

BRIEF COMMUNICATION

Application of spectrally resolved fluorescence induction to study light-induced nonphotochemical quenching in algae

R. KAŇA

Institute of Microbiology, Czech Academy of Sciences, Třeboň, Czech Republic

Abstract

The light-induced nonphotochemical quenching (NPQ) can safely dissipate excess of absorbed light to heat. Here we describe an application of spectrally resolved fluorescence induction (SRFI) method for studying spectral variability of NPQ. The approach allows detection of spectrally-resolved nonphotochemical quenching (NPQ_λ) representing NPQ dependency on fluorescence emission wavelength in the whole spectral range of fluorescence emission. The experimental approach is briefly described and NPQ_λ is studied for the cryptophyte alga *Rhodomonas salina* and for green alga *Chlorella* sp. We confirm presence of NPQ_λ only in membrane-bound antennae (chlorophyll *a/c* antennae) and not in phycobiliproteins in lumen in cryptophyte and show that NPQ_λ is inhibited in the whole spectral range by NPQ inhibitors in *Chlorella* sp. We discuss variability in the quenching in the particular spectral ranges and applicability of the NPQ_λ parameter to study quenching locus *in vivo*.

Additional key words: fluorescence parameters; light-harvesting complex; photoprotection; photosynthesis; photosystem II.

Light-induced nonphotochemical quenching of chlorophyll (Chl) *a* fluorescence (NPQ) represents a regulated feedback mechanism allowing photoprotection of thylakoid membrane proteins during short periods (minutes) of excessive irradiation (*see e.g.*, Ruban *et al.* 2012, Derks *et al.* 2015). The process protects PSII and proceeds either in PSII reaction centrum (*see, e.g.*, Bruce *et al.* 1997, Komura *et al.* 2010, Krupnik *et al.* 2013) or in the associated antenna proteins (Belgio *et al.* 2014, Holzwarth *et al.* 2009, Xu *et al.* 2015). On a molecular basis, regulatory NPQ in antennae is connected with reversible switch of antenna proteins between light-harvesting and photoprotective mode (Krüger *et al.* 2011, 2013). The so-called energy

dependent component (q_E) of NPQ is triggered by lumen acidification (Briantais *et al.* 1979, Gilmore and Yamamoto 1992, Belgio *et al.* 2013); however, there are some other factors (*e.g.*, role of ions) that need to be taken into account (Kaňa and Govindjee 2016). The pH sensitivity is controlled by several other allosteric regulators including xanthophylls (Niyogi *et al.* 1997, Kaňa *et al.* 2016) or PsbS protein in vascular plants (Li *et al.* 2000). Once the regulatory NPQ is induced, the quantum yield of Chl *a* fluorescence is lowered due to dissipation of excess excitation energy into heat, which can be detected as a change in sample temperature (Kaňa and Vass 2008).

Received 19 May 2017, accepted 10 October 2017, published as online-first 10 January 2018.

E-mail: kana@alga.cz

Abbreviations: CAC – chlorophyll *a/c* antennae complexes of cryptophyte; Chl – chlorophyll; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_t – fluorescence intensity at particular irradiance/time of measuring protocol; F₀ – minimal fluorescence intensity for open reaction center; F_M – maximal fluorescence intensity for closed reaction center measured with dark-adapted sample; F_{M'} – maximal fluorescence intensity for closed reaction center measured with light-adapted sample; NPQ – nonphotochemical quenching of chlorophyll *a* fluorescence; NPQ_λ – spectrally resolved nonphotochemical quenching of fluorescence; RC – reaction center; Rfd – fluorescence decrease ratio.

Acknowledgement: This research was supported by the Czech Science Foundation (project GACR 16–10088S) and by institutional projects Algatech Plus (MSMT LO1416) by the Czech Ministry of Education, Youth and Sport. I'm thankful to Ondřej Prášil for jointly developing the Spectrally Resolved Fluorescence Induction (SRFI) method described previously (Kaňa *et al.* 2009a, 2012a), and to Govindjee for long-term cooperation and useful discussion of the topic. I also highly appreciate technical assistance, software development and various methodical and physiological measurements that have been done by several members of our lab (namely Jaroslav Krafel, Barbora Šedivá, Jiří Šetlík, Eva Kotabová, and Gabor Steinbach) that allowed continual improvement of the SRFI method and its applicability for different organisms/questions. I would like to thank Erica Belgio for critical reading of the manuscript.

However, direct measurement of the NPQ based on changes in the sample temperature is not well established [compare Kaňa and Vass (2008) with Kulasek *et al.* (2016)], therefore, the method of variable fluorescence is often used as a versatile and easy-to-use method to detect the extent of NPQ (*see, e.g.*, Bilger and Björkman 1990, Papageorgiou and Govindjee 2004, Demmig-Adams *et al.* 2014). In typical experimental approach, NPQ is calculated and measured based on a decrease in maximal Chl *a* fluorescence after illumination by actinic light (*i.e.*, as a decrease from dark level F_M to F_M' level on light) using Stern–Volmer formalism for collisional quenching [*see, e.g.*, Lakowicz (2006) or Holzwarth *et al.* (2013)]. An extent of the process of nonphotochemical quenching can be also defined in terms of quantum yields of nonphotochemical quenching, where regulatory and nonregulatory (basal) nonphotochemical quenching needs to be considered [for more details *see* Kramer *et al.* (2004) and review of Lazár (2015)]. Recently (Lazár 2015), the NPQ parameter has been found to be a ratio of quantum yield of the regulatory nonphotochemical quenching to quantum yield of nonregulatory (basal) nonphotochemical quenching.

In a standard (spectrally not resolved) experimental set-up, the decrease in the Chl *a* fluorescence is calculated as an integral value over the wide range of Chl *a* emission band (usually above 700 nm) and does not take into account changes at particular wavelengths. There are only few attempts that have tried to address fluorescence quenching spectrally. A spectrally resolved fluorescence spectroscopy at low temperature has allowed to attribute NPQ locus into light-harvesting antennae (Ruban and Horton 1994). Later, spectral resolution of NPQ at room temperature has allowed detection of different quenching components in *Arabidopsis* leaves (Lambrev *et al.* 2010) and role of red-shifted antennae in NPQ of *Chromera velia* alga (Kotabová *et al.* 2014) or absence of fluorescence quenching in phycoerythrins in cryptophyte (Kaňa *et al.* 2012b).

All these methods required application of a certain type of a spectrally resolved fluorescence induction (SRFI) method. The SRFI method can address regulation of light-harvesting in general. Initially, the method was used to study mechanism of state transitions and decoupling of phycobilisomes in the cyanobacteria *Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803 (Kaňa *et al.* 2009a, 2012a). Later, it has been adapted to more specific projects dealing with regulation of light harvesting in cyanobacteria *Synechocystis* sp. 6803 and its mutants (Kaňa *et al.* 2012a, Acuña *et al.* 2016, Bernat *et al.* 2017). Recently, it has been applied for several algal strains including *Chromera velia* (Kotabová *et al.* 2014) and cryptophyte algae (Kaňa *et al.* 2012b, Cheregi *et al.* 2015) to study NPQ locus and mechanism of state transitions. Here, the spectrally resolved nonphotochemical quenching (NPQ_λ) is characterized in more details for cryptophyte and green algae in terms of NPQ_λ kinetics and a role of inhibitors. The data reported here indicate that application

of SRFI to detect NPQ_λ is a very promising technique for future research in photosynthesis as it allows detection of spectral changes occurring *in vivo* during natural energy equilibration/quenching processes.

The cryptophyte alga *Rhodomonas salina* (strain CCAP 978/27) and the green alga *Chlorella* sp. (Novotný *et al.* 2017) were grown in the cell suspension in artificial seawater medium with f/2 nutrient addition. The cultures were continually bubbled with air in a temperature-controlled bath (18°C) and illuminated by dimmable fluorescence tubes [30 μmol(photon) m⁻² s⁻¹, day–night cycle of 12/12 h). Each sample was dark-adapted for 20 min before measurements. The measurements were carried out in the exponential growing phase (OD_{760nm} was in the range 0.2–0.3).

Nonmodulated technique of SRFI was measured with spectrometer SM-9000 (Photon Systems Instruments, Brno, Czech Republic) with spectral bandwidth of 0.8 nm (in the range of 200–980 nm), the dark current of the instrument was automatically subtracted before measurements, based on the method described previously for cyanobacteria (Kaňa *et al.* 2012a) and algae (Kotabová *et al.* 2014). Samples were dark-adapted for 20 min before applying low-intensity measuring light [2 μmol(photon) m⁻² s⁻¹] for the detection of fluorescence of open PSII reaction center measured with the dark-adapted sample – F_0 . Maximal fluorescence intensities for the dark-adapted (F_M) and light-adapted sample (F_M') have been measured at the end of 200-ms multiple turnover saturating actinic flashes [464 nm, 2,350 μmol (photon) m⁻² s⁻¹]. The kinetic changes in spectra during actinic irradiation [464 nm, 1,100 μmol(photon) m⁻² s⁻¹] were measured in the whole fluorescence spectrum with millisecond time-resolution scale (every 50 ms). The spectra of maximal fluorescence in the light $F_M'(\lambda)$ were detected after at different time of actinic irradiation (*see* description in figure legends). The spectrally resolved NPQ_λ was calculated based on the Stern–Volmer formalisms as $NPQ_\lambda = [F_M(\lambda) - F_M'(\lambda)] / [F_M'(\lambda)]$ for every wavelength. For comparison, the standard Chl *a* fluorescence induction has been detected simultaneously in the spectral range 690–710 nm (Fig. 1S, *supplement available online*) by FL 100 spectrophotometer (Photon System Instrument, Brno, Czech republic) as described before (Kaňa *et al.* 2012a).

A measurement of NPQ_λ requires application of SRFI that has been described previously (Kaňa *et al.* 2012a). The SRFI method allows detection of the whole fluorescence spectrum in a wide spectral range (200–980 nm) with relatively high spectral resolution (wavelength accuracy of 0.8 nm, relative resolution reflecting FWHM $\Delta\lambda = 3$ nm, *see* Kaňa *et al.* 2012a), and with a fast detection rate (every 50 ms in our case). Evolution of the fluorescence spectra in time is plotted in a 3D graph (Fig. 1A) and as a 2D color-coded graph (Fig. 1B). There, the fluorescence emission spectra are represented as sections parallel to the wavelength axis (x-axis) at a given time of the measuring protocol (*see* top part of Fig. 1B labeled as “Spectrum”),

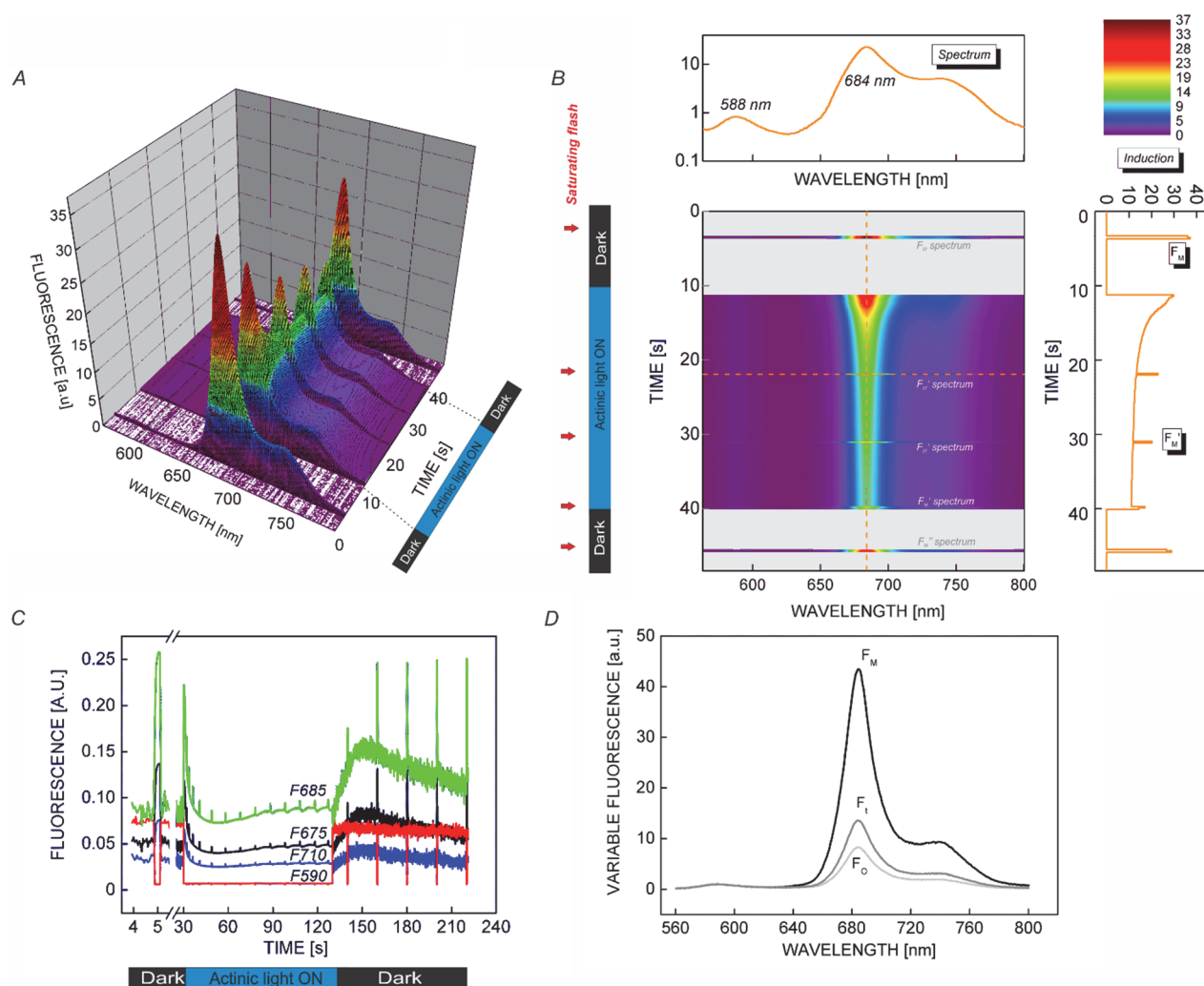


Fig. 1. The spectrally resolved fluorescence induction (SRFI) method applied to *Rhodomonas salina* cells. To explain the method, two different protocols were used; a short one (60 s, panels A,B) and the long one (220 s, panels C,D). Cells were dark-adapted for 20 min and blue light [464 nm; $1,100 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] was used to induce nonphotochemical quenching. The entire emission spectrum was measured every 50 ms. (A): 3D graph of fluorescence induction during a short protocol (60 s) with three saturating flashes applied during light period (F_M') and one before (see F_M) and after the light period. (B): Two dimensional (2D) reploting of SRFI data presented in panel A. The graph shows dependency of intensity of variable fluorescence $F = f(\lambda, t)$, different intensity of fluorescence at particular time/wavelength is represented by artificial colors (see the scale). The timing of actinic light irradiation and application of saturating flashes are shown by bars and red arrows, respectively. Spectra are presented in the range between 560 and 800 nm, measured during the 60-s long protocol. The typical fluorescence spectrum at constant time (at $t = 30$ s of measurements) is presented (see insert "Spectrum" on the top) together with typical fluorescence induction at 685 nm (see inset "Induction"). (C): Examples of other fluorescence inductions measured with 220-s long protocol. The fluorescence induction at selected wavelengths (F590, F685, F675, F710) are presented, the fluorescence intensity was corrected for different intensities of actinic light used for detection of F_0 , F_M , and F_t . Data resembled simultaneous single wavelength range (690–710 nm) measurement by FL 100 fluorimeter presented in Fig. 1S. (D): Fluorescence spectra measured at different stages of fluorescence induction, they were used for calculation of F_0 , F_M (measured after 2 min on light during saturating flash), and F_t spectra (measured after 2 min in light) obtained from long protocol (220 s). The fluorescence intensity was corrected for different intensities of actinic light used for detection of F_0 , F_M , and F_t .

a section parallel to the time axis (y-axis) shows fluorescence induction at a particular wavelength (see left part of Fig. 1B labeled as "Induction"). The approach allows us continual detection of the fluorescence induction at different wavelengths (Fig. 1C). The kinetic data (i.e., fluorescence induction) are comparable with fluorescence measurements by other commercial spectrophotometers

including pulse-modulated fluorimeters (Schreiber 2004) or other fluorimeters (see, e.g., Kaftan *et al.* 1997 and data in Fig. 1S). The main advantage of the SRFI method is represented by simultaneous detection of fluorescence kinetics (kinetics of fluorescence quantum yield) in any selected wavelengths (see Fig. 1C, and previous applications in Kaňá *et al.* 2009a, 2012a; Kotabová *et al.* 2014).

As the method continuously detects spectra of all particular fluorescence parameters (F_t , F_0 , F_M spectra in Fig. 1D), all fluorescence parameters defined for pulse-modulated fluorimeters (Rfd , F_v/F_M , Φ_{PSII} , q_P , q_N , Φ_{NPQ} , Φ_{NO} , etc., see, e.g., Lazar 2015, Schreiber 2004) can be detected spectrally by the SRFI method. Here, we have applied the SRFI method to study spectral variability in NPQ_λ (Fig. 2).

The spectra of maximal fluorescence for closed PSII reaction center in dark-adapted sample (F_M) and during actinic irradiation (F_M') were acquired during saturating flashes (Fig. 1A,C). NPQ_λ was calculated for all wavelengths (see Materials and methods) for two organisms, the

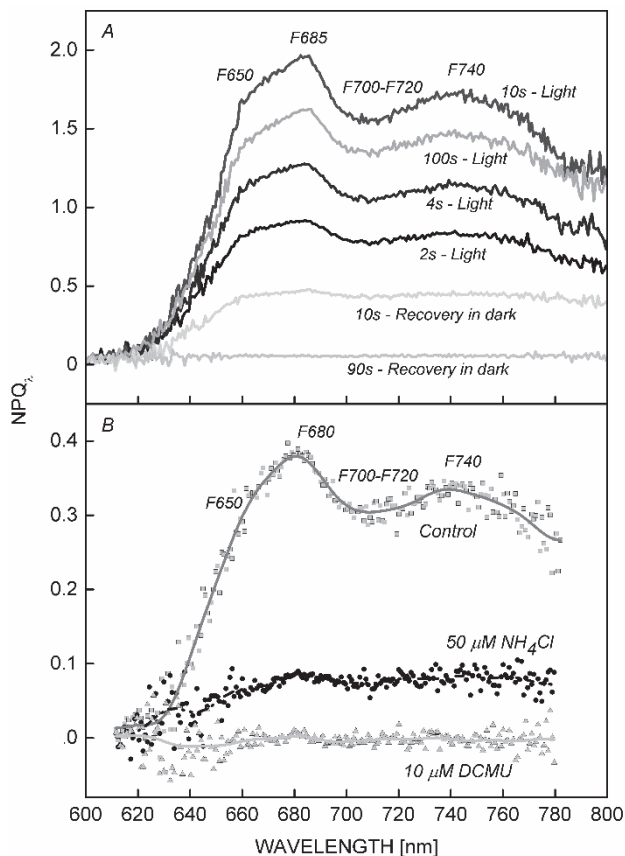


Fig. 2. Spectral variability of nonphotochemical quenching in *Rhodomonas salina* and *Chlorella* sp. NPQ was calculated based on Stern–Volmer equation from the SRFI data and defined the spectrally-resolved nonphotochemical quenching parameter NPQ_λ . (A): Induction of spectrally resolved NPQ on blue actinic light in cryptophyte algae *R. salina*, dark adapted cells were irradiated by blue actinic light [464 nm ; $1,000\text{ }\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$] and fluorescence changes were observed during light (for 100 s) and dark period after irradiation. (B): Effect of inhibitors for spectrally resolved NPQ detected in green algae *Chlorella* sp.. Data represent control cells, cells with NH_4Cl (50 μ M) and DCMU (5 μ M) added before measurements. The spectrally resolved NPQ was calculated after 100 s of blue light irradiation [464 nm , $300\text{ }\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$].

cryptophyte alga *R. salina* and the green alga *Chlorella* sp. (Fig. 2). In *Chlorella* sp., we proved that NPQ_λ is inhibited in the whole spectral range after addition of DCMU (PSII inhibitor) or ammonium chloride that efficiently inhibits NPQ in various algae (Ruban *et al.* 2004, Kotabová *et al.* 2011, Kaňa *et al.* 2012b). The other model organism, which we used, cryptophyte alga *R. salina*, photosynthetic algae from red clade of photosynthesis evolved from a red alga ancestor (Zimorski *et al.* 2014). This alga is a very useful model organism for studying NPQ_λ as it contains two types of antenna systems (MacPherson and Hiller 2003) with different fluorescence emission: (1) phycobiliproteins (typical for red algae and cyanobacteria) located in thylakoid membrane lumen with fluorescence emission peak at 589 nm, which are tightly packed in the thylakoid lumen (Kaňa *et al.* 2009b); (2) membrane-embedded Chl antennae typical for green algae and higher plants, emitting in the 670–740 nm range, with maximum at 681 nm (Kaňa *et al.* 2012b). The Chl *a/c* antenna complexes (CAC) of cryptophyte consist of Lhcr and Lhcz proteins (Büchel 2015), and there is no xanthophyll cycle (no violaxanthin, no diadinoxanthin) (Funk *et al.* 2011, Kaňa *et al.* 2012b) that would affect the pH-dependent NPQ (Kaňa *et al.* 2012b). Our data show kinetics of NPQ_λ evolution on light, and confirm previous observations that there is no NPQ in phycobiliproteins (see F589 in Fig. 2A) and the fluorescence is quenched mostly in Chl-binding proteins (660–800 nm) in line with previous results proposing NPQ locus into CAC antennae (Kaňa *et al.* 2012b). We further suggest that the high NPQ_λ in this range could be attributed to some changes in CAC aggregation as antennae aggregation results in a shift of fluorescence emission maxima (e.g., by 5 nm in LHCIIs of higher plants, see Johnson and Ruban 2009). The connection between the red shift of the F_{685} band and NPQ in antennae has been also proposed for intact leaves (Franck *et al.* 2005). However, a fluorescence quenching in the PSII inner antennae or in PSII RC cannot be excluded as a possible reason of NPQ_λ between F_{681} and F_{685} . It could include a quenching in the inner antennae, either by $P680^+$ (Bruce *et al.* 1997) or quenching in the inner PSII core antennae by Chl Z^+ (Komura *et al.* 2010, Miyake *et al.* 2011). Alternatively, some nonradiative dissipation directly in the reaction center has been also proposed as a mechanism of photoprotection (Cser and Vass 2007, Vass 2011). The importance of RC-type of quenching is species dependent, it has been proposed to be crucial in extremophilic red algae (Krupnik *et al.* 2013) and cyanobacteria (Ohad *et al.* 2010), in mesophilic red algae (Delphin *et al.* 1998), and in desiccated lichens (Komura *et al.* 2010, Miyake *et al.* 2011). On the other hand, it seems to be rather small in higher plants (Belgio *et al.* 2014). However, Fig. 2A indicates that emission from F_{685} is interconnected with F_{740} . It is visible in the similar changes in NPQ_λ at F_{685} and F_{740} at 4, 10, and 100 s. This seems to indicate energy equilibration and presence of one quenching mechanism across the whole emission band. In

any case, to assess the importance of the quenching mechanism in the RC of PSII in cryptophyte, more experiments are required. Interestingly, we have shown also high NPQ_λ in the blue-shifted region (between F₆₅₀–F₆₈₁) similarly with NPQ_λ observed for *C. velia* (Kotabová *et al.* 2014). We tend to think that the blue-shifted NPQ_λ could be partially attributed to the quenching in the weakly coupled Chl pigments of light-harvesting antennae (Santabarbara and Jennings 2005). These pigments were described for LHCIIs and are characterized by blue-shifted emission maxima (650–675 nm, *see, e.g.*, Santabarbara and Jennings 2005).

We have also repeatedly confirmed a small drop in NPQ_λ between 700–720 nm (Kaňa *et al.* 2012b, Kotabová *et al.* 2014), which is also visible here in the data for cryptophyte and green algae (Fig. 2A,B). Recently, it has been shown that the relative fluorescence increase in the red fluorescence components around 720 nm (Lambrev *et al.* 2010) is a characteristic marker of NPQ conditions *in vivo* for higher plants; in fact, the actual quenching locus is located around 682 nm (Lambrev *et al.* 2010, Miloslavina *et al.* 2008). The low importance of the fluorescence emission around 720 nm to actual NPQ has been confirmed also for Lhca antenna in red conformation because of their longest fluorescence lifetime (Passarini *et*

al. 2010). There, F₇₂₀ has been shown not to be involved in quenching, in contrast with antennae with blue-shifted fluorescence emission (F₆₈₁), which exhibits short fluorescence lifetimes. Similarly, a relative increase in the fluorescence of F₇₁₀ in comparison to F₆₈₅ component has been also observed for dry lichens in the quenched state (Miyake *et al.* 2011). As there is usually increase in the fluorescence between 700–720 nm during stimulation of NPQ, the decrease of NPQ_λ between 700–720 nm could be caused by this fluorescence rise. An alternative explanation could be a higher involvement of fluorescence originating from PSI core (or their antennae) in this range (Franck *et al.* 2002, Rizzo *et al.* 2014, Giovagnetti *et al.* 2015), that usually does not show any NPQ even though it has kind of variable fluorescence as shown in theoretical models (Lazár 2013). Finally, the most red-shifted NPQ_λ (the F740 quenching, Fig. 2) can be attributed to aggregated forms of CAC antenna complexes in line with previous data (Komura *et al.* 2010, Miyake *et al.* 2011). However, these two possible quenching loci seem to be tightly interconnected as quenching at F₆₈₅ and F₇₄₀ behave similarly during fluorescence induction (Fig. 2A). In conclusion, we have shown applicability of SRFI method to measure spectrally resolved NPQ_λ in green algae and in cryptophyte.

References

- Acuña A.M., Kaňa R., Gwizdala M. *et al.*: A method to decompose spectral changes in *Synechocystis* PCC 6803 during light-induced state transitions. – *Photosynth. Res.* **130**: 237–249, 2016.
- Belgio E., Duffy C.D.P., Ruban A.V.: Switching light harvesting complex II into photoprotective state involves the lumen-facing apoprotein loop. – *Phys. Chem. Chem. Phys.* **15**: 12253–12261, 2013.
- Belgio E., Kapitonova E., Chmeliov J. *et al.*: Economic photoprotection in photosystem II that retains a complete light-harvesting system with slow energy traps. – *Nat. Commun.* **5**: 4433, 2014.
- Bernat G., Steinbach G., Kaňa R. *et al.*: On the origin of the slow M to T chlorophyll a fluorescence decline in cyanobacteria: interplay of short-term light-responses. – *Photosynth. Res.* DOI: 10.1007/s11120-017-0458-8, *in press*, 2018.
- Bilger W., Björkman O.: Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. – *Photosynth. Res.* **25**: 173–185, 1990.
- Briantais J.M., Verrotte C., Picaud M. *et al.*: Quantitative study of the slow decline of chlorophyll alpha-fluorescence in isolated-chloroplasts. – *Biochim. Biophys. Acta* **548**: 128–138, 1979.
- Bruce D., Samson G., Carpenter C.: The origins of nonphotochemical quenching of chlorophyll fluorescence in photosynthesis. Direct quenching by P680+ in photosystem II enriched membranes at low pH. – *Biochemistry* **36**: 749–755, 1997.
- Büchel C.: Evolution and function of light harvesting proteins. – *J. Plant Physiol.* **172**: 62–75, 2015.
- Cheregi O., Kotabová E., Prášil O. *et al.*: Presence of state transitions in the cryptophyte alga *Guillardia theta*. – *J. Exp. Bot.* **66**: 6461–6470, 2015.
- Cser K., Vass I.: Radiative and non-radiative charge recombination pathways in Photosystem II studied by thermoluminescence and chlorophyll fluorescence in the cyanobacterium *Synechocystis* 6803. – *BBA-Bioenergetics* **1767**: 233–243, 2007.
- Delphin E., Duval J.C., Etienne A.L. *et al.*: Delta pH-dependent photosystem II fluorescence quenching induced by saturating, multiturnover pulses in red algae. – *Plant Physiol.* **118**: 103–113, 1998.
- Demmig-Adams B., Garab G., Adams III W. *et al.*: Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria. Springer, Dordrecht 2014.
- Derks A., Schaven K., Bruce D.: Diverse mechanisms for photoprotection in photosynthesis. Dynamic regulation of photosystem II excitation in response to rapid environmental change. – *BBA-Bioenergetics* **1847**: 468–485, 2015.
- Franck F., Dewez D., Popovic R.: Changes in the room-temperature emission spectrum of chlorophyll during fast and slow phases of the Kautsky effect in intact leaves. – *Photoch. Photobio.* **81**: 431–436, 2005.
- Franck F., Juneau P., Popovic R.: Resolution of the Photosystem I and Photosystem II contributions to chlorophyll fluorescence of intact leaves at room temperature. – *BBA-Bioenergetics* **1556**: 239–246, 2002.
- Funk C., Alami M., Tibiletti T. *et al.*: High light stress and the one-helix LHC-like proteins of the cryptophyte *Guillardia theta*. – *BBA-Bioenergetics* **1807**: 841–846, 2011.
- Gilmore A.M., Yamamoto H.Y.: Dark induction of zeaxanthin-dependent nonphotochemical fluorescence quenching mediated by ATP. – *P. Natl. Acad. Sci. USA* **89**: 1899–1903, 1992.

- Giovagnetti V., Ware M.A., Ruban A.V.: Assessment of the impact of photosystem I chlorophyll fluorescence on the pulse-amplitude modulated quenching analysis in leaves of *Arabidopsis thaliana*. – Photosynth. Res. **125**: 179-189, 2015.
- Holzwarth A.R., Lenk D., Jahns P.: On the analysis of non-photochemical chlorophyll fluorescence quenching curves: I. Theoretical considerations. – BBA-Bioenergetics **1827**: 786-792, 2013.
- Holzwarth A.R., Miloslavina Y., Nilkens M. *et al.*: Identification of two quenching sites active in the regulation of photosynthetic light-harvesting studied by time-resolved fluorescence. – Chem. Phys. Lett. **483**: 262-267, 2009.
- Johnson M.P., Ruban A.V.: Photoprotective Energy dissipation in higher plants involves alteration of the excited state energy of the emitting chlorophyll(s) in the light harvesting antenna II (LHCII). – J. Biol. Chem. **284**: 23592-23601, 2009.
- Kaftan D., Trtilek M., Kroon B. *et al.*: Fast-response double-modulation fluorometer. – In: Garab G. (ed.): International Congress on Photosynthesis, Vol. 5. Pp. 4297-4300. Kluwer Academic Publishers, Budapest 1997.
- Kaňa R., Govindjee: Role of ions in the regulation of light-harvesting. – Front. Plant Sci. **7**: 1849, 2016.
- Kaňa R., Kotabová E., Komárek O. *et al.*: The slow S to M fluorescence rise in cyanobacteria is due to a state 2 to state 1 transition. – BBA-Bioenergetics **1817**: 1237-1247, 2012a.
- Kaňa R., Kotabová E., Kopečná J. *et al.*: Violaxanthin inhibits nonphotochemical quenching in light-harvesting antenna of *Chromera velia*. – FEBS Lett. **590**: 1076-1085, 2016.
- Kaňa R., Kotabová E., Sobotka R. *et al.*: Non-photochemical quenching in cryptophyte alga *Rhodomonas salina* is located in chlorophyll *a/c* antennae. – PLoS ONE **7**: e29700, 2012b.
- Kaňa R., Prášil O., Komárek O. *et al.*: Spectral characteristic of fluorescence induction in a model cyanobacterium, *Synechococcus* sp (PCC 7942). – Biochim. Biophys. Acta **1787**: 1170-1178, 2009a.
- Kaňa R., Prášil O., Mullineaux C.W.: Immobility of phycobilins in the thylakoid lumen of a cryptophyte suggests that protein diffusion in the lumen is very restricted. – FEBS Lett. **583**: 670-674, 2009b.
- Kaňa R., Vass I.: Thermoimaging as a tool for studying light-induced heating of leaves: Correlation of heat dissipation with the efficiency of photosystem II photochemistry and non-photochemical quenching. – Environ. Exp. Bot. **64**: 90-96, 2008.
- Komura M., Yamagishi A., Shibata Y. *et al.*: Mechanism of strong quenching of photosystem II chlorophyll fluorescence under drought stress in a lichen, *Physciella melanchla*, studied by subpicosecond fluorescence spectroscopy. – BBA-Bioenergetics **1797**: 331-338, 2010.
- Kotabová E., Jarešova J., Kaňa R. *et al.*: Novel type of red-shifted chlorophyll *a* antenna complex from *Chromera velia*. I. Physiological relevance and functional connection to photosystems. – Biochim. Biophys. Acta **1837**: 734-743, 2014.
- Kotabová E., Kaňa R., Jarešova J. *et al.*: Non-photochemical fluorescence quenching in *Chromera velia* is enabled by fast violaxanthin de-epoxidation. – FEBS Lett. **585**: 1941-1945, 2011.
- Kramer D.M., Johnson G., Kiirats O. *et al.*: New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. – Photosynth. Res. **79**: 209-218, 2004.
- Krüger T.P.J., Iliaia C., Johnson M.P. *et al.*: The specificity of controlled protein disorder in the photoprotection of plants. – Biophys. J. **105**: 1018-1026, 2013.
- Krüger T.P.J., Wientjes E., Croce R. *et al.*: Conformational switching explains the intrinsic multifunctionality of plant light-harvesting complexes. – P. Natl. Acad. Sci. USA **108**: 13516-13521, 2011.
- Krupnik T., Kotabová E., van Bezouwen L.S. *et al.*: A reaction centre-dependent photoprotection mechanism in a highly robust photosystem II from an extremophilic red alga *Cyanidioschyzon merolae*. – J. Biol. Chem. **288**: 23529-23542, 2013.
- Kulasek M., Bernacki M.J., Ciszak K. *et al.*: Contribution of PsbS Function and stomatal conductance to foliar temperature in higher plants. – Plant Cell Physiol. **57**: 1495-1509, 2016.
- Lakowicz J.R.: Principles of Fluorescence Spectroscopy. Springer, New York 2006.
- Lambrev P.H., Nilkens M., Miloslavina Y. *et al.*: Kinetic and spectral resolution of multiple nonphotochemical quenching components in *Arabidopsis* leaves. – Plant Physiol. **152**: 1611-1624, 2010.
- Lazár D.: Simulations show that a small part of variable chlorophyll *a* fluorescence originates in photosystem I and contributes to overall fluorescence rise. – J. Theor. Biol. **335**: 249-264, 2013.
- Lazár D.: Parameters of photosynthetic energy partitioning. – J. Plant Physiol. **175**: 131-147, 2015.
- Li X.P., Björkman O., Shih C. *et al.*: A pigment-binding protein essential for regulation of photosynthetic light harvesting. – Nature **403**: 391-395, 2000.
- MacPherson A.N., Hiller R.G.: Algae with chlorophyll *c* in light-harvesting antennae in photosynthesis. – In: Green B.R. and Parson W.W. (ed.): Light-Harvesting Antennas in Photosynthesis. Pp. 323-352. Kluwer Acad. Publ., Dordrecht 2003.
- Miloslavina Y., Wehner A., Lambrev P.H. *et al.*: Far-red fluorescence: A direct spectroscopic marker for LHCII oligomer formation in non-photochemical quenching. – FEBS Lett. **582**: 3625-3631, 2008.
- Miyake H., Komura M., Itoh S. *et al.*: Multiple dissipation components of excess light energy in dry lichen revealed by ultrafast fluorescence study at 5 K. – Photosynth. Res. **110**: 39-48, 2011.
- Niyogi K.K., Björkman O., Grossman A.R.: The roles of specific xanthophylls in photoprotection. – P. Natl. Acad. Sci. USA **94**: 14162-14167, 1997.
- Novotný J.P., Chugtai A.A., Kostrouchová M. *et al.*: *Trichoplax adhaerens* reveals a network sensitive to 9-cis-retinoic acid at the base of metazoan evolution. – Peer J. **5**: e3789, 2017.
- Ohad I., Raanan H., Keren N. *et al.*: Light-Induced changes within photosystem ii protects microcoleus sp in biological desert sand crusts against excess light. – PLoS ONE **5**: e11000, 2010.
- Papageorgiou G.C., Govindjee: Chlorophyll *a* Fluorescence. A Signature of Photosynthesis. Springer, Dordrecht 2004.
- Passarini F., Wientjes E., van Amerongen H. *et al.*: Photosystem I light-harvesting complex Lhca4 adopts multiple conformations: Red forms and excited-state quenching are mutually exclusive. – BBA-Bioenergetics **1797**: 501-508, 2010.
- Rizzo F., Zucchelli G., Jennings R. *et al.*: Wavelength dependence of the fluorescence emission under conditions of open and closed Photosystem II reaction centres in the green alga *Chlorella sorokiniana*. – BBA-Bioenergetics **1837**: 726-733, 2014.
- Ruban A.V., Horton P.: Spectroscopy of nonphotochemical and photochemical quenching of chlorophyll fluorescence in leaves; evidence for a role of the light-harvesting complex of

- photosystem-ii in the regulation of energy dissipation. – *Photosynth. Res.* **40**: 181-190, 1994.
- Ruban A.V., Johnson M.P., Duffy C.D.P.: The photoprotective molecular switch in the photosystem II antenna. – *BBA-Bioenergetics* **1817**: 167-181, 2012.
- Ruban A.V., Lavaud J., Rousseau B. *et al.*: The super-excess energy dissipation in diatom algae: comparative analysis with higher plants. – *Photosynth. Res.* **82**: 165-175, 2004.
- Santabarbara S., Jennings R.C.: The size of the population of weakly coupled chlorophyll pigments involved in thylakoid photoinhibition determined by steady-state fluorescence spectroscopy. – *BBA-Bioenergetics* **1709**: 138-149, 2005.
- Schreiber U.: Pulse-Amplitude-Modulation (PAM) fluorometry and saturation pulse method: An overview. – In: Papageorgiou G.C. and Govindjee (ed.): *Chlorophyll *a* Fluorescence: A Signature of Photosynthesis*. Pp. 279-319. Springer, Dordrecht 2004.
- Vass I.: Role of charge recombination processes in photodamage and photoprotection of the photosystem II complex. – *Physiol. Plantarum* **142**: 6-16, 2011.
- Xu P.Q., Tian L.J., Klotz M. *et al.*: Molecular insights into zeaxanthin-dependent quenching in higher plants. – *Sci. Rep.* **5**: 13679, 2015.
- Zimorski V., Ku C., Martin W.F. *et al.*: Endosymbiotic theory for organelle origins. – *Curr. Opin. Microbiol.* **22**: 38-48, 2014.