
74 PSII: photosystem II  
75 PUFAs: polyunsaturated fatty acids  
76 qE: energy dependent quenching  
77 SQDG: sulphoquinovosyldiacylglycerol  
78 *T. pseudonana*: *Thalassiosira pseudonana*  
79 XC: xanthophyll cycle  
80

81

## 82 **Introduction**

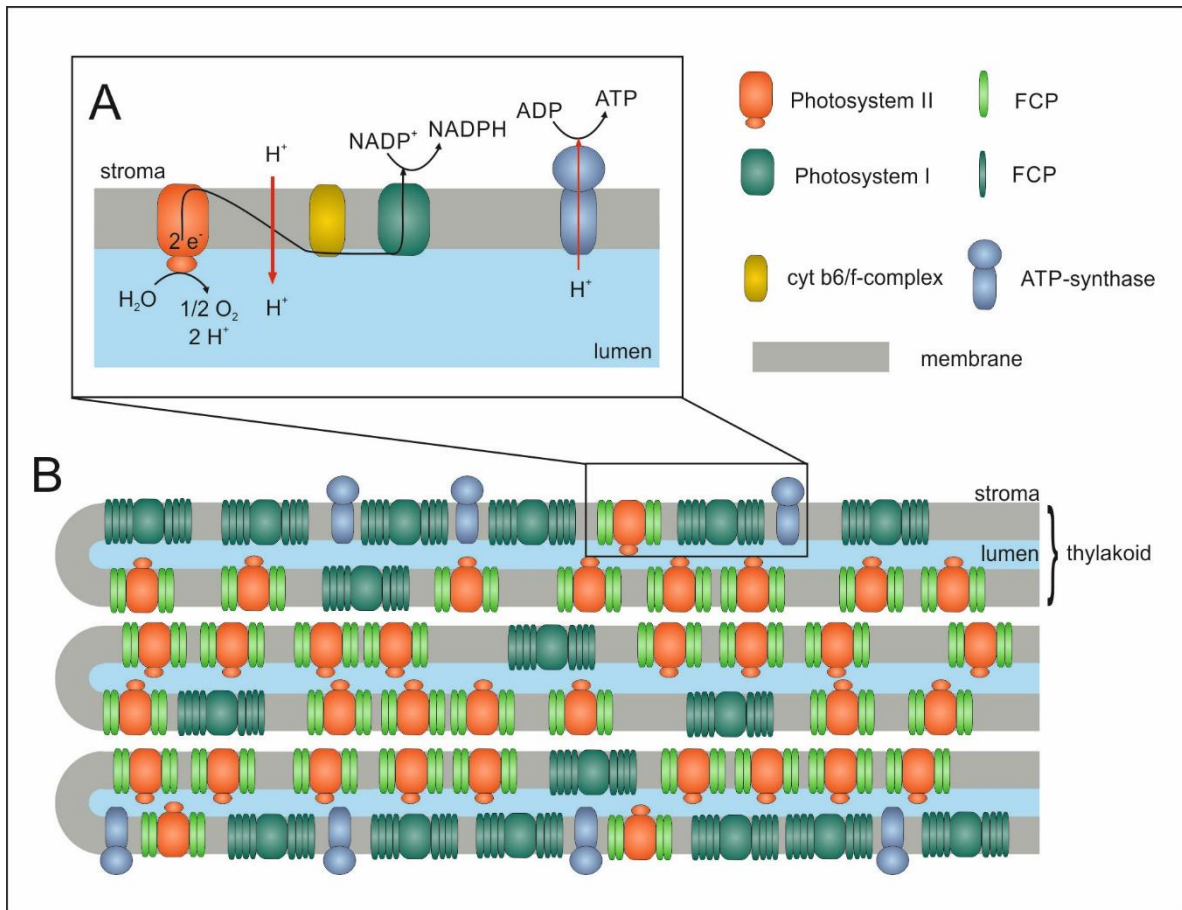
83 Diatoms perform oxygenic photosynthesis, whereby the basic reactions are identical in all  
84 eukaryotes: light energy is absorbed by the pigments bound to photosystem (PS) I and II and  
85 their associated light-harvesting complexes (LHC). It is transferred to special chlorophyll (Chl)  
86 *a* molecules in the PS, the so-called reaction center Chls, where charge separation takes place.  
87 This fuels the photosynthetic electron transport, where electrons are subtracted from water and  
88 eventually used for the generation of NADPH (Fig. 1A). These photosynthetic light reactions  
89 take place in the thylakoid membranes, and the coupling of the electron transfer to a  
90 unidirectional transport of protons leads to accumulation of protons in the lumen that is used  
91 by an ATP-Synthase to generate ATP (for details see “*Photosynthetic light reactions in diatoms.*  
92 *II. The dynamic regulation of the various light reactions*”). NADPH and ATP are then mainly  
93 used by the Calvin cycle in the chloroplast stroma to fix CO<sub>2</sub>. Thus, diatoms share the same  
94 elementary photosynthetic modules with other organisms performing oxygenic photosynthesis  
95 (Falkowski et al. 2008), which are fairly similar in all eukaryotes, with one exception: Light-  
96 harvesting systems differ significantly amongst the different taxa (Neilson and Durnford 2010,  
97 Büchel 2015, Büchel 2020, Croce and van Amerongen, 2020). In addition, there are further  
98 subtle changes concerning photosystems and their functional organization. For example, the  
99 PSII core recently crystallized from *Chaetoceros gracilis* contains four more Chl *a* molecules  
100 than the plant PSII core, and one additional subunit shielding the oxygen evolving complex  
101 (Nagao et al. 2019a). With more than 100,000 species of diatoms present on earth, exhibiting a  
102 huge phylogenetic diversity (Mann and Vanormelingen 2013), we may also assume some  
103 diversity among diatom species even within these elementary modules.

104 The photosynthetic modules are embedded in the thylakoid membrane. In contrast to the green  
105 lineage, diatom thylakoids are organized in homogeneous stacks of three which run along the  
106 whole diatom plastid (Berkaloff et al. 1990) (Fig. 1B). They do not show the grana stroma  
107 differentiation which imposes the lateral segregation of the PSII and PSI in plants (Anderson  
108 1999). In diatoms, immunolocalization studies, lipid analyses, and 3D reconstruction showed  
109 that PSI are mostly found in the stroma-facing external thylakoids whereas PSII are embedded  
110 in the core of the stack, at the interface of two thylakoids (Pyszniak and Gibbs 1992; Lepetit et  
111 al. 2012; Flori et al. 2017). This heterogeneity is enforced under red light conditions, which  
112 induce an unusual stacking of diatom thylakoids. Here, large areas were revealed which are  
113 exclusively occupied by PSI supercomplexes consisting of PSI cores with their PSI-specific  
114 antenna of Lhc proteins (Bína et al. 2016). In line with this, clusters of PSII cores including  
115 their FCP antennae have been revealed by cryo-electron tomography in *Phaeodactylum*

116 *tricornutum* recently (Levitani et al. 2019). For steric reasons the ATP synthase is located in the  
117 outer thylakoids. The vicinity of PSII and PSI, as well as some connections between thylakoids,  
118 ensures a fast diffusion of electron shuttles between the two photosystems (Flori et al. 2017),  
119 whereas their diffusion can limit the overall rate of the linear electron flow in plants (Kirchhoff  
120 et al. 2004; Kirchhoff et al. 2011). This peculiar segregation also has important consequences  
121 for the regulation of the light capture by the two photosystems, by preventing the share of  
122 excitons between them (Flori et al. 2017), a process found in cyanobacteria and red algae, and  
123 called spillover (Biggins and Bruce 1989). Whether based on immunoblot quantification or  
124 functional measurements, diatoms seem to possess more PSII than PSI, in contrast to plants and  
125 green algae where PSI occurs at least in the same amount as PSII (Smith and Melis 1988;  
126 Strzepak and Harrison 2004; Thamatrakoln et al. 2013). With this higher amount of PSII,  
127 diatoms may compensate their slightly lower maximum PSII efficiency, which is usually  
128 around 0.6-0.7, while in plants it reaches values of ~0.84 (Kalaji et al. 2014).

129 Lipids and pigmented proteins are the major components of the diatom thylakoid membrane.  
130 Usually, the proteins occupy an area of 70-80 % of the thylakoid membrane, while lipids  
131 correspondingly contribute to 20-30 % (Kirchhoff 2014). Compared to the green lineage, both  
132 the respective amounts of the different thylakoid lipid classes and the respective fatty acids  
133 composition is considerably different in diatoms. Moreover, the structure and composition of  
134 the light harvesting system, the dominant protein constituent of the thylakoid membranes,  
135 shows various diatom specific peculiarities. Certainly one reason for these differences is based  
136 on the complex evolutionary history of diatoms - with plastids originating from secondary  
137 endosymbiosis with red algae, while considerable amounts of 'green' genes are also found in  
138 the diatom genome (Dorrell et al. 2017).

139 In this review article, we will summarize current knowledge on lipid composition (part 1) and  
140 antennae structures of diatom thylakoids (part 2).



141

142 **Figure 1:** (A) General, simplified scheme of the light reactions of photosynthesis. Only PSII  
 143 (red) cytb6/f complex (yellow), PSI (green) and ATPsynthase (blue) are shown. Light-  
 144 harvesting systems have been omitted since they differ significantly between the different algal  
 145 groups and higher plants. For more detail on electron and proton transfer refer to Figure 1 in  
 146 chapter “*Photosynthetic light reactions in diatoms. II. The dynamic regulation of the various*  
 147 *light reactions*”. (B) Scheme of the thylakoid membrane structure of diatoms (B). The  
 148 thylakoids are organized in stacks of three that span the whole plastid. The outer membranes of  
 149 such stack are enriched in PSI, whereas PSII is predominantly found in the inner four  
 150 membranes. Cytb6/f complexes that are not preferentially localized (Flori et al. 2017) have been  
 151 omitted as well as connections between thylakoids of one stack. In diatoms the light-harvesting  
 152 complexes are called fucoxanthin-chlorophyll proteins (FCP). FCPs tightly bound to PSII are  
 153 shown in light green, FCPs belonging to PSI are shown in dark green.

154

155

## 156 1. Thylakoid lipids

### 157 1.1 The lipid composition of thylakoid membranes

158 Before the lipid and fatty acid composition of diatom thylakoid membranes is presented in  
 159 detail, it should be mentioned that most of the depicted results have been derived from  
 160 laboratory experiments under defined growth conditions (for details please refer to the cited  
 161 references). However, in their natural environment diatoms are exposed to extreme differences

162 in temperature and light intensities. To cope with these extreme abiotic conditions diatoms  
163 adjust the lipid and fatty acid composition of the thylakoids in order to maintain the membrane  
164 in a fluid working state (see also 1.2 and 1.3).

165 The diatom thylakoid membranes are composed of the two neutral galactolipids  
166 monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), the  
167 negatively charged sulfolipid sulphoquinovosyldiacylglycerol (SQDG) and the anionic  
168 phospholipid phosphatidylglycerol (PG) (Vieler et al. 2007; Goss et al. 2009; Lepetit et al.  
169 2012; Abida et al. 2015). These lipids are also found in higher plants, but in addition the diatom  
170 thylakoid membranes can contain low amounts of betaine lipids like  
171 diacylglycerylcarboxyhydroxymethylcholine (DGGC) and diacylglycerylhydroxymethyl-  
172 N,N,N-trimethyl- $\beta$ -alanine (DGTA) (Vieler et al. 2007; Canavate et al. 2016). A third betaine  
173 lipid, namely diacylglyceryl-N-trimethylhomoserine (DGTS), which acts as precursor in the  
174 synthesis of DGTA, can be found in trace amounts (Canavate et al. 2016). Phosphatidylcholine,  
175 which in higher plant thylakoid preparations is supposed to represent a contamination with  
176 chloroplast envelope membranes, seems to be a general constituent of diatom thylakoid  
177 membranes (Vieler et al. 2007; Goss et al. 2009; Lepetit et al. 2012).

178 Although the main lipids of diatom thylakoids are comparable to those of the green lineage,  
179 their contribution to the overall lipid content of the membrane is significantly different. While  
180 in higher plants and green algae the neutral galactolipids dominate the lipid content of the  
181 thylakoid membrane and amount to 70 to 80% of the total lipid (Murata and Siegenthaler 1998),  
182 the concentrations of MGDG and DGDG are strongly reduced in the thylakoids of pennate (*P.*  
183 *tricornutum*) and centric (*Cyclotella meneghiniana*) diatoms (Goss et al. 2009; Lepetit et al.  
184 2012; Abida et al. 2015). The reduction of MGDG and DGDG is accompanied by simultaneous  
185 increases of the two negatively charged lipids SQDG and PG. This is especially obvious in  
186 thylakoid membranes of *P. tricornutum* or *C. meneghiniana* purified from high light grown  
187 cultures (Lepetit et al. 2012). They contain SQDG as the most abundant lipid, and the combined  
188 negatively charged lipids SQDG and PG contribute to more than 50% of the total thylakoid  
189 lipids. The ratio of neutral to negatively charged lipids lies between one and two and is thus  
190 significantly lower than the typical values between three and four observed in higher plant and  
191 green algal thylakoid membranes. High concentrations of the negatively charged lipids SQDG  
192 and PG in diatom thylakoid membranes are supported by the study of Yan et al. (2011) who  
193 determined the photosynthetic lipid and fatty acid profile of three different strains of the marine  
194 diatom *Skeletonema* sp.. Two of the three strains show high SQDG concentrations (35-40% of

195 the photosynthetic lipids) and, in combination with PG, the negatively charged lipids amount  
196 to almost 50% of the total photosynthetic lipids of *Skeletonema*.

### 197 ***1.2 Fatty acid composition of thylakoid membrane lipids***

198 Diatoms are characterized by the synthesis of very long-chain polyunsaturated fatty acids  
199 (PUFAs) with chain lengths of up to 28 C atoms (Guschina and Harwood 2006). The presence  
200 of long-chain PUFAs is also reflected by the fatty acid composition of the thylakoid membrane  
201 lipids. In centric diatoms (*Skeletonema marinoi*, *Thalassiosira weissflogii*) and the pennate *P.*  
202 *tricornutum* MGDG contains the main long-chain PUFA of diatoms, i.e. eicosapentaenoic acid  
203 (EPA, 20:5) (Yongmanitchai and Ward 1993; Yan et al. 2011; Dodson et al. 2013; Dodson et  
204 al. 2014; Abida et al. 2015). EPA is preferentially bound to the sn-1 position of the glycerol  
205 backbone whereas C16 fatty acids with different amounts of double bonds (16:1, 16:2, 16:3,  
206 16:4) can be observed at the sn-2 position. MGDG with C20:5 and C16:3 fatty acids seems to  
207 represent the majority of MGDG molecules in diatom thylakoids. Interestingly, EPA at the sn-  
208 1 position of the MGDG molecule can be replaced by other C20 fatty acids with a lower amount  
209 of double bonds like eicosatrienoic acid (20:3) or eicosatetraenoic acid (20:4). In addition to  
210 MGDG molecules with C20/C16 fatty acids, the recent analyses have presented evidence for  
211 the presence of MGDG molecules with C16 fatty acids at both the sn-1 and sn-2 position.  
212 Longer-chain PUFAs, like docosahexaenoic acid (DHA, 22:6), or C18 fatty acids are usually  
213 not found or detected in only minor concentrations in MGDG of centric diatoms and *P.*  
214 *tricornutum*.

215 The analyses by Yongmanitchai and Ward (1993), Yan et al. (2011), Dodson et al. (2013),  
216 Dodson et al. (2014) & Abida et al. (2015) also pointed out that the fatty acid composition of  
217 the main galactolipids MGDG and DGDG is comparable. EPA is the main fatty acid species at  
218 the sn-1 position of the DGDG molecule. The sn-2 position of DGDG is usually occupied by  
219 C16 fatty acids, and DGDG forms with C16 fatty acids at both the sn-1 and sn-2 positions can  
220 be observed. Like MGDG, DGDG molecules with the two fatty acids EPA (C20:5) and  
221 hexadecatrienoic acid (C16:3) seem to represent the majority of DGDG molecules in the diatom  
222 thylakoid membrane. Other pennate diatoms (*Haslea ostrearia* and *Navicula perminuta*) seem  
223 to exhibit a different fatty acid composition of the MGDG and DGDG molecules compared  
224 with the centric diatoms and *P. tricornutum* (Dodson et al. 2013). In these species, an  
225 enrichment of EPA cannot be detected and C18/C16 and C18/C18 forms of MGDG and DGDG  
226 are typically observed. The major forms of MGDG and DGDG seem to contain linolenic acid  
227 (18:3) and hexadecatrienoic acid (16:3) at the sn-1 and sn-2 positions, respectively. The fatty

228 acid composition of these pennate diatoms thus is comparable to the fatty acid composition of  
229 MGDG and DGDG of higher plants and green algae (Browse et al. 1986; Cho and Thompson  
230 1987). However, this different lipid profile may be related to acclimation to environmental  
231 temperatures. Indeed, Dodson et al. (2014) could detect significant amounts of EPA in *H.*  
232 *ostrearia* at the sn-1 position of the MGDG and DGDG molecules under normal temperature  
233 conditions, whereas at high temperatures (30 °C) no EPA or other C20 fatty acids, but high  
234 concentrations of C18 fatty acids could be observed at the sn-1 position of MGDG and DGDG  
235 both in *H. ostrearia* and also *P. tricornutum*.

236 With regard to the anionic membrane lipids of diatoms, the fatty acid composition of SQDG of  
237 *P. tricornutum* (Yongmanitchai and Ward 1993; Abida et al. 2015) and that of SQDG and PG  
238 of three *Skeletonema* species has been analyzed (Yan et al. 2011). In contrast to MGDG and  
239 DGDG, the main part of SQDG present in diatom thylakoid membranes is enriched in fatty  
240 acids with a shorter chain length and C14 and C16 fatty acids are usually observed in both the  
241 sn-1 and sn-2 positions. The C14 and C16 fatty acids include C14:0, C16:0, C16:1 and C16:3  
242 species. PG in *Skeletonema*, on the other hand, contains C18:1 fatty acids as the main molecular  
243 species.

### 244 ***1.3 Function of thylakoid membrane lipids***

245 Thylakoid membrane lipids exert their functions on different structural levels. First, they build  
246 up the membrane bilayer in which the photosynthetic pigment protein complexes and the  
247 components of the electron transport chain are incorporated. Changes in the lipid and/or fatty  
248 acid composition of the thylakoid membrane as a reaction to changes in the environmental  
249 conditions, like e.g. temperature or light fluctuations, keep the membrane in a fluid state and  
250 thus maintain efficient electron transport and other membrane related processes such as the  
251 operation of the diadinoxanthin cycle (see below). The importance of a fluid state for the  
252 operation of biological membranes has first been described by Singer and Nicolson (1972).  
253 Recently, advances in our understanding of the fluid mosaic model with a special focus of  
254 different lipid phases in thylakoid membranes have been presented by Wilhelm et al. (2020).

255 With respect to the environmental factor light, Mock and Kroon (2002) observed an increase  
256 of the non-bilayer lipid MGDG accompanied by higher concentrations of EPA in sea ice  
257 diatoms which were grown under low light illumination. They proposed that the high contents  
258 of EPA were responsible for membrane fluidity and the velocity of the photosynthetic electron  
259 transport. Rousch et al. (2003) observed that the thermo-tolerant diatom *Chaetoceros muelleri*  
260 reacted with stronger changes in the fatty acid profile to temperature changes than the thermo-

261 intolerant *P. tricornutum*. Recently, Bojko et al. (2017) could show that the centric diatom  
262 *Thalassiosira pseudonana* reacted with an increase of PUFAs to a decrease of the cultivation  
263 temperature. The higher content of PUFAs resulted in a stabilization of the membrane fluidity  
264 and the PSII quantum yield at low temperatures. In addition to their role as membrane building  
265 blocks, thylakoid lipids serve as structural elements of the pigment proteins and play a role in  
266 the oligomerization of photosystems and light-harvesting complexes. Through an enrichment  
267 at the monomer-monomer interface MGDG seems to play a role in the establishment and  
268 maintenance of PSII dimers which represent the native state of PSII in the thylakoid membrane  
269 (Kern and Guskov 2011; Nagao et al. 2019a). PG is most likely involved in the trimerization of  
270 the PSII light-harvesting complex of higher plants, the LHCII, whereas DGDG is important for  
271 the structural integrity of the complex (Kühlbrandt et al. 1994).

272 Recently, the first molecular structure of a diatom pigment protein complex determined by x-  
273 ray crystallography has been published, namely that of the dimeric fucoxanthin chlorophyll  
274 binding protein (FCP) complex of *P. tricornutum* (Wang et al. 2019) (see “2.5 Molecular  
275 structure of FCPs”). Within the dimeric FCP two lipid molecules have been resolved, a PG and  
276 a DGDG molecule. Although the PG and DGDG molecules have not been assigned specific  
277 roles in the dimerization of the FCP, it was proposed that the two lipid molecules serve to  
278 stabilize the FCP dimers. The determination of the structure of a PSII-FCP supercomplex from  
279 the pennate diatom *Ch. gracilis* by cryo-electron microscopy revealed the presence of more  
280 than 100 lipid molecules in the PSII-FCP dimer (Pi et al. 2019; Wang et al. 2020). The majority  
281 of the lipids, comprising 20 DGDG, 42 MGDG, 16 SQDG and 30 PG molecules, are located at  
282 the interfaces of the PSII protein subunits which, according to Wang et al. (2020), argues for  
283 an important role in the establishment and stabilization of protein subunit interactions. A  
284 stabilizing role of thylakoid membrane lipids for the native structure of FCP complexes has also  
285 been reported in another study (Schaller-Laudel et al. 2017). In these experiments, the neutral  
286 galactolipids MGDG and DGDG exhibited the highest capacity for the stabilization of FCP  
287 complexes. For higher plants and cyanobacteria it has been shown that MGDG plays a role in  
288 targeting  $Q_B$  to its binding site at the stromal side of the PSII reaction center and that DGDG is  
289 important for the stabilization of the Oxygen Evolving Complex at the luminal side of PSII  
290 (Kern and Guskov 2011). Although the first results concerning the structure of the diatom PSII  
291 core complex have been published (Wang et al. 2020), a detailed assignment of the lipid  
292 molecules within the core complex to specific functions is still missing. However, the structural  
293 and functional similarities between the diatom and higher plant and cyanobacterial PSII and  
294 PSI core complexes suggest a similar role of the thylakoid lipids in photosystems of diatoms.

295 Besides the lipids that build up the main lipid phase of the thylakoid membrane and those that  
296 serve as structural lipids embedded into the protein matrix, a third class of membrane lipids  
297 exist. These lipids are closely associated with the pigment protein complexes and form a lipid  
298 shield surrounding the complexes. In the LHCII of higher plants (Schaller et al. 2010) and the  
299 FCP complexes of the diatoms *P. tricornutum* and *C. meneghiniana* (Lepetit et al. 2010) the  
300 lipid shields are highly enriched in MGDG. MGDG serves to solubilize the hydrophobic  
301 xanthophyll cycle (XC) pigments violaxanthin, antheraxanthin and zeaxanthin in higher plants  
302 and diadinoxanthin (Dd) and diatoxanthin (Dt) in diatoms, thereby making these pigments  
303 accessible to the enzymes violaxanthin or diadinoxanthin de-epoxidase (Goss et al. 2005; Goss  
304 et al. 2007). (*Side note: The diadinoxanthin de-epoxidase is usually annotated as a violaxanthin*  
305 *de-epoxidase in diatom genomes. This reflects the fact that this enzyme can also de-epoxidize*  
306 *violaxanthin, which is a precursor in synthesis of the main diatom carotenoids fucoxanthin and*  
307 *Dd (Lohr and Wilhelm 1999).)* The XC has a dominant role in photoprotection of plants and  
308 diatoms (see “*Photosynthetic light reactions in diatoms. II. The dynamic regulation of the*  
309 *various light reactions*”). It has also been proposed that the local enrichment of MGDG leads  
310 to the establishment of a special lipid phase, the so-called inverted hexagonal (H<sub>II</sub>) phase. This  
311 non-bilayer phase seems to be essential for the efficient conversion of XC pigments (Latowski  
312 et al. 2002; Latowski et al. 2004; Goss et al. 2005; Goss et al. 2007). The H<sub>II</sub> phase together  
313 with the LHCII/FCP, the de-epoxidases and the XC pigments can be described as a special  
314 thylakoid membrane domain with a specific lipid and protein composition and a specific  
315 function, namely the enzymatic de-epoxidation of the XC pigments (Goss et al. 2017).  
316 The XC pigments also influence the properties of the lipid phase of the membrane. The  
317 conversion of Dd to Dt leads to a stable increase of the rigidity of the hydrophobic core of the  
318 diatom thylakoid membrane and a dynamic stabilization of the peripheral membrane parts  
319 which can be observed during the ongoing de-epoxidation reaction (Bojko et al. 2019). The  
320 stabilization of diatom thylakoids by XC pigments has been suggested to play a role in the  
321 adaptation of the membrane to rapid changes in temperature.

322

#### 323 ***1.4 Localization of thylakoid membrane lipids***

324 Based on the findings that SQDG strongly inhibits the de-epoxidation of Dd to Dt (Goss et al.  
325 2009) and that MGDG forms a lipid shield around the FCP complexes, which incorporates a  
326 large part of the XC pigments (Lepetit et al. 2010), a model for the lipid and protein distribution  
327 in the thylakoid membranes of diatoms was proposed (Lepetit et al. 2012). The model predicts

328 that the inner membranes of the typical stacks of three thylakoids are enriched in PSII and its  
329 associated FCP complexes (Fig. 1). The lipid composition of the inner membranes is dominated  
330 by MGDG and represents the place where efficient operation of the XC is taking place. SQDG,  
331 on the other hand, is enriched in the outer membranes of the thylakoid stacks so as not to  
332 interfere with the diadinoxanthin de-epoxidase. Outer membranes and margin regions of the  
333 stacks contain mainly PSI and the ATP synthase which thus gains access to the chloroplast  
334 stroma. Although the model was mainly based on physiological and biochemical data, recent  
335 structural data have confirmed several important aspects and predictions (Flori et al. 2017) (see  
336 also "*Introduction*"). Revealing also connections between the different thylakoid lamellae, the  
337 authors concluded that the three-dimensional network of the thylakoid membrane of diatoms is  
338 far more complex than the simple layout of three loosely connected membranes.

339

340

## 341 **2. Light harvesting**

342 Diatoms, like vascular plants, harvest light using membrane intrinsic proteins that belong to the  
343 light-harvesting complex (Lhc) protein family. Lhcs are characterized by three membrane  
344 spanning  $\alpha$ -helices, whereby helix 1 and 3 form a cross-like superhelical structure. These  
345 proteins non-covalently attach chlorophylls (Chl) as well as carotenoids. Lhcs are connected  
346 with the photosystem core complexes, forming so-called photosystem supercomplexes. Major  
347 differences compared to plants can be found in the number of Lhcs associated with the  
348 photosystems, their specific structure, and the number and identity of pigments bound to the  
349 diatom light harvesting apparatus. In recent years there have been major advances in  
350 understanding the light harvesting machinery of diatoms which we will review here.

351

### 352 ***2.1 The diatom light harvesting systems***

353 In diatoms Lhcs, Chl *a* and Chl *c*<sub>1</sub> (and little Chl *c*<sub>2</sub>) (Fawley 1989; Kraay et al. 1992) are  
354 accompanied by the major carotenoid fucoxanthin (Fx), and usually by minor amounts of Dd  
355 and Dt. The diatom genome encodes large numbers of *Lhc* genes whose translated proteins fall  
356 into three major and some minor groups. The major groups include i) the main group of proteins  
357 working in light harvesting, named *Lhcf*, ii) Lhcs most closely related to those of red algae  
358 called *Lhcr*, and iii) the photoprotective proteins called LI818 or *Lhcx*, related to green algal  
359 *LhcSR* (Eppard and Rhiel 1998; Bailleul et al. 2010; Ghazaryan et al. 2016). The minor groups  
360 comprise iv) a small group of genes called *Lhcz*, v) the so called *RedCaps* (Engelken et al.  
361 2012), and vi) some other sequences that do not belong to either group. *Lhcf* and *Lhcr* genes  
362 constitute the biggest groups with 8-17 and 9-14 members, respectively, when comparing the  
363 three best studied diatoms *P. tricornutum* (Bowler et al. 2008), *T. pseudonana* (Armbrust et al.  
364 2004), and *Cyclotella meneghiniana* (Gundermann et al. 2019). Concerning *Lhcx*, numbers are  
365 smaller with 4-6 genes. However, numbers are even higher for e.g. *Fragilariopsis cylindrus*  
366 (Mock et al. 2017). For *P. tricornutum* as well as for *T. pseudonana*, expression of all those  
367 genes was proven either on mRNA or on protein level in cells and pigment-protein complexes  
368 (Nymark et al. 2009; Lepetit et al. 2010; Grouneva et al. 2011; Schober et al. 2019; Kansy et  
369 al. 2020), and for *C. meneghiniana* all *Lhcf* and some *Lhcx* proteins were found in different  
370 light harvesting complexes (Gundermann et al. 2019). Most of the proteins encoded for by *Lhc*  
371 genes assemble into multi-subunit complexes that are then called fucoxanthin-chlorophyll-  
372 protein complexes (FCP). Note that single Lhc proteins of diatoms sometimes are also called

373 FCP mainly for historical reasons, since the Lhc nomenclature was only introduced when the  
374 first whole genome sequences became available.

375

## 376 **2.2 Subunit compositions of light harvesting complexes**

377 As in vascular plants, the photosystems of diatoms are surrounded by light harvesting  
378 complexes, whereby some are more tightly bound to either PSII or PSI, forming PSII-FCP or  
379 PSI-FCP supercore complexes/supercomplexes, respectively. Besides, there is an additional  
380 pool of more loosely bound FCPs, which will be considered first. Early on pools of FCPs were  
381 isolated and characterized from the pennate diatom *P. tricornutum* (Alberte et al. 1981;  
382 Friedman and Alberte 1984; Gugliemelli 1984; Fawley and Grossman 1986; Owens and World  
383 1986; Caron and Brown 1987; Owens 1988; Berkaloff et al. 1990; Lavaud et al. 2003;  
384 Guglielmi et al. 2005). Later, centric diatoms like *C. meneghiniana* or *T. pseudonana* were  
385 studied as well, and sub-populations of FCP complexes were differentiated. In the pennate *P.*  
386 *tricornutum*, only trimeric FCP complexes were found (Joshi-Deo et al. 2010; Grouneva et al.  
387 2011) that, nonetheless, could interact to form yet larger complexes (Lepetit et al. 2007; Gardian  
388 et al. 2014). Further separation demonstrated the presence of three major trimers, with Lhcf5,  
389 Lhcf10 and Lhcf2, or Lhcf4 as main subunits, respectively, together with different other Lhcf  
390 polypeptides (Gundermann et al. 2013). Later, homodimers of Lhcf4 were found in crystals  
391 used for X-ray crystallography (Wang et al. 2019, see below). No members of the other Lhc  
392 families (Lhcr or Lhcx) were found in purified trimeric FCPs of pennates so far (Gundermann  
393 et al. 2013), although the photoprotective Lhcx as well as Lhcr proteins are present when  
394 isolating the whole pool of FCP complexes (Lepetit et al. 2010; Nagao et al. 2013a; Taddei et  
395 al. 2018). In contrast, trimeric and specific higher oligomeric complexes were isolated from *C.*  
396 *meneghiniana*, a centric diatom (Büchel 2003), and two different complexes were later verified  
397 for other centrics as well, *T. pseudonana* (Grouneva et al. 2011) and *Ch. gracilis* (Nagao et al.  
398 2012; Nagao et al. 2013a), respectively. In the centrics *C. meneghiniana* and *T. pseudonana*,  
399 Lhcf proteins accompanied by Lhcx1 proteins defined the major trimeric complex, named  
400 FCPa. The oligomeric complex with distinct Lhcf composition, named FCPb, was proven to be  
401 a nonamer (Röding et al. 2018). In *Ch. gracilis*, the oligomeric complex was named FCP-A and  
402 the trimeric complex FCP-B/C due to their subunit composition (Nagao et al. 2012; Nagao et  
403 al. 2013a). Recently, FCPa and FCPb complexes of *C. meneghiniana* were further divided into  
404 sub-complexes (Gundermann et al. 2019). Four different trimeric FCPa complexes were shown  
405 to exist, whereby FCPa3 and FCPa4 were the most abundant forms. Lhcf1 was the major  
406 polypeptide in both, accompanied by Lhcx1 in case of FCPa4. For FCPb two sub-complexes

407 were distinguished: FCPb1 that is built almost solely of Lhcf3, and FCPb2 that in addition  
408 contains Lhcx1 and Lhcx6\_1 when isolated from cells grown under high light. When using  
409 sucrose gradient centrifugation to separate solubilized thylakoid membrane proteins, two bands  
410 were obtained, whereby the upper band contained associations of FCPa4, FCPa1 and FCPb2,  
411 i.e. those complexes that contain Lhcx1, whereas the lower band was an association of the  
412 remaining FCPa trimers and FCPb nonamers devoid of Lhcx1.

413 Lhcx1 is very similar in sequence in centrics and pennates, but only in centrics it was found in  
414 FCP trimers so far. It was proven to be involved in photoprotection in whole cells in both groups  
415 (Bailleul et al. 2010; Zhu and Green 2010; Ghazaryan et al. 2016; Buck et al. 2019) (see  
416 *Photosynthetic light reactions in diatoms. II. The dynamic regulation of the various light*  
417 *reactions*). For *C. meneghiniana* it could also be shown that suppression of the fluorescence  
418 quenching of FCPa depends on Lhcx1 content, pH and aggregation (Gundermann and Büchel  
419 2012), whereby Lhcx1 *in vivo* does not seem to act as direct quencher, but probably promotes  
420 FCPa aggregation (Ghazaryan et al. 2016), in line with the proposed uncoupling of the  
421 peripheral antennae upon induction of qE in pennates (Buck et al. 2019). Regarding the other  
422 Lhcx proteins, very little is known about their specific localization in the thylakoid membrane.  
423 For Lhcx3 of *P. tricornutum*, some evidence exists for a binding to the FCP complexes and to  
424 PSI, but further experiments are needed to corroborate this result (Taddei et al. 2018).

425 Only pennate diatoms like *P. tricornutum* have a ‘red antenna’ when cultivated under red light  
426 (Herbstová et al. 2015; Herbstová et al. 2017), characterized by a long wavelength fluorescence  
427 at around 710 nm at room temperature that is attributed to PSII. The main component of this  
428 red fluorescing antenna is Lhcf15, a protein which is not closely related to the other main Lhcf  
429 proteins that constitute the trimers and which has no close homologue in centrics. This red-  
430 shifted antenna was interpreted as an evolutionary adaptation towards survival in shaded  
431 environments. Recently, however, results demonstrated that the ‘red antenna’ may also be  
432 induced under weak green and yellow light (Oka et al. 2020).

433

### 434 **2.3 FCPs associated with PSI**

435 PSI-FCP supercores were isolated before sequences of Lhcs became known, and accordingly  
436 no detailed attribution of protein isoforms to complexes was initially possible (Berkaloff et al.  
437 1990; Brakemann et al. 2006; Veith and Büchel 2007; Ikeda et al. 2008). Later, the homology  
438 of Lhcr with the red algal PSI antenna proteins gave rise to the assumption that Lhcr  
439 polypeptides fulfill the same function. Indeed, Lhcr proteins can be found in PSI isolates (Veith  
440 et al. 2009; Lepetit et al. 2010; Grouneva et al. 2011). Depending on the isolation procedure,

441 Lhcr are the sole PSI antennae components (Lepetit et al. 2010), or Lhcf (and sometimes Lhcx)  
442 proteins were present in the PSI antenna as well (Brakemann et al. 2006; Veith and Büchel  
443 2007; Ikeda et al. 2008; Veith et al. 2009; Grouneva et al. 2011; Juhas and Büchel 2012; Ikeda  
444 et al. 2013; Calvaruso et al. 2020; Nagao et al. 2020). As in vascular plants, PSI of diatoms is  
445 a monomer (Veith and Büchel 2007; Ikeda et al. 2008; Nagao et al. 2020). In *Ch. gracilis*, the  
446 antenna complexes that serve PSI are characterized by long wavelength absorbing Chls that  
447 rapidly equilibrate with Chls in the PSI core through uphill energy excitation transfer (Nagao  
448 et al. 2018; Nagao et al. 2019b).

449 From the same organism the structure of a PSI-FCP supercomplex at 2.4 Å resolution became  
450 available recently (Nagao et al. 2020). Sixteen monomeric Lhcs surround the highly conserved  
451 core, i.e. much more Lhc subunits than in any other organism studied so far. Ten of those are  
452 Lhcr proteins and the others belong to a group that was named Lhcq and is closely related to  
453 Lhcf. The Lhc subunits are called Fcpa in the corresponding publication (Nagao et al. 2020),  
454 but the respective Lhcr/Lhcq names can be found in the protein data bank file (pdb file 6L4U).  
455 Nine Fcpa are surrounding the core in a circle that is almost closed (Fcpa1-9), whereby only  
456 the PsaL/I side is not binding any Lhc. These Lhc all belong to the Lhcr family. On the PsaA  
457 side, Fcpa10-16 are found in addition, whereby only Fcpa10 belongs to the Lhcr group, and the  
458 others are Lhcq proteins. Pigment binding differs strongly between the Fcpa subunits: five to  
459 thirteen Chl *a*, none to seven Chl *c*, one to seven Fx and one to four Dd are found. For example,  
460 the subunit called Fcpa15 has the highest pigment load with 13 Chl *a*:0 Chl *c*:7 Fx:2 Dd,  
461 whereas Fcpa1 carries only a small amount of pigments (6 Chl *a*:2 Chl *c*:2 Fx:1 Dd). Fcpa13 is  
462 very unusual with a high Chl *c* and low Fx content (6:6:2:1). More recently, the structure of an  
463 even bigger PSI-FCP complex from *Ch. gracilis* was solved (Xu et al. 2020). Here, 24  
464 monomeric FCP surround the monomeric PSI cores. Unfortunately, neither protein nor pigment  
465 attribution are identical for the FCP complexes present in both structures.

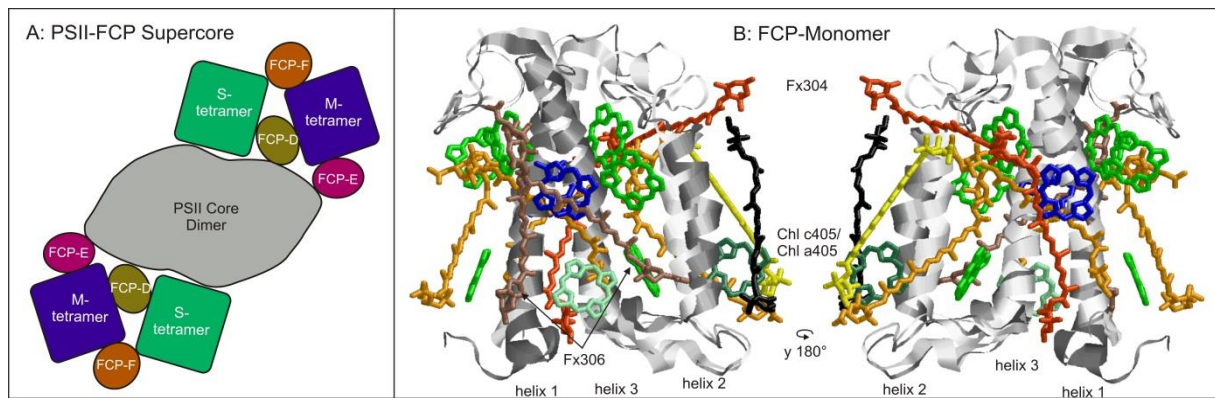
466

#### 467 ***2.4 FCPs associated with PSII***

468 A lot of detail is also known about PSII-FCP supercores, since the structure of such a complex  
469 at high resolution from *Ch. gracilis* became also available recently (Nagao et al. 2019a; Pi et  
470 al. 2019). The core dimer of PSII is highly homologous to that of vascular plants and especially  
471 red algae, but the organization of the surrounding FCPs differs. Diatoms lack homologues to  
472 the minor antenna proteins CP24, CP26, and CP29 of plants, but nonetheless monomeric Lhc  
473 are found, albeit in totally different positions. Comparable to the situation in vascular plants,  
474 where light-harvesting complex II (LHCII) is found in the supercores, two strongly bound (S)

475 and two less tightly bound (M) oligomers of FCPs are present in the dimeric PSII supercores  
476 (Fig. 2A). However, these FCPs are tetrameric in *Ch. gracilis*, in contrast to the trimers or  
477 nonamers reported for the free pool of FCPs in the same species. Since *Ch. gracilis* is not  
478 sequenced, only some Lhc sequences could be used for model building. According to Pi et al.  
479 (2019), the tetramers consist of FCP-A, i.e. the polypeptide found in the nonamers of the free  
480 FCP pool before. Due to the lack of sequences, the homologous sequence from *T. pseudonana*  
481 (Lhcf8) was used for modelling. In contrast, Nagao et al. (2019a) attributed the subunits of the  
482 tetramer to Lhcf1, a constituent of the trimeric complexes in other centrics. However, since the  
483 resolution of the PSII-FCP supercore structures is at 3.0 and 3.6 Å, respectively, slight  
484 variations in the electron densities between monomers might suggest that the tetramers consist  
485 of several, very similar Lhc proteins. Concerning the monomeric subunits, one (FCP-D) turned  
486 out to be an antenna protein of the Lhcr group (Pi et al. 2019). The others could not be  
487 unambiguously identified. Very recently, a low resolution structure of the PSII-FCP complex  
488 of *T. pseudonana* was published (Arshad et al. 2021). The overall arrangement with three  
489 monomeric and two oligomeric FCP complexes resembled closely the structure of PSII from  
490 *Ch. gracilis*. However, the oligomeric FCPs appeared as trimers, not as tetramers, in accordance  
491 with the trimeric FCPs from the free FCP pool of *T. pseudonana*.

492 In summary, surprisingly, three different oligomeric states of FCPs exist besides monomeric  
493 Lhcs when considering pennate and centric diatoms together, whereas in plants only trimeric  
494 complexes (LHCII) and dimers (LHCI) are found. The recently available structures of a  
495 supercore from centrics (*Chaetoceros*) (Nagao et al. 2019a; Pi et al. 2019) and an FCP from  
496 pennates (*Phaeodactylum*) (Wang et al. 2019) were combined by Wang et al. (2020) into a  
497 model where the supercore having tetrameric FCPs bound is surrounded by a pool of dimeric  
498 FCPs. However, for the centrics *C. meneghiniana* as well as *T. pseudonana* trimeric FCPs were  
499 demonstrated by imaging methods to constitute the pool of peripheral FCPs and the PSII  
500 associated FCPs, respectively (Röding et al. 2018, Arshad et al. 2021). In addition, no data are  
501 available about the oligomeric state of the remaining FCPs in pennates (built of other subunits  
502 than Lhcf4), and no structure of the PSII supercomplex is available. Using biochemical  
503 analyses, trimers were found in both groups of diatoms. In addition, in centric diatoms  
504 nonamers of specific polypeptide composition are present (Beer et al. 2006; Grouneva et al.  
505 2011; Nagao et al. 2013a). For PSI the situation is much more clear, since only monomeric  
506 Lhcs are attached (Veith and Büchel 2007; Nagao et al. 2019b; Nagao et al. 2020, Xu et al.  
507 2020).



508

509

510 **Figure 2**

511 A: Scheme of the PSII-FCP supercore complex according to Nagao et al. (2019a) and Pi et al.  
 512 (2019). The core of PSII, with the reaction center proteins D1 and D2 and the inner antenna  
 513 proteins CP43 and CP47, is almost identical in plants and diatoms and shown in grey. PSII is a  
 514 dimer, surrounded by FCP complexes on both sides. FCP-A, the subunit of both tetramers (ST  
 515 and MT) was modelled using *T. pseudonana* Lhcf8 in case of Pi et al. (2019) and identified as  
 516 Lhcf1 by Nagao et al. (2019a). Note that in *T. pseudonana* these complexes are trimeric (Arshad  
 517 et al. 2021). FCP-D corresponds to Lhca2, a protein of the Lhcr group. FCP-E and FCP-F could  
 518 not be identified.

519 B: Overlay of one of the monomers of the S-tetramer (taken from pdb 6JLU, 3.0 Å resolution,  
 520 Pi et al. 2019) with one monomer of the dimeric FCP out of Lhcf4 of *P. tricornutum* (pdb  
 521 6A2W, 1.8 Å resolution, Wang et al. 2019). Since the structure of the M-tetramers as well as  
 522 the tetramers by Nagao et al. (2019a) (pdb 6J40, 3.6 Å) closely resembles the shown structure,  
 523 they are omitted from the comparison. The protein backbone is depicted in grey (6JLU) and  
 524 white (6A2W), respectively. The pigments in (nearly) identical sites in both complexes are  
 525 omitted for the dimeric FCP and shown in colors for the ST-FCP: Chl *a* in green and Chl *c* in  
 526 blue, whereby Chl 405, which was attributed to Chl *a* in the dimeric FCP and to Chl *c* in the  
 527 tetramers, is shown in blue-green. The Chl *a* not found in the dimeric FCP (and M-tetramers),  
 528 but in S-tetramers is depicted in light-green. The four Fx molecules present in identical  
 529 locations in the dimer and the tetramers are shown in orange, whereas the Fx molecule present  
 530 only in the tetrameric FCPs is shown in yellow. Fx306 is oriented more perpendicular to the  
 531 membrane plane in the FCP dimer, whereas it adopts a different orientation in some subunits  
 532 of the tetramers; both orientations are given in brown color. The additional Fx molecules and  
 533 the Dd only present in the dimer are shown in red and black, respectively. The left panel shows  
 534 the same complexes as the right panel, but turned by 180° around the y-axis.  
 535

536

537 **2.5 Molecular structure of FCPs**

538 The first molecular structure at high resolution (1.8 Å) of a dimeric FCP of Lhcf4 from *P.*  
 539 *tricornutum* became available recently (Wang et al. 2019), and the overall features were  
 540 confirmed for the FCPs within the PSII-FCP and PSI-FCP supercomplexes (Nagao et al. 2019a;

541 Pi et al. 2019; Nagao et al. 2020) (Fig. 2B). The overall protein scaffold structure of FCP  
542 monomers is similar to that of LHCII monomers (for an overview see Büchel 2019). The helices  
543 where sequence conservation is high, i.e. helix 1 and 3, adopt an almost identical configuration  
544 in FCP vs. LHCII, whereas helix 2 has a slightly different location and tilt that also differs  
545 between the FCP-dimer from *P. tricornutum* and the FCP-tetramers from *Ch. gracilis* found in  
546 PSII-supercomplexes. The complex from *P. tricornutum* was crystallized as a dimer, whereby  
547 the connection between the monomers was due to strong interactions between helices 2. This  
548 ‘head to head’ configuration has not been shown before for any member of the Lhc family,  
549 since e.g. Lhca1/Lhc4 dimers of plant PSI interact in a ‘head to tail’ configuration (Ben-Shem  
550 et al. 2003). Similarly, the monomers of the M- and S-tetramer in the PSII-FCP supercore  
551 complexes are associated ‘head to tail’. In contrast to LHCII, where helices 3 come close to the  
552 stromal surface, the subunits of the tetramers are turned by 180° in comparison to LHCII, so  
553 that helices 1 approach closest to the stromal side (Nagao et al. 2019a; Pi et al. 2019). Although  
554 helices 1 and 3 are almost identical in FCP and LHCII, the dimer and the tetramers are arranged  
555 in a way that these helices adopt a slightly different tilt with respect to the membrane plane  
556 compared to the arrangement in the trimeric LHCII (Liu et al. 2004; Standfuss et al. 2005;  
557 Büchel 2019).

558 Seven Fx, one Dd, seven Chl *a*, and two Chl *c* per monomer were fitted into the electron density  
559 map of the *P. tricornutum* FCP-dimer (Wang et al. 2019), whereas in the tetrameric FCPs of  
560 *Ch. gracile* only six Fx, but nine (M-tetramer) or ten Chl molecules (S-tetramer) were found  
561 (Pi et al. 2019; Wang et al. 2019) (Fig. 2B), and, as stated above, the variability is even larger  
562 in PSI associated Lhc. In general, the Chl:carotenoid ratio is much lower than in plant LHCII.  
563 As already anticipated from sequence comparisons and based on spectroscopy data  
564 (Premvardhan et al. 2010), six of the Chl binding sites are highly conserved between LHCII  
565 and FCP, with the central Fx molecules arranged close to helix 1 and 3 similarly to the luteins  
566 in LHCII (Liu et al. 2004; Standfuss et al. 2005). One Dd was fitted in a more peripherally  
567 located density in the *P. tricornutum* FCP-dimer, but was not found in any of the *Ch. gracilis*  
568 tetramers (Fig. 2B). The porphyrin rings of Chl *c* and Chl *a* are rather similar in structure, so  
569 Chl *c* can only be distinguished from Chl *a* due to the planarity of the C18=C17 double bonds  
570 and the lack of phytol in Chl *c*, which requires a high resolution electron density map to resolve.  
571 In the diatom FCP-tetramers close to PSII, three Chl *c* were assigned, the additional one being  
572 Chl *c*405 (nomenclature according to Wang et al. 2019), a binding site that had been assigned  
573 to Chl *a* in the FCP-dimer of *P. tricornutum* only for reasons of pigment stoichiometry (Wang  
574 et al. 2019). Surprisingly, Chl *c*403 and Chl *c*408 reside in the strongly conserved binding sites

575 603 (Chl *c*403) and 612 (Chl *c*408), occupied by Chl *a* in case of LHCII. Both Chl *c*'s are in  
576 close contact with Fx molecules, which are bound in places where no carotenoids are found in  
577 case of LHCII. All carotenoid molecules in FCP are in *all-trans* configuration, and all have Chl  
578 *a* molecules in close vicinity (3.1 to 3.6 Å). Whereas the Chl binding sites are highly conserved  
579 between the FCP-dimer and the FCP-tetramer structures, only four Fx are found in identical  
580 places to LHC carotenoids (Fig. 2B), including those in the lutein binding sites. The Fx  
581 interacting with one of the Chl *c* in the Chl *a*/Chl *c*/Chl *a* clusters (Fx306) is oriented almost  
582 perpendicular to the membrane plane in the FCP-dimers and in some subunits of the tetramers,  
583 whereas it is oriented in an angle of almost 45° in others. Two Fx are found in totally different  
584 locations in tetramers compared to the dimer. The most peculiar Fx is Fx304 in the FCP-dimer,  
585 running almost parallel to the membrane plane on the stromal side. This Fx is missing in the  
586 FCP-tetramers.

## 587 **2.6 Excitation energy transfer in FCPs**

588 Pigment ratios of around 6-8 Chl *a* : 2-3 Chl *c* : 6-8 Fx were reported for the different diatom  
589 FCP complexes (which represented rather the main, free pool of FCP complexes), depending  
590 on growth condition, isolation procedure and species (Papagiannakis et al. 2005; Beer et al.  
591 2006; Lepetit et al. 2007; Joshi-Deo et al. 2010; Lepetit et al. 2010; Gundermann and Büchel  
592 2012; Nagao et al. 2013b). More evident differences were reported concerning the PSI  
593 associated Lhc antennae proteins: For both centric and pennate diatoms the Chl *c*/Fx ratio was  
594 lower, whereas the (Dd+Dt)/Fx ratio was strongly increased (Lepetit et al. 2007; Veith and  
595 Büchel 2007; Veith et al. 2009; Lepetit et al. 2010; Juhas and Büchel 2012). The Chl *c*/Fx ratio  
596 was even lower when *P. tricornutum* was grown under red light (Bína et al. 2016). Whereas  
597 this pigmentation is obviously due to the presence of the different Lhcf and Lhcr polypeptides,  
598 respectively, nothing is known so far about the pigmentation of the gene products of *RedCaps*,  
599 *Lhcy* or *Lhcz* that have been solely analyzed on genetic level so far. Even for Lhcx proteins the  
600 question of whether they are pigmented remains open, since they have not been isolated as  
601 single proteins so far. However, their high similarity to the pigmented LhcSR proteins of green  
602 algae (Bonente et al. 2011) is a strong argument for diatom Lhcx proteins binding pigments  
603 (see "*Photosynthetic light reactions in diatoms. II. The dynamic regulation of the various light*  
604 *reactions*").

605 In FCPa and FCPb complexes of *C. meneghiniana*, two differently bound Chl *c* molecules were  
606 identified by Resonance Raman spectroscopy (Premvardhan et al. 2010) and also in 2D  
607 spectroscopy by their different transfer times (Songaila et al. 2013; Gelzinis et al. 2015), in

608 agreement with the numbers although not the locations found in the crystal structure for *P.*  
609 *tricornutum* Lhcf4 (Wang et al. 2019). In all FCPs isolated so far, the Q<sub>Y</sub> absorption of FCPs is  
610 at relatively short wavelength (~671 nm), excluding close excitonic interactions between Chl *a*  
611 molecules as verified by CD spectra, where no excitonic interactions are visible in the Q<sub>Y</sub>  
612 (Büchel 2003; Szábo et al. 2008; Joshi-Deo et al. 2010). The fact that the strong Chl *a*  
613 interaction of a610/a611/a612 visible in LHCII (Novoderezhkin et al. 2004) is broken by Chl *c*  
614 in FCPs, also due to slightly different arrangement of Chl *a*401 (FCP) compared to Chl *a*611  
615 (LHCII), is also in line with the lack of excitonic interactions. Thus, delocalized excited states  
616 are missing, which contribute to the robustness of energy transfer in the flexible protein  
617 environment e.g. in LHCII of vascular plants. FCP complexes, in contrast, seem to use subtle  
618 changes in protein scaffold conformation to switch frequently into low-energy states with  
619 improved light-harvesting properties as shown for FCPa from *C. meneghiniana* (Krüger et al.  
620 2017).

621 Fx is actively involved in energy transfer to Chl *a*. Fx is special since it has a carbonyl moiety  
622 in conjugation with the polyene backbone. Carotenoids are in principle able to transfer energy  
623 from both their lowest singlet excited states, i.e. S<sub>1</sub> and S<sub>2</sub>, but direct absorption into S<sub>1</sub> is  
624 forbidden due to their symmetry. In Fx an additional excited state with an intramolecular charge  
625 transfer (ICT) character exists. This ICT state can be coupled to the S<sub>1</sub> state, and plays a major  
626 role in carotenoid-Chl energy transfer (Bautista et al. 1999; Vaswani et al. 2003; Zigmantas et  
627 al. 2004; Papagiannakis et al. 2005; Premvardhan et al. 2005; Gelzinis et al. 2015; West et al.  
628 2018). In addition, Fx displays an extreme bathochromic shift upon protein binding, extending  
629 the absorption from 390 nm up to 580 nm. More ‘blue’, ‘green’ and ‘red’ absorbing Fx  
630 molecules were detected in FCPa as well as in FCPb from *C. meneghiniana* (Premvardhan et  
631 al. 2008; Premvardhan et al. 2009; Premvardhan et al. 2010) using Stark and Resonance Raman  
632 spectroscopy, but could so far not be attributed to certain Fx molecules in the structure. ‘Blue’  
633 and ‘red’ Fx were also demonstrated for whole cells of *C. meneghiniana* and *P. tricornutum*  
634 using electrochromic shift measurements (Szábo et al. 2010). Excitation energy transfer from  
635 Fx to Chl *a* proceeds mainly via the S<sub>1</sub>/S<sub>ICT</sub> state in FCPs, whereby the transfer to the Q<sub>Y</sub> state  
636 of Chl *a* has a time constant of 0.6 ps in FCPa of *C. meneghiniana* (Papagiannakis et al. 2005;  
637 Gildenhoff et al. 2010a). The transfer for the Fx S<sub>2</sub> state directly into the Q<sub>X</sub> state of Chl *a* is  
638 even faster with <150 fs (Gildenhoff et al. 2010a). For *P. tricornutum*, two routes using the  
639 S<sub>1</sub>/S<sub>ICT</sub> state were determined recently, whereby the slower (~ 6 ps) route uses the S<sub>1</sub> part of the  
640 potential surface of the S<sub>1</sub>/S<sub>ICT</sub> equilibrium (West et al. 2018). The fast Fx to Chl *a* transfer is

641 possible due to the arrangement of Fx and Chl *a*: each Fx has an Chl *a* molecule in close vicinity  
642 as stated above. Fx also acts as an efficient quencher of Chl *a* triplets (Di Valentin et al. 2012).  
643 Fx-Fx transfer with a time constant of 25 ps was measured using FCPa of *C. meneghiniana*  
644 (Gildenhoff et al. 2010a; Gildenhoff et al. 2010b). However, no pair of Fx molecules can be  
645 pinpointed in the structures responsible for this route of excitation energy transfer.

646 No energy transfer from Fx to Chl *c* was observed within the limit of instrumentation of pump-  
647 probe as well as 2D spectroscopy for *C. meneghiniana* FCPs (Papagiannakis et al. 2005;  
648 Gildenhoff et al. 2010a; Songaila et al. 2013; Gelzinis et al. 2015). 2D spectroscopy also  
649 revealed that the two Chl *c* have different time constants for energy transfer into Chl *a*, both  
650 reactions being extremely fast with 60 fs and less, respectively (Songaila et al. 2013; Gelzinis  
651 et al. 2015). This is in agreement with the close contact of Chl *c* and Chl *a*, allowing fast  
652 excitation energy transfer between these pigments. Judging from the structure, also the Fx/Chl  
653 *c* arrangement is, however, perfectly suited for fast excitation energy transfer. If present, it has  
654 to be even faster than Chl *c*-Chl *a* transfer in order to have escaped detection.

655 The differences might be due to the fact that spectroscopically mainly the trimeric FCPs from  
656 *C. meneghiniana* were analyzed, whereas structural data come from the dimeric FCP of *P.*  
657 *tricornutum*. The contradictions between the structure and the wealth of spectroscopic data were  
658 recently highlighted (Gelzinis et al. 2020). More structures are evidently needed in order to  
659 fully understand excitation energy transfer in FCP.

660 In the PSII-FCP supercore complex, energy pathways have only been deduced from the  
661 structure so far. The distances between Chls imply that the S-tetramers funnel energy into CP47,  
662 one of the inner antenna proteins of PSII, via a Chl *a* binding subunit called PsbG that is not  
663 present in vascular plant PSII, and into FCP-D (Fig. 2A). From the latter the energy can be  
664 transferred to CP43, the inner antenna on the opposite side of the PSII reaction center compared  
665 to CP47. The M-tetramer might transfer energy via FCP-E and FCP-D also into the Chls bound  
666 to CP43. FCP-F is rather peripheral and thus probably delivers energy mainly to the M-tetramer.

667 Also for the PSI-FCP complexes an excitation energy transfer network was proposed, with  
668 pathways in between the inner ring of Lhc proteins as well as pathways from the more  
669 peripheral antenna into the inner ring and from there to the core. Since the methods to prepare  
670 supercore complexes in sufficient amounts for ultrafast spectroscopy are available now, these  
671 pathways will probably be elucidated in more detail in future.

672

673

## 674 **Outlook**

675 The recently obtained structures of PSII and PSI supercomplexes allow for the first time  
676 detailed insights into particular steric features of the whole light harvesting system and the  
677 excitation energy routes in diatoms. We need more of these structures, obtained from diatoms  
678 in different acclimation states but also from different species. This way, we may reveal the  
679 localization of the Lhcx proteins as well of those proteins which so far have only been found  
680 on the genetic level, such as *Lhcz* or *Redcap*. More of these studies will also enable us to resolve  
681 some so far existing discrepancies about the composition and oligomerisation states of FCPs  
682 obtained either by classical biochemical preparations or by crystallization approaches. In  
683 addition, the dynamic interaction between thylakoid lipid domains and thylakoidal protein  
684 complexes in diatoms is a field which is as yet largely understudied and would also profit from  
685 sophisticated imaging approaches. In this regard, we also need to better understand biogenesis  
686 of thylakoid membranes and photosynthetic complexes. Recently, first insights regarding the  
687 insertion of FCPs in *P. tricornutum* have been obtained. Alb3b, a protein functioning in the  
688 insertion and assembly of thylakoid membrane protein complexes in plants, was demonstrated  
689 to be essential for FCP assembly (Nymark et al. 2019). On the other hand, the chloroplast signal  
690 recognition particle protein CpSRP54, involved in targeting LHC to the chloroplast membrane  
691 in plants and green algae, is not involved in FCP accumulation but important for the insertion  
692 of plastid encoded thylakoid membrane proteins (Nymark et al 2021). This paves the way for  
693 future studies on FCP assembly, but to fully understand this process, also the enzymes working  
694 in Fx and Chl *c* synthesis still need to be identified.

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697 **References**

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1050 **Acknowledgements**

1051 BB acknowledges financial support from the European Research Council (ERC) under the  
1052 European Union Horizon 2020 research and innovation program (grant agreement no. 715579).  
1053 BL thanks the Deutsche Forschungsgemeinschaft (LE3358/3-2) and the Baden-Württemberg  
1054 Stiftung (Elite program) for financial support. CB acknowledges support by the European  
1055 Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie  
1056 grant agreement No 675006 and from the Deutsche Forschungsgemeinschaft, grant Bu 812 10-  
1057 1. DAC thanks the Canada Research Chairs and Natural Science and Engineering Research  
1058 Council of Canada for support. JL thanks the Centre National de la Recherche Scientifique-  
1059 CNRS, the Natural Sciences and Engineering Research Council of Canada-NSERC (Discovery  
1060 and Northern Supplement grants), the Canada First Research Excellence Fund-Sentinelle Nord,  
1061 and the strategic research cluster Québec-Océan for their financial support. All authors thank  
1062 the reviewer for valuable suggestions.