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Functional heterogeneity of the fucoxanthins and fucoxanthin-chlorophyll proteins in diatom cells revealed by their electrochromic response and fluorescence and linear dichroism spectra

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Abstract

In this work, by analyzing the electrochromic transient spectra, the 77 K fluorescence emission and excitation, as well as the linear dichroism (LD) and circular dichroism (CD) spectra of low-light (LL) and high-light (HL) grown *Phaeodactylum tricornutum* cells, we show that the fucoxanthins (Fx) and fucoxanthin-chlorophyll proteins (FCP) exhibit marked functional heterogeneity. Electrochromic transients reveal that LL and HL cells differ substantially in their relative contents of two Fx forms, which absorb at 501 and 550 nm; they exhibit distinct LD signals but are CD silent. Fluorescence emission and excitation spectra at 77K reveal that although both forms efficiently transfer excitation energy to Chl *a*, the red form feeds somewhat more energy to photosystem II than to photosystem I. Similar data obtained in *Cyclotella meneghiniana* cells suggest that the heterogeneity of the FCP pool, with different Fx, forms plays a role in the regulation of energy utilization in FCP-containing organisms.

Keywords: diatoms, fucoxanthin, electrochromic absorbance changes, fucoxanthin chlorophyll *a/c* proteins, Stark spectroscopy

Introduction

Diatoms are an important photosynthesizing algal group of the phytoplankton community, contributing to almost one quarter of the global primary production [1]. To maximize photosynthetic efficiency, diatoms adapt their pigment composition to their aquatic environment according to the energy of the photons from the incident sunlight that are able to penetrate different water depths. As a result of such chromatic adaptation, the diatom pigment composition differs considerably from that of higher plants. They possess chlorophyll *a* (Chl *a*), but Chl *b* is replaced by Chl c_1 and Chl c_2, whose ratio differs among diatom species [2]. Instead of lutein, diatoms incorporate fucoxanthin (Fx) as the carotenoid. The presence of a carbonyl group in conjugation with the isoprenoid chain is responsible for the unique spectroscopic features of Fx [3], which also allows it to function as the main harvester of light. Instead of viola-, anthera- and zeaxanthin, the main photoprotective pigments in diatoms are diadinoxanthin (Ddx) and diatoxanthin (Dtx)[4]. Moreover, the stoichiometry of chlorophylls and carotenoids in the light harvesting antennae of diatoms differs from that of higher plants, with the Chl *a*:Fx ratio being 1:1[5].

These carotenoids, and most of the Chls, are found in the main light-harvesting complexes, the fucoxanthin-chlorophyll *a/c* proteins (FCPs), which serve as antennae for both photosystems (PSs) [6]. FCP belongs to the LHC-superfamily and possesses many structural and functional similarities to LHCII, the main Chl *a/b* complex of PSII of higher plants [7,8] (LHC, light harvesting complex). FCPs span the membrane with three transmembrane helices, and are thought to possess a three-dimensional structure similar to LHCII in higher plants [5,9,10]. It has been shown that FCP complexes are arranged into trimers and higher oligomers, and as in higher plants, the oligomeric forms seem to be the prevailing structure *in vivo* [11,12]. This is corroborated by the observation that the pigment-protein complexes in diatoms, similar to higher plants, are arranged into structurally flexible, chirally ordered macrodomains [13].

Steady state and ultrafast spectroscopic methods, during the past decade, have revealed important features on the molecular architecture of isolated FCP and established the role of Fx molecules in excitation energy migration [3,5]. Recently, Stark

spectroscopy revealed the existence of different Fx forms, with noticeably different static dipole moments; this heterogeneity of Fx forms was confirmed by resonance Raman spectroscopy [14,26].

In contrast to detailed studies on isolated FCP complexes, much less is known about the heterogeneity and function of Fx and FCP in intact diatom cells. Recently we used „in vivo Stark spectroscopy", flash-induced electrochromic absorbance transients, to identify the field-indicating pigment molecules [13] that exhibit large static dipoles [14-16]. We found that *Phaeodactylum tricornutum* cells exhibit strong electrochromic signals between 470 and 570 nm, and observed an intense band peaking at an unusually long wavelength position, at 564 nm, and a somewhat weaker band at around 510 nm, which were assigned to originate from two different Fx transitions [13].

In this work we investigated the origin of these two field-sensitive Fx forms. Our data reveal large variations in their relative amplitudes upon varying the light intensity during growth, indicating a heterogeneity in the FCP pool; fluorescence excitation and emission spectra show that the FCP containing the long wavelength form of Fx or enriched in it, transfers somewhat more excitation energy to PSII than to PSI.

Materials and Methods

Phaeodactylum tricornutum (1090-1a) and *Cyclotella meneghiniana* (1020-1a) cells, obtained from the Culture Collection of Algae, Göttingen (SAG, FRG), were grown in ASP-medium according to [18] using the modifications described in [19] - for *C. meneghiniana* the medium was supplemented with 1 mM silica. *P. tricornutum* cells were cultivated as batch cultures at a photon flux density of 15 μmol m^{-2} s^{-1} (low light, LL) and of 180 μmol m^{-2} s^{-1} (high light, HL) photosynthetically active radiation (PAR) in a light/dark regime of 16 h/8 h; *C. meneghiniana* cells were grown at 40 μmol m^{-2} s^{-1}. The temperature of the growth chamber was set to 19 °C.

Absorbance spectra were recorded with a Shimadzu UV-3000 spectrophotometer, in double-beam mode, in the wavelength range of 400 – 750 nm. The optical pathlength was 1 cm and the bandwidth was set to 2 nm. The chlorophyll concentrations of the HL and LL cultures were set to obtain the same OD values at 673 nm (the maximum of the Q_y band of Chl *a*). In order to minimize the spectral artefacts caused by light scattering of turbid cell suspensions, the spectra were measured in a sample holder designed to correct for scattering: the cuvette is placed as close as possible to the photomultiplier tube, in front of which a quartz diffuser was placed.

Pigments were isolated by filtering 10 ml of algal cell suspension. After removal of ASP-2 medium by using a vacuum pump, 1 ml of HPLC medium (90% methanol/0.2 M ammonium acetate (90/10, v/v) and 10% ethylacetate) and a small quantity of glass beads were added. The cells were disrupted in a cell homogenizer (Braun, FRG), centrifuged, and the pigments separated and quantified by HPLC as described in [11].

Electrochromic absorbance changes induced by single turnover saturating flashes were measured in 5 nm steps between 470 and 570 nm in a setup described previously [20]. The time constant was set to 100 μs, and 32 kinetic traces, collected with a repetition rate of 1 s^{-1}, were averaged. The measurements were performed in the presence of 7% Ficoll to avoid rapid sedimentation of cells during the measurement. In order to reveal the absorbance bands of the electrochromically shifted pigments, the transient spectra were fitted with the first derivatives of gaussians [20]. Prior to fitting the spectra were shifted by a constant value, to zero at 470 nm [13]. The origin of the baseline

anomaly is unknown, but electrochromic absorbance changes have been shown to be accompanied by scattering transients, which are physically correlated with the absorption changes [21].

Linear dichroism (LD) spectra were measured at room temperature in a Jobin-Yvon CD6 dichrograph, equipped with LD boards, in the wavelength range of 400 to 730 nm with a bandwidth of 2 nm. Cells or thylakoid membranes, isolated from *P. tricornutum* or *C. meneghiniana* were oriented with the gel-squeezing method [22]. The samples were embedded in 5% polyacrylamide gel and the gel cube was squeezed to half the original width (M=2, where M is the squeezing factor). The Chl concentrations of the samples, before embedding in the gel, were set to obtain the same OD values, about 1, at 673 nm (the maximum of the Q_y band of Chl *a*). In the gel, in the direction of the measuring beam, the sample exhibited $OD_{673-750}$ = 0.35. Experiments were also carried out on magnetically aligned *P. tricornutum* cells, which yielded similar results to those with gel squeezing; in these experiments, we used a permanent magnet of 0.5 T field strength and an optical cell with a pathlength of 1 cm and a width of 0.5 cm. The LD spectra are plotted in delta-absorbance units.

Circular dichroism spectra were measured at room temperature in a Jobin-Yvon CD6 dichrograph in the wavelength range of 400 to 750 nm, with a bandwidth of 2 nm. The Chl concentrations of the HL and LL cultures were set to obtain the same OD values at 673 nm (the maximum of the Q_y band of Chl *a*). The optical pathlength was 1 cm. The CD-spectra are plotted in delta-absorbance units.

The 77K fluorescence spectra were recorded with a Horiba Jobin-Yvon Fluorolog 3 spectrofluorimeter. The Chl content was set to about 0.5 μg/ml ($OD_{674-750}$ = 0.04) to avoid re-absorption. (Samples containing 60% (v/v) glycerol yielded similar fluorescence spectra as those without this cryoprotectant (not shown).) Fluorescence emission spectra were measured between 600 and 800 nm, using 510 and 550 nm excitation wavelengths with excitation and emission bandwidths of 5 and 2 nm, respectively. The fluorescence excitation spectra were recorded between 400 and 600 nm with excitation and emission bandwidths of 2 and 5 nm, respectively; the emissions were collected at 689 or 713 nm.

Results and Discussion

Upon the excitation of photosynthetic membranes with a single turnover saturating flash a uniform transmembrane electrical field of 10^5 V/cm magnitude is built up [23]. This field is known to induce electrochromic absorbance changes, which can be used to monitor the flash-induced kinetics of membrane potential *in vivo*. The large electrochromic absorbance changes of carotenoids in chloroplast thylakoid membranes as well as in photosynthetic bacteria originate from an almost homogeneous shift of the absorption bands of the so-called field-indicating pigment molecules; the magnitude of the shift, a few Ångstroms, when measured in energy units is linearly proportional to the field strength [15,16]. Hence, the resulting light-induced electrochromic absorbance transient spectra are composed of the first derivatives of the absorbance bands of the participating electronic transitions. This specificity can be used to identify the field-indicating pigment molecules and determine their concentrations. Further, since the electrochromic shift depends on the difference between the ground- and excited state dipole moments and polarizabilities, these transients provide unique information on the microenvironment of the given molecule. This information is essentially the same that can be obtained from Stark spectroscopy on randomly oriented protein complexes [17,24,25].

In the case of the external-field induced change in the absorption (Stark signal) of FCP isolated from *Cyclotella meneghiniana*, three main Fx forms, Fx_{blue}, Fx_{green} and Fx_{red}, were identified, with the lower-energy Fx_{red} and Fx_{green} forms exhibiting large changes in (static) dipole moment on photon absorption, of about 40 and 15 D, respectively [14]. These Fx's were also shown to be structurally distinct, a conclusion from resonance Raman spectroscopy [26].

In whole *P. tricornutum* cells, as shown earlier [13], two main electrochromic absorbance band-shifts can be identified, with positive/negative peaks at around 565/535 nm and 515/485 nm (Fig. 1a) that overlap the 0-0 and 0-0/0-1 bands of Fx_{red} and Fx_{green}, respectively [14]. The occurrence of the electrochromic absorbance transients in this diatom is consistent with the Stark signal of isolated FCP in the external field. The long wavelength form, as determined by fitting the transient curve, is identified to originate

from a 550 nm absorbance band with a full width at half maximum (*fwhm*) of about 22-23 nm (Fig. 1b). This Fx form has a considerably larger amplitude than the Fx that gives rise to the shorter wavelength transition at around 501 nm, also with an 22-23 nm *fwhm* (Fig. 1b). The energetic locations of the Fx species that give rise to the two electrochromic transitions in *P. tricornutum* cell are close to the 0-0 bands of Fx_{red} and Fx_{green}, respectively, previously identified in the FCP complexes isolated from *C. meneghiniana*. In this context, it is important to note that we have found essentially the same electrochromic transients in *C. meneghiniana* cells, where Fx_{red} and Fx_{green} were found at 553 and 502 nm, respectively (Supplementary Figure 1).

Fig. 1.

At the given signal to noise ratio, no other electrochromic band could be identified, suggesting that the static dipole moments (more precisely the difference between the ground- and excited-state dipole moments) of all other pigment molecules absorbing between 470 and 570 nm are significantly weaker. The difference spectra, calculated between the measured and fitted transient spectra, i.e. between the data points and the sum of the two first derivative gaussians, were quite featureless, except below 5 ms, where the transient of oxidized cytochrome f could be clearly identified. In other terms, in the measured transient spectra or in the calculated difference spectra, no spectral variations could be identified either from scattering [cf. 21] or absorbance changes; in particular, no flash-induced absorbance change could be assigned to originate from Ddx or Dtx although the amount of these xanthophylls was four times higher in HL cells than in LL cells, calculated on the basis of the same Chl *a* content; the Fx content on the same basis was also found to remain unchanged, as verified by HPLC (data not shown).

For the electrochromic transients in whole cells at room temperature, the relatively poor signal to noise ratio, partly due to sedimentation of the cells during the measurements, do not allow the determination of the band structure of different Fx forms, in contrast to Stark spectroscopy at 77K on isolated FCP. Nevertheless, the fact that the electrochromic transients of intact cells are in general agreement with the Stark spectroscopy data on isolated FCP proves that the light-induced transmembrane field can

also be used to investigate the electro-optical properties of the field-indicating pigment molecules. Thus, our measurements of electrochromic absorbance changes on intact cells provide an important tool for discerning the local environments of pigments, and verifying that they undergo charge-transfer reactions, in the pigment- protein complexes *in vivo*.

As shown in Fig. 1, the electrochromic transients in LL and HL cells differed significantly from each other: LL cells contained significantly larger contributions from Fx_{red} than HL cells. As determined from the transient spectra, in HL cells the amplitude of the Fx_{red} band was 2.2 ± 0.2 times larger than that of Fx_{green}. In LL cells, this ratio was 5.8 ± 0.7. Assuming no change in the electrostatic properties of the two forms, this difference must originate from an accumulation of Fx_{red} in LL cells. This means that Fx_{red} and Fx_{green} originate from different FCPs. In other words, the fact that the ratio of the two electrochromic bands varies in a broad range in HL and LL cells indicate that the two Fx forms are bound to different FCPs.

These two Fx forms were also clearly discernible in the LD spectra shown in Fig. 2, in particular Fx_{red} exhibited an intense signal.

Fig. 2.

Using the squeezing parameter M=2 and the calculated reduced LD [27], the orientation angle of the transition dipole of Fx_{red} at 550 nm in membranes isolated from LL cells was found to be 29° with respect to the membrane plane. In comparison, the orientation angle of the Q_y transition of Chl *a* was calculated to be 17° in the same membranes (for the red spectral region, see Supplementary Figure 2). In contrast to the large signal at 550 nm, the LD signal at 500 nm was weaker , showing that the transition dipoles around 500 nm, with an estimated 34-36° orientation angle, i.e. tend to tilts out somewhat more from the membrane plane than those at 550 nm. Unlike the Stark signals, where the overlapping 0-0 and 0-1 bands of the different Fx's cannot be differentiated with absolute certainty at 500 nm, the LD spectrum most clearly signals the presence of the so-called Fx_{green} being quite different from Fx_{red}, with the former exhibiting a much weaker signal. Similar LD spectra were obtained in *C meneghiniana* thylakoid

membranes (Supplementary Figure 2), showing that the orientation of Fx_{red} and Fx_{green} are essentially the same as in *P. tricornutum*. (Whole *C. meneghiniana* cells could not be aligned by gel squeezing.) These data agree well with those published earlier by Hiller and Breton [28].

The orientations of the Fx transition dipole moments relative to the membrane plane can also be used to understand the nature of the electrochromic signals in the transmembrane field: larger signals are expected not only on the basis of the intrinsic dipolar properties of the pigments but also when the pigments are aligned to have their static dipole moments, $\boldsymbol{\mu}$, (close to) parallel to the transmembrane field, **F**. The large electrochromic transient, proportional to $\mu F\cos\theta$ (θ is the angle between $\boldsymbol{\mu}$ and **F**), from Fx_{red} therefore indicates that its static dipole moment must be close to parallel to the trans-membrane field vector (**F**). Notably, the angle between the transition dipole and the change in static-dipole moments was measured to be ~ 20^{o} in *solution* [14], which in conjunction with the LD data, an orientation angle of 29° of the transition dipole with respect to the membrane plane, would mean that the Fx_{red} could be oriented at ~ 49° with respect to the membrane plane; hence, ~ 41° between $\boldsymbol{\mu}$ and **F**, which can yield more than 70% of the maximum possible electrochromic response to the trans-membrane field. For Fx_{green} this value can be as high as 83 %. It must also be noted that the angle between the transition dipole and static dipole could be even larger in the protein for Fx_{red} if it has a planar 'S' shape conformation, as proposed in [26].

In order to obtain information about the functional significance of the heterogeneity of the Fx forms and the FCPs, low-temperature fluorescence excitation and emission spectra were recorded.

Fig. 3.

The excitation spectra revealed that energy transfer to Chl *a* occurs from both the short- and long-wavelength Fx forms (Fig. 3a and b). Carotenoids absorbing at shorter wavelengths, corresponding to Fx_{blue} [14,26], displayed less efficient excitation energy transfer, with higher absorbance and lower fluorescence excitation bands compared to Fx_{green} and Fx_{red}. This finding is in agreement with earlier data [5]. Our data also show

that excitations in the Fx_{red}-absorbing region 520 – 560 nm range enhance the short wavelength emission band, around 689 nm, compared to the excitation of Fx_{green}, at around 500 nm; this was true both in both LL (Fig. 3a) and HL (Fig. 3b) cells but was more pronounced in LL cells, which were enriched in Fx_{red}. Fluorescence emission spectra (Fig. 3c and d) also indicate that upon excitation at 550 nm, both LL and HL cells exhibit slightly stronger fluorescence emission at 689 nm, indicating that Fx_{red} favours energy transfer to F_{689} (PSII). In contrast, excitation at 510 nm of both LL and HL cells exhibit relatively less intense fluorescence at 689 nm, indicating that Fx_{green} favours energy transfer to F_{713} (PSI) or has no preference for ET to either one of the PSs. Again, very similar data were obtained in *C. meneghiniana* cells, albeit in this diatom the intensity of F_{713} was much lower than in *P. tricornutum* (Supplementary Figure 3).

The above data on the heterogeneity of the Fx molecules and of the FCP pool are in reasonable agreement with biochemical analyses. The presence of different FCP pools was indicated by Western blot analysis, showing that the FCPs of the antenna are different from the FCPs connected to the photosystems [29,30]. In addition, the FCP composition of the antenna varied if the algae were grown under LL or HL conditions [31] . Heterogeneity of FCPs can also be seen in the ultrafast fluorescence decay kinetics [32], which showed that two different pools of FCPs are responsible for the generation of steady-state non-photochemical fluorescence quenching (NPQ) in the diatoms *P. tricornutum* and *C. meneghiniana*. One pool of FCPs, detached from the photosystems, forms a fluorescence-quenching site, whereas the second quenching site consists of FCPs that remain in contact with the PSII core complex [32]. (NPQ at this site furthermore depends on the presence of the de-epoxidized xanthophyll cycle pigment Dtx.) The heterogeneous lipid distribution in the thylakoid membranes of diatoms also supports the existence of different FCP pools and macrodomains of the pigment-protein complexes [33], although no variability of stacked to exposed membranes is observed by electron microscopy [34].

To gain information about the pigment-pigment interactions on cells grown in either HL or LL, CD spectra were also obtained and shown in Fig. 4.

Fig. 4.

In both HL and LL cells, no CD signatures are associated with the field-sensitive Fx forms, which are essentially CD silent as in isolated FCP [11,12]. Nevertheless, it is worth mentioning that LL cells exhibit a more intense psi-type band at (+)698 nm than HL cells, which is most probably due to the accumulation of the light harvesting complexes in LL membranes. Similar data were obtained with *C. meneghiniana* cells as well as in another Chl *a/c*-containing organism, *Pleurochloris meiringensis* [35].

In conclusion, our data provide direct experimental evidence for the heterogeneity of the Fx forms in intact diatom cells, similar to the heterogeneity earlier shown to occur in isolated FCP preparations. We propose that this heterogeneity originates from the heterogeneity of the FCP pool: different FCPs appear to contain Fx_{green} and Fx_{red} in different proportions and they are associated to different extents with the two PSs, with the FCP enriched in Fx_{red} being bound in slightly greater amount to PS II than to PSI. It is also worth noting that the electrochromic transients given rise by the flash-induced transmembrane electric field, in combination with *in vitro* Stark spectroscopy, provide unique information on the electro-optical properties of FCP embedded in the native thylakoid membranes.

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References

[1] P.G. Falkowski, R.T. Barber, V. Smetacek, Science 281 (1998) 200.

[2] S.W. Jeffrey, Biochim. Biophys. Acta 177 (1969) 456.

[3] D. Zigmantas, R.G. Hiller, F. P. Sharples, H.A. Frank, V. Sundström, T. Polívka, Phys. Chem. Chem. Phys. 6 (2004) 3009.

[4] J. Lavaud, Funct. Plant. Sci. Biotech. 1 (2007) 267.

[5] E. Papagiannakis, I.H.M. van Stokkum, H. Fey, C. Büchel, R. van Grondelle, Photosynth. Res. 86 (2005) 241.

[6] T.G. Owens, Plant Physiol. 80 (1986) 739.

[7] D.G. Durnford, R. Aebersold, B.R. Green, Mol. Gen. Genet. 253 (1996) 377.

[8] A. Grossman, Mol. Gen. Genet. 224 (1990) 91.

[9] C. Wilhelm, C. Büchel, J. Fisahn, R. Goss, T. Jakob, P. Kroth, J. LaRoche, J. Lavaud, M. Lohr, U. Riebesell, K. Stehfest, K. Valentin, Protist 157 (2006) 91.

[10] M. Westermann, E. Rhiel, Protoplasma 225 (2005) 217.

[11] B. Lepetit, D. Volke, M. Szabó, R. Hoffmann, G. Garab, C. Wilhelm, R. Goss, Biochemistry 46 (2007) 9813.

[12] C. Büchel, Biochemistry 42 (2003) 13027.

[13] M. Szabó, B. Lepetit, R. Goss, C. Wilhelm, L. Mustárdy, G. Garab, Photosynth. Res. 95 (2008) 237.

[14] L. Premvardhan, D. Sandberg, H. Fey, R. Birge, C. Büchel, R. van Grondelle, J. Phys. Chem. B 112 (2008) 11838.

[15] W. Junge, Annu. Rev. Plant Phys. 28 (1977) 503.

[16] W. Cramer, A.R. Crofts, in: Govindjee (Ed.), Photosynthesis: Energy Conversion by Plants and Bacteria, Academic Press, Inc. New York, 1982, p. 387.

[17] S.G. Boxer, J. Phys. Chem. B 113 (2009) 2972.

[18] L. Provasoli, J.J.A. McLaughin, M.R. Droop, Arch. Microbiol. 25 (1957) 392.

[19] M. Lohr, C. Wilhelm, Planta 212 (2001) 382.

[20] C. Büchel, G. Garab, Photosynth. Res. 43 (1995) 49.

[21] G. Garab, G. Paillotin, P. Joliot, Biochim. Biophys. Acta 545 (1979) 445.

[22] G. Garab, H. van Amerongen, Photosynth. Res. 101 (2009) 135.

[23] T. Kakitani, B. Honig, A.R. Crofts, Biophys. J. 39 (1982) 57.

[24] M.A. Steffen, K. Lao, S.G. Boxer, Science 264 (1994) 810.

[25] B. Chance, L. Smith, Nature 175 (1955) 803.

[26] L. Premvardhan, L. Bordes, A. Beer, C. Büchel, B. Robert, J. Phys. Chem. B 113 (2009) 12565.

[27] G. Garab, in: J. Amesz, A.J. Hoff (Eds.), Linear and Circular Dichroism, Biophysical Techniques in Photosynthesis, Advances in Photosynthesis, Vol. 3, Kluwer Academic Publishers, Dordrecht/Boston/London, 1996, p. 11.

[28] R. G. Hiller, J. Breton, Biochim. Biophys. Acta 1102 (1992) 365.

[29] T. Veith, J. Brauns, W. Weisheit, M. Mittag, C. Büchel, Biochim. Biophys. Acta 1787 (2009) 905.

[30] T. Brakemann, W. Schlormann, J. Marquardt, M. Nolte, E. Rhiel, Protist 157 (2006) 463.

[31] A. Beer, K. Gundermann, J. Beckmann, C. Büchel, Biochemistry 45 (2006) 13046.

[32] Y. Miloslavina, I. Grouneva, P.H. Lambrev, B. Lepetit, R. Goss, C. Wilhelm, A.R. Holzwarth, Biochim. Biophys. Acta 1787 (2009) 1189.

[33] R. Goss, J. Nerlich, B. Lepetit, S. Schaller, A. Vieler, C. Wilhelm, J. Plant Physiol. 166 (2009) 1839.

[34] A.M. Pyszniak, S.P. Gibbs, Protoplasma 166 (1992) 208.

[35] C. Büchel, G. Garab, J. Photochem. Photobio. B 37 (1997) 118.

Appendix. Supplementary material

Figure legends

Fig. 1. Electrochromic absorbance transients of HL (open squares) and LL (closed squares) cells of *P. tricornutum* (a), and absorbance bands of the field-sensitive pigments obtained from the fits of the transients (b) of HL (solid line) and LL (dashed line) cells. Measured datapoints were obtained from absorbance transients induced by saturating single turnover flashes (see Materials and Methods). In the transient spectra, the data points at 5 ms after the flash were fitted with first derivatives of gaussians of HL (solid line) and LL (dashed line) cells. Samples were adjusted to the same Chl *a* Q_y absorption, $OD_{673\text{-}750} \approx 1$.

Fig. 2. Linear dichroism spectra of HL (solid line) and LL (dashed line) cells. The Chl contents of the two samples were equal. (For further details se Materials and Methods.)

Fig. 3. Low temperature (77K) fluorescence excitation (a and b) and emission (c and d) spectra of LL (a and c) and HL (b and d) *P. tricornutum* cells. The fluorescence excitation spectra, normalized at 438 nm, were recorded for the 689 nm (solid line) and the 713 nm (dashed line) bands. For the emission spectra, normalized at 713 nm, the cells were excited at 510 nm (solid line) or at 550 nm (dotted line). The room temperature absorbance spectra of LL (a) and HL (b) cells (dotted line) are also normalized to the 438 nm band of the excitation spectra. (The wavelength for normalization, 438 nm, was selected because of the maximum of Chl *a* absorbance and virtually no overlap with the two Fx forms in question, i.e. with Fx_{red} and Fx_{green}).

Fig. 4. Circular dichroism spectra of HL (solid line) and LL (dashed line) cells. The samples were adjusted to the same Q_y absorbance of Chl *a*, $OD_{673\text{-}750} \approx 0.5$, the optical pathlength was 1 cm.

Figure 1

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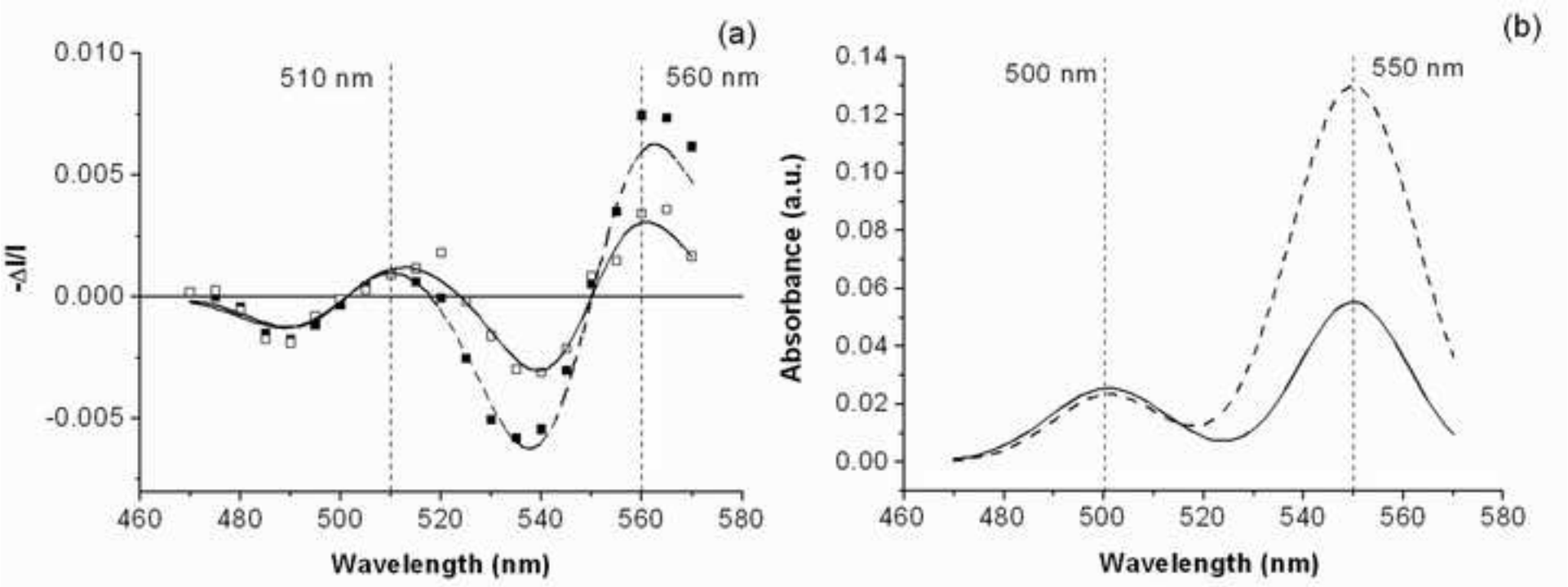

Figure 2

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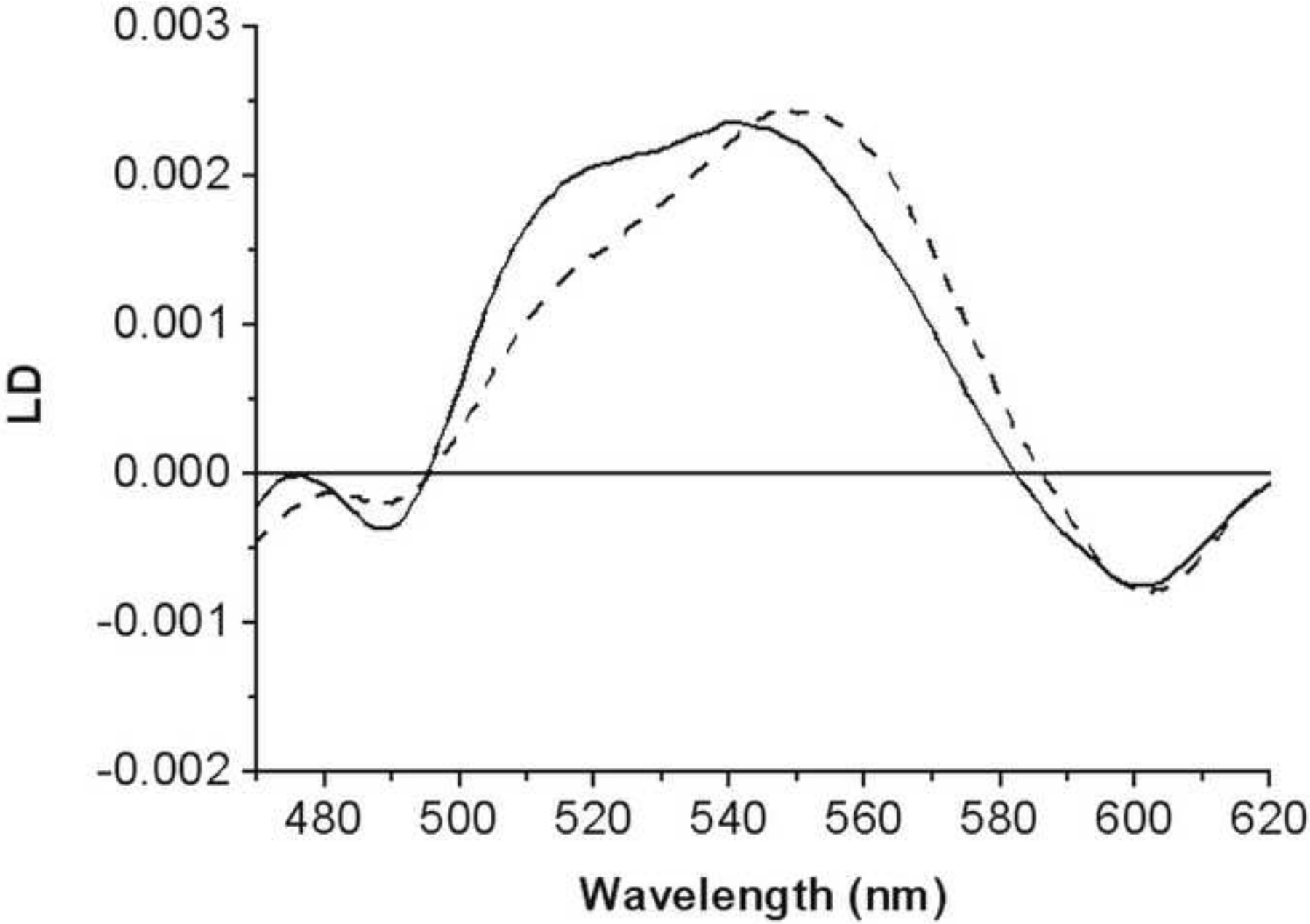

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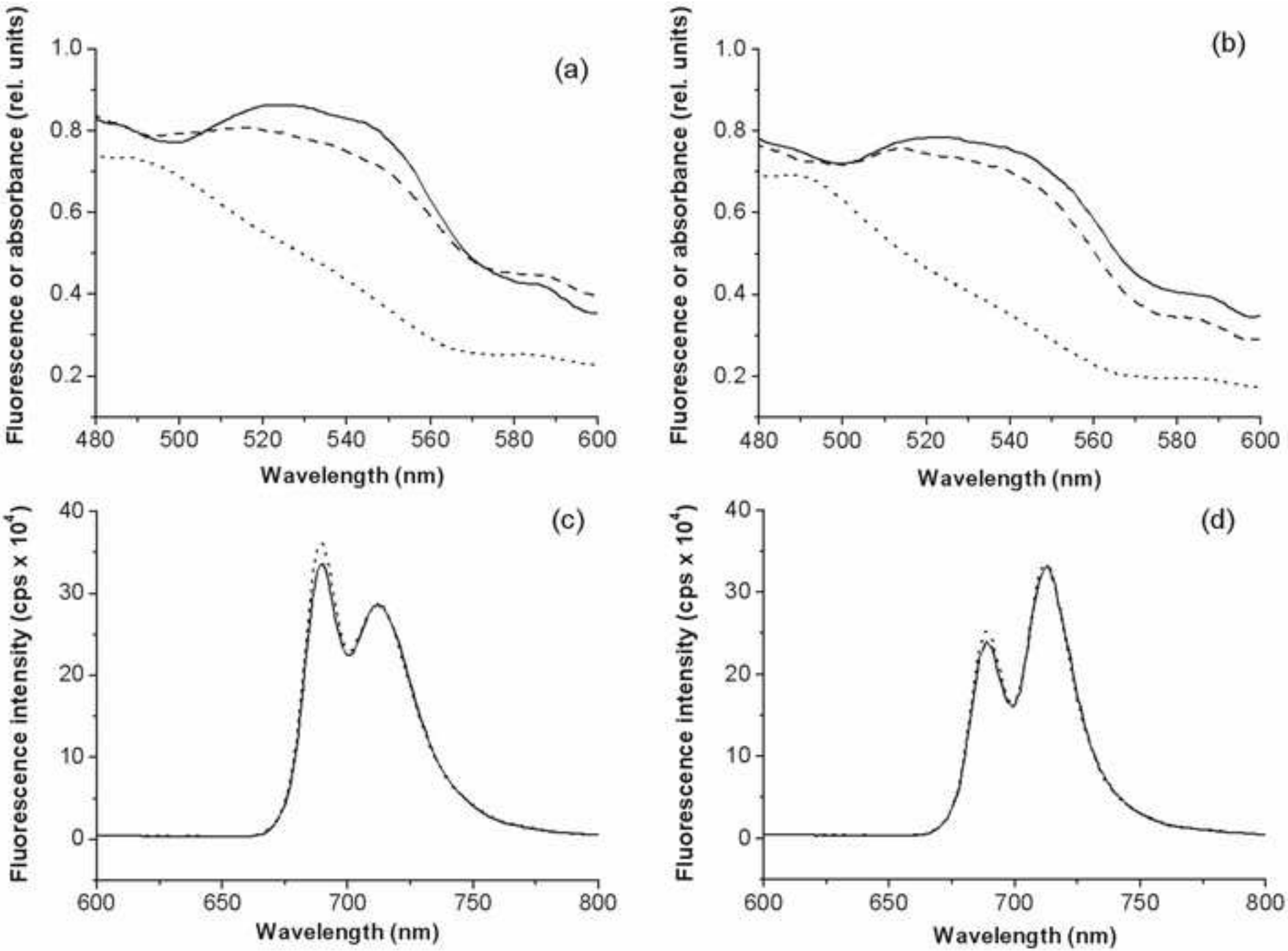

Figure 4

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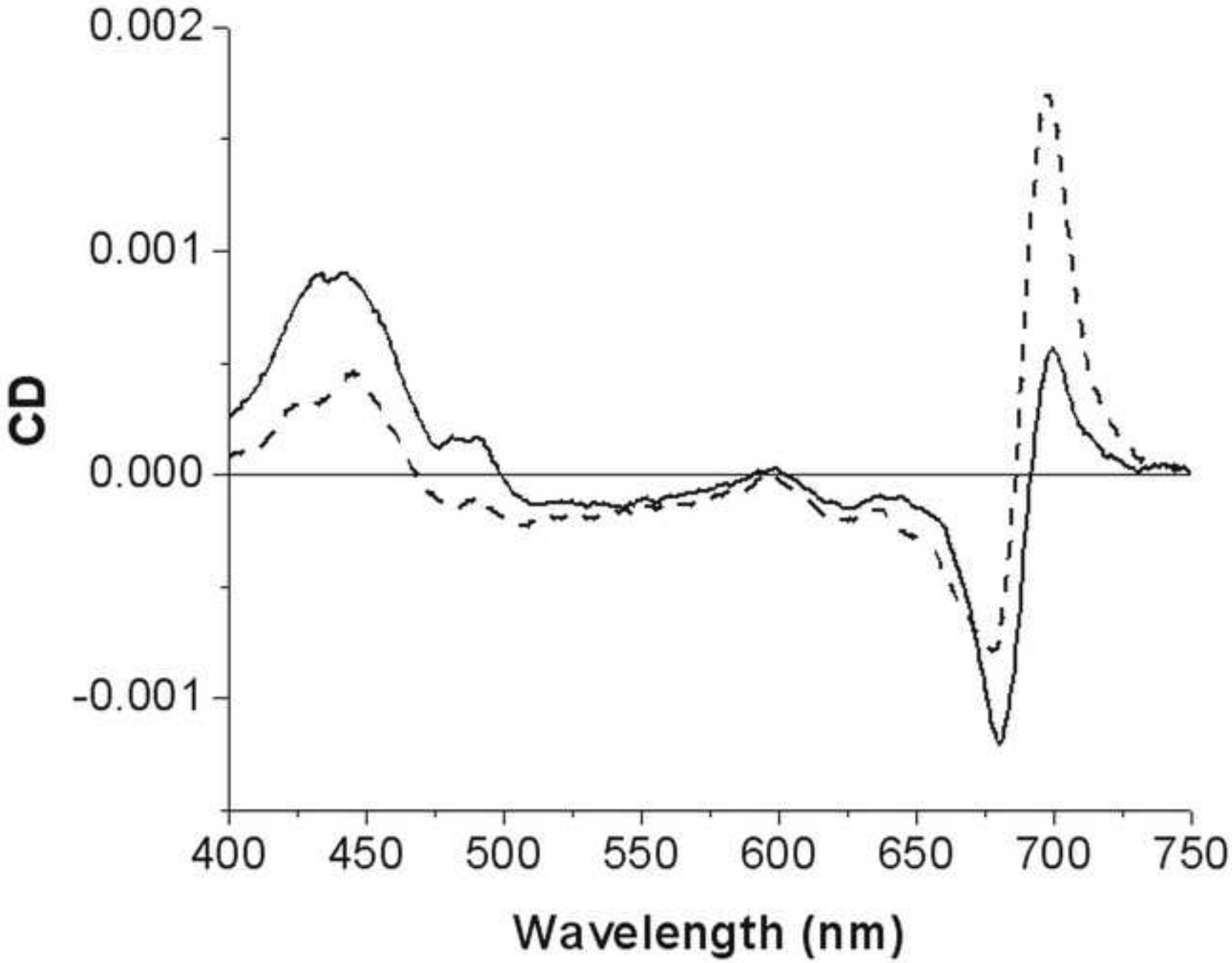

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