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

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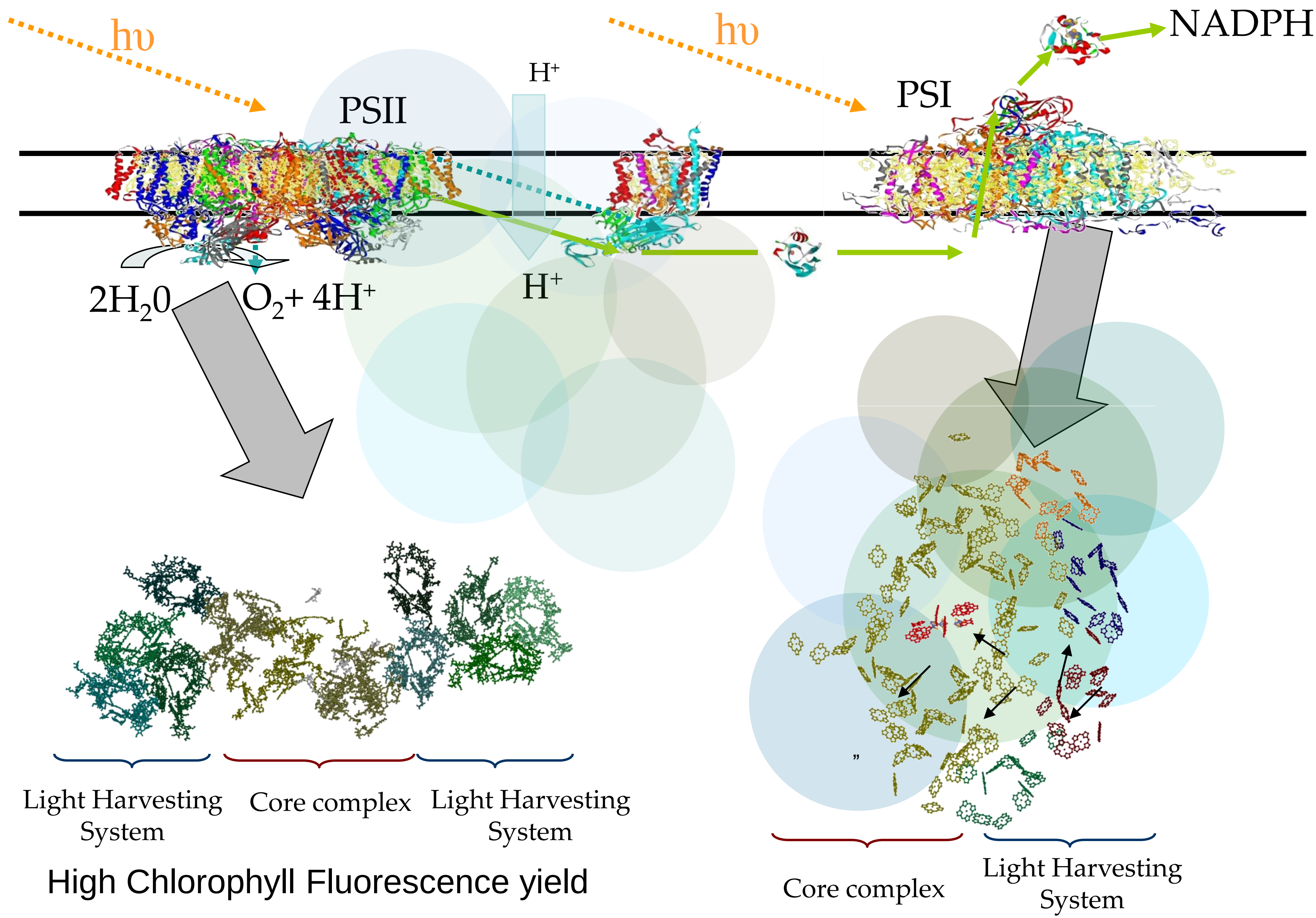
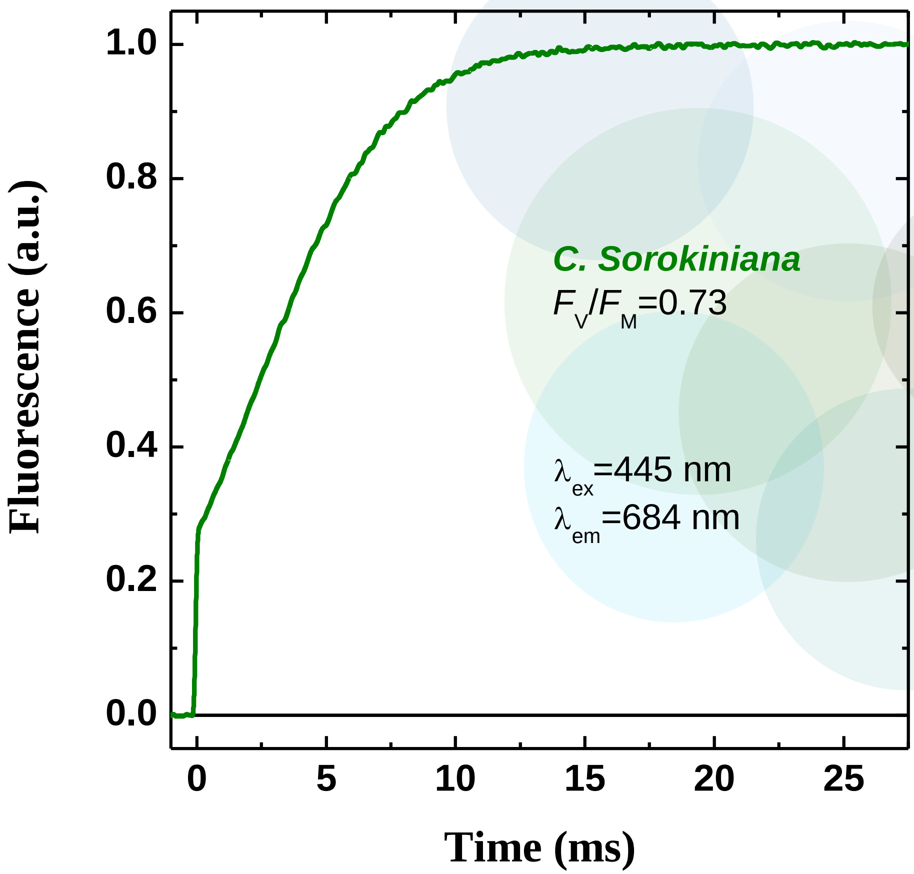


High Chlorophyll Fluorescence yield

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

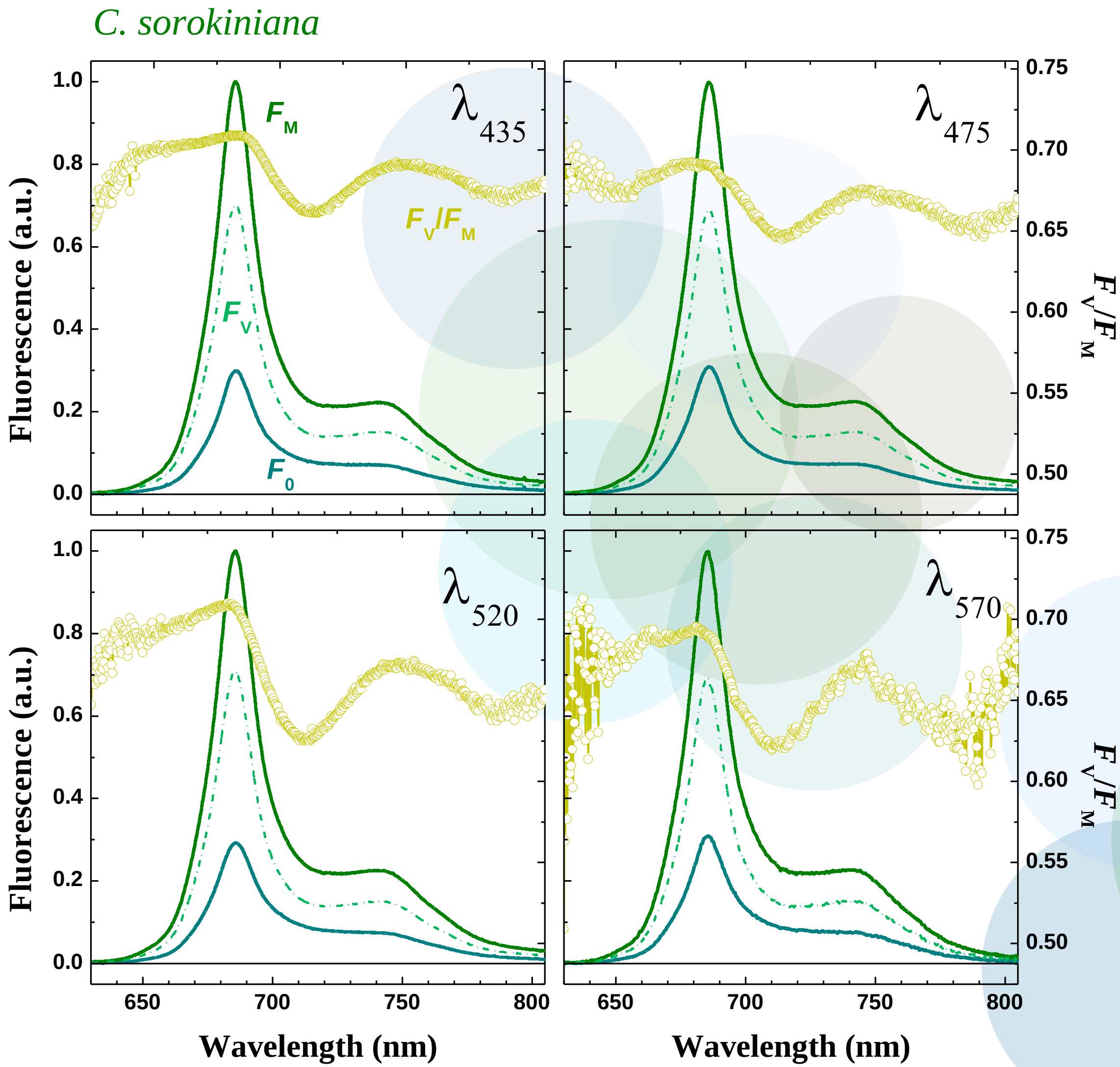


$$\frac{F_V}{F_M} \simeq \Phi_{PSII}^{Max} = \frac{K_{PC}}{\sum_i k_i + K_{PC}}$$

Caveats:

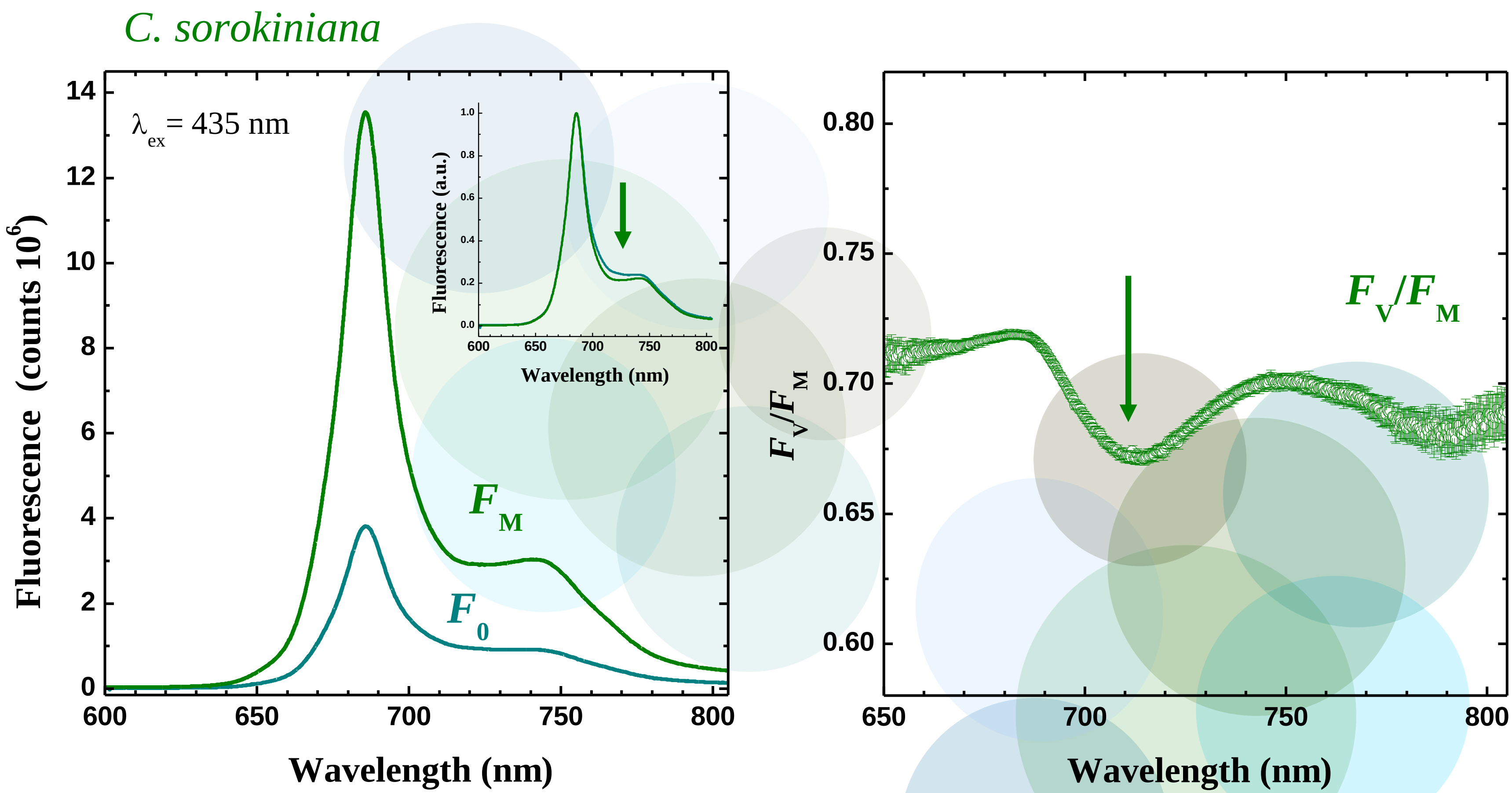
- **emission is exclusively (or almost) from PSII**
- **quantum yields are independent on excitation and emission wavelengths.**

F_V/F_M spectral dependence: emission and excitation



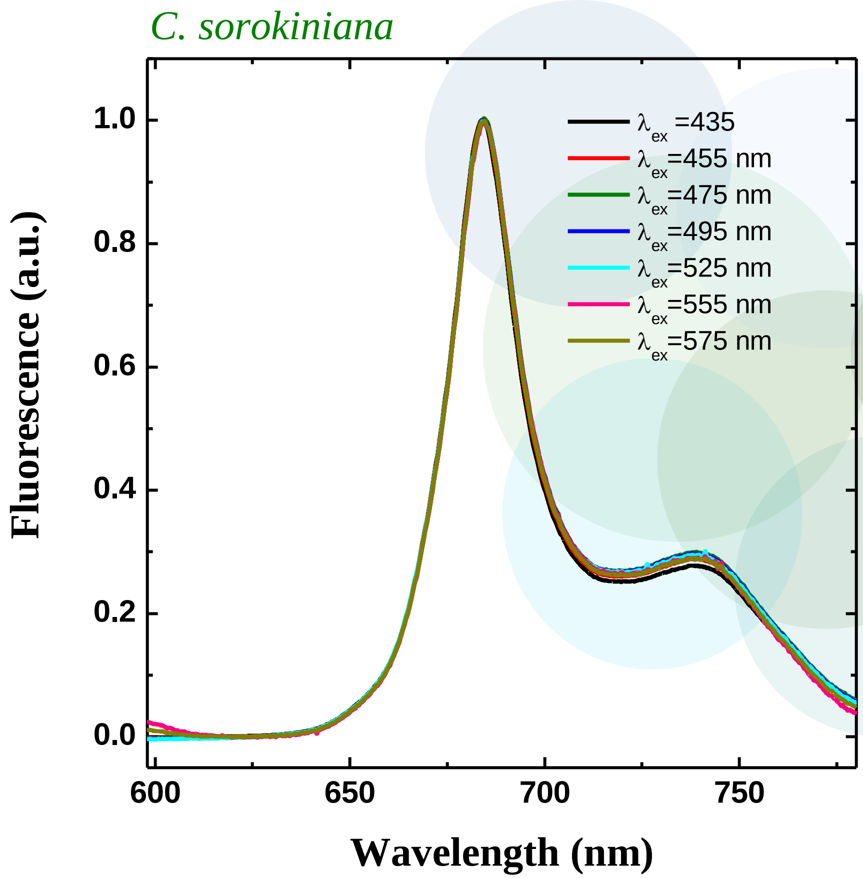
- limited spectra variation between F_0 and F_M
- also limited spectral dependence of F_V/F_M

F_V/F_M spectral dependence: emission



- 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



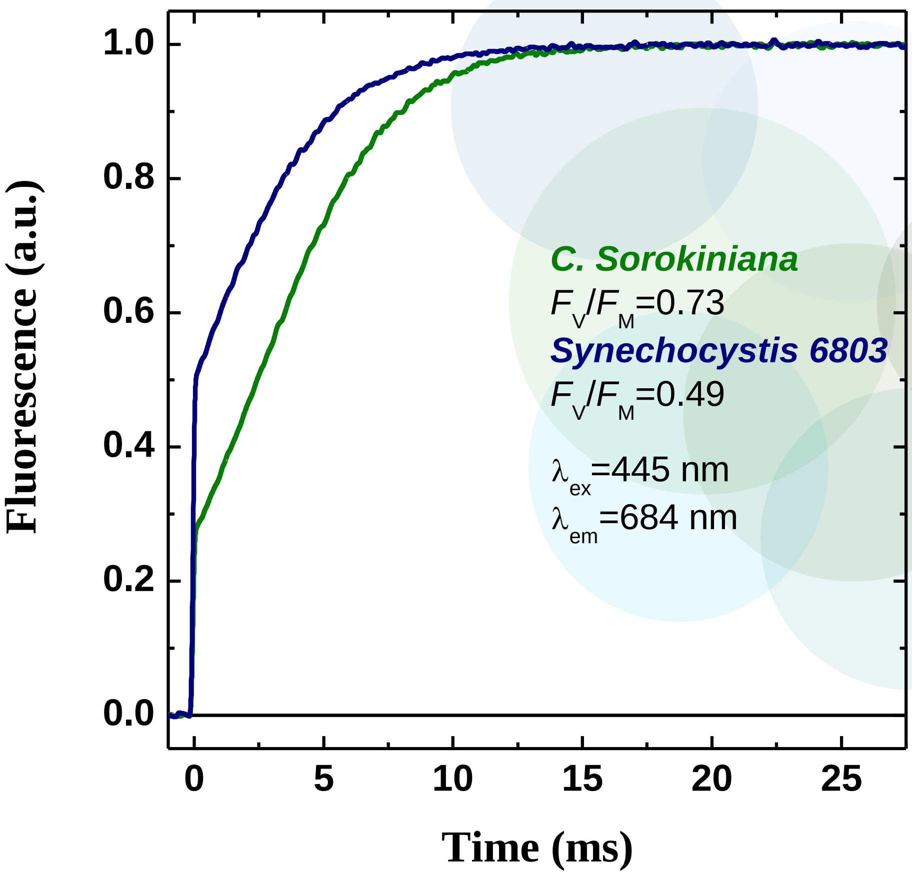
For green algae
(same in *C. reinhardtii*)

- **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**

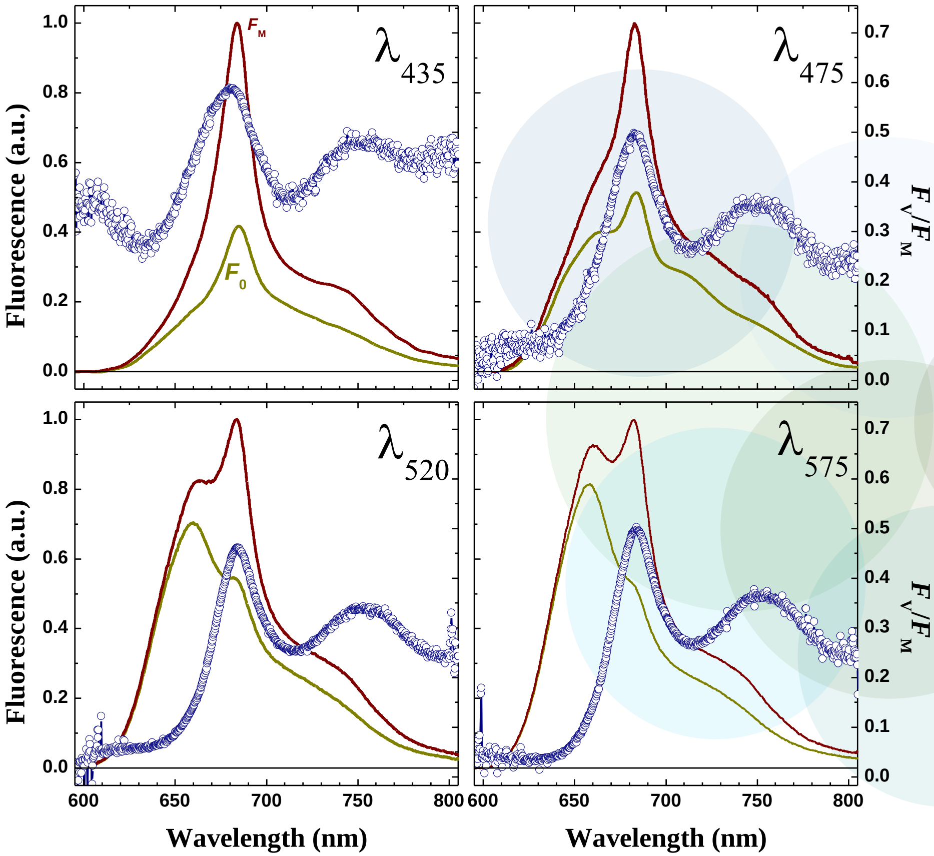
How comes? Is

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

not valid?

F_V/F_M spectral dependence: emission/excitation

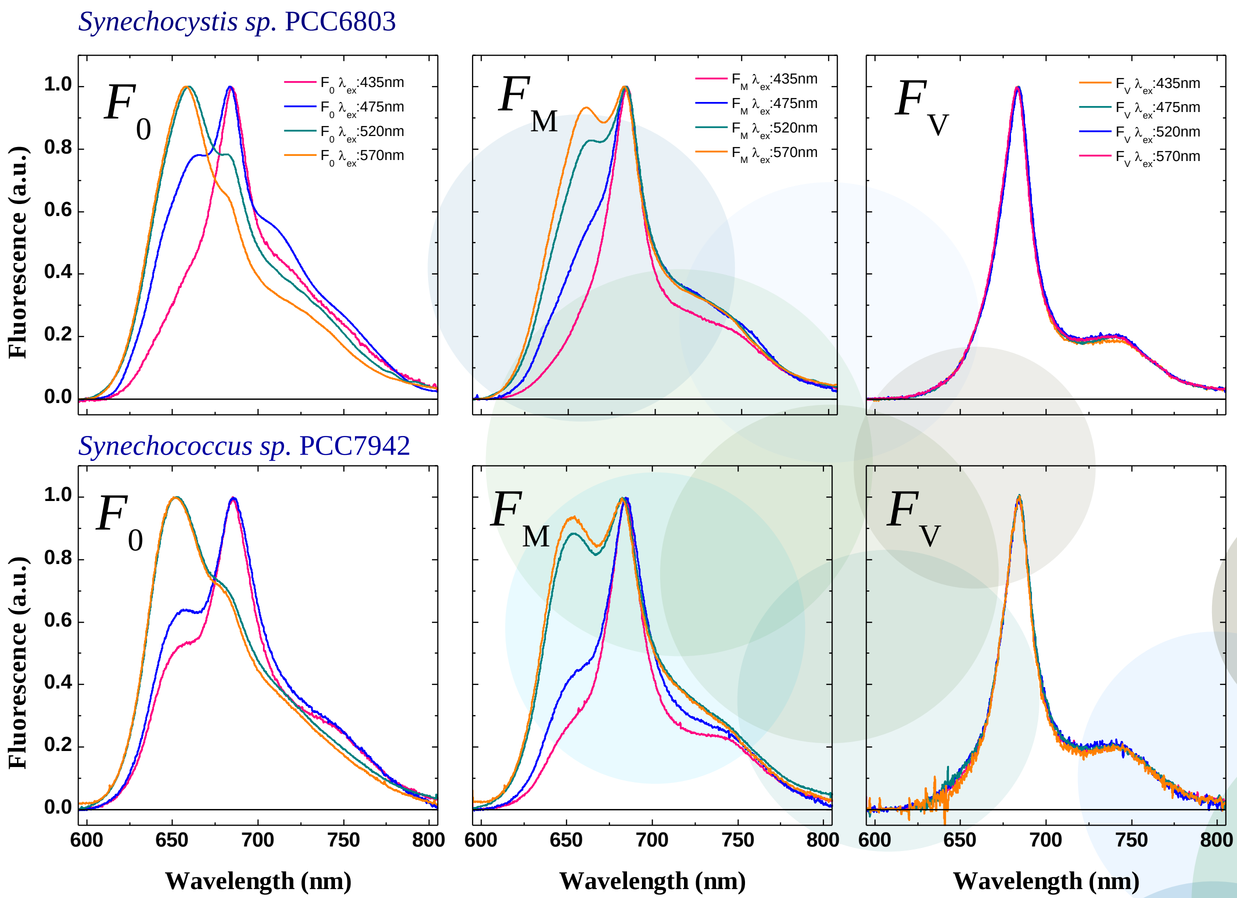
Synechocystis sp. PCC6803



In *Synechocytis sp.* PCC6803

- **large spectral variation between F_0 and F_M**
- **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



In *Synechocytis sp.* PCC6803 & *Synechococcus sp.* PCC7942

- **large spectral variation between F_0 and F_M**
- **both F_0 and F_M spectra depend on the excitation wavelength**
- **the F_V/F_M ratio is largely dependent on both the excitation and the emission wavelength**
- **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)**

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

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

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

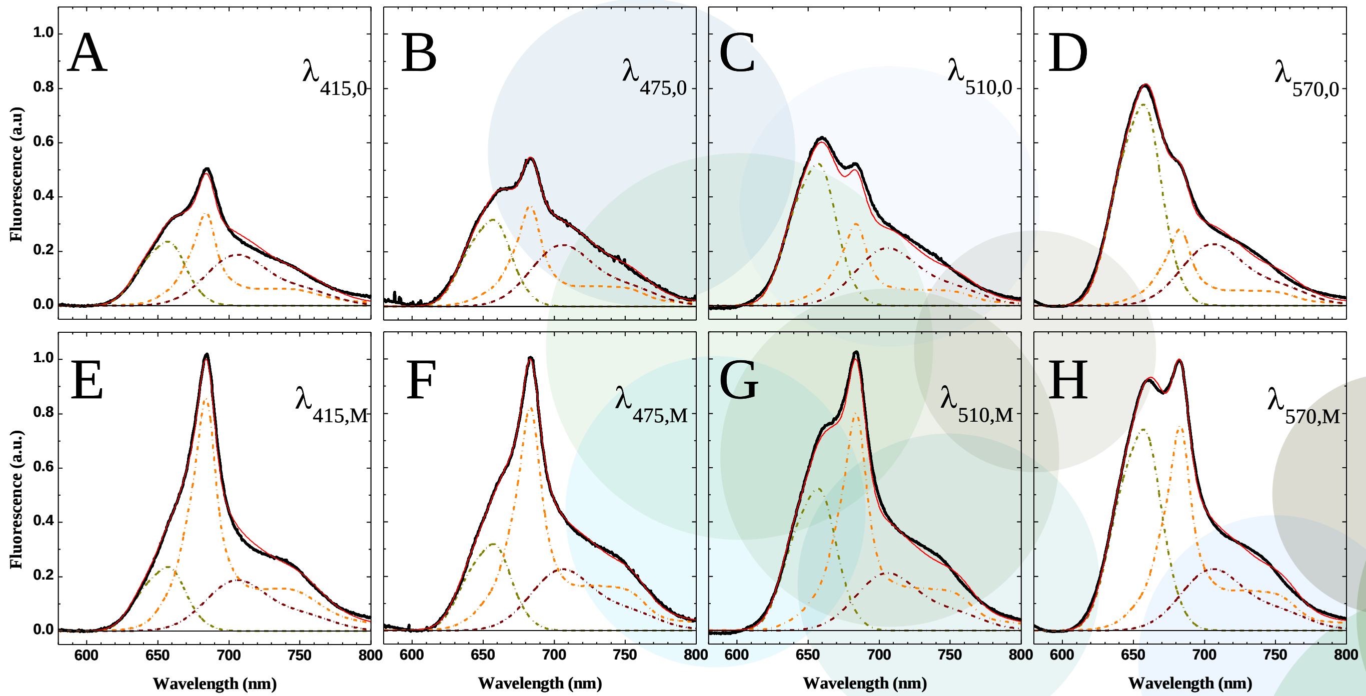
then

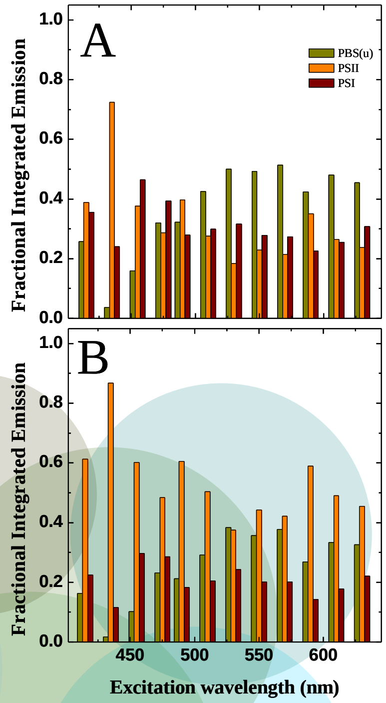
$$\begin{cases} F_0(\lambda_{em},\lambda_{ex}) = \phi_{II,0}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em}) + \phi_{I}\sigma_{I}(\lambda_{ex})\rho_{I}(\lambda_{em}) + \phi_{PBU}\sigma_{PBU}(\lambda_{ex})\rho_{PBU}(\lambda_{em}) \\ F_M(\lambda_{em},\lambda_{ex}) = \phi_{II,M}\sigma_{II}(\lambda_{ex})\rho_{II}(\lambda_{em})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

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 (cyanobacteria)





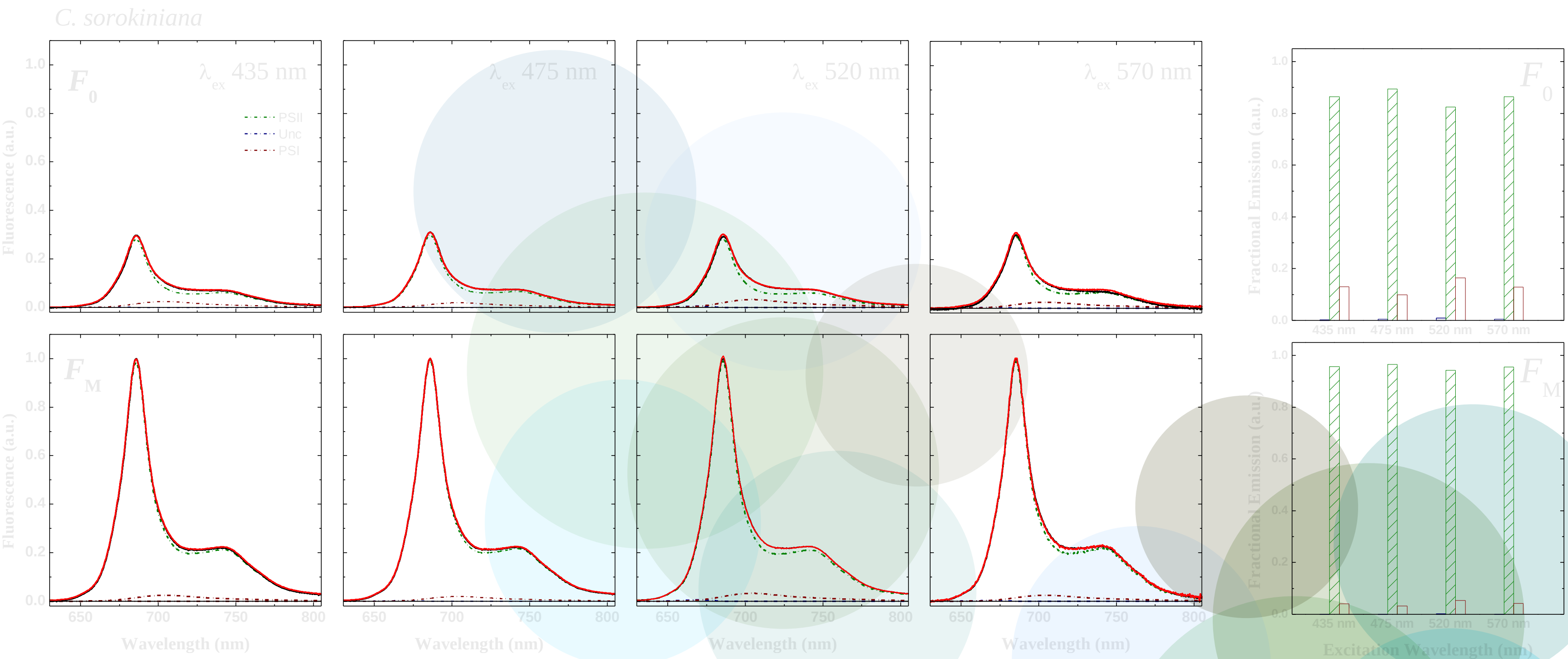
o highlights the different contribution of PSII, PSI and uncoupled PBS at each set of excitation/emission wavelengths

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

o from which spectra can be *simulated*

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

Decomposition of spectra into components (green algae)

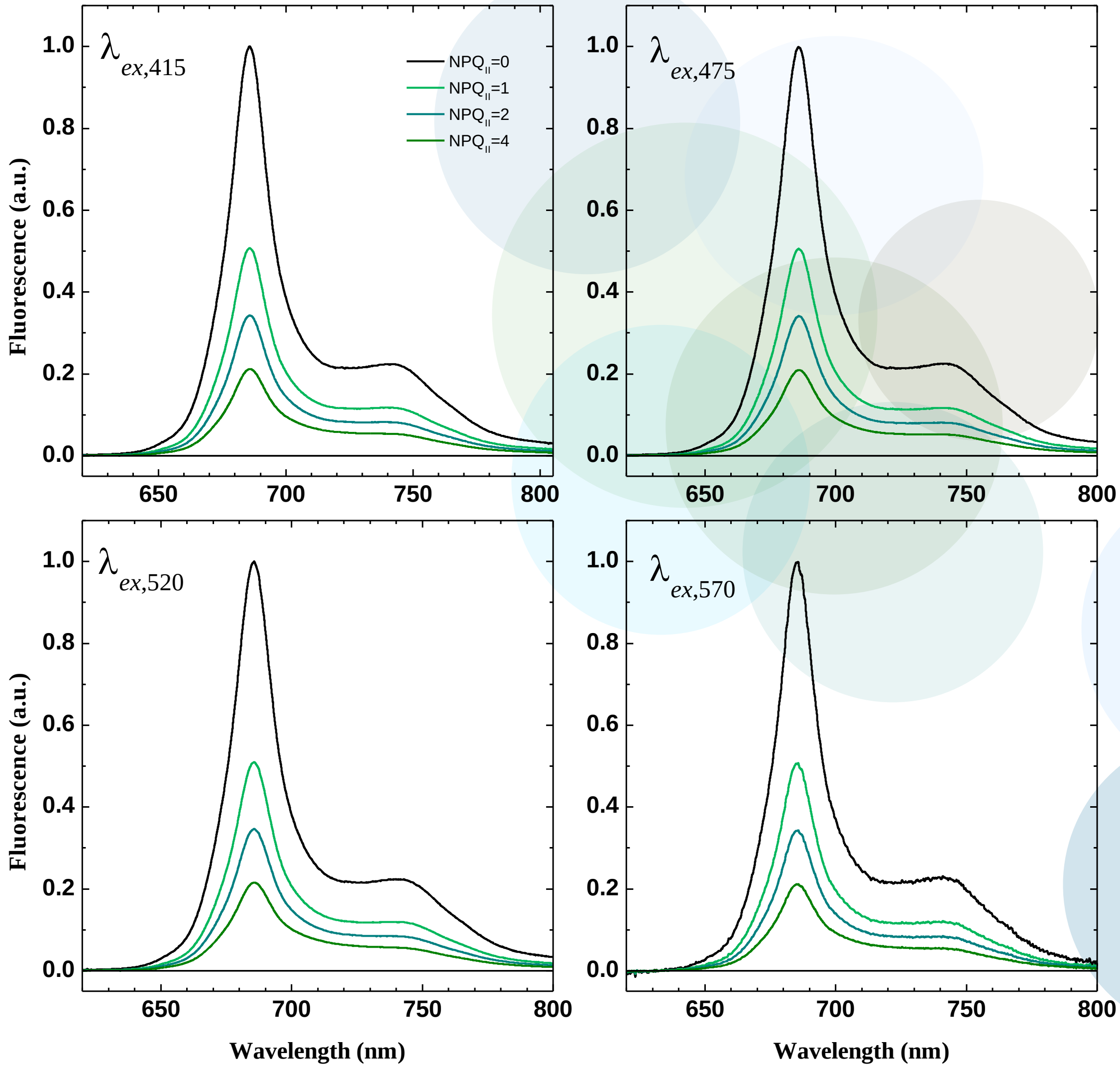


- more homogeneous contribution of PSII, PSI and uncoupled antenna almost excitation-wavelength independent

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

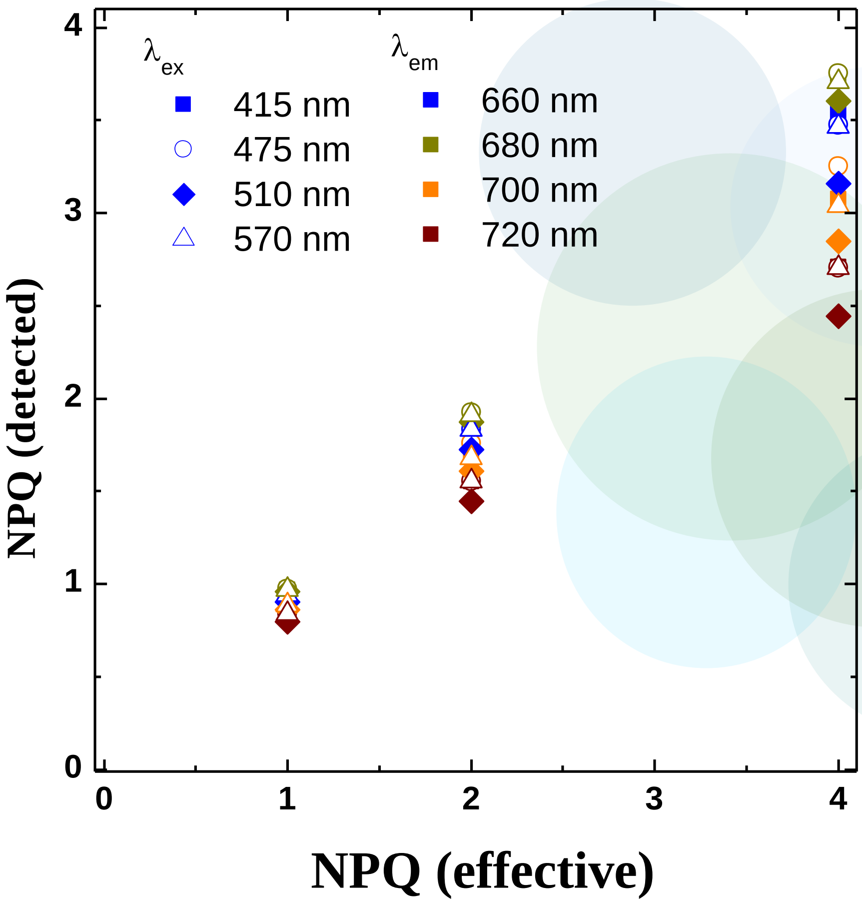
$$\Phi_{PSII}^{Max} = 0.725$$

Impact on other parameters estimation: NPQ (green algae)



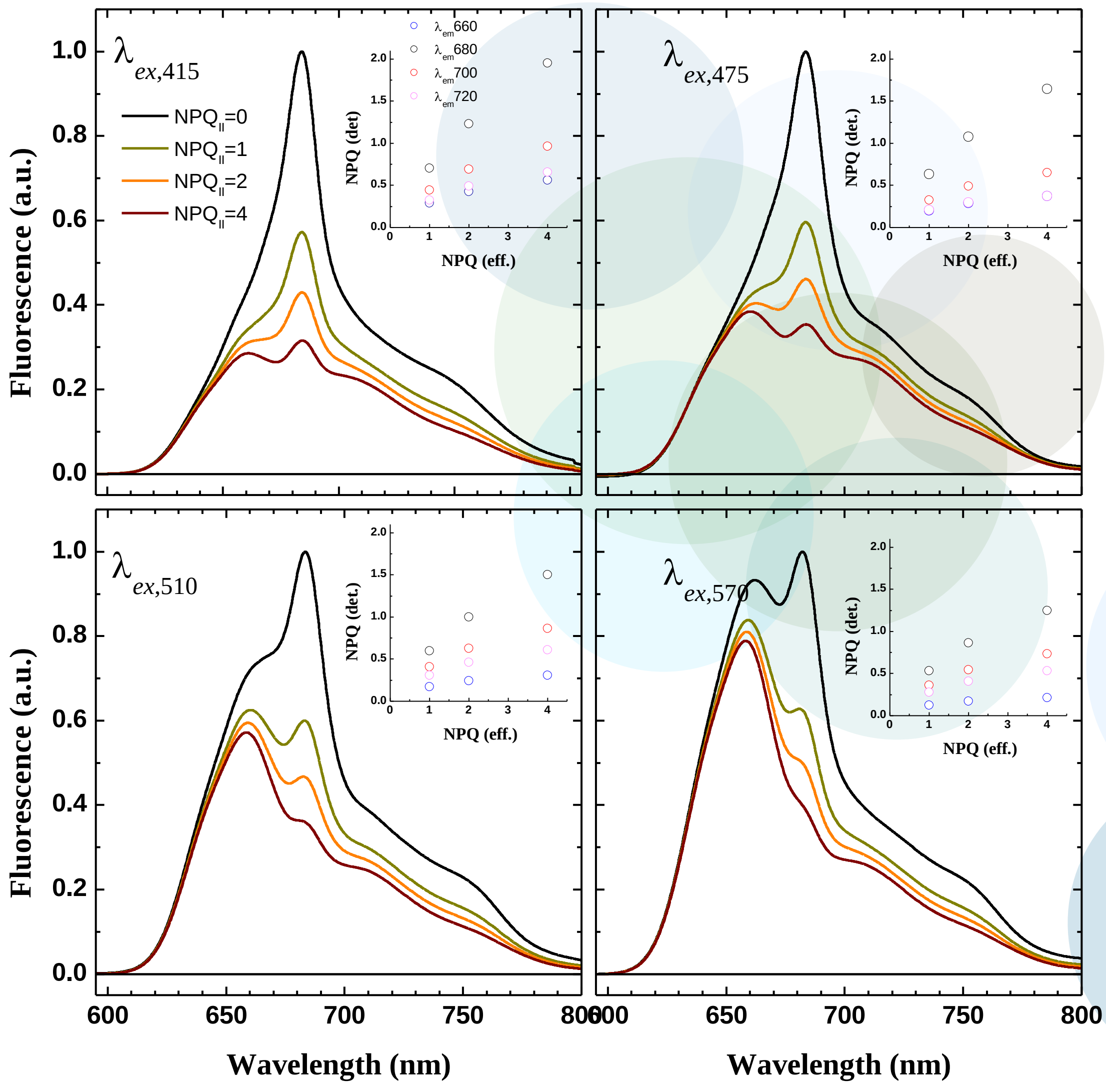
- spectra simulated for increasing levels of NPQ (0-4) for different excitations for PSII-LHCII only
- NPQ=1-F_M/F_M' computed after convolving for 10 nm interferential

Impact on other parameters estimation: NPQ (green algae)



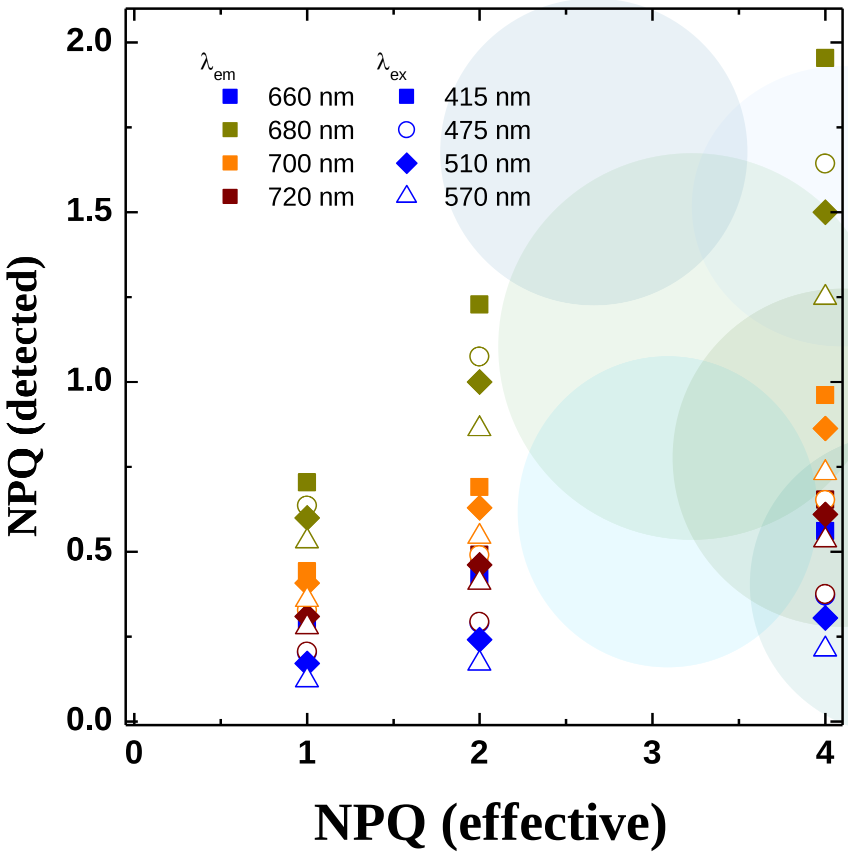
- o **some underestimation, particularly when non-photochemical quenching is rather large (i.e >2.5-3)**
- o **lowest deviations when monitoring PSII maximal emission (~680 nm) and preferential excitation (~475 nm)**
- o **lowest estimated (up to ~50% underestimated) at wavelengths where PSI emits the most**

Impact on other parameters estimation: NPQ (cyanobacteria)



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

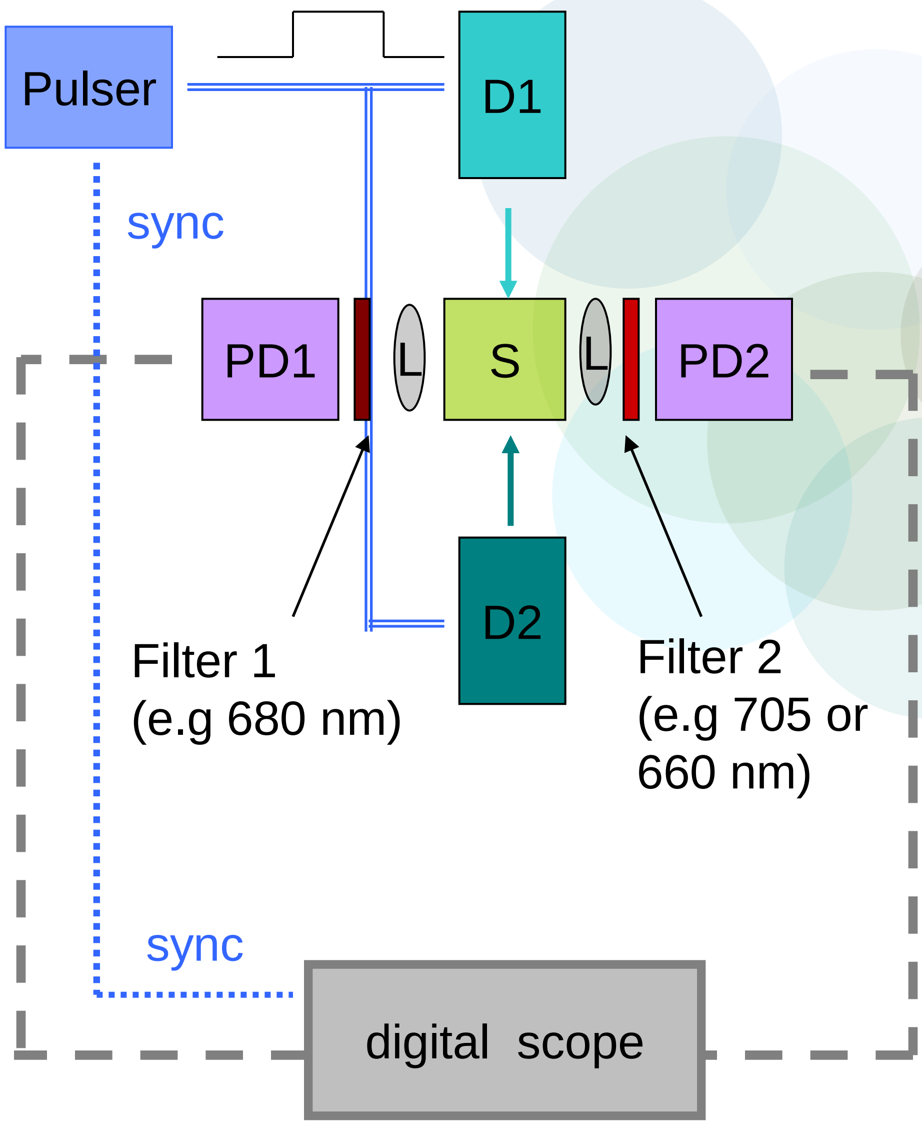
Impact on other parameters estimation: NPQ (Cyanobacteria)



- sensible underestimations at most excitation/emission conditions
- larger values (50% underestimated) for PSII max detection/Soret Excitation
- the most severe bias, and the lower values simulated (>80% underestimated) are for PBS detection/PBS Excitation

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

Direct improvement by multiple-wavelength detection



(CW) Prototype

- **simultaneous detection at two emission wavelength with T-geometry. Filters allow rapid tuneability**
- **PIN-Phodiodes (PD1, PD2) used for detection + transimpedence amplifier**
- **signal collected directly by an oscilloscope (25 MHz band, sufficient)**
- **excitation provided by LEDs (D1 and D2), operated individually or jointly.**
- **we opted for integrated drivers, so that they can be switched without acting on the pulser**

Conclusion

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

In cyanobacteria *(Synechocystis 6803 and Synechococcus 7942)*

- the emission band-shape at RT depends on the excitation wavelength
- the emission band-shape varies differently at F_0 with respect to F_M
- the value of F_V/F_M depends on both the excitation and emission wavelengths
- the F_V spectra are excitation wavelength independent
- 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

- F_V/F_M is generally underestimated and need to be corrected to obtain meaningful information
- Similar bias in the fluorescence-based indicators affects also other parameters such as NPQ. It can leas to dramatic underestimation of this process.

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

Thank You For the Attention !

Photosynthesis Research Unit
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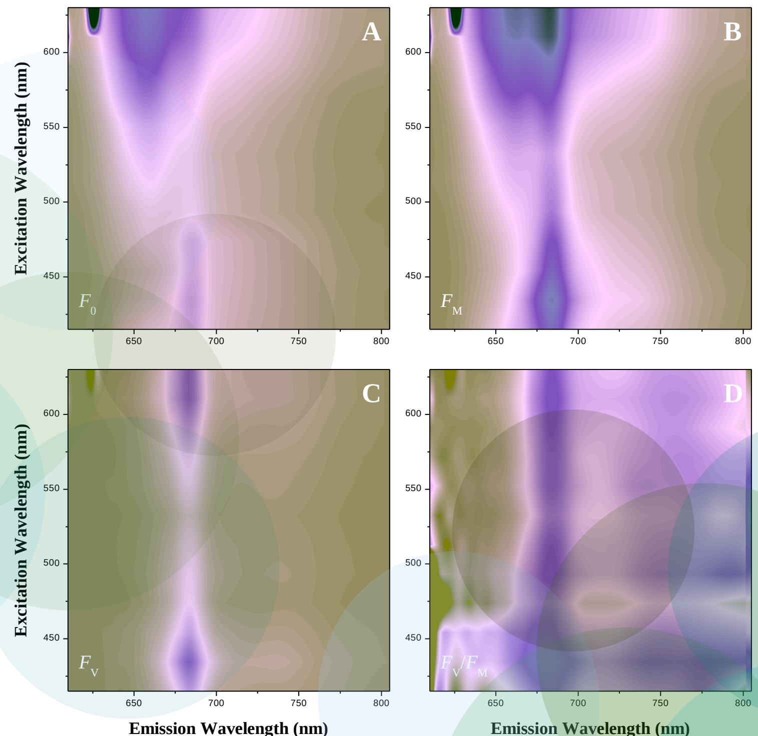

Collaborators:

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http://www.cyaoproject.org

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