PHOTOSYNTHETICA 37 (2): 225-237, 1999

# Excitation-emission map as a tool in studies of photosynthetic pigment-protein complexes

Mika KERÄNEN, Eva-Mari ARO, and Esa TYYSTJÄRVI*

*University of Turku, Department of Biology, Laboratory Plant Physiology and Molecular Biology, Biocity A, FIN-20520 Turku, Finland*

## Abstract

Excitation-emission maps were constructed by measuring emission spectra from tobacco thylakoids and from thylakoids and intact cells of the cyanobacterium *Synechocystis* 6803. The measurement of such maps is greatly facilitated by the current diode-array detector technology. We show that excitation-emission maps are valuable tools for studies of the structure and energy transfer pathways in photosynthetic systems.

*Additional key words*: CP43 chlorophyll *a* binding protein; D1 protein mutant; 77 K fluorescence emission; *Synechocystis* 6803.

## Introduction

Emission of fluorescence from photosynthetic pigments can be used as a tool in both structural and functional studies (for reviews see Bose 1982, Govindjee 1995, Sauer and Debreczeny 1996). An emission spectrum reflects the pigment composition of the sample and may show energy transfer pathways within the sample (Govindjee and Yang 1966, Gantt and Lipschultz 1973, Strasser and Butler 1977). Induction kinetics of chlorophyll (Chl) *a* fluorescence can also be used as a probe of electron transfer in photosystem 2 (PS2) (for reviews see Krause and Weis 1984, 1991, Dau 1994; for a quantitative model see Trissl and Lavergne 1994). In the present contribution, we use excitation-emission maps as tools in the study of antenna and reaction centre

*Received* 5 May 1999, *accepted* 1 November 1999.
*Author for correspondence: fax +358 2 333 8075; e-mail: esatyy@utu.fi
*Abbreviations*: APC - allophycocyanin; Chl - chlorophyll; CP43 and CP47, 43 and 47 kDa Chl *a* protein complexes of photosystem 2; EEM - excitation-emission matrix; $F_{Exc}^{Em}$ - fluorescence intensity at emission wavelength *Em* [nm] and excitation wavelength *Exc* [nm]; LHC2 - light-harvesting Chl *a/b* complex of photosystem 2; PS2 and PS1, photosystems 2 and 1, respectively; PC - phycocyanin; PPFD - photosynthetic photon flux density; WT - wild type.
*Acknowledgements*: This study was funded by a grant from Kone Foundation to MK. E-MA and ET were supported by the Academy of Finland. We would like to thank Marja Hakala for preparing the tobacco thylakoids. The Center for Scientific Computing (Espoo, Finland) is thanked for permission to use their computer facilities.

complexes of higher plants and cyanobacteria. An excitation-emission map, also called an excitation-emission matrix (EEM, Beltrán *et al.* 1998) is a representation of the wavelength dependencies of both excitation and emission of fluorescence. In practice, such a map can be obtained by combining fluorescence emission spectra measured with several different excitation wavelengths.

Excitation-emission spectroscopy is useful primarily because it shows how excitation of an energy donor leads to fluorescence from an energy acceptor. Historically, energy transfer from the auxiliary antenna pigments to Chl *a* in a diatom (Dutton *et al.* 1943), and from Chl *b* to Chl *a* in solution (Duysens 1952) were discovered with excitation-emission spectroscopy. The technique of selective excitation has also been essential in assignments of emission peaks to photosystems (PS) 1 and 2 (Govindjee and Yang 1966). Böddi *et al.* (1993) present a low-temperature excitation-emission map of isolated wheat etioplast membranes. A time-resolved EEM was used by Mullineaux and Holzwarth (1991) to study the kinetics of excitation energy transfer in cyanobacterial phycobilisome-PS2 complex.

Thylakoid membranes of higher plants contain Chl *a*, Chl *b*, and carotenoids while cyanobacteria have Chl *a*, carotenoids, and phycobilins as light-harvesting pigments. Only Chl *a* and phycobilins show a significant fluorescence yield in intact systems. In higher plant membranes practically all fluorescence is emitted by Chl *a*; the low fluorescence yield of Chl *b* of higher plants is attributed to efficient energy transfer from Chl *b* to Chl *a* (Tomita and Rabinowitch 1962, Govindjee 1995).

The shape of the fluorescence emission spectrum is strongly temperature dependent (Sauer and Debreczeny 1996). At room temperature, the fluorescence yield of PS1 is very low but lowering the temperature to 77 K brings about a large increase in fluorescence emission peaking at 715-735 nm (Brody 1958). The peak wavelength depends on species: in higher plants the PS1 peak is at 735 nm and in cyanobacteria and green algae between 715 and 735 nm (Bose 1982). In *Synechocystis* 6803, PS1 emission peaks at 722 nm. Lowering the temperature also minimizes broadening of spectral bands due to phonons and other excitations, and therefore the emission peaks become narrower and better resolved (Moerner 1988). The emission from Chl *a* of PS2 splits at 77 K into two distinct peaks emitted mainly by the CP43 Chl *a*-protein complex (685 nm peak) and the CP47 Chl *a*-protein complex (peak or shoulder at 695 nm). Because practically all Chl *b* is in the light-harvesting complex of PS2 (LHC2), the PS2 emission bands at 685 and 695 nm of higher plants are sensitized by Chl *a*, Chl *b*, and carotenoids while PS1 emission is sensitized only by Chl *a* and carotenoids. Chl *a* of LHC2 itself has a very low emission yield due to efficient energy transfer to PS2 reaction centres, and a distinct emission peak of LHC2 Chl *a* at 680 nm can normally only be seen below 15 K (Rijgersberg *et al.* 1979). An intense 77 K emission peak at 680 nm was also found in senescent thylakoids of the stay-green soybean mutant *cytG* (J. Guiamet, E. Tyystjärvi, I. John, E. Pichersky, and L. Noodén, unpublished) in which LHC2 is retained during the senescence-induced breakdown of other thylakoid proteins.

In addition to Chl *a* fluorescence peaking at 685, 695, and 715-735 nm, cyanobacteria show also phycobilisome emission. Cyanobacterial phycobilisomes consist of phycobiliproteins carrying one of four main spectral types of open-chain

tetrapyrrole pigments, phycobilins, covalently linked to the protein. The absorption maximum depends on the pigment type. Phycoerythrocyanin absorbs at 575 nm, phycoerythrin has absorption maxima at 492 and 543 nm, phycocyanin (PC) at 550-640 nm, and allophycocyanin (APC) at 642-655 nm. Phycoerythrin emits below 600 nm whereas the main emission peak of PC is at 646 nm and APC emits at 658 nm. Emission spectra of both PC and APC also have a shoulder above 700 nm (Sidler 1994). In cyanobacteria, the 685 and 695 nm emission peaks are sensitized by phycobilin pigments and Chl *a* whereas the far-red PS1 emission is sensitized mainly by Chl *a*.

The position and function of a particular phycobiliprotein component in the phycobilisome structure may affect its optical properties. In particular, the APC-containing terminal emitters that connect the phycobilisome to photosynthetic reaction centres, have an emission peak at 685 nm at 77 K (van Thor *et al.* 1998). This peak contributes to the 685 nm emission band which in higher plants originates from CP43 alone. The far-red shoulders of emission bands from PC and APC, in turn, overlap with the emission from PS1.

Energy transfer between photosynthetic pigment complexes has traditionally been studied with excitation spectra while emission spectra are more relevant for structural studies. Current diode-array detector technology has, however, facilitated the measurement of emission spectra so much that excitation and emission spectra can easily be drawn as excitation-emission maps that combine structural values to energy transfer pathways in an illustrative way. We constructed excitation-emission maps by measuring emission spectra as a function of the excitation wavelength.

As research objects we used tobacco thylakoids and intact cells or isolated thylakoid membranes of the cyanobacterium *Synechocystis* sp. PCC 6803. We measured excitation-emission maps from a wild type (WT) strain of *Synechocystis* 6803 and compared the results to spectra measured from a PS2-less mutant LC (Mulo *et al.* 1997). Although the LC mutant lacks a functional PS2 reaction centre and contains only traces of the D1, D2, and CP47 proteins in the membranes, it has a normal phycobilisome content. Immunoblots of thylakoid proteins of the LC strain also show that this PS2 deficient mutant retains large amounts of the CP43 Chl *a* binding protein (Mulo *et al.* 1997). We addressed the question whether the 'orphan' CP43 protein is connected to phycobilisomes in the LC strain.

We also asked how much PS1 fluorescence is sensitized by phycobilisomes, and to which extent the far-red shoulders of PC and APC fluorescence emission peaks affect the small far-red PS1 emission peak observed when phycobilisomes are specifically excited. The question is relevant for understanding of so called state 1-state 2 transitions (for review see van Thor *et al.* 1998). In state 1, reached during prolonged selective excitation of PS1, phycobilisomes are thought to transfer their energy preferentially to PS2, and in state 2, phycobilisomes serve PS1 as well. The intensity of the PS1 emission band around 722 nm under selective excitation of phycobilisomes can be used as a measure of energy transfer from phycobilisomes to PS1. We present a simple method to correct the far-red fluorescence peak for emission from PC and APC in the excition-emission map of *Synechocystis* 6803. A principally similar correction method was used by Strasser and Butler (1977), who

applied the difference between the 514 nm and 633 nm excited fluorescence spectra to calculate a pure PS1 emission spectrum in flashed leaves of dark-grown bean.

## Materials and methods

**Strains and growth conditions**: Tobacco (*Nicotiana tabacum* L.) was grown in a 12 h light/dark rhythm (PPFD 300 μmol $m^{-2}$ $s^{-1}$) in a phytotron at 22 °C. Cells of the photoautotrophic glucose-tolerant wild type strain of the cyanobacterium *Synechocystis* sp. PCC 6803 were grown in liquid BG-11 growth medium (Rippka *et al.* 1979, Williams 1988) under the PPFD of 40 μmol $m^{-2}$ $s^{-1}$ at 30 °C. The heterotrophic D1 polypeptide mutant LC (Mulo *et al.* 1997) with a deletion of amino acids T227-Y246 from the stromal loop between the fourth and fifth membrane spanning helices of the D1 protein was grown in BG-11 medium in the presence of 5 mM glucose and appropriate antibiotics (Mulo *et al.* 1997) at the same physical conditions as the wild type *Synechocystis* 6803. The cells were harvested during the logarithmic growth phase.

**Isolation of thylakoid membranes and phycobilisomes**: Fluorescence spectra were measured both from cell suspensions and isolated thylakoids. Thylakoid membranes were isolated according to Tyystjärvi *et al.* (1994) by grinding cyanobacterial cells with glass beads, removing the deep blue supernatant and collecting, after removal of the beads, a thylakoid-containing pellet. Chl *a* was determined according to Arnon (1949).

The deep-blue cell-free supernatant obtained as a side product of the thylakoid isolation was used to isolate phycobilisomes by washing 120 $cm^3$ of supernatant with 1.35 $cm^3$ of acetone to remove remaining Chl. The phycobilisomes were then collected by centrifuging for 2 min at 16 000×*g*, dried, and resuspended to 110 $cm^3$ of buffer containing 50 mM Hepes-NaOH (pH 7.5), 800 mM sorbitol, 30 mM $CaCl_2$, and 1 mM ε-amino-*n*-caproic acid (Gombos *et al.* 1994). Then the pellet was prepared for the 77 K spectrum measurement in the same way as the intact cells.

Tobacco thylakoids were isolated as described by Pätsikkä *et al.* (1998). The fluorescence measurements were done in buffer containing 0.33 M sorbitol, 40 mM Hepes, pH 7.6, 5 mM NaCl, 5 mM $MgCl_2$, and 1 M glycine betaine.

**Measurement of 77 K fluorescence emission spectra**: The liquid surface of the cell suspension [160 $cm^3$, 3.75 g(Chl) $m^{-3}$, 25 % glycerol] in an Eppendorf tube was touched with a quartz rod and this combination was frozen in liquid nitrogen. The quartz rod was then removed and replaced by the light guide of the fluorometer. The thylakoid suspensions were frozen in the same way as the cells except that the thylakoid membranes were suspended in buffer containing 10 mM Tris-HCl pH 8.0, 0.4 M sucrose, 10 mM NaCl, and 20 mM Na-EDTA.

Fluorescence emission spectra were measured at 77 K with a diode array spectrophotometer (*S2000, Ocean Optics*, Dunedin, Florida, USA) equipped with a reflectance probe. The spectrophotometer has 2048 diodes arranged behind a monochromator between 594 and 857 nm. A 50 mm built-in slit guarantees a nominal

5 nm wavelength resolution. Excitation was with a 250 W slide projector (*Royal AF, Zeiss Ikon*, Germany) through a 600 nm short pass filter (*LS-600-S, Corion*, USA) and a f/3.4 monochromator (*Applied Photophysics*, Surrey, U.K.). The PPFD at the sample surface varied from 2 μmol $m^{-2}$ $s^{-1}$ at 400 nm to 9.3 μmol $m^{-2}$ $s^{-1}$ at 560 nm. Fluorescence emission was recorded from 600 to 800 nm using different excitation wavelengths from 400 to 585 nm with 5 nm steps. Each spectrum was recorded with an optimum integration time of 0.2-28.0 s and the spectra were then corrected by dividing the integrated irradiances by the integration time. The irradiance at each excitation wavelength was measured with a *Lambda LI-185A* quantum meter between 400 and 600 nm, and the spectra were corrected by dividing the fluorescence intensity with the exciting irradiance at each wavelength. Finally, a minor correction for scattered radiation was done by subtracting a baseline obtained from a cuvette filled with water.

**Notation**: We denote locations on excitation-emission maps by the symbol $F_{Exc}^{Em}$ with the excitation wavelength *Exc* [nm] as a subscript and the emission wavelength *Em* [nm] as a superscript.

**Data processing**: A linear interpolation of the original fluorescence emission values was used to obtain a 0.5 nm grid in the excitation axis. All maps were scaled to the Chl-sensitized PS1 emission peak by dividing each emission intensity by the mean value found in the rectangle $F_{405-415}^{719-723}$ in (cyanobacterial samples) or $F_{405-415}^{733-737}$ (tobacco thylakoids). All processing of data and graphics were done with *Matlab* version *5.3* (*Mathworks*, USA) run in *Compaq AlphaServer 8400*.

## Results and discussion

**Excitation-emission maps of tobacco and *Synechocystis* 6803**: Fig. 1 shows an excitation-emission map of tobacco thylakoids. In the spectrum, the 685 nm emission peak is intense and the 695 nm emission peak is only a shoulder (Fig. 1, *insert*). Our experience is that in addition to the phosphorylation-induced changes in the ratio of fluorescence from PS2 and PS1 (Bennett *et al.* 1980), also the relative intensities of the 685 and 695 nm bands vary a lot in higher plants when the phosphorylation status of the thylakoid proteins is varied. The sharpness of the 685 nm peak and the 695 nm shoulder show that the 5 nm nominal wavelength resolution of the diode array spectrometer is sufficient for the separation of these two peaks (Fig. 1, *insert*). The PS1 emission peaking at 735 nm has approximately the same amplitude as the 685 nm peak emitted by the CP43 Chl *a* binding protein of PS2. Both PS1 and PS2 emission peaks decrease in intensity when the wavelength of excitation increases above 440 nm. However, while the PS1 peak steeps monotonously down, the PS2 emission shows a shoulder between 440 and 490 nm. That is attributed to sensitisation of PS2 Chl *a* fluorescence by Chl *b* of LHC 2.

Compared to the excitation-emission map of tobacco, the excitation-emission map of *Synechocystis* 6803 cells (Fig. 2*A*) is more complex. In addition to the PS1

emission peak at 722 nm and the peaks of the PS2 pigment complexes CP43 (685 nm) and CP47 (695 nm), the phycobilisome emission peaks $F_{>550}^{645}$ (PC) and $F_{>550}^{658}$ (APC) can be clearly seen with phycobilisome excitation above 550 nm. The low ratio of the PS2 emission to the huge PS1 emission peak with Chl *a* excitation is contrast with the high PS2:PS1 emission ratio when the actinic radiation above 550 nm preferentially excites phycobilisomes instead of Chl *a*. An interesting difference to the higher-plant map is the steep decrease in PS1 emission at 722 nm between excitation wavelengths 440 and 470 nm. This difference may reflect a difference in the carotenoid content of tobacco and *Synechocystis* PS1. Part of PS1 emission may be sensitized by PS2 *via* the spillover mechanism (Butler and Kitajima 1975) and absorption by Chl *b* may extend the excitation of PS1 emission in the 440-490 nm region in tobacco (Fig. 1).

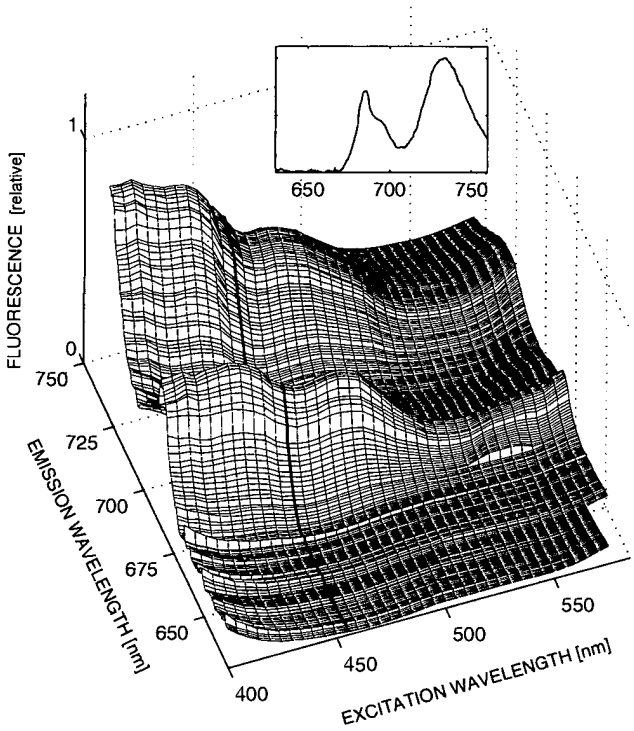


Fig. 1. 77 K excitation-emission map measured from isolated thylakoids of tobacco (*Nicotiana tabacum*). The fluorescence emission spectra were measured with a diode array spectrometer using different excitation wavelengths and the values were combined to a map. The insert shows emission spectrum measured with 455 nm excitation; the same path is shown with a thick line in the excitation-emission map. A small artefact caused by the subtraction of background emission can be seen in the range $F_{530-570}^{680}$.

The relative importance of phycobilisomes as the antenna structures of PS2 in *Synechocystis* 6803 is immediately revealed by the amplitude of phycobilisome-excited $F_{>550}^{685}$ and $F_{>550}^{695}$ peaks compared to the smaller amplitudes observed when Chl *a* is selectively excited at 400–450 nm (Fig. 2*A*).

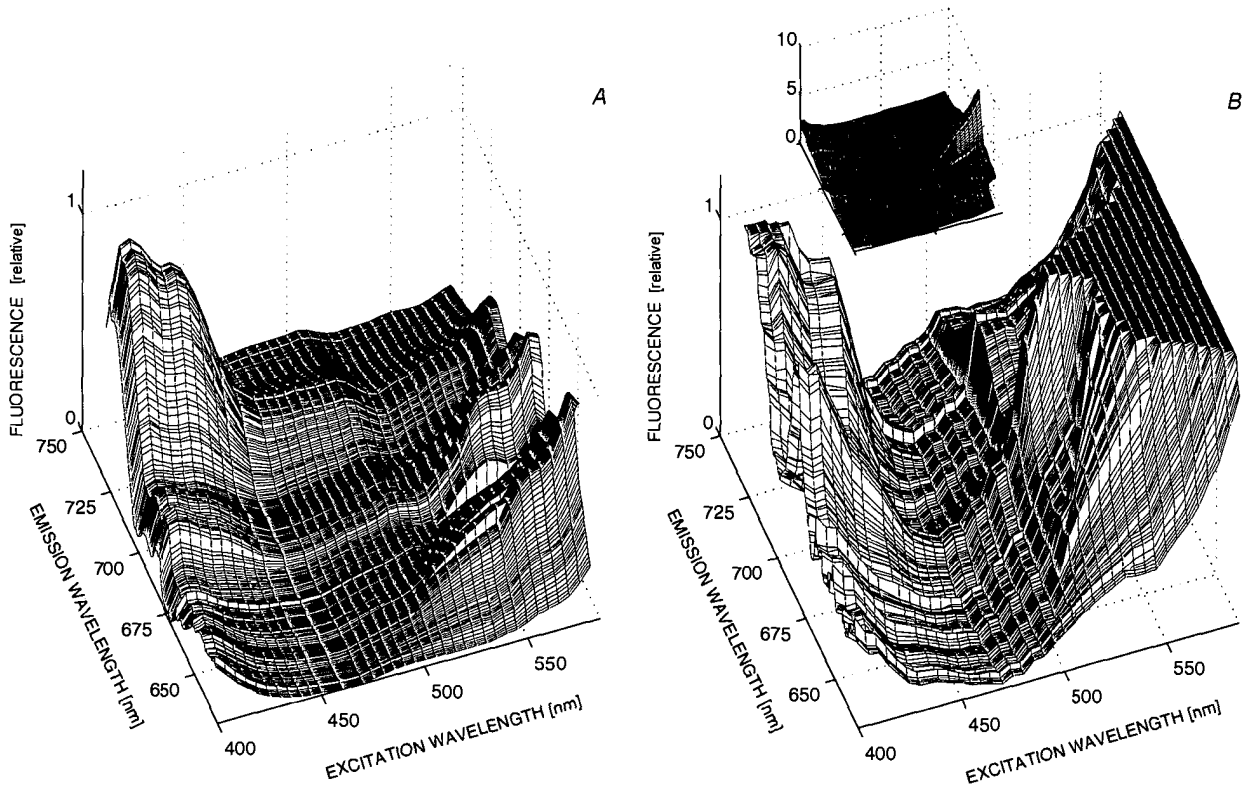


Fig. 2. 77 K excitation-emission map for (*A*) wild type cells and (*B*) cells of the PS2-less LC mutant strain of *Synechocystis* 6803. The spectra were measured from intact *Synechocystis* cells with a diode array spectrometer using different excitation wavelengths and combined to a map. The values of LC are cut to show the structure of the 400–500 nm excitation range. The insert in (*B*) illustrates the complete peak in the LC strain.

$F_{>550}^{685}$ **in the PS2-less mutant LC**: The excitation-emission map measured from cells of the PS2-less LC strain is dominated by an intense phycobilisome-excited peak $F_{>550}^{685}$ while emission at 695 nm is lacking (Fig. 2*B*). Other emission peaks are similar in the excitation-emission map of the wild type cells (Fig. 2*A*) and in the map of the LC strain (Fig. 2*B*). The phycobilisome-excited $F_{>550}^{685}$ peak is approximately ten times higher in LC than in WT (*insert* of Fig. 2*B*), and this high intensity may have three possible reasons: (*1*) The concentration of the CP43 Chl *a* binding protein is extremely high, (*2*) a functional PS2 reaction centre acts as a fluorescence quencher at 77 K, and the CP43 fluorescence is high because the LC mutant lacks this quencher, or (*3*) changed interactions between the phycobilisome antenna and the remainders of PS2 reaction centre favour energy transfer from PC to CP43, resulting in the intense $F_{>550}^{685}$ in the LC strain.

The Chl *a* sensitized $F_{400-470}^{685}$ peak in LC, compared to Chl *a* sensitized PS1 emission at $F_{405-415}^{719-723}$, is approximately three times as intense as in the WT (Fig. 2). This difference is in line with three times higher CP43 content of LC than WT thylakoids revealed by immunoblots of thylakoid proteins (Mulo *et al.* 1997), supporting assumption (*1*). This consistency between the amount of CP43 and Chl *a* sensitized fluorescence yield of CP43 rules out the possibility (*2*) that the orphan CP43 of LC has a high fluorescence yield because LC lacks reaction centres quenching PS2 fluorescence. However, the three-fold higher CP43 content of LC is not big enough to explain the ten-fold higher phycobilisome-sensitized $F_{>550}^{685}$ in LC than in WT, and we conclude that the high emission results from changed interactions between phycobilisomes and the remaining PS2 proteins in the PS2-less LC strain (*3*). Either energy transfer from phycobilisomes to CP43 is exceptionally efficient in LC, leading to high phycobilisome-sensitized emission from CP43, or the terminal emitters of the phycobilisomes of LC have an exceptionally high fluorescence yield. A high emission peak at 685 has been found earlier in cyanobacterial mutants that lack functional PS2 reaction centres but retain CP43 (Vermaas *et al.* 1986, Nilsson *et al.* 1992). This high peak has often been attributed to CP43 alone, but the specific requirement for phycobilisomes as sensitizers in the LC mutant (Fig. 2*B*) may suggest a contribution of both CP43 and the terminal emitters of the phycobilisome antenna in $F_{>550}^{685}$. We tried the technique developed by Weber (1961) to determine the number of fluorescence sources in the 685 nm region in LC, but the results remained ambiguous, probably due to energy transfer processes involved.

**Thylakoid isolation leads to a partial dissociation of phycobilisomes**: In the excitation-emission map of isolated *Synechocystis* 6803 thylakoids (Fig. 3), the PC emission peak $F_{>550}^{645}$ was relatively more intense than in the map measured from intact cells (Fig. 2*A*). The difference indicates detachment of some of the phycobilisomes from

the membranes during isolation of thylakoids, and indeed the isolation procedure contains a step at which a dark blue supernatant is removed from a Chl-enriched pellet. Only the PC emission peaking at 645 nm increases when the connection between the phycobilisomes and the membrane is lost, whereas the APC emission peaking at 658 nm is rather lowered, suggesting that energy transfer from PC to APC decreases when the phycobilisome is detached from the membrane. The ratio of the phycobilisome-excited 685 nm emission peak to the corresponding 695 nm peak is lower in isolated thylakoids than in intact cells, suggesting again that the terminal APC emitters of the phycobilisomes contribute significantly to the 685 nm emission peak. Another possibility is that the thylakoid isolation procedure specifically lowers the efficiency of energy transfer from phycobilisomes to CP43. The relative intensities of the Chl *a* excited emission peaks at 685, 695, and 722 nm were not affected by thylakoid isolation (Fig. 3).

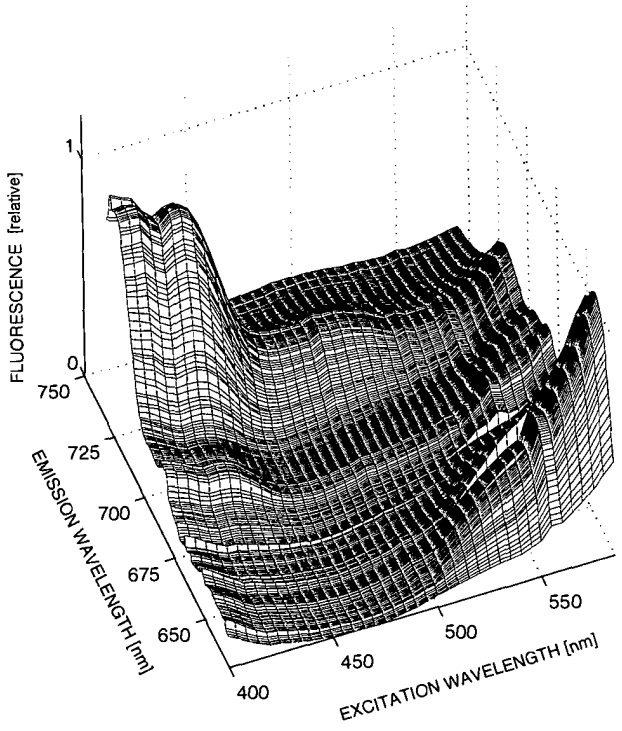


Fig. 3. 77 K excitation-emission map measured from isolated thylakoids from wild type *Synechocystis* 6803 cells. The spectra were measured with a diode-array spectrometer and combined to a map.

**Correction for phycobilisome contribution to** $F_{>550}^{>715}$: Both PC and APC have a strong shoulder in their emission peaks extending to approximately 720 nm (Sidler 1994) and these shoulders inevitably contribute to $F_{>550}^{>715}$ in *Synechocystis* 6803. Because the long-wavelength edge of the phycobilisome emission shoulder is at a shorter

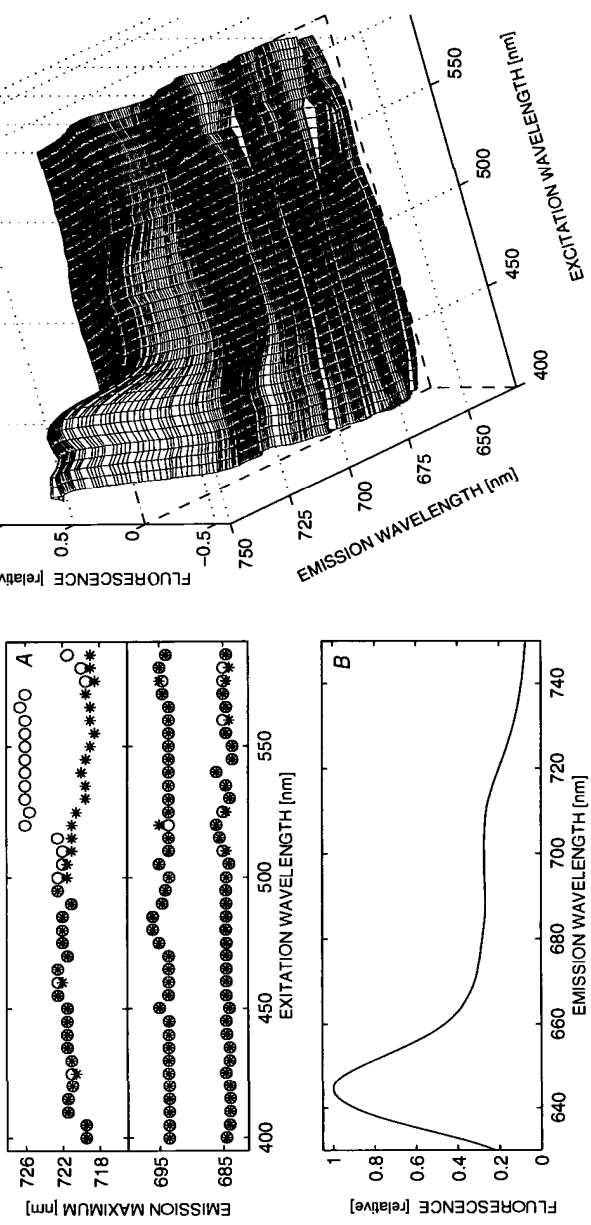


Fig. 4. (*A*) Positions of the emission maxima around 722, 685, and 695 nm, measured from the excitation-emission map of *Synechocystis* 6803 thylakoids. Original emission maxima (*) and maxima obtained after subtraction of PC and APC emission spectra (O). (*B*) The emission spectrum measured from isolated PC complex with 585 nm excitation at 77 K. (*C*) 77 K emission-excitation map of *Synechocystis* 6803 after subtraction of phycobilisome emission. PC fluorescence was first subtracted by assuming that all emission at 645 nm is from PC and that the fluorescence has the profile shown in (*B*). The remaining APC fluorescence was thereafter subtracted according to the published emission spectrum of APC (Sidler 1994) by assuming that all remaining fluorescence at 658 nm is emitted by APC.

wavelength than the 722 nm peak of PS1 emission observed with Chl *a* excitation, the phycobilisome contribution is expected to induce a blue-shift in the far-red emission. Indeed, the excitation-emission map shows that the position of the far-red emission peak changes from 722 to 720 nm above 520 nm (Fig. 4*A*). The stability of the wavelengths of the PS2 emission maxima at 685 and 695 nm indicates that the shift is not a technical artefact. We estimated the size of the PS1 contribution in the phycobilisome-excited far-red emission peak by subtracting the phycobilisome contribution.

To correct for the phycobilisome contribution in $F_{>550}^{>715}$ we needed the emission spectrum of isolated phycobilisomes. We used the 77 K emission spectra of isolated PC and APC for the correction. The spectra have been published (Sidler 1994), and we checked, by washing a phycobilisome-enriched thylakoid preparation with acetone, that the mobile PC-containing part of the phycobilisomes of our *Synechocystis* 6803 strain indeed has the same form as the published PC spectrum (Fig. 4*B*). Both PC and APC can be clearly seen in all excitation-emission maps at the emission wavelengths of 645 and 658 nm, respectively (Figs. 2 and 3). From the excitation-emission map of WT *Synechocystis* 6803 thylakoids (Fig. 2*A*) we subtracted the emission of PC (Fig. 4*B*) by assuming that all emission at 645 nm comes from PC. From the result, we subtracted the APC emission, scaling the APC emission spectrum to remaining 658 nm emission intensity. This method partially neglects the overlap of the APC and PC emission around 645 nm, and therefore slightly exaggerates the subtraction, but a strong exaggeration is ruled out by the finding that the resulting phycobilisome-fluorescence-free excitation-emission map does not show negative fluorescence values (Fig. 4*C*). Although the far-red emission peak was not completely removed, the correction for phycobilisome emission lowered the far-red fluorescence peak to the same low intensity as seen between excitation wavelengths 500 and 550 nm. The subtraction procedure also shifted the emission maximum of the far-red peak PS1 from 718 to 726 nm (Fig. 4*A*). It is possible that this shift of the maximum to 726 nm instead of 722 nm is caused by a slight overestimation in the subtraction of the spectra of APC and PC. We conclude that a correction for the far-red emission from the phycobilisomes is essential if the 77 K emission spectra are used to measure energy transfer from phycobilisomes to the two photosystems in *Synechocystis* 6803, and that phycobilisome emission causes a shift of PS1 emission peak towards shorter wavelengths when phycobilisomes are selectively excited.

## References

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.

Beltrán, J.L., Ferrer, R., Guiteras, J.: Multivariate calibration of polycyclic aromatic hydrocarbon mixtures from excitation-emission fluorescence spectra. - Anal. chim. Acta **373**: 311-319, 1998.

Bennett, J., Steinback, K.E., Arntzen, C.J.: Chloroplast phosphoproteins: Regulation of excitation energy transfer by phosphorylation of thylakoid membrane polypeptides. – Proc. nat. Acad. Sci. USA 77: 5253-5257, 1980.

Böddi, B., Ryberg, M., Sundqvist, C.: Analysis of the 77 K fluorescence emission and excitation spectra of isolated etioplast inner membranes. – J. Photochem. Photobiol. **B 21**: 125-133, 1993.

Bose, S.: Chlorophyll fluorescence in green plants and energy transfer pathways in photosynthesis. – Photochem. Photobiol. **36**: 725-731, 1982.

Brody, S.S.: New excited state of chlorophyll. - Science **128**: 838-839, 1958.

Butler, W.L., Kitajima, M.: Energy transfer between photosystem II and photosystem I in chloroplasts. – Biochim. biophys. Acta **396**: 72-85, 1975.

Dau, H.: Molecular mechanisms and quantitative models of variable photosystem II fluorescence. – Photochem. Photobiol. **60**: 1-12, 1994.

Dutton, H.J., Winston, M.M., Duggar, B.M.: Chlorophyll fluorescence and energy transfer in the diatom *Nitzschia closterium*. – J. phys. Chem. **46**: 308-313, 1943.

Duysens, L.N.M. Transfer of Excitation Energy in Photosynthesis. – Ph.D. Thesis. State University of Utrecht, Utrecht 1952.

Gantt, E., Lipschultz, C.A.: Energy transfer in phycobilisomes from phycoerythrin to allophycocyanin. – Biochim. biophys. Acta **292**: 858-861, 1973.

Gombos, Z., Wada, H., Murata, N.: The recovery of photosynthesis from low-temperature photoinhibition is accelerated by the unsaturation of membrane lipids: A mechanism of chilling tolerance. - Proc. nat. Acad. Sci. USA **91**: 8787-8791, 1994.

Govindjee: Sixty-three years since Kautsky: Chlorophyll *a* fluorescence. – Aust. J. Plant Physiol. **22**: 131-160, 1995.

Govindjee, Yang, L.: Structure of the red fluorescence band in chloroplasts. – J. gen. Physiol. **49**: 763-780, 1966.

Krause, G.H., Weis, E.: Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. – Photosynth. Res. **5**: 139-157, 1984.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Moerner, W.E.: Introduction. – In: Moerner, W.E. (ed.): Persistent Spectral Hole-Burning: Science and Applications. Pp. 1-15. Springer-Verlag, Berlin - Heidelberg 1988.

Mullineaux, C.W., Holzwarth, A.R.: Kinetics of excitation energy transfer in the cyanobacterial phocobilisome-Photosystem II complex. - Biochim. biophys. Acta **1098**: 68-78, 1991.

Mulo, P., Tyystjärvi, T., Tyystjärvi, E., Govindjee, Mäenpää, P., Aro, E.-M.: Mutagenesis of the D-E loop of photosystem II reaction centre protein D1. Function and assembly of photosystem II. - Plant mol. Biol. **33**: 1059-1071, 1997.

Nilsson, F., Simpson, D.J., Jansson, C., Andersson, B.: Ultrastructural and biochemical characterization of a *Synechocystis* 6803 mutant with inactivated *psbA* genes. – Arch. Biochem. Biophys. **295**: 340-347, 1992.

Pätsikkä, E., Aro, E.-M., Tyystjärvi, E.: Increase in the quantum yield of photoinhibition contributes to copper toxicity *in vivo*. – Plant Physiol. **117**: 619-627, 1998.

Rijgersberg, C.P., Amesz, C.P., Thielen, A.P.G.M., Swager, J.A.: Fluorescence emission spectra of chloroplasts and subchloroplast preparations at low temperature. – Biochim. biophys. Acta **545**: 473-482, 1979.

Rippka, R., Deruelles, J., Waterburg, J.B., Herdman, M., Stanier, R.Y.: Genetic assignments, strain histories and properties of pure cultures of cyanobacteria. – J. gen. Microbiol. **111**: 1-61, 1979.

Sauer, K., Debreczeny, M.: Fluorescence. – In: Amesz, J., Hoff, A.J. (ed.): Biophysical Techniques in Photosynthesis. Pp. 41-61. Kluwer Academic Publ., Dordrecht - Boston - London 1996.

Sidler, W.A.: Phycobilisome and phycobiliprotein structures. - In: Bryant, D.A. (ed.): The Molecular Biology of Cyanobacteria. Pp. 139-216. Kluwer Academic Publ., Dordrecht 1994.

Strasser, R.J., Butler, W.L.: Fluorescence emission spectra of Photosystem I, Photosystem II and the light-harvesting chlorophyll *a/b* complex of higher plants. - Biochim. biophys. Acta **462**: 307-313, 1977.

Tomita, G., Rabinowitch, E.: Excitation energy transfer between pigments in photosynthetic cells. - Biophys. J. **2**: 483-499, 1962.

Trissl, H.-W., Lavergne, J.: Fluorescence induction from Photosystem II: Analytical equations for the yields of photochemistry and fluorescence derived from analysis of a model including exciton-radical pair equilibrium and restricted energy transfer between photosynthetic units. - Aust. J. Plant Physiol. **22**: 183-93, 1994.

Tyystjärvi, T., Aro, E.-M., Jansson, C., Mäenpää, P.: Changes of amino acid sequence in PEST-like area and QEEET motif affect degradation of D1 polypeptide in photosystem II. - Plant mol. Biol. **25**: 517-526, 1994.

Van Thor, J.J., Mullineaux, C.W. Matthijs, H.C.P., Hellingwerf, K.J.: Light harvesting and state transitions in cyanobacteria. - Bot. Acta **111**: 430-443, 1998.

Vermaas, W.F.J., Williams, J.G.K., Rutherford, A.W., Mathis, P., Arntzen, C.J.: Genetically engineered mutant of the cyanobacterium *Synechocystis* 6803 lacks the photosystem II chlorophyll-binding protein CP-47. - Proc. nat. Acad. Sci. USA **83**: 9474-9477, 1986.

Weber, G.: Enumeration of components in complex systems by fluorescence spectroscopy. – Nature **190**: 27-29, 1961.

Williams, J.G.K.: Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering methods in *Synechocystis* 6803. – In: Abelson, J.N., Simon, M.I. (ed.): Methods in Enzymology. Vol. **167**. Pp. 766-778. Academic Press, San Diego - New York - Berkeley - Boston - London - Sydney - Tokyo - Toronto 1988.