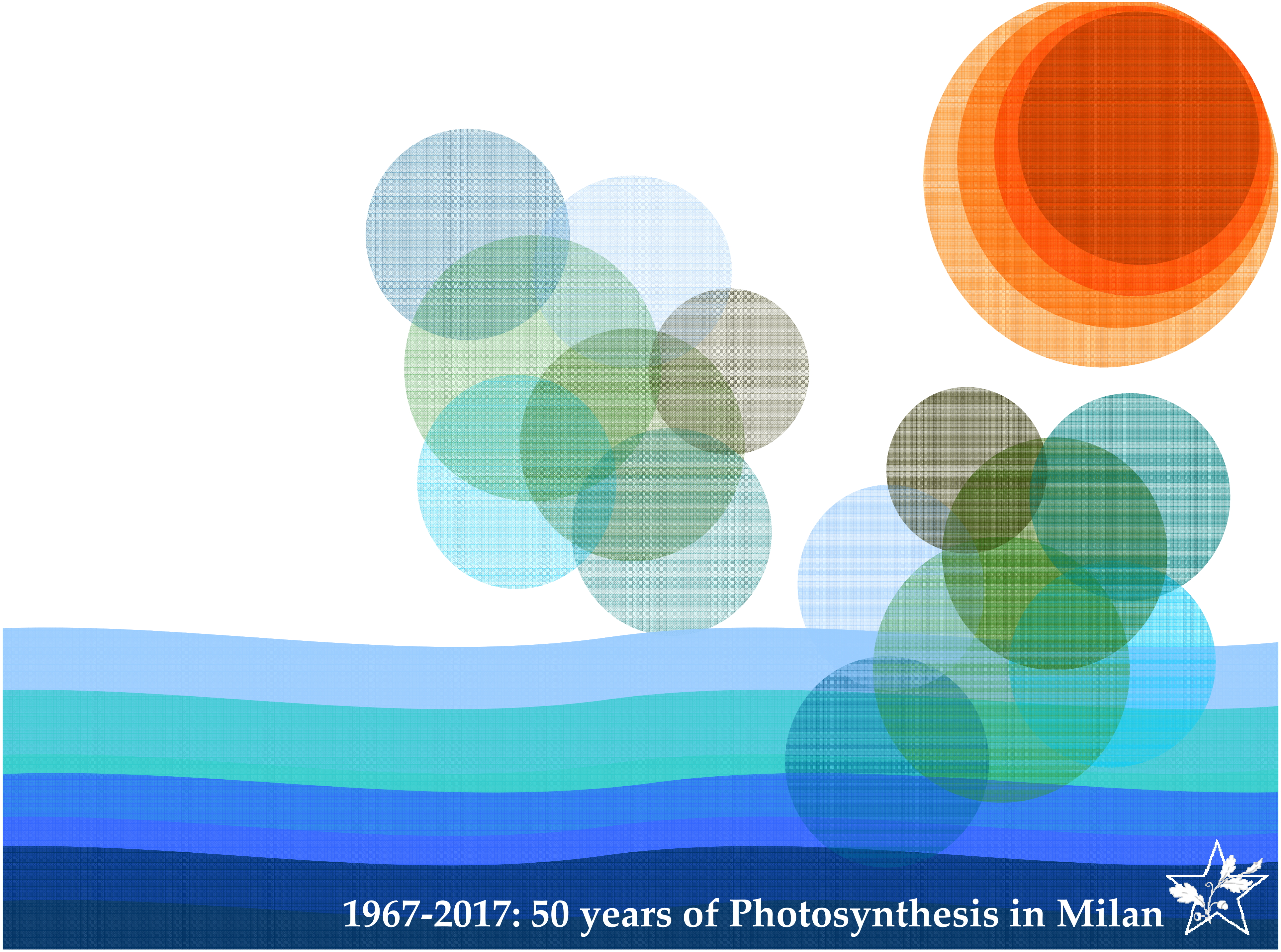
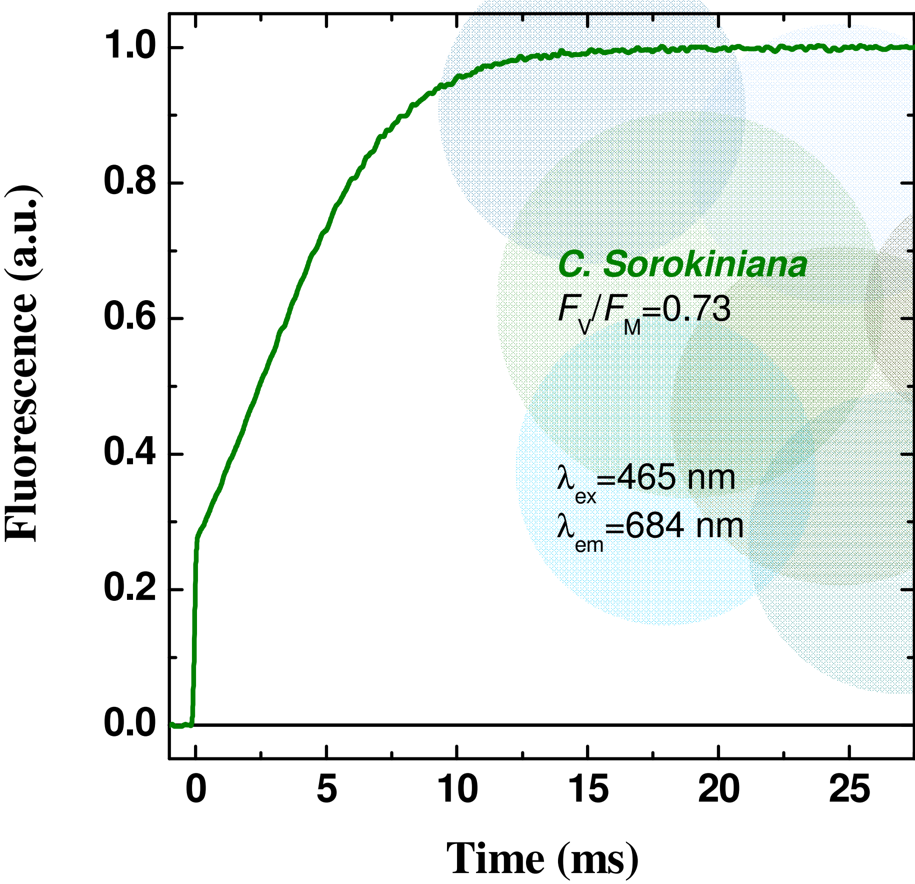

1967-2017: 50 years of Photosynthesis in Milan

Comparative excitation-emission dependence of the F_V/F_M ratio in model green algae and cyanobacterial strains.

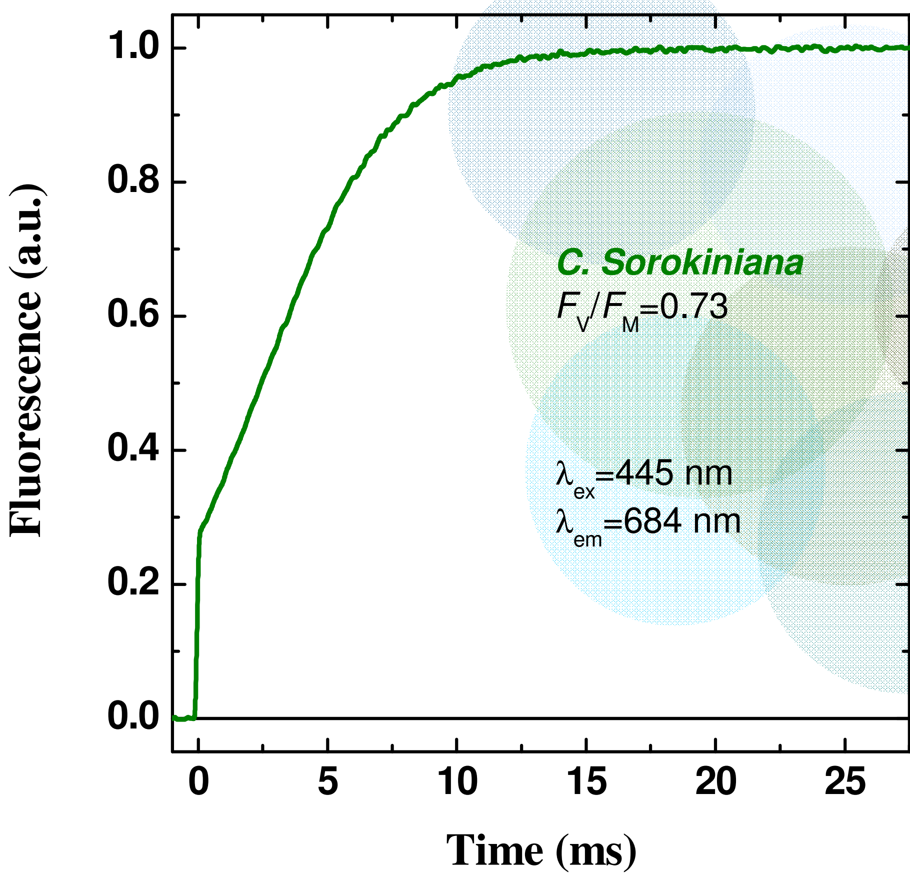
Stefano Santabarbara
Photosynthetic Research Unit
Institute of Biophysics,
National Research Council
stefano.santabarbara@cnr.it

F_V/F_M a rapid and simple indicator of PSII quantum yield



$$\frac{F_V}{F_M} \simeq \Phi_{PSII}^{Max} = \frac{K_{PC}}{\sum_i k_i + K_{PC}}$$

F_V/F_M a rapid and simple indicator of PSII quantum yield



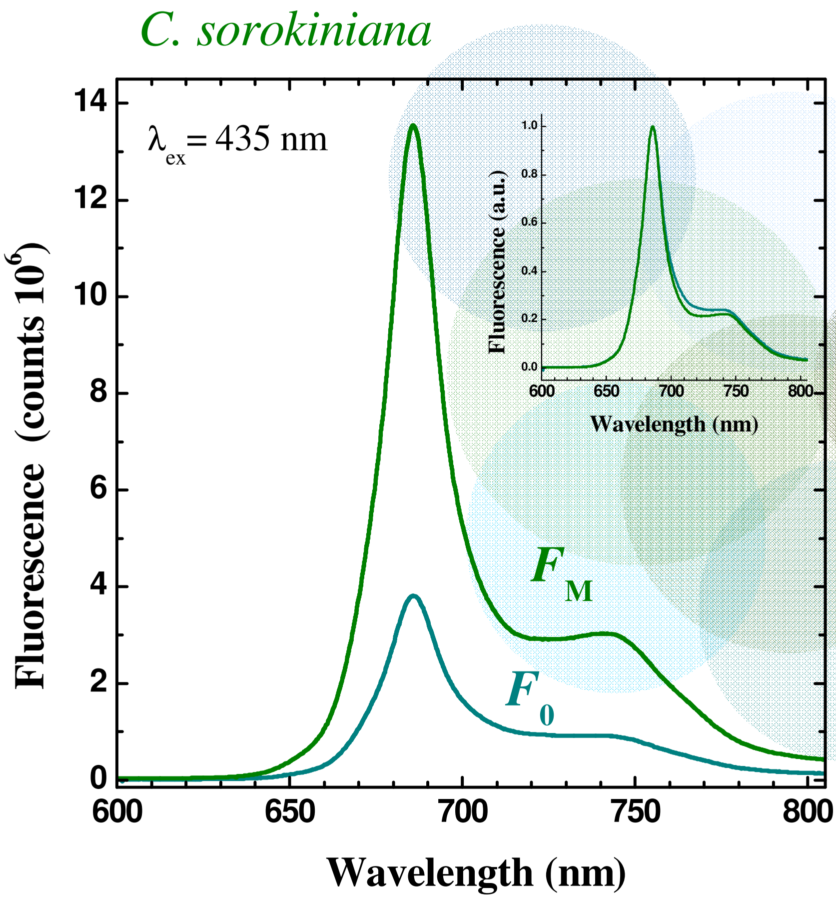
$$\frac{F_V}{F_M} \simeq \Phi_{PSII}^{Max} = \frac{K_{PC}}{\sum_i k_i + K_{PC}}$$

Caveats:

o emission is exclusively (or almost) from PSII

o quantum yields are independent on excitation and emission wavelengths.

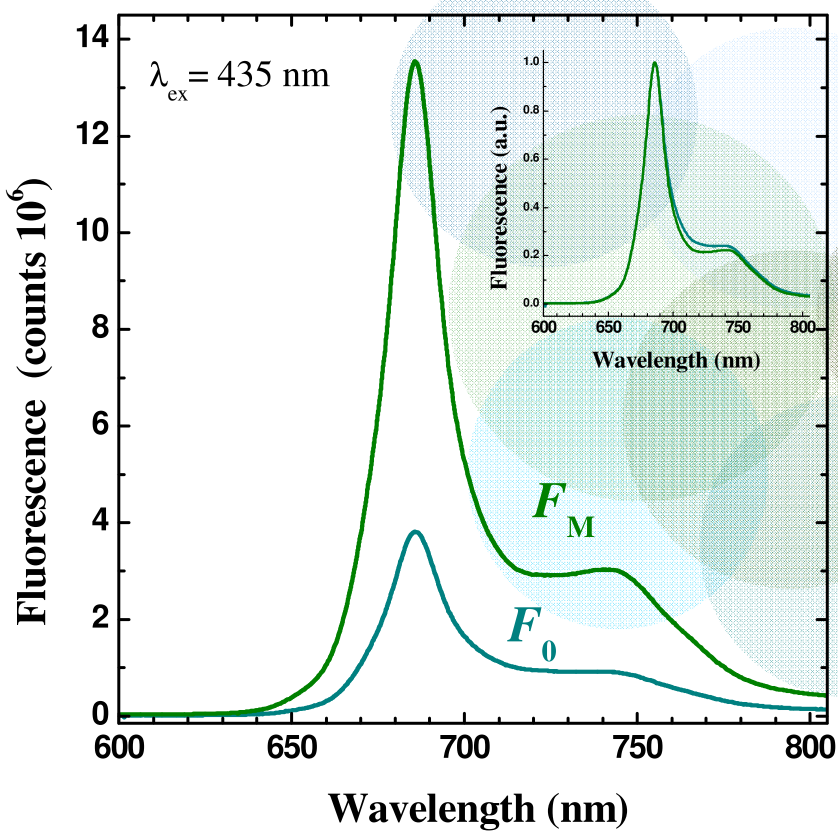
F_V/F_M spectral dependence: emission

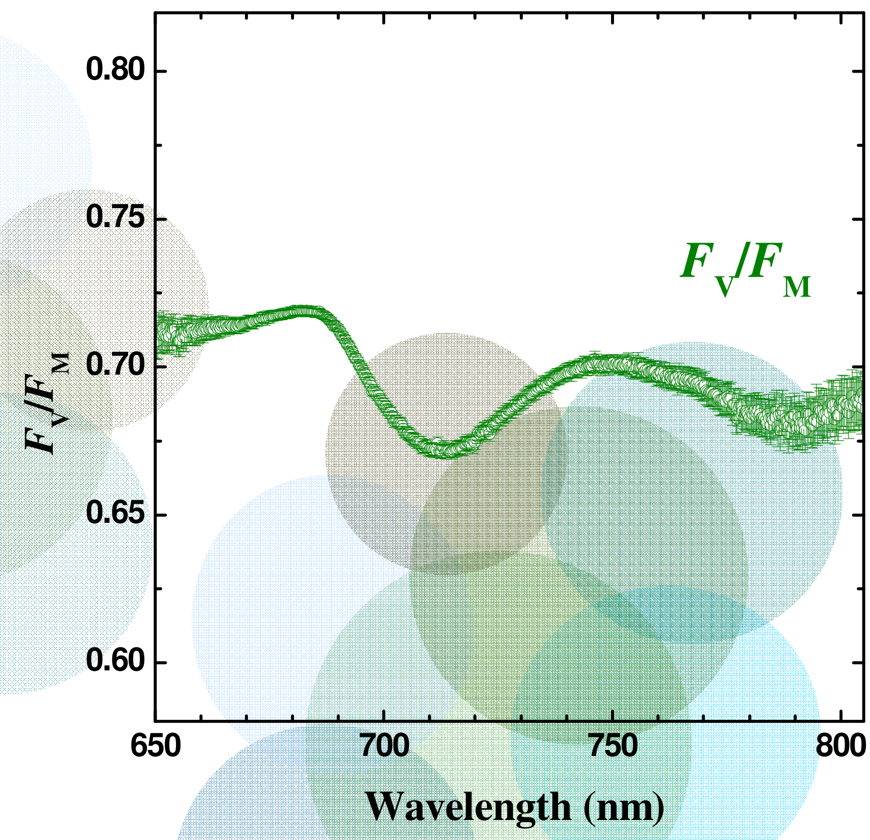


o **limited spectra variation between F_0 and F_M**

F_V/F_M spectral dependence: emission

C. sorokiniana

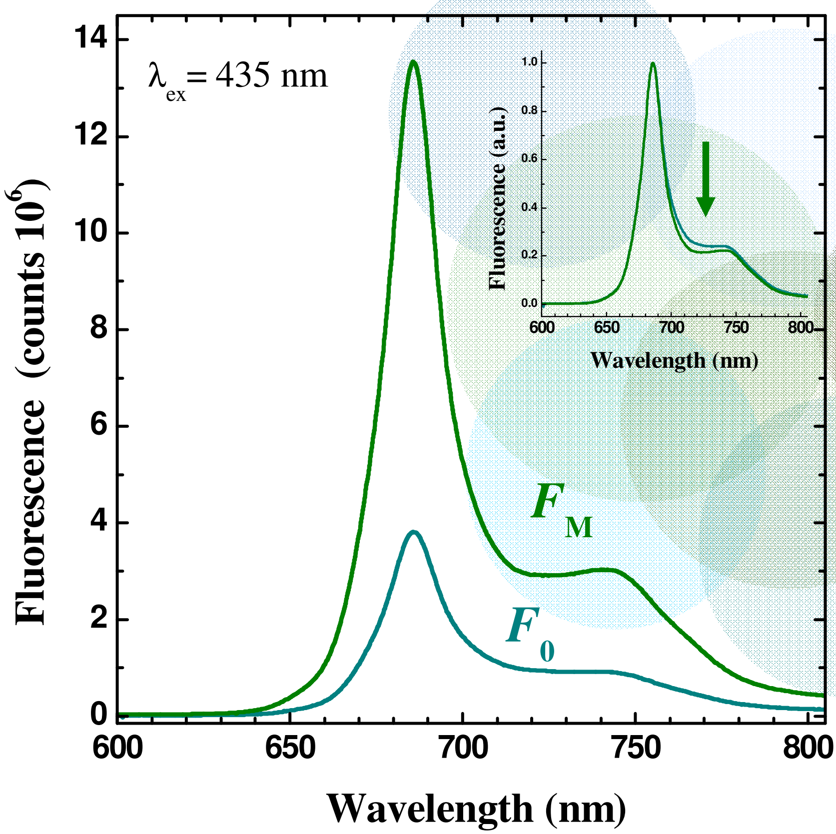


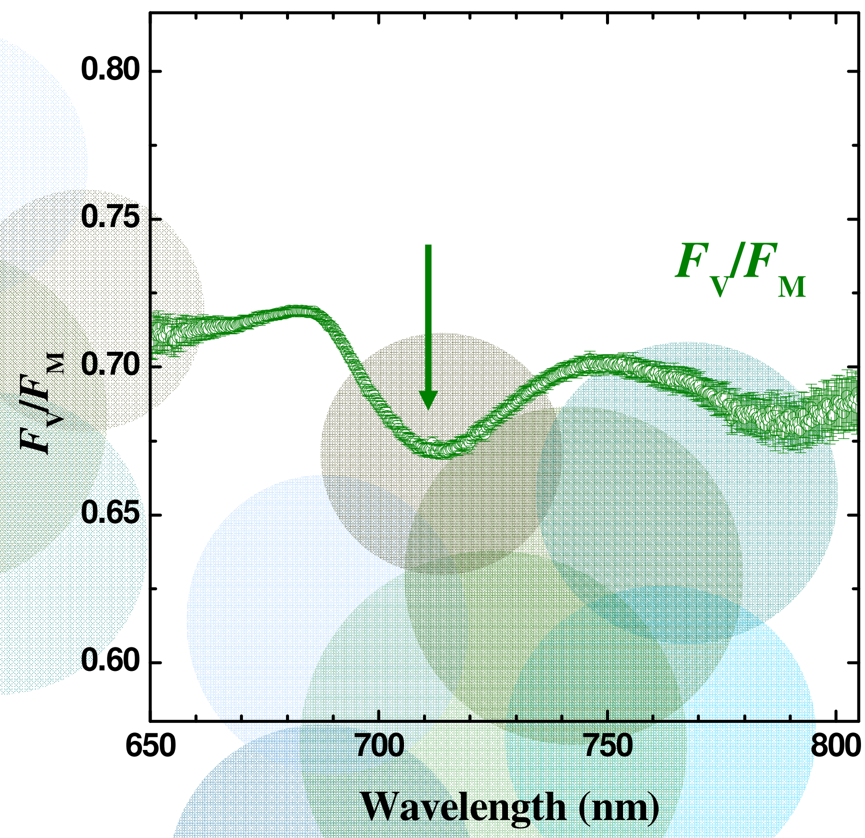


- limited spectra variation between F_0 and F_M
- also limited spectral dependence of F_V/F_M

F_V/F_M spectral dependence: emission

C. sorokiniana

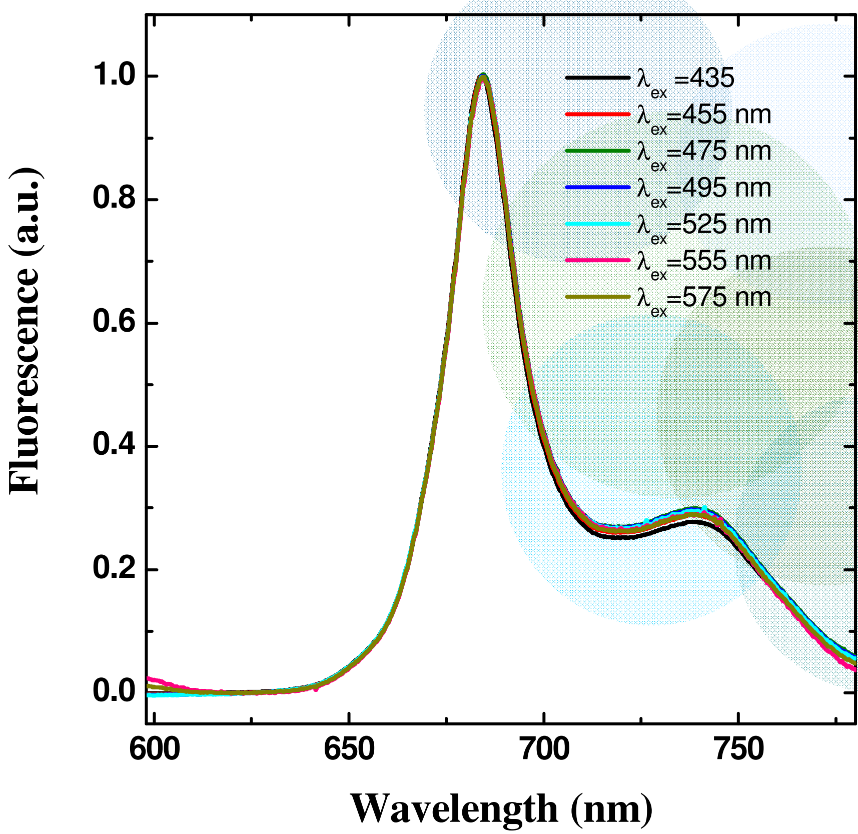




- limited spectra variation between F_0 and F_M
- also limited spectral dependence of F_V/F_M
- largely due to PSI emission

F_V/F_M spectral dependence: excitation

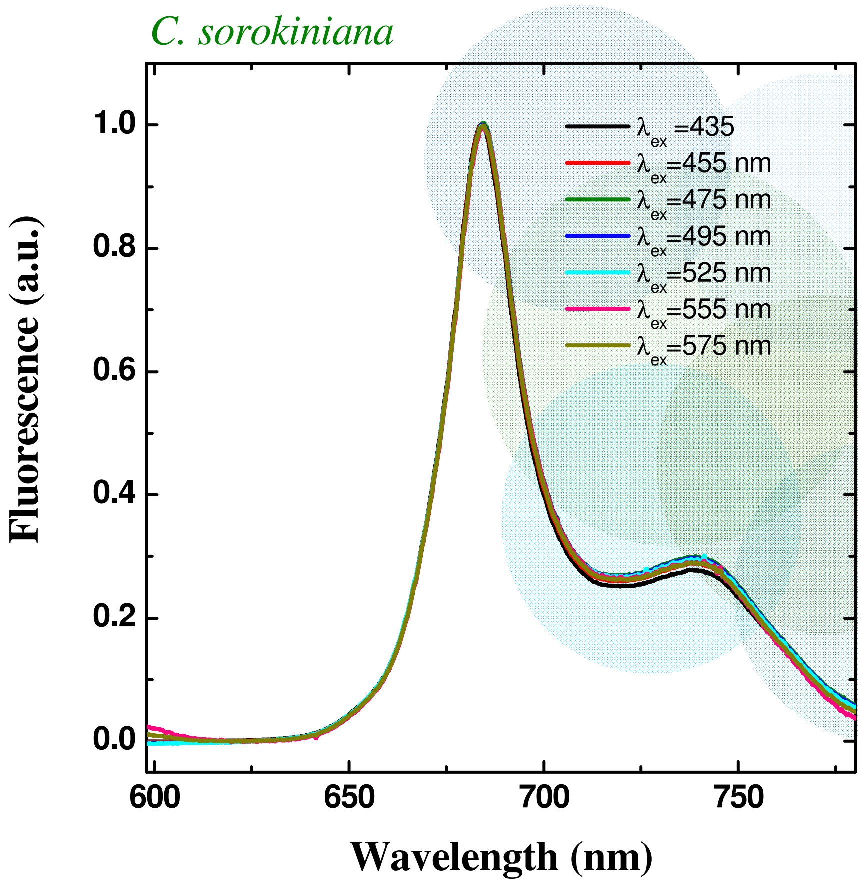
C. sorokiniana



For green algae
(same in *C. reinhardtii*)

- **very limited excitation wavelength dependence.**
- **limited spectra variation between F_0 and F_M**
- **limited spectral dependence of F_V/F_M (due to PSI emission)**

F_V/F_M spectral dependence: excitation



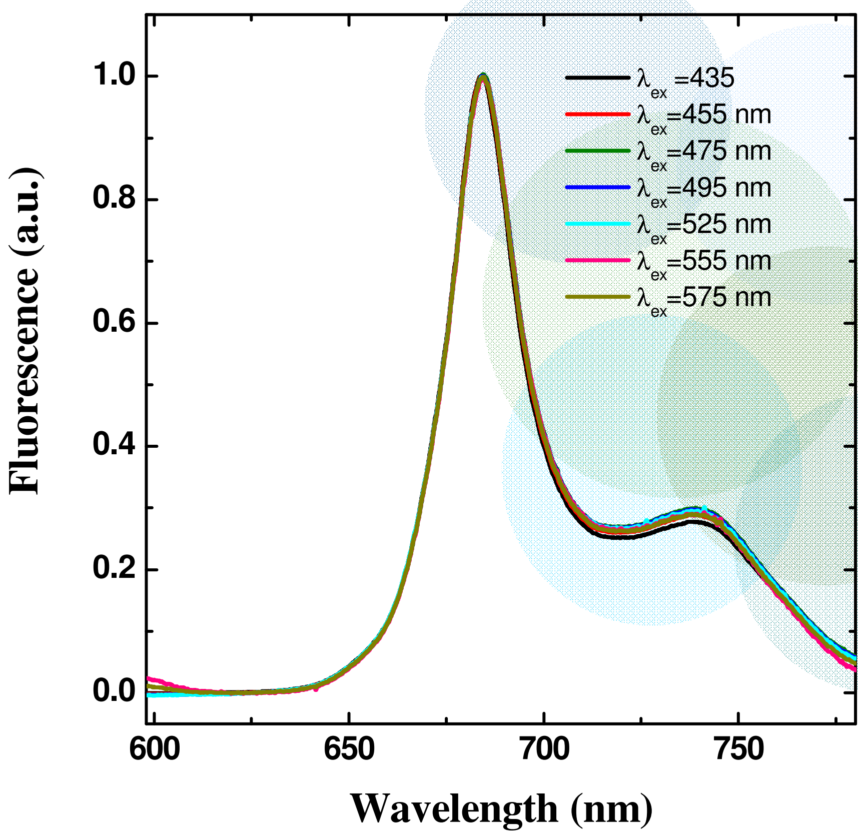
For green algae
(same in C. reinhardtii)

- **very limited excitation wavelength dependence.**
- **limited spectra variation between F_0 and F_M**
- **limited spectral dependence of F_V/F_M (due to PSI emission)**

$$\frac{F_V}{F_M} \simeq \Phi_{PSII}^{Max} = \frac{K_{PC}}{\sum_i k_i + K_{PC}}$$

F_V/F_M spectral dependence: excitation

C. sorokiniana



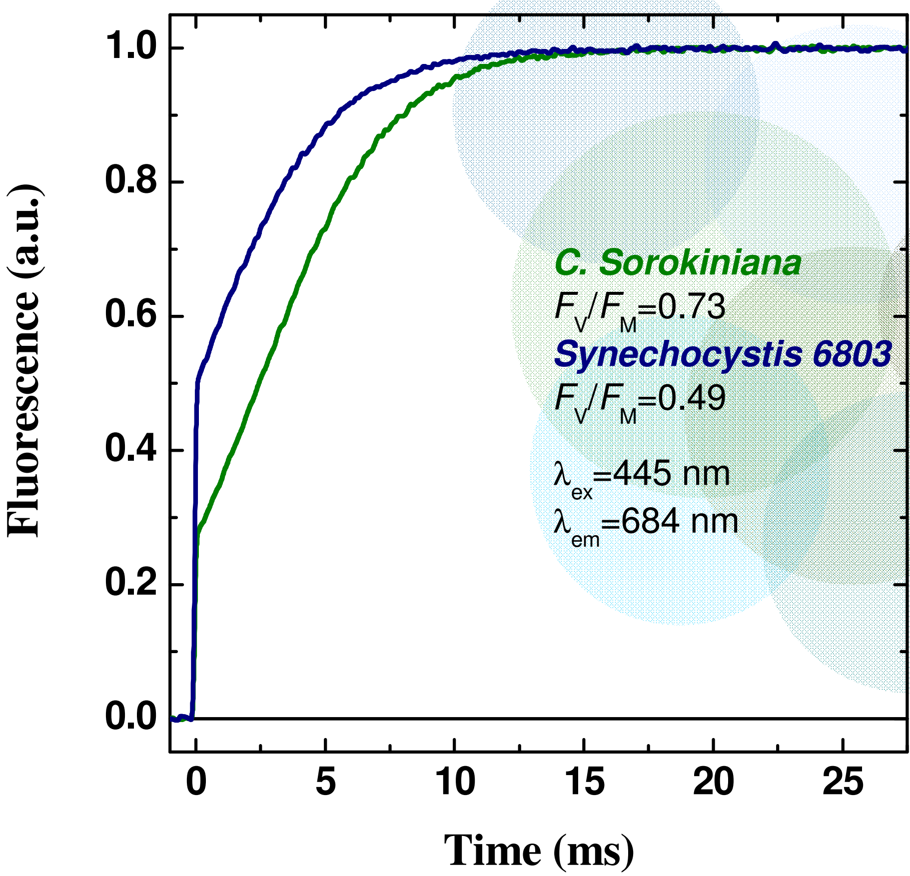
For green algae (same in C. reinhardtii)

- **very limited excitation wavelength dependence.**
- **limited spectra variation between F_0 and F_M**
- **limited spectral dependence of F_V/F_M (due to PSI emission)**

$$\frac{F_V}{F_M} \simeq \Phi_{PSII}^{Max} = \frac{K_{PC}}{\sum_i k_i + K_{PC}}$$

$$\frac{F_V}{F_M}(\lambda_{em}) \simeq \frac{(\phi_m - \phi_m)\cdot \rho_{II}(\lambda_{em})}{\phi_m \cdot \rho_{II}(\lambda_{em}) + \phi_i \cdot \rho_I(\lambda_{em})}$$

F_V/F_M: comparison with cyanobacteria



comparison:

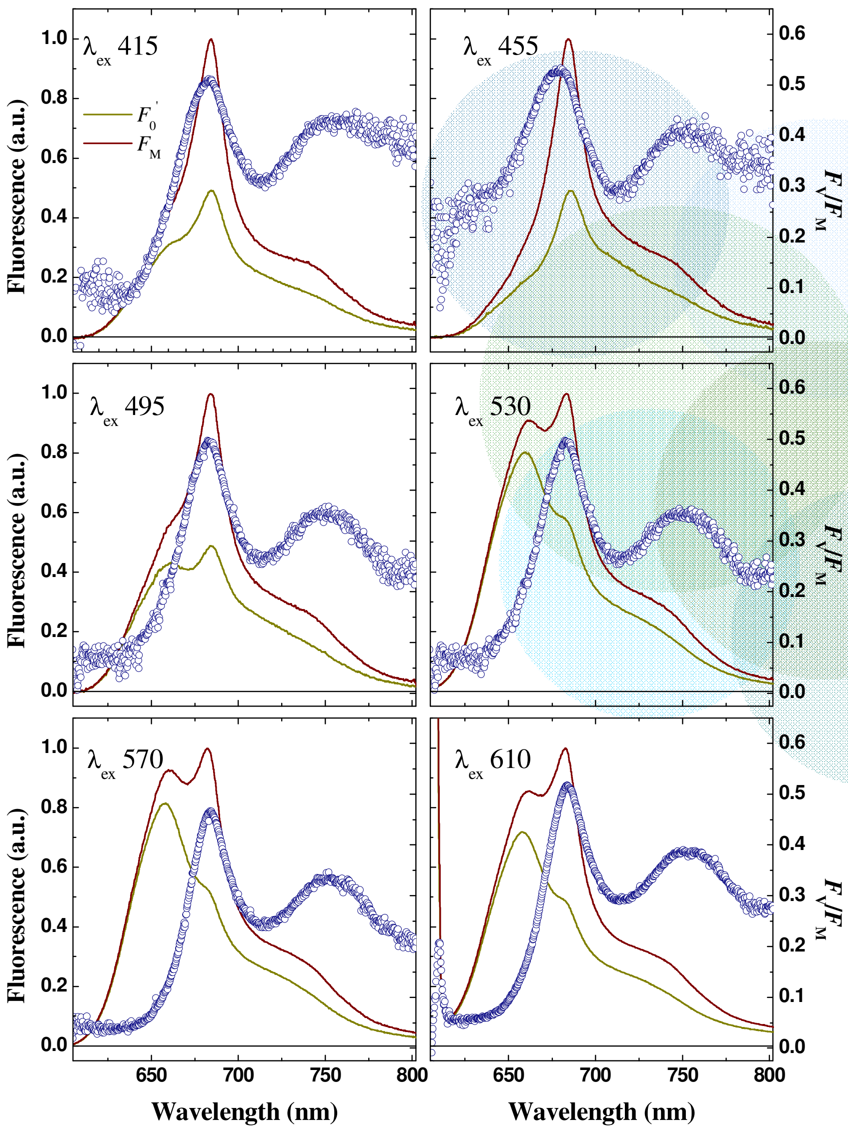
- **lower F_V/F_M (down to 0.2-0.3)**
- **also from literature data**

How comes? Is

$$\frac{F_V}{F_M} \simeq \Phi_{PSII}^{Max} = \frac{K_{PC}}{\sum_i k_i + K_{PC}}$$

not valid?

F_V/F_M spectral dependence: emission/excitation



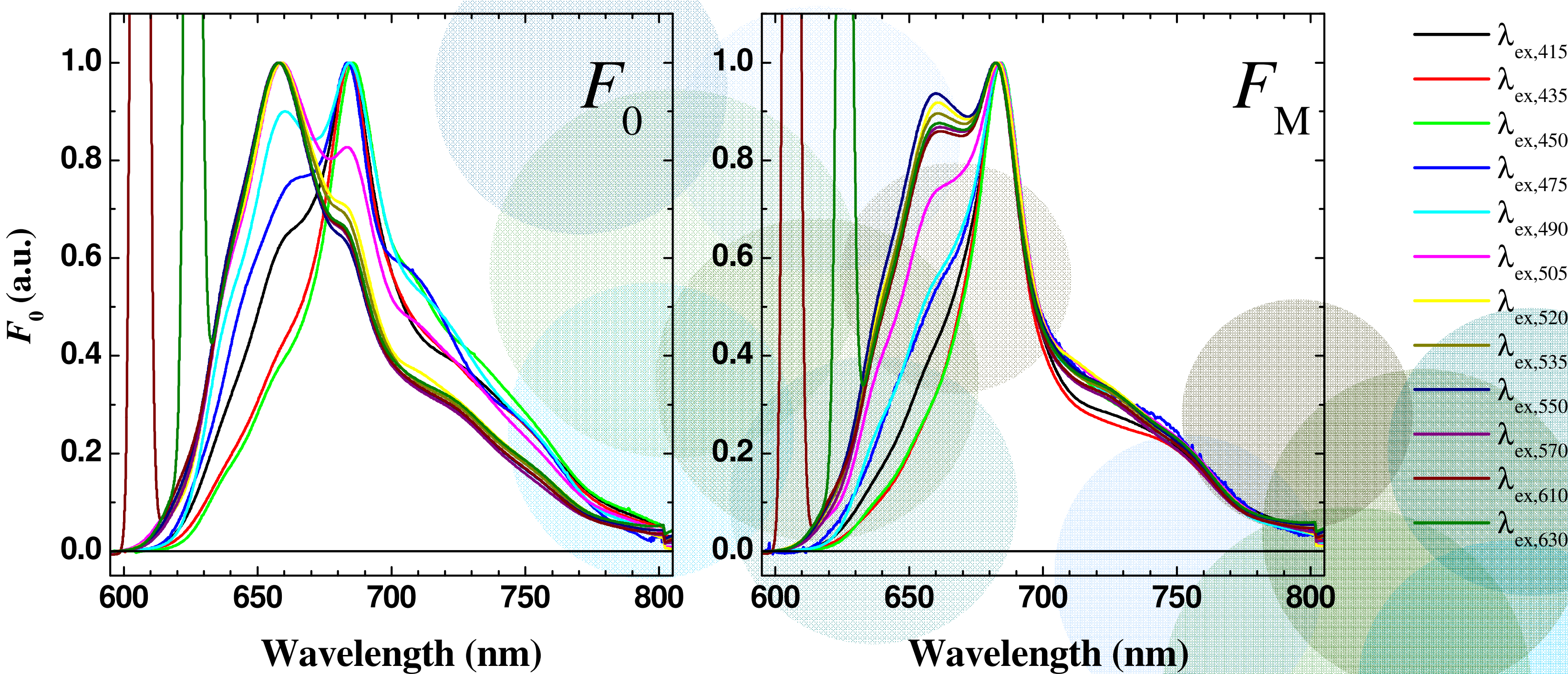
In *Synechocytis sp.* PCC6803

o large spectral variation between F_0 and F_M

o the F_V/F_M ratio is largely dependent on BOTH the excitation and the emission wavelength

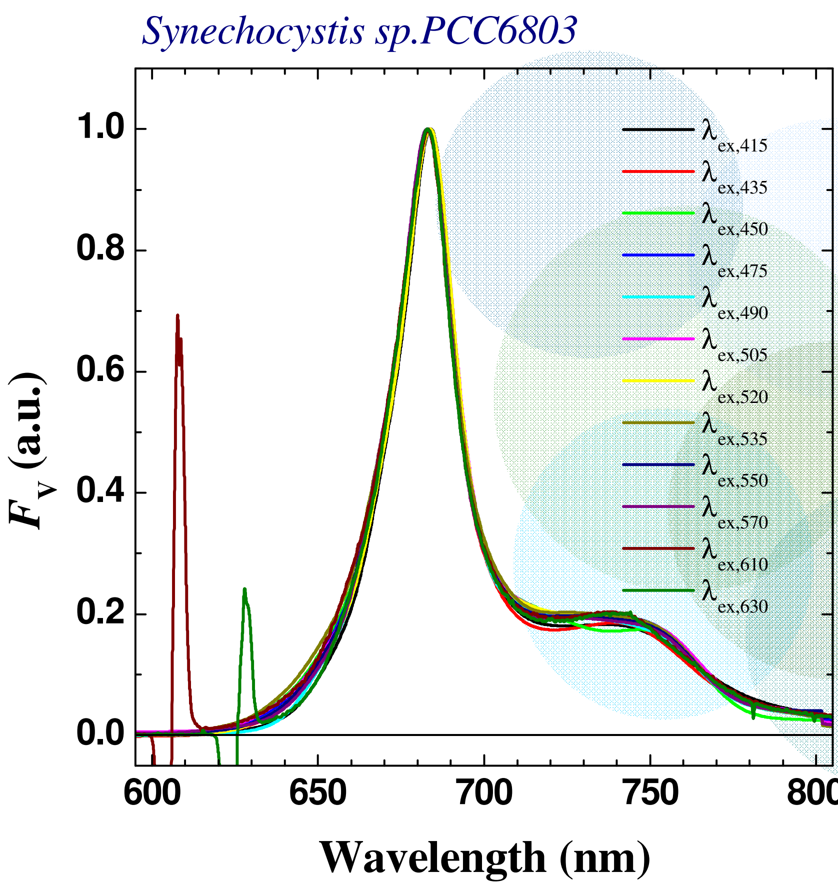
F_V/F_M spectral dependence: emission/excitation

Synechocystis sp. PCC6803



- both F_0 and F_M spectra depend on the excitation wavelength

F_V/F_M spectral dependence: emission/excitation



In *Synechocytis sp.* PCC6803

o large spectral variation between F_0 and F_M

o both F_0 and F_M spectra depend on the excitation wavelength

o the F_V/F_M ratio is largely dependent on BOTH the excitation and the emission wavelength

but

o the F_V spectra are (close to) excitation wavelength independent

F_V/F_M spectral dependence: how to rationalise it?

Considering thee independent emitting components

- **PSII-PBS super-complexes (showing variable fluorescence)**
- **PSI(-PBS) super-complexes (no variable fluorescence)**
- **an uncoupled population of PBS (no variable fluorescence)**

$$F_{PSII(0,M)}(\lambda_{em},\lambda_{ex}) = \phi_{II,(0/M)}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em})$$

$$F_{PSI}(\lambda_{em},\lambda_{ex}) = \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em})$$

$$F_{PBU}(\lambda_{em},\lambda_{ex}) = \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho$$

F_V/F_M spectral dependence: how to rationalise it?

Considering thee independent emitting components

- **PSII-PBS super-complexes (showing variable fluorescence)**
- **PSI(-PBS) super-complexes (no variable fluorescence)**
- **an uncoupled population of PBS (no variable fluorescence)**

$$F_{PSII(0,M)}(\lambda_{em},\lambda_{ex}) = \phi_{II,(0/M)}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em})$$

$$F_{PSI}(\lambda_{em},\lambda_{ex}) = \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em})$$

$$F_{PBU}(\lambda_{em},\lambda_{ex}) = \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho$$

then

$$\begin{cases} F_0(\lambda_{em},\lambda_{ex}) = \phi_{II,0}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) + \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em}) + \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho_{PBU}(\lambda_{em}) \\ F_M(\lambda_{em},\lambda_{ex}) = \phi_{II,M}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) + \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em}) + \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho_{PBU}(\lambda_{em}) \end{cases}$$

F_V/F_M spectral dependence: how to rationalise it?

Considering thee independent emitting components

- **PSII-PBS super-complexes (showing variable fluorescence)**
- **PSI(-PBS) super-complexes (no variable fluorescence)**
- **an uncoupled population of PBS (no variable fluorescence)**

$$F_{PSII(0,M)}(\lambda_{em},\lambda_{ex}) = \phi_{II,(0/M)}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em})$$

$$F_{PSI}(\lambda_{em},\lambda_{ex}) = \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em})$$

$$F_{PBU}(\lambda_{em},\lambda_{ex}) = \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho$$

then

$$\begin{cases} F_0(\lambda_{em},\lambda_{ex}) = \phi_{II,0}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) + \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em}) + \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho_{PBU}(\lambda_{em}) \\ F_M(\lambda_{em},\lambda_{ex}) = \phi_{II,M}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) + \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em}) + \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho_{PBU}(\lambda_{em}) \end{cases}$$

and

$$\begin{cases} F_V(\lambda_{em},\lambda_{ex}) = (\phi_{II,M}-\phi_{II,0})\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) \\ \dfrac{F_V}{F_M}(\lambda_{em},\lambda_{ex}) = \dfrac{(\phi_{II,M}-\phi_{II,0})\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em})}{\phi_{II,M}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) + \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em}) + \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho_{PBU}(\lambda_{em})} \end{cases}$$

F_V/F_M spectral dependence: how to rationalise it?

Considering thee independent emitting components

- **PSII-PBS super-complexes (showing variable fluorescence)**
- **PSI(-PBS) super-complexes (no variable fluorescence)**
- **an uncoupled population of PBS (no variable fluorescence)**

$$F_{PSII(0,M)}(\lambda_{em},\lambda_{ex}) = \phi_{II,(0/M)}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em})$$

$$F_{PSI}(\lambda_{em},\lambda_{ex}) = \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em})$$

$$F_{PBU}(\lambda_{em},\lambda_{ex}) = \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho$$

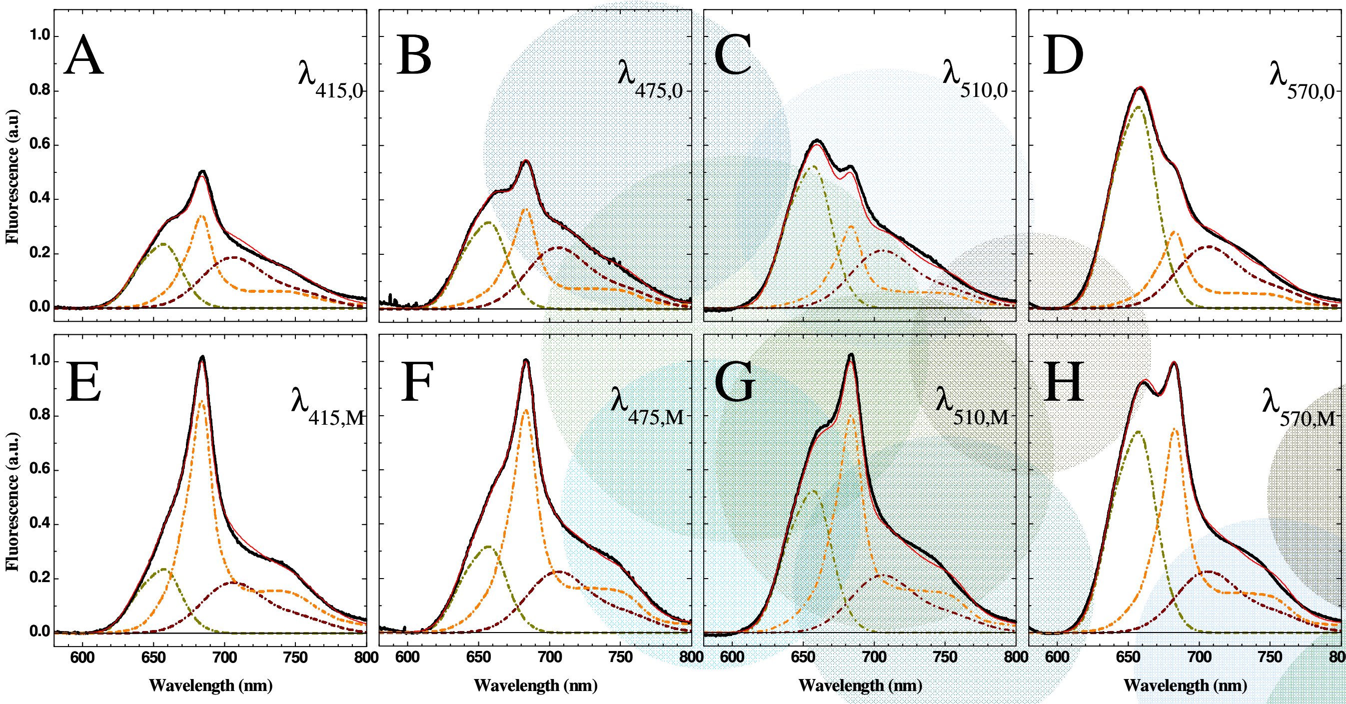
then

$$\begin{cases} F_0(\lambda_{em},\lambda_{ex}) = \phi_{II,0}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) + \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em}) + \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho_{PBU}(\lambda_{em}) \\ F_M(\lambda_{em},\lambda_{ex}) = \phi_{II,M}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) + \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em}) + \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho_{PBU}(\lambda_{em}) \end{cases}$$

and

$$\begin{cases} F_V(\lambda_{em},\lambda_{ex}) = (\phi_{II,M} - \phi_{II,0})\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) \\ \dfrac{F_V}{F_M}(\lambda_{em},\lambda_{ex}) = \dfrac{(\phi_{II,M} - \phi_{II,0})\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em})}{\phi_{II,M}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) + \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em}) + \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho_{PBU}(\lambda_{em})} \end{cases}$$

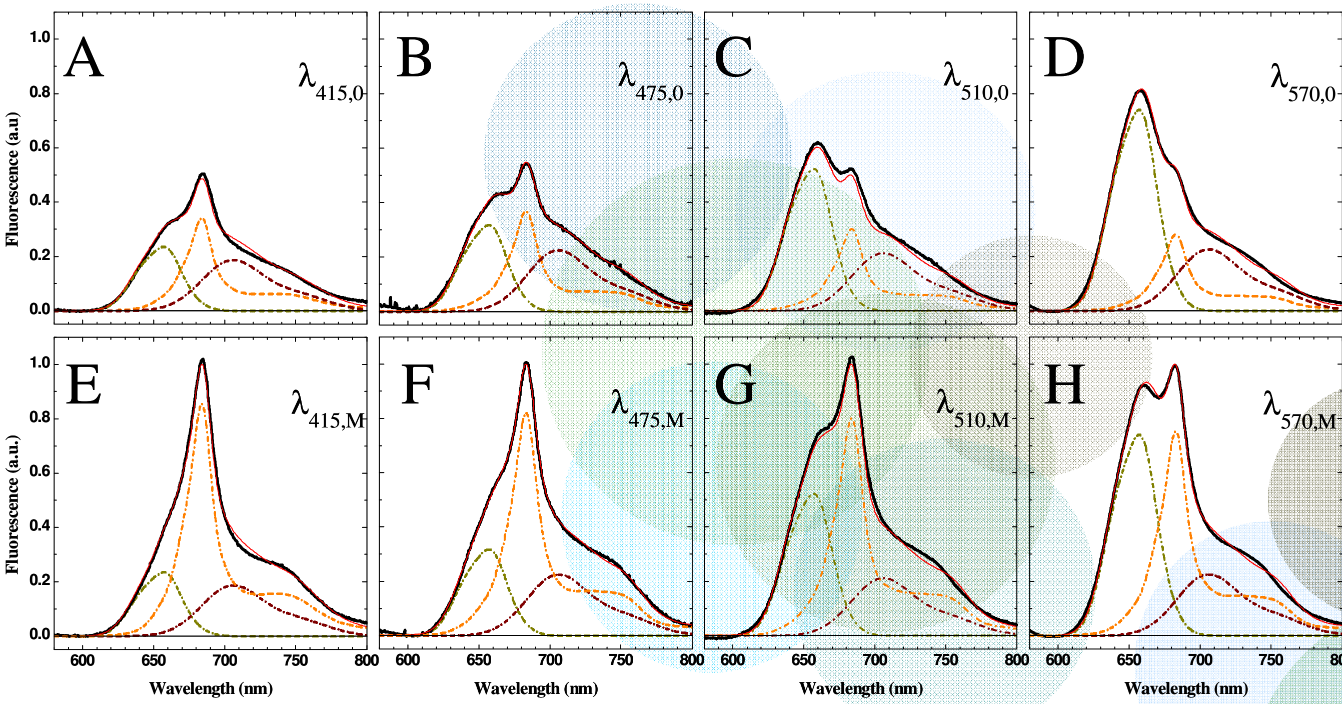
Decomposition of spectra into components

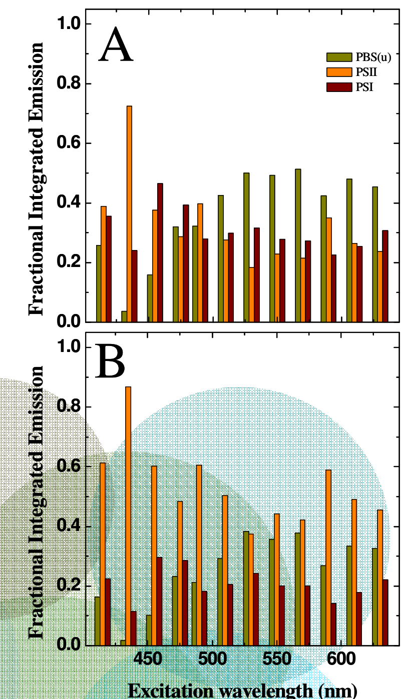


$$\Phi_{PSII}^{Max} = 0.62$$

o **highlights the different contribution of PSII, PSI and uncoupled PBS at each set of excitation/emission wavelengths**

Decomposition of spectra into components



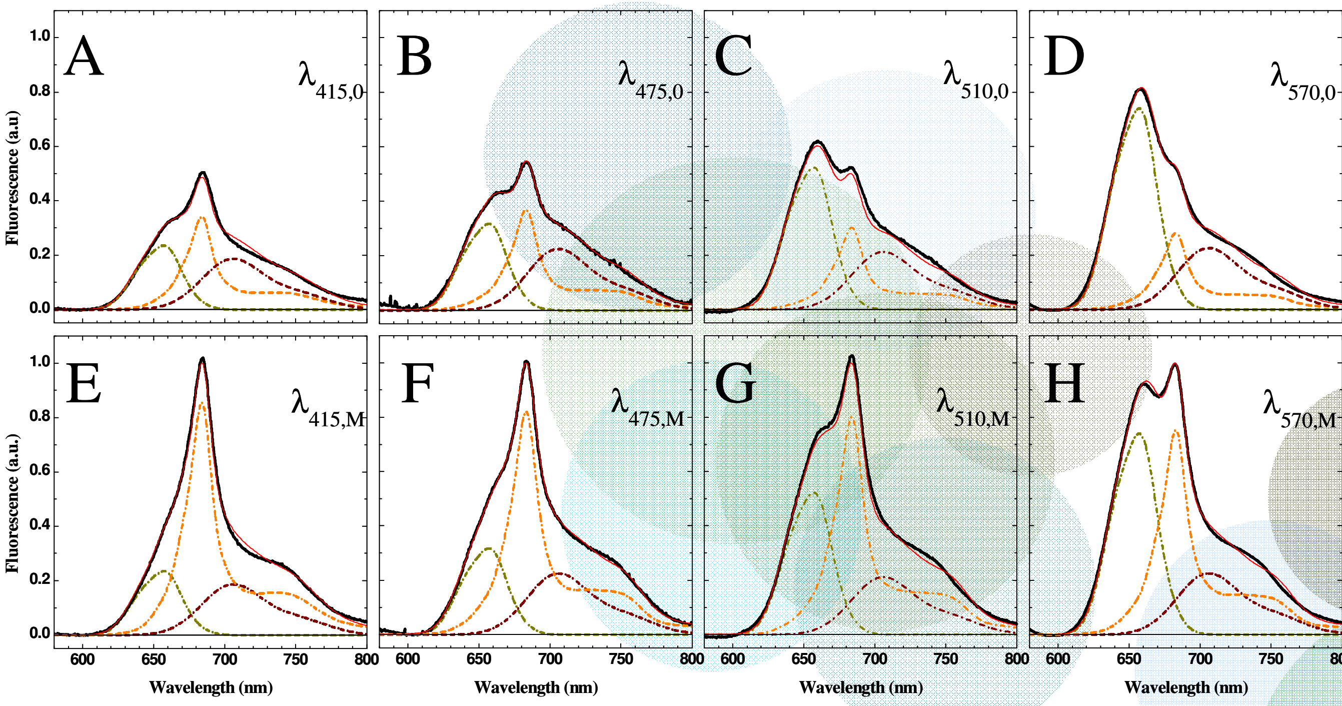


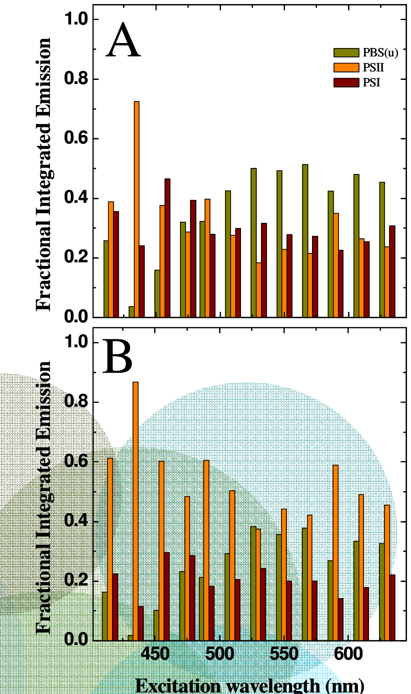
o **highlights the different contribution of PSII, PSI and uncoupled PBS at each set of excitation/emission wavelengths**

o **allows to determine the relative absorption cross-section and emission bandwidth**

$$\Phi_{PSII}^{Max} = 0.62$$

Decomposition of spectra into components

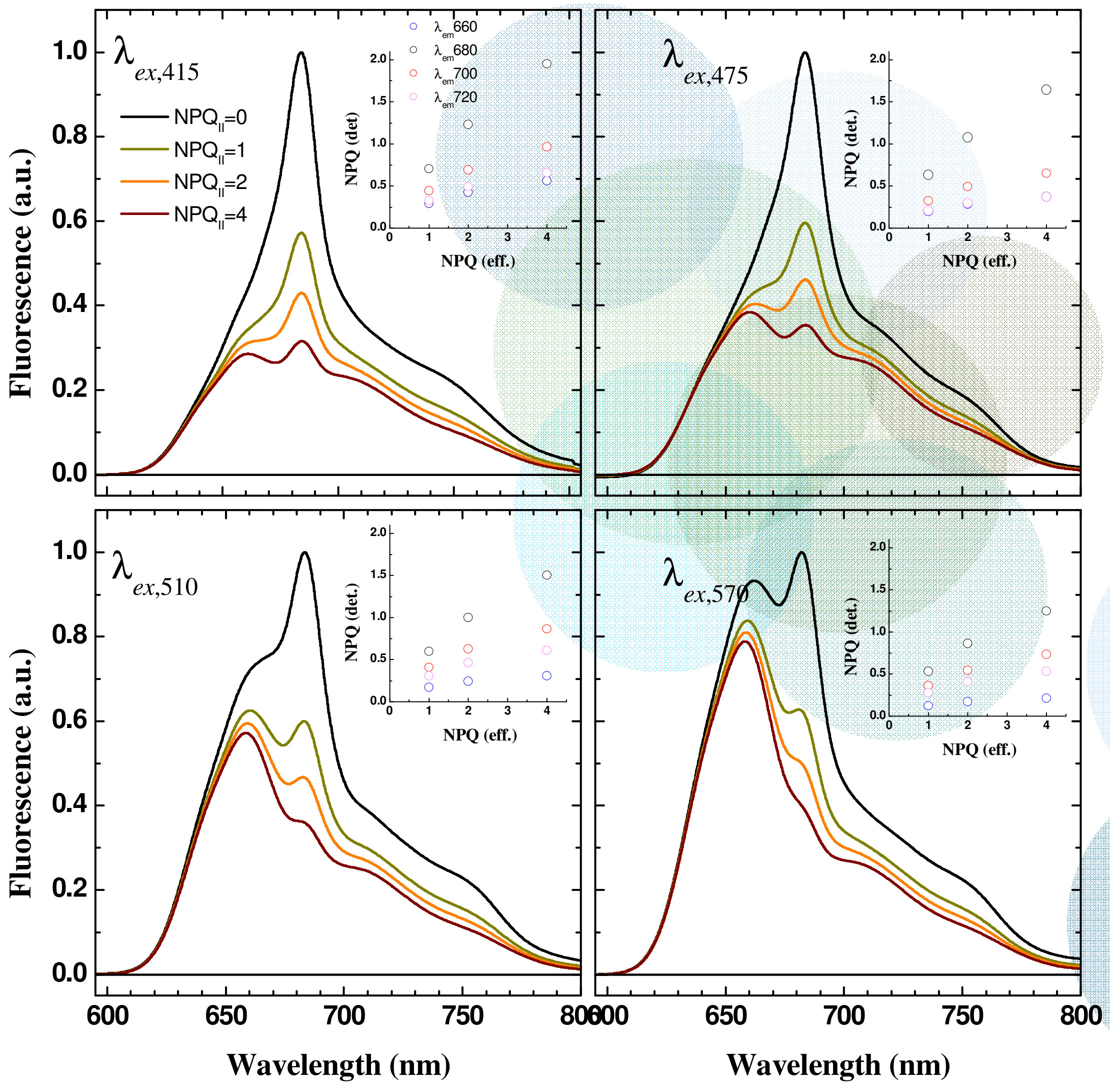




o highlights the different contribution of PSII, PSI and uncoupled PBS at each set of excitation/emission wavelengths
o allows to determine the relative absorption cross-section and emission bandwidth

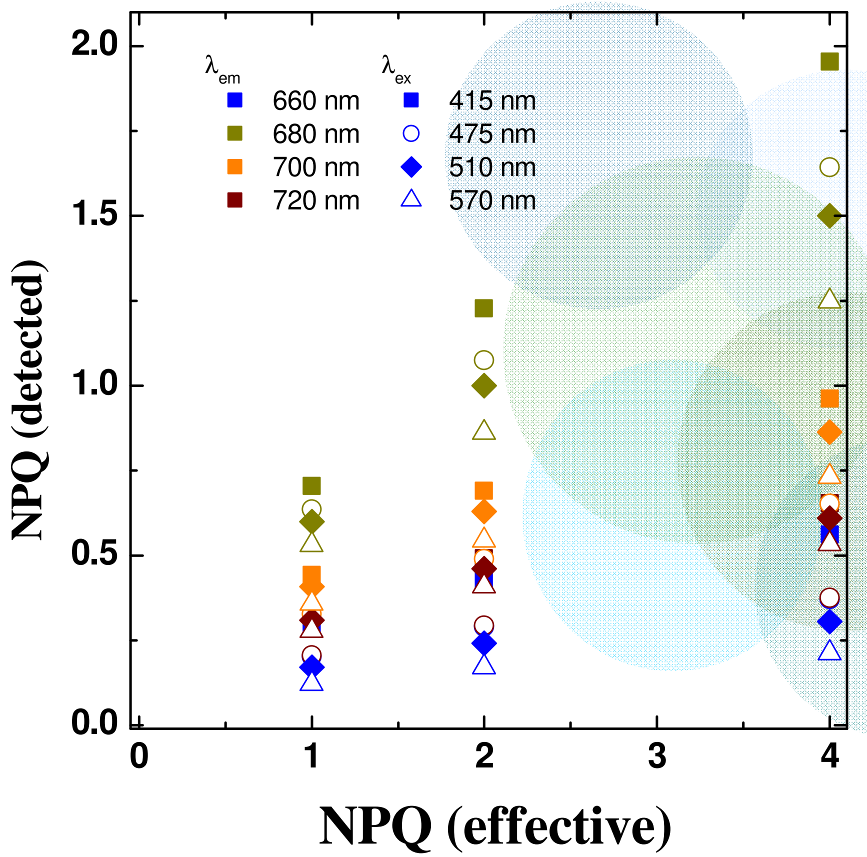
o from which spectra can be *simulated*

Impact on other parameters estimation: NPQ



- spectra simulated for increasing levels of NPQ (0-4) for different excitations for **PSII-PBS only!**
- NPQ=1-F_M/F_M' computed after convolving for 10 nm interferential
- **largely underestimated!**

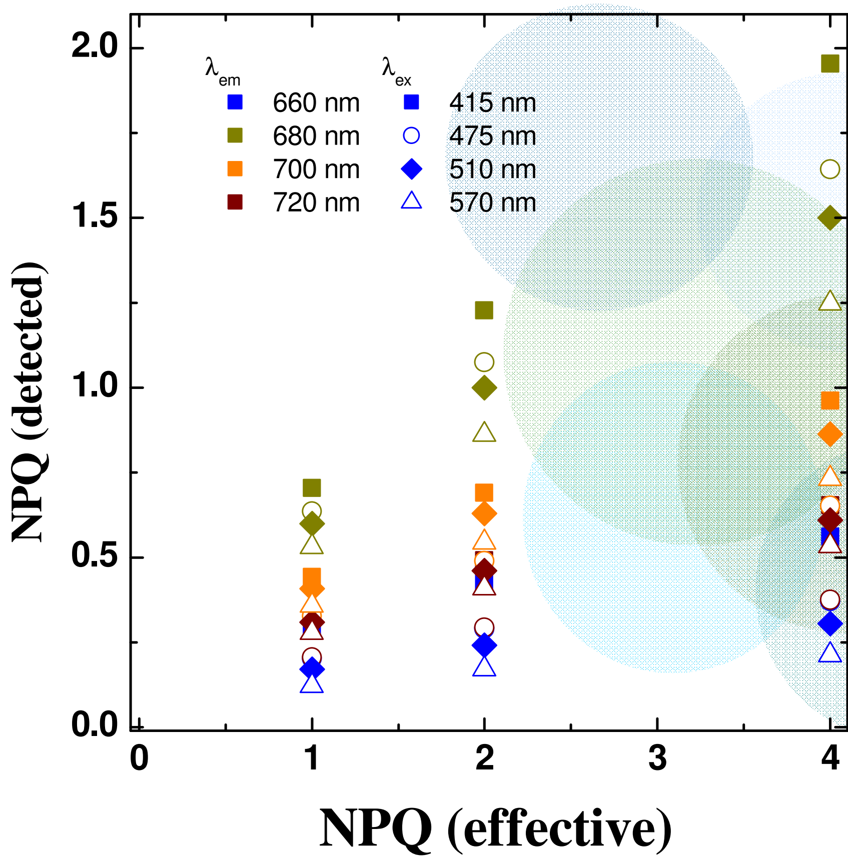
Impact on other parameters estimation: NPQ



- larger values (50% underestimated) for PSII max detection/Soret Excitation

- lower values (>80% underestimated) for PBS detection/PBS Excitation

Impact on other parameters estimation: NPQ

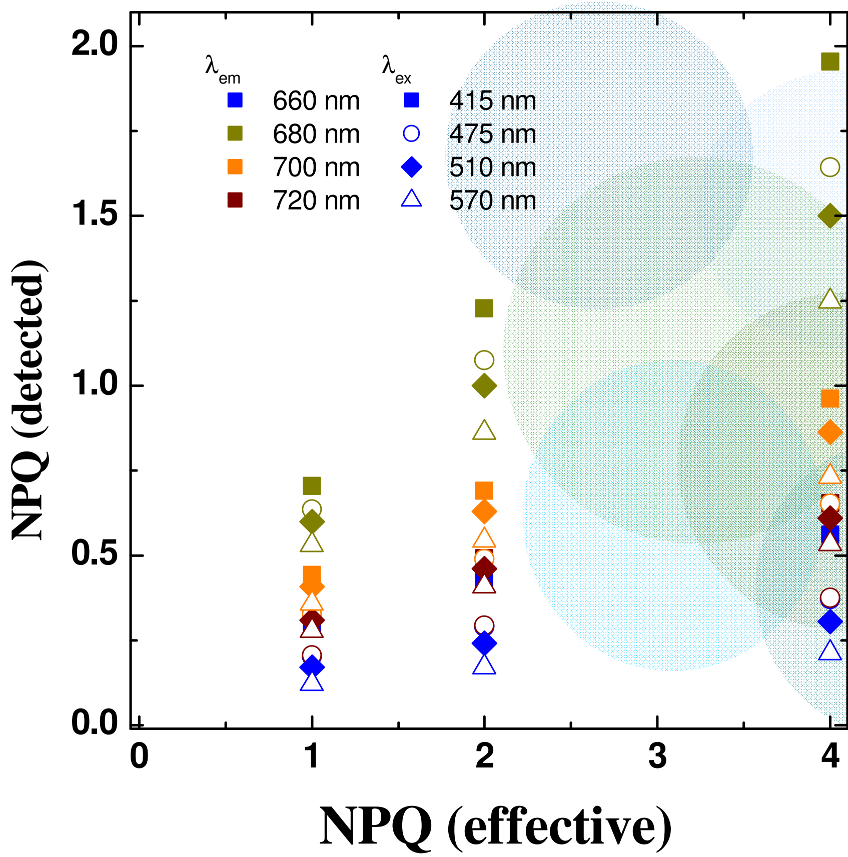


o larger values (50% underestimated) for PSII max detection/Soret Excitation

o lower values (>80% underestimated) for PBS detection/PBS Excitation

o it can be corrected, knowing the super-complex absorption/emission cross-sections

Impact on other parameters estimation: NPQ



o larger values (50% underestimated) for PSII max detection/Soret Excitation

o lower values (>80% underestimated) for PBS detection/PBS Excitation

o it can be corrected, knowing the super-complex absorption/emission cross-sections

o <u>can be useful to distinguish different mechanisms/sites of quenching</u>

Conclusion

o F_V/F_M perfectly fine but "surrounding conditions" need to be verified
o It is necessary to be carefully choosing the measurements conditions

In cyanobacteria *(Synechocystis 6803 and Synechococcus 7942)*
o the emission band-shape at RT depends on the excitation wavelength
o the emission band-shape varies differently at F_0 with respect to F_M
o the value of F_V/F_M depends on both the excitation and emission wavelengths
o the F_V spectra are excitation wavelength independent
o this can be explained by a super-imposition of three emitters, PSI-PBS, PSII-PBS and an uncoupled PBS fraction (PBS_U)
As a result
o F_V/F_M is generally underestimated and need to be corrected to obtain meaningful information
o Similar bias in the fluorescence-based indicators affects also other parameters such as NPQ. It can leas to dramatic underestimation of this process.

o In ***green algae*** the issue are less relevant. Distortion from actual values less than 10%

Thank You For the Attention !

Photosynthesis Research Unit

William Remelli

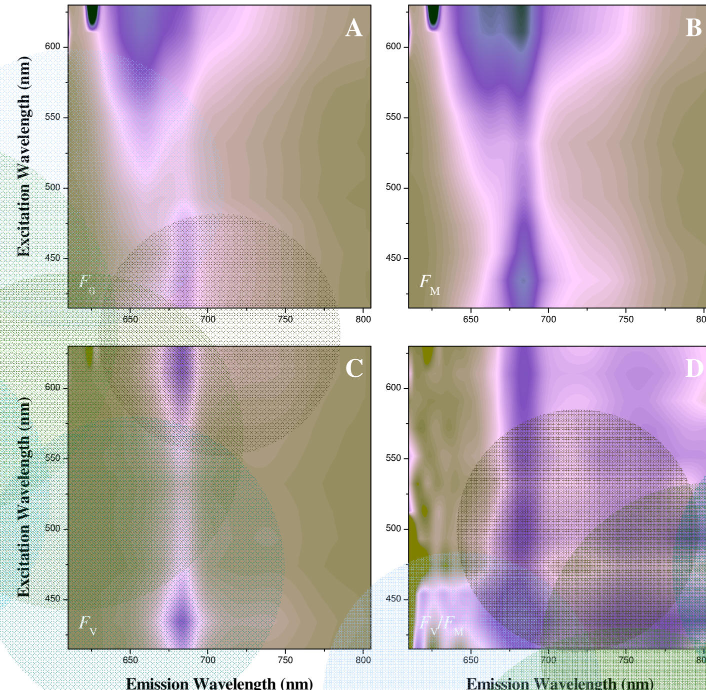
Federico Rizzo

Francesca Villafiorita-Monteleone

Anna Paola Casazza

Barbara Menin

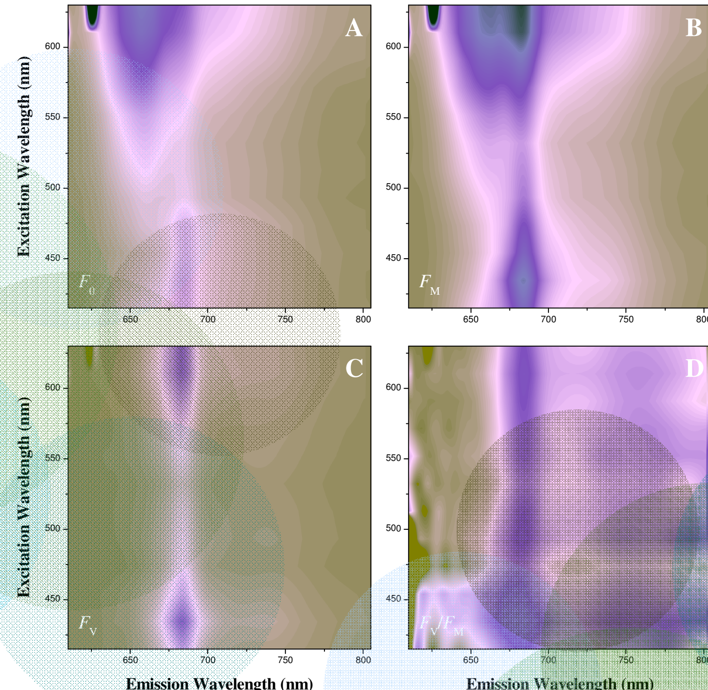
Emiliano Grigis



Thank You For the Attention !

Photosynthesis Research Unit
William Remelli
Federico Rizzo

Francesca Villafiorita-Monteleone
Anna Paola Casazza
Barbara Menin



Collaborators:

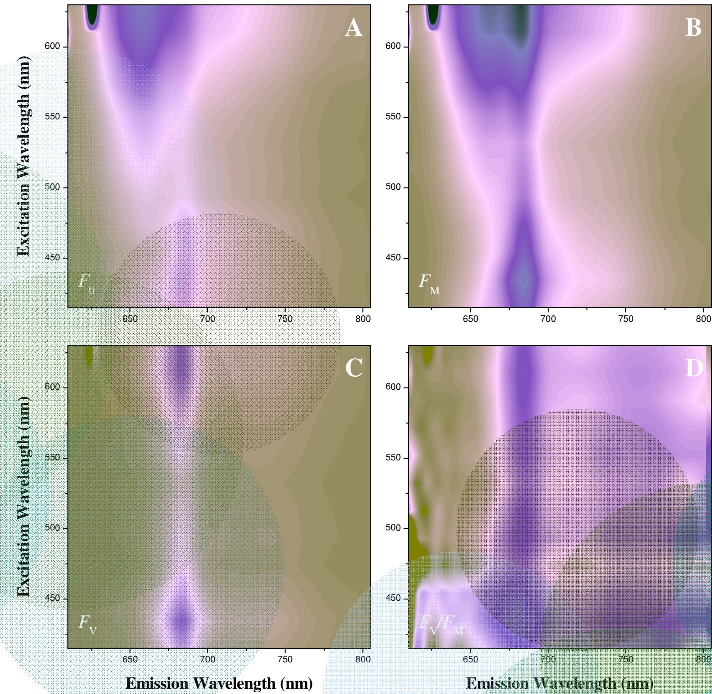
Ondrej Prasil, Radek Kana

Benjamin Bailleul, Francis-Andre Wolmann, Stefania Viola

Thank You For the Attention !

Photosynthesis Research Unit
William Remelli
Federico Rizzo

Francesca Villafiorita-Monteleone
Anna Paola Casazza
Barbara Menin



Collaborators:

ALGATECH

Ondrej Prasil, Radek Kana

IBPC

Benjamin Bailleul, Francis-Andre Wolmann, Stefania Viola

acknowledgment

http://www.cyaoproject.org

fondazione cariplo