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The structure of FCPb, a light-harvesting complex in the diatom *Cyclotella meneghiniana*

Anja Röding¹ · Egbert Boekema² · Claudia Büchel¹

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Abstract Diatoms possess fucoxanthin chlorophyll proteins (FCP) as light-harvesting systems. These membrane intrinsic proteins bind fucoxanthin as major carotenoid and Chl c as accessory chlorophyll. The relatively high sequence homology to higher plant light-harvesting complex II gave rise to the assumption of a similar overall structure. From centric diatoms like *Cyclotella meneghiniana*, however, two major FCP complexes can be isolated. FCPa, composed of Fcp2 and Fcp6 subunits, was demonstrated to be trimeric, whereas FCPb, known to contain Fcp5 polypeptides, is of higher oligomeric state. No molecular structure of either complex is available so far. Here we used electron microscopy and single particle analysis to elucidate the overall architecture of FCPb. The complexes are built from trimers as basic unit, assembling into nonameric moieties. The trimer itself is smaller, i.e. more compact than LHCII, but the main structural features are conserved.

Keywords Electron microscopy · Fucoxanthin chlorophyll protein · Oligomeric state · Single particle analysis

Abbreviations

BN-PAGE Blue native polyacrylamide gel electrophoresis

Chl	Chlorophyll
DDM	Dodecyl maltoside
FCP	Fucoxanthin chlorophyll protein
Fx	Fucoxanthin
IEX	Ion exchange chromatography
LHC	Light-harvesting complex

Introduction

Diatoms are unicellular, eukaryotic phototrophs that are responsible for about one quarter of the yearly primary production on earth (Field et al. 1998). Due to their evolutionary history the chloroplast structure differs from that of higher plants, and thus no grana/stroma differentiation is found. Nonetheless, diatoms contain membrane intrinsic light-harvesting complexes, whereby the sequences are similar to higher plant light-harvesting complex II (LHCII). The proteins are smaller but probably show the major structural elements of three membrane-spanning α -helices as well (Green and Kühlbrandt 1995). Pigmentation, however, is considerably different. About eight chlorophyll (Chl) a, two Chl c and up to eight fucoxanthin (Fx) molecules are bound to each monomer (Papagiannakis et al. 2005; Premvardhan et al. 2010), shifting the carotenoid to Chl ratio considerably in comparison to LHCII. Chl c and Fx are much more polar than their functional counterparts in higher plants, i.e. Chl b and lutein, and differ in spectroscopic features. Fx molecules are bound in three different environments, and especially two Fx display a huge bathochromic shift and are thus responsible for the extended absorbance of the complexes around 480–550 nm (Premvardhan et al. 2008, 2010). Because of its pigmentation the light-harvesting complexes of diatoms are also called fucoxanthin-chlorophyll-proteins (FCP). Different

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FCPs have been isolated from a range of diatoms. In the case of centric diatoms like *Cyclotella meneghiniana*, two major complexes, FCPa and FCPb, could be purified that differ in their subunit composition and oligomeric state (Beer et al. 2006; Grouneva et al. 2011; Gundermann and Büchel 2008). FCPa is built from Fcp1–3 and Fcp6/7 proteins, whereas Fcp5 is a constituent of FCPb complexes. Based on size exclusion chromatography FCPa is assumed to be trimeric, whereas FCPb has a higher oligomeric state (Büchel 2003). No molecular structure of either complex is available up to now. Here we demonstrate that (i) FCPa is indeed a trimer, that (ii) the trimers are also the basic unit of FCPb, that (iii) these trimers resemble the LHCII structure, and that (iv) FCPb also assembles into nonamers that are less sensitive to solubilisation than to electric charges, in accordance with the high polarity differences inside this membrane protein.

Materials and methods

Growth conditions

Cyclotella meneghiniana

Batch cultures of *Cyclotella meneghiniana* (Culture Collection Göttingen, strain 1020-1a) were grown for 7 days under continuous shaking (120 rpm) and a light/dark cycle ($35 \mu\text{mol m}^{-2} \text{s}^{-1}$) of 16h/8h at a constant temperature of 18 °C in ASP medium (86 mM NaCl, 4 mM Tris, 21 mM KCl, 8.1 mM MgSO_4 , 11.8 mM, NaNO_3 , 0.58 mM K_2HPO_4 , and 0.16 mM H_3BO_3 , adjusted with H_2SO_4 to a pH of 7.7, autoclaved and supplemented with the following trace metals: 2.72 mM CaCl_2 , 0.092 mM $\text{Na}_2\text{-EDTA}$, 0.012 mM FeCl_3 , 0.02 mM MnCl_2 , 0.002 mM ZnCl_2 , 50 pM CoCl_2 , 25 pM Na_2MoO_4 , 22.5 pM CuCl_2 and 1 mM silica), a modified version of the protocol published by Provasoli et al. (1957).

Nicotiana tabacum

Nicotiana tabacum var. *xanthi* seeds were kindly provided by Prof G. Sandman, Goethe University, Frankfurt, from a collaboration with Prof. Dr. Feng Chen, University of Hongkong (Huang et al. 2012). *N. tabacum* was grown in a day/night cycle of 16/8 h at 25 °C and under a white fluorescent illumination of 100–140 $\mu\text{E}/(\text{s}\cdot\text{m}^2)$, depending on plant height.

FCPa and FCPb preparation

The algal cultures were harvested on day 7 an hour after the onset of light and thylakoids isolated according to Beer

et al. (2006). Chlorophyll determination was performed according to Jeffrey and Humphrey (1975). To separate the photosynthetic complexes, thylakoids were solubilised at 4 °C with 20 mM β -dodecyl maltoside (β -DDM) at 0.25 mg/ml Chl a in buffer B1 (25 mM Tris, pH 7.4, 2 mM KCl) under gentle, constant shaking for 20 min. Unsolubilised material was removed by spinning the solution for 5 min at 13,000 rpm and 4 °C in a table-top centrifuge. The supernatant was filtered with a 0.2 μm Filtropur S and loaded on an ion exchange (IEX) column. The IEX was carried out according to Beer et al. (2006) in buffer B1a (buffer B1 supplemented with 0.03% β -DDM). For further purification, the FCP containing fractions were concentrated and FCPs further purified on discontinuous sucrose density gradients (SDG) according to Gundermann and Büchel (2008).

Nicotiana tabacum LHCII preparation

Nicotiana tabacum leaves were harvested at an age of 6–8 weeks at the end of the dark period. Thylakoid isolation took place at 4 °C in the dark, modified after Burke et al. (1978). Using 4.4% Triton-X-100 at a Chl a concentration of 2 mg/ml, BBYs were prepared according to Berthold et al. (1981). BBYs were solubilised at 1 mg/ml Chl a with 20 mM β -DDM in buffer B1 using the method described by Eshaghi et al. (1999) and LHCII were separated on a sucrose gradient as described for solubilised *C. meneghiniana* thylakoids.

Size exclusion chromatography

Purified FCP fractions were loaded with a maximum Chl a content of 100 μg in 100 μl on a 0.66 cm x 33 cm Superdex200 column for analytical size exclusion chromatography, with a flow rate of 0.3 ml/min and detection at 280 nm (protein) and 437 nm (Chl a), adapted from Büchel (2003). To disassemble the oligomeric FCPa and FCPb complexes, additional samples were incubated with 20 and 100 mM β -DDM, respectively, for 1 h at room temperature before loading them on the column. For calibration, LHCII was run as well, using the same Chl concentration.

Spectral analysis

FCPa and FCPb complexes were analysed by fluorescence spectra using a Jasco FP-6500 fluorospectrometer. Emission spectra were recorded at room temperature with 465 nm for Chl c excitation. Excitation and emission bandwidths were set to 5 and 3 nm, respectively. Spectra were corrected using a calibrated lamp spectrum.

Gel electrophoresis

Blue native polyacryl amide gel electrophoresis (BN-PAGE)

Blue native gels consisting of a 4–12.5% gradient separation gel and a 5% stacking gel modified after Schagger and von Jagow (1991) were used. The anode buffer consisted of 0.05 M BisTris, pH 7, and the cathode buffer contained 0.05 M Tricine, 1 mM BisTris, 0.01% Serva Blue G, pH 7. Thylakoids (25 µg Chl a) were solubilised with 20 mM β-DDM for 5 min at 4 °C. A final amount of 7 µg Chl a in sample buffer was then loaded onto the gel. The run was performed at 4 °C with 10 and 30 mA for stacking and separation gel, respectively.

Immunodetection

Western Blots were carried out according to the method of Towbin et al. (1979) with minor modifications. Since the Serva Blue dye used for the BN gels caused a strong background signal during immunodetection, the membrane was incubated for 30 min at 50 °C in 100 mM β-mercaptoethanol, 67.5 mM Tris (pH 6.7) and 2% SDS. This solution was also used when removing the first antibody to allow for reusing of the membrane for a second immunodetection on the same membrane. Immunodetection was carried out by 1 h incubation of the membrane with the primary antibody (FCPa: α-FCP2 (E. Rhiel, University of Oldenburg, Oldenburg, Germany), or FCPb: α-lhcf1-11 (Juhas and Büchel 2012)) followed by 45 min incubation with a goat-α-rabbit secondary antibody (Calbiochem). The blots were developed using the chemoluminescence reaction and images were recorded on X-ray film.

Electron microscopy and single particle analysis

Purified samples were prepared for transmission electron microscopy by 1:20 dilution in buffer B1a and subsequent negative staining using 2% uranyl acetate on either glow-discharged (0.1 mbar/10 mA, Cressington Power Unit 208) or untreated carbon-coated copper grids. Electron microscopy was performed in a Philips CM120 equipped with a LaB₆ tip operating at 120 kV. Data acquisition for single particle analysis was carried out using GRACE, a system for semi-automated specimen selection and data acquisition (Oostergetel et al. 1998). Images were recorded at 133,000× calibrated magnification with a Gatan 4000 SP 4 K slow-scan CCD camera. Pixel size was 0.225 nm. A total of 54,000 particle projections were collected. Instead of picking particles manually we used EMAN2 software for automated particle picking with a box size of 64×64 pixel and 128×128, respectively (Tang et al. 2007).

Single particle analysis was performed using Groningen Image Processing (“GRIP”) software packages on a PC cluster including band pass filtering, non-reference and multireference alignments, multivariate statistical analysis and classification as in Boekema et al. (1999). Finally two characteristic views were obtained from 4344 and 2661 images, respectively, that were summed up without imposing symmetry.

Results

The oligomeric state of FCP complexes

Size exclusion chromatography of *Cyclotella meneghiniana* FCPs (Figs. 1a, 2a) and *Nicotiana tabacum* LHCII (Fig. 2a) underlined previous results (Büchel 2003) about the oligomeric state of FCP complexes. FCPa eluted after the trimeric LHCII, in agreement with its smaller size. In contrast, FCPb eluted before the trimeric LHCII and FCPa, indicating its higher oligomeric state. To further analyse the supramolecular built of the FCPb oligomers, disassembly by strong solubilisation with 100 mM β-DDM was carried out, and as a control FCPa was solubilised using 20 or 100 mM β-DDM. Figure 1a shows two oligomeric states for FCPa, which can be ascribed to monomers (I) and trimers (II), whereby the monomeric fraction increased when using 100 mM β-DDM (data not shown). In contrast, three different oligomeric states for FCPb are discernible, with the original oligomers (III) and monomers (I) as well as trimers (II) of the same size as for FCPa. Spectroscopic analysis of the fractions demonstrated that all these fractions contain functional FCP proteins (Fig. 1b, c), except for the monomers of FCPb, where a relatively high Chl c emission is indicative of loss of energy transfer from Chl c to Chl a due to the strong solubilisation.

Despite the results from size exclusion chromatography, blue native gel electrophoresis of solubilised thylakoids of *C. meneghiniana* and *N. tabacum* showed only FCPs of lower apparent molecular weight than LHCII (Fig. 2b). Immunodetection was carried out on the same membrane first for FCPa and after stripping for FCPb, revealing a very distinguished signal for FCPb below the LHCII trimer (Fig. 2b, c) and one signal for FCPa far below the trimeric LHCII and FCPb (Fig. 2c). Thus, under the conditions of an electric field, FCP complexes run at positions different from what was expected from gel filtration, where FCPb is shown to be larger than LHCII. One explanation would be an unusual running behaviour due to higher overall charge of FCPs compared to LHCs. The other explanation is that FCP complexes disassemble into sub-complexes that are otherwise only obtained by treatment with high detergent concentrations.

Fig. 1 **a** Size Exclusion Chromatogram at 437 nm of purified FCPa (grey solid line) and FCPb (black solid line). Samples incubated with 20 mM β -DDM (FCPa, dashed grey line) and 100 mM β -DDM (FCPb, dashed black line) are shown to demonstrate the different sub-

complexes. **b** and **c** demonstrate the emission spectra of FCPb (**b**) and FCPa (**c**) (sub)-complexes from elution maxima I (dotted lines), II (dashed lines) and III (solid line), respectively, recorded upon excitation at 465 nm

Fig. 2 Size comparison of FCPa, FCPb and LHCII. **a** Size exclusion chromatography of FCPa (green dashed line), LHCII (black dotted line), and FCPb (red solid line). **b** Blue native gel electrophoresis of *N. tabacum* thylakoids (lane 1) and *C. meneghiniana* thylakoids (lane 2). The black dotted line shows LHCII, the red solids line depict

FCPb, and the green dashed lines point to FCPa. **c** Blue native gel electrophoresis of *C. meneghiniana* thylakoids (lane 1) and immunodetection with α -FCP2 (lane 2), an antibody specific for FCPa complexes, and with α -lhcf1-11 (lane 3), an antibody specific for FCPb complexes

Transmission electron microscopy of FCP complexes

Electron microscopy of isolated FCPa, FCPb and LHCII complexes was carried out on carbon-coated, glow-discharged copper grids at a magnification of 300,000 $\times$ (Fig. 3a–c). Surprisingly, FCPa, LHCII and FCPb were all imaged as roundish particles of similar size (with roughly 10 nm in diameter) under those conditions and most probably represent top views of the complexes. Since, however, all complexes were of roughly the same size, the FCPb complexes under those conditions most probably represent

trimers as well, although higher oligomers were used for grid preparation.

If the same FCPb sample was used on grids that had not been glow-discharged before (Fig. 3d), only very few particles were bound to the carbon surface. Those represented a longish type of particles with about 6 nm along the short axis. When measuring the long axis for 229 FCPb particles, two major sizes became evident (Fig. 3e). About 48% of the complexes measured around 14 nm (± 1.3 nm) and another 31% had a length in the range of 20 nm (± 1.1 nm). An additional minor fraction of 7% was even longer with

Fig. 3 Transmission electron microscopy of negatively stained FCPb (a), LHCII (b) and FCPa (c) on glow-discharged carbon-coated copper grids. Bar corresponds to 10 nm. **d** Different side views of FCPb particles on untreated carbon-coated copper grids. Bar corresponds to 20 nm. **e** 229 side views of FCPb complexes imaged on untreated carbon copper grids as in **d** were analysed for their size along the long axis. Particles were then sorted into 1 nm bins and relative occurrence calculated (diamonds). When summing the most abundant particle sizes (overlaid in grey), about 48% of the particles had a size of around 14 nm (square), whereas 31% of the particles showed a higher length of roughly 20 nm (triangle) and a minor population (7%) was even larger with about 25 nm on average

about 25 nm (± 1 nm). Thus, the particles were all significantly larger than the trimeric particles seen on glow-discharged grids and the long axis most probably reflects the longest dimension of an oligomeric arrangement of FCPb in side view position.

Single particle analysis of FCPb

Single particle analysis was performed on about 54,000 FCPb particle images obtained on glow-discharged grids with a magnification factor of 133,000. Although we initially isolated FCPb higher oligomers via size exclusion chromatography, these seem to be unstable under the preparation conditions as demonstrated above, disassembling in

trimeric units. Two characteristic classes of FCPb trimer top view images were summed (Fig. 4, upper panel). Both showed particles of 6–7 nm in size and P3 symmetry. The size and the symmetry strongly argue for trimeric top views again. The occurrence of two particles cannot be explained by different proteins, since preparations were clean and only one protein band was visible on SDS–PAGE (data not shown). Negative staining is partly sensitive to the surface of a protein, since staining is better on top and less between carbon and protein. Assuming that FCPb complexes can arrange themselves with either the stromal or the luminal surface to the grid, two views become explainable. When comparing the structure with the calculated 2D projection of a trimer of LHCII truncated at 10 Å as shown in Caffarri

Fig. 4 Upper panel FCPb trimer of *C. meneghiniana*. Single particle analysis, sum of 4344 (left) and 2661 (right) different particles. Bar corresponds to 10 nm. Lower panel Fitting of a FCPb model (Premvardhan et al. (2010), adopted from the LHCII X-ray structure (Liu et al. 2004)) into the top views

et al. (2009), especially the left particle shown in Fig. 4 has some similarities: three strong densities at the rim for each monomer and a stain-filled cavity in the middle. The right particle is more structured, in addition displaying an inner ring of densities, which is visible in the calculated structure of LHCII as well. The size of both views also allows for overlaying a recent model of FCPs including pigments (Premvardhan et al. 2010) that was built using the LHCII protein scaffold (Liu et al. 2004) but exchanging the pigments. Whereas for the particle sum shown on the left of Fig. 4 the fit is tight but still reasonable (Fig. 4, lower panel), the model exceeds the smaller particle on the right side in size. Thus, the structure of FCPb seems to be more compact than that of LHCII, in agreement with the smaller

molecular weight due to smaller connecting loops between the three membrane spanning α -helices, and in agreement with the results of the size exclusion chromatography (Fig. 2). In summary, the particles are smaller than LHCII and most probably represent trimeric FCPb. From this analysis the arrangement of helices cannot be visualised, but the density distribution points to a similar scaffold as for LHCII as predicted from sequence similarities.

When using glow-discharged grids, very few FCPb complexes amongst the vastly trimeric particles showed a higher oligomeric state, representing top views. Some of those could be aligned during the analysis process (Fig. 5), but did not allow for higher resolution analysis. However, the sum of a few particles still allowed for an idea of the potential oligomeric state of FCPb. Thus, the outlines of the trimeric particles were now used to explain the oligomeric structure by fitting them into the particles, yielding a nonameric structure (Fig. 5).

Discussion

Stability of the FCP complexes

Using methods that do not rely on electric forces for separation like size exclusion chromatography, FCP complexes are either trimeric (FCPa) or of higher oligomeric state (FCPb). Only treatment with enhanced detergent concentrations is able to destroy the oligomeric structure under those conditions, leading to monomers in case of FCPa and trimers and monomers in case of FCPb. Nonetheless the preparation of grids by glow discharging (i.e. depositing charge) had tremendous influence on the size of the particles found, as had gel electrophoresis. On BN-gels, even FCPb showed more mobility than LHCII, arguing for a disassembly to trimers, whereas FCPa ran even faster and thus the band on BN-PAGE most probably resembles a monomer. It has to be emphasized that the banding pattern of BN-PAGE resembles that published before (Grouneva et al. 2011; Nagao et al. 2013), but in those cases no LHCII was run

Fig. 5 Single particle analysis of the higher oligomer of FCPb (left, sum of 13 particles) and possible arrangement of FCPb trimers (as outlined on the right) within the nonameric structure (middle). The size bar corresponds to 10 nm

as reference. The higher instability of the FCP complexes compared to LHCII under conditions employing electric forces can easily be explained by the general build of FCPs: whereas LHCII binds Chl b besides Chl a, the former is replaced by Chl c in FCPs. Due to the lack of a phytol ester, Chl c has a carbonyl group instead and is a very polar molecule, much more polar than even fucoxanthin. Nonetheless Chl c has to be organised in the membrane part of the complex, since the loops are much too small (Premvardhan et al. 2010). Accommodating a polar substance in the membrane part of the complex might thus easily explain the susceptibility of FCPs against electric charges/forces.

Overall structure of the FCPb complex

For FCPs no molecular structures are available so far. However, sequence analysis allowed for modelling the structure based on LHCII (Premvardhan et al. 2010). Two out of three membrane helices are well conserved and the third was assumed to take on a similar position as in LHCII. The same holds for some of the Chl molecules, especially the Chl a cluster around helices 1 and 3. When comparing the result of our single particle analysis with the molecular model by Premvardhan et al. (2010) it becomes immediately obvious that the trimeric scaffold is the same for FCPb and LHCII, and thus most probably for FCPa as well. However, the structure of FCPb is slightly smaller or tighter, in accordance with the smaller loops in FCPb. However, the overall pigment load is almost the same for FCPs and LHCII, but FCPs carry more carotenoids than Chls, which take up less space in comparison to the relatively bulky Chl molecules.

To our knowledge, only two other publications dealt with the structure of FCPs and related complexes using electron microscopy. An FCP complex of the brown alga *Petalonia* (Katoh and Ehara 1990) appeared as disc with an average diameter of 11.2 nm and an average height of 10.2 nm, but no single particle analysis was done at that time. The structure nonetheless seemed to be based on a higher aggregate, since 128 molecules Chl a molecules were reported, which would account for 16 FCP monomers or about five trimers. The height argues for a stack of two membrane proteins each, since membranes usually have a height of around 5 nm (Nagle and Tristram-Nagle 2000). This publication as well as our structure is in sharp contrast to Gardian et al. (2014), who reported ring-like structures for FCPs of *C. meneghiniana*, in contrast to FCPs from the pennate diatom *Phaeodactylum tricornutum*. The latter complexes were roughly comparable to our trimers and from biochemical studies trimers were reported as well, whereas no higher oligomers of specific polypeptide composition were found (Grouneva et al. 2011; Gundermann et al. 2013). However, the FCPs

from *C. meneghiniana* used by Gardian et al. (2014) were prepared using only sucrose gradients, whereby in contrast to Büchel (2003) only one band was obtained. Thus, the fraction contained FCPb as well as FCPa and probably more lipids than our complexes purified using IEX additionally. The structure might thus resemble a supramolecular arrangement of the different FCP complexes, but does not show any features that allow for recognition of single trimers. The high similarity between FCPa, FCPb and LHCII demonstrated here clearly argues for a trimeric complex as the basic unit for both FCP complexes. This is further supported by the P3 symmetry as well as the arrangement of areas of higher density that fit well to the presumed α -helical regions of FCPb (Fig. 4).

When using glow-discharged grids a minor fraction of FCPs in higher oligomeric states became visible as top views. From size estimation three trimers of FCPb would fit into these structures, pointing to a nonameric arrangement. However, the side views visible on untreated grids displayed three different lengths, whereby only the smallest, 14 nm, is in accordance with the nonameric top-view. An explanation is outlined in Fig. 6. Here the formation of doublets via one of the edges of the nonamers is assumed, yielding a rhombus. Artificial adhesion of membrane complexes under the conditions of negative staining is a well-known phenomenon, e.g. for photosystem II often doublets were found (Nield et al. 2000). The additional lengths of about 20 and 25 nm seen in the side views can then easily be explained by the view along the longest axis of the rhombus (roughly 25 nm) and by the view rotated by 30° (about 20 nm). However,

Fig. 6 Model of FCPb nonamers (triangles) composed of trimers (circles) of two different subunits (blue and white) that can be arranged in doublets. The possible viewing angles (arrows) in side-view projections are shown as well, including the lengths of the resulting views (blue lines)

a stable nonamer, as well as a doublet of nonamers, cannot be built of identical subunits, since the attracting forces for interactions would then be the same along all axes. In *C. meneghiniana* only one subunit, Fcp5, was described so far, but the genome of *C. meneghiniana* was not sequenced up to now. In the sequenced sister species *Thalassiosira pseudonana* three genes for the identical polypeptides (Lhcf3, 8, 9) are present (Gundermann et al. 2013), but when using BN-PAGE and mass spectrometry Lhcf4, 5, 7 and Lhcf1/2 were found as well (Grouneva et al. 2011). If FCPb of *C. meneghiniana* is also composed of at least two subunits, the stronger forces within a nonamer as opposed to the forces between nonamers can be explained as outlined in Fig. 6. One subunit would then be responsible for nonamer formation, whereas the others would only allow for doublets/multimolecular arrangements by less strong/specific binding.

One of the unsolved questions is the arrangement of FCPa and FCPb around the photosystems. FCPb was shown by several methods to transfer excitation energy predominantly into photosystem II (Chukhutsina et al. 2013; Szabó et al. 2010), but no idea exists about supercomplexes of photosystem II so far, especially since no ‘minor’ antenna proteins can be found in the genomes or were identified biochemically. Whereas photosystem I of diatoms seems to be organised in a similar way to that of green algae (Drop et al. 2011; Kargul et al. 2003; Veith and Büchel 2007), especially the physical connection between FCPb and photosystem II remains to be elucidated.

In conclusion, trimers are the basic unit for FCPa as well as for FCPb. These trimers share the general structure with LHCII, but are of tighter built. Whereas FCPa does not seem to form larger oligomers, we conclude from the data presented above that the main oligomeric state of FCPb is nonameric. Both the trimers of FCPa and the oligomeric FCPb are sensitive against electric fields, and disassemble to the next smaller oligomeric state, i.e. monomers and trimers, respectively.

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