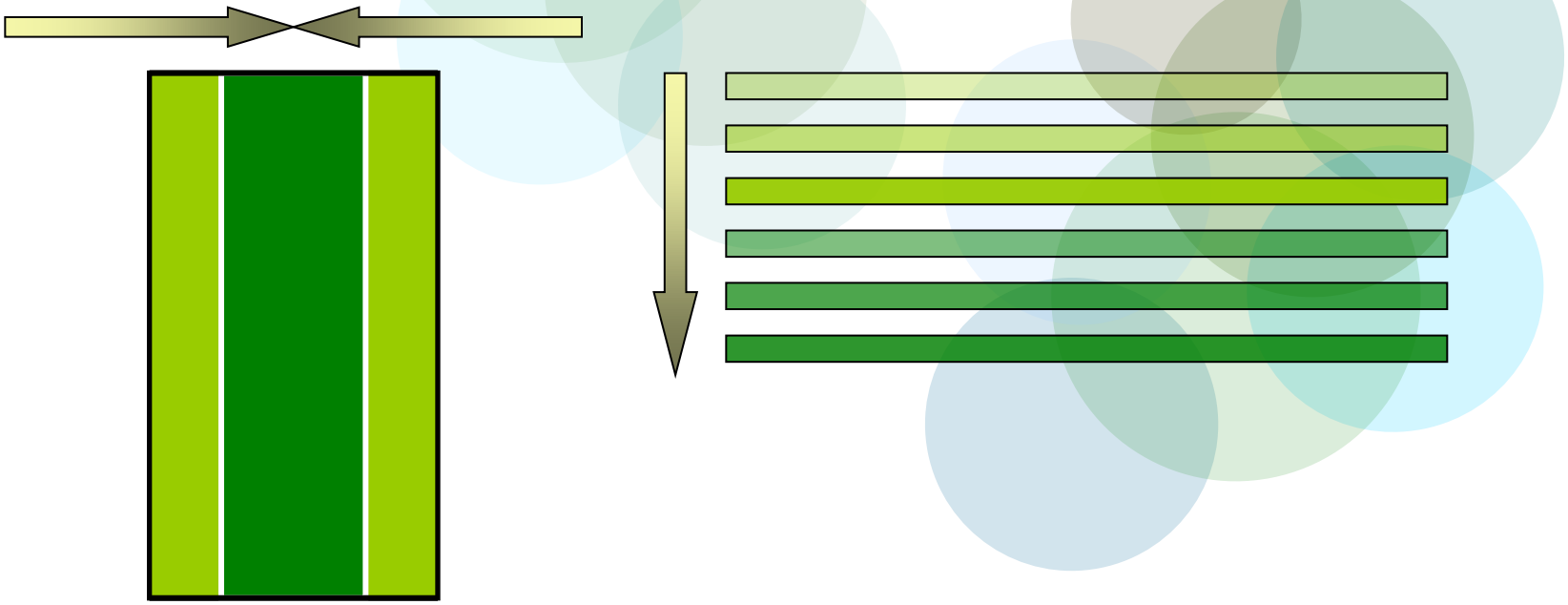
CYAO: Summary of Activities, Open Questions & Perspectives

Stefano Santabarbara
Photosynthetic Research Unit
National Research Council

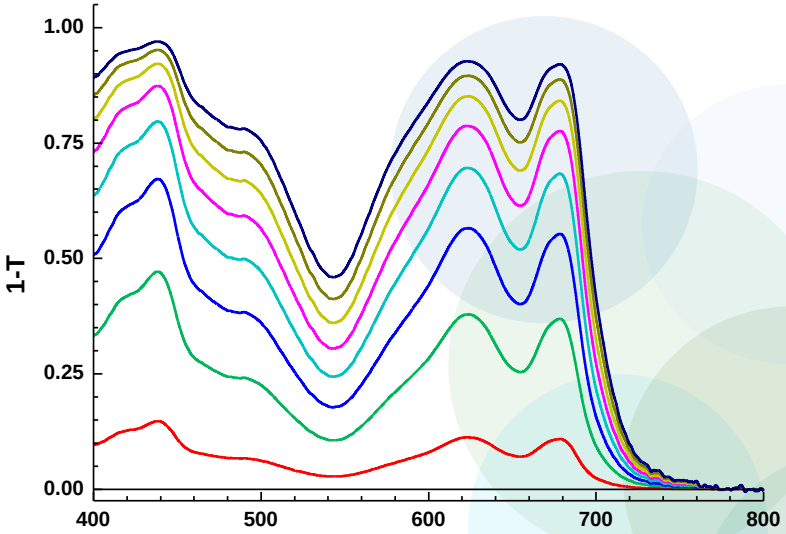
stefano.santabarbara@cnr.it

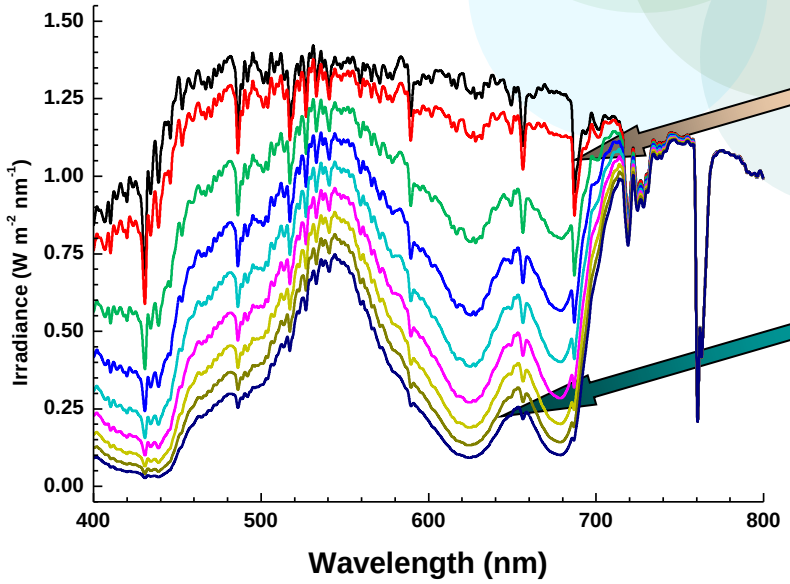
General problem: Limit to productivity due to self-shading:

- Phototrophic photosynthetic organisms use light to grow
- Upon growth light is absorbed by the culture, the inner layers experience a “shaded” environment
- As the culture get dense, light intensity and light quality change and become inhomogeneous



Self Shading





Sunlight

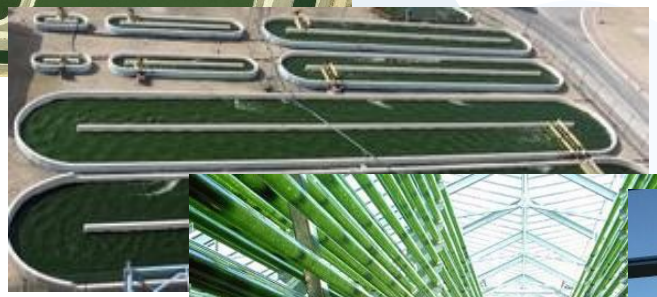
Culture-shaded

Culture-shaded light:

- **Far Red enriched**
- **Low Intensity (standard)**

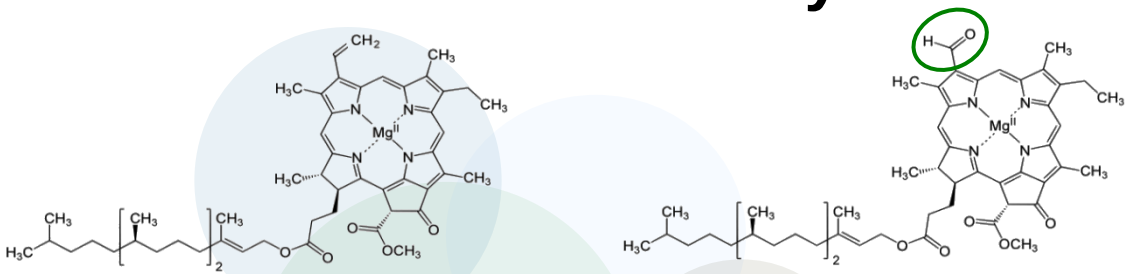
One strategy is to act on PBR architecture

open ponds

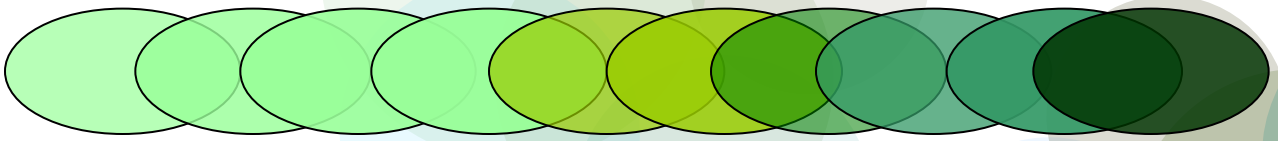


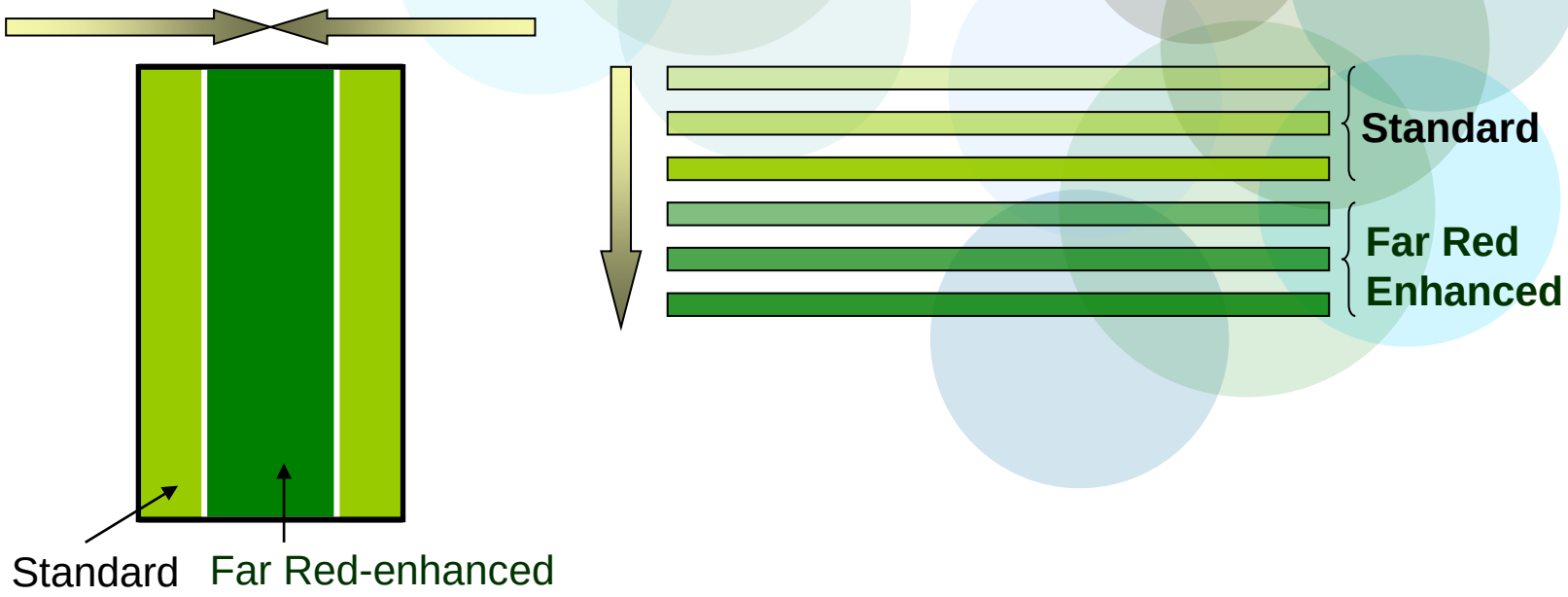
closed systems

Proposed solution in the CYAO Project: controlled activation of Chl *d* biosynthesis

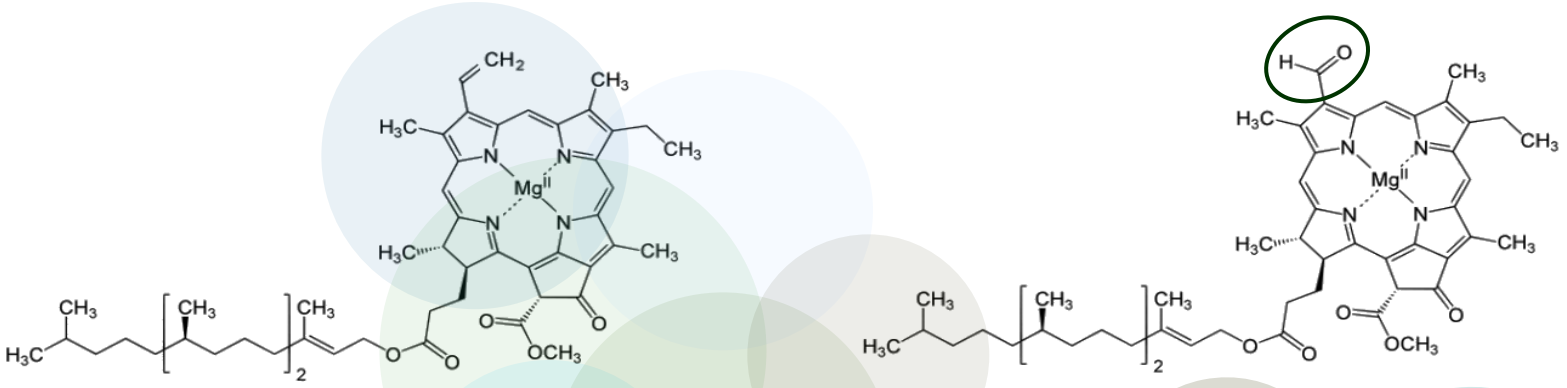


Chlorophyll *a* Chlorophyll *d*



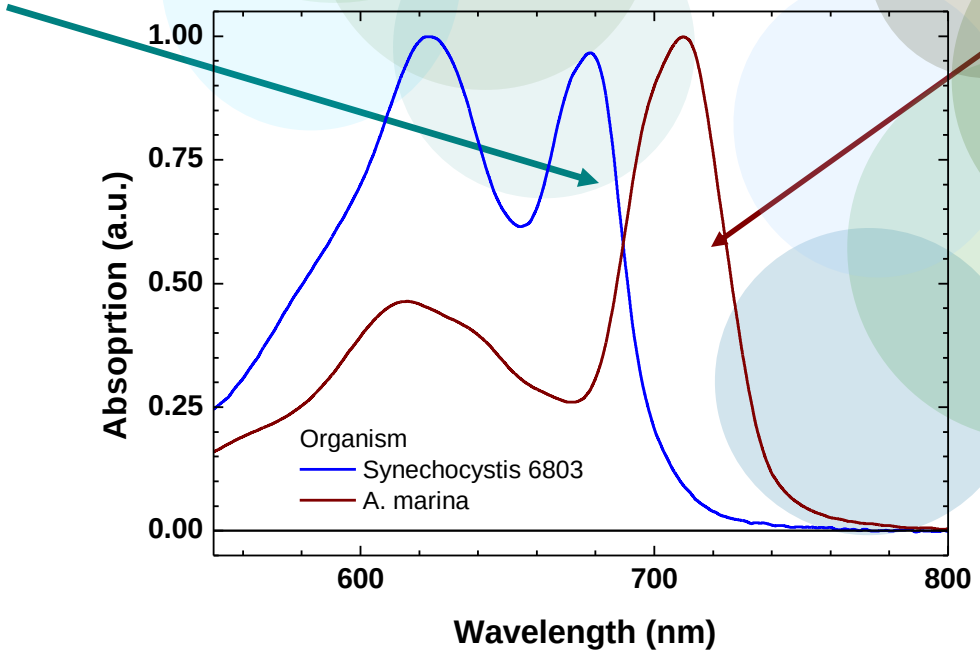


Why Chlorophyll *d*?



Chlorophyll *a*

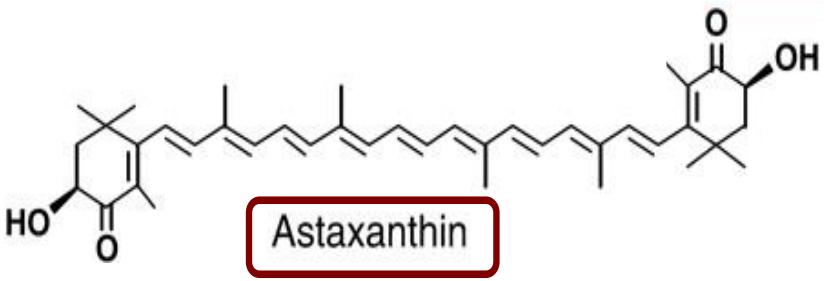
Chlorophyll *d*



Constitutive.
Both PSI & PSII

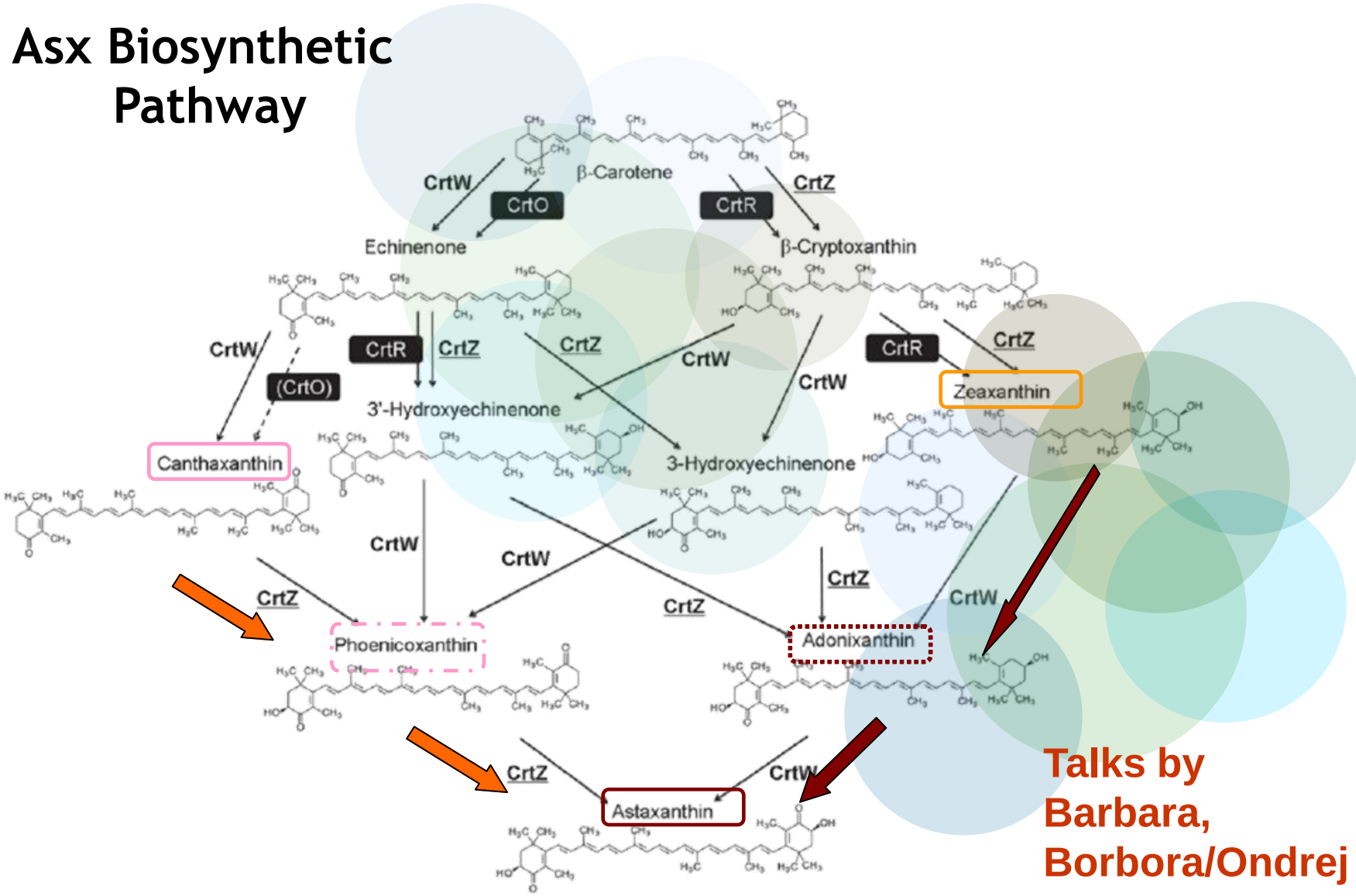
Limits to economic viability of micro-algae cultivation:

- **Even with an efficient PBR, the sole biomass for biofuels (ethanol or diesel) has limited economic benefits (but has ecological benefits!)**
- **Second generation plants: combine biomass + added value bio-products**
- **Suggested solution in CYAO project: biosynthesis of the carotenoid Astaxantin (ASX) in "model" cyanobacteria**



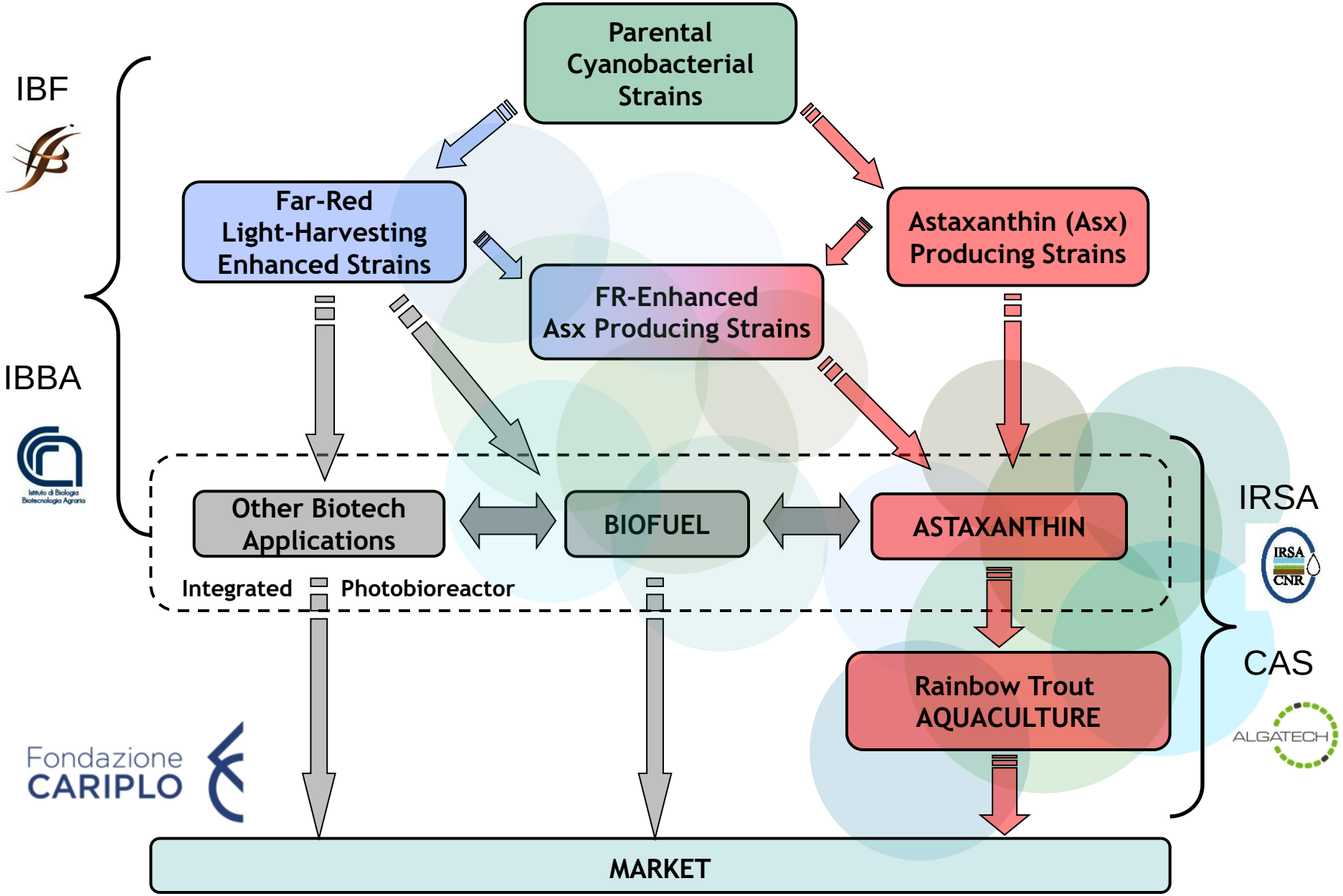
This was the proposed strategy:

Asx Biosynthetic Pathway



Talks by Barbara, Borbora/Ondrej

On-Paper work-flow: combining the two strategies

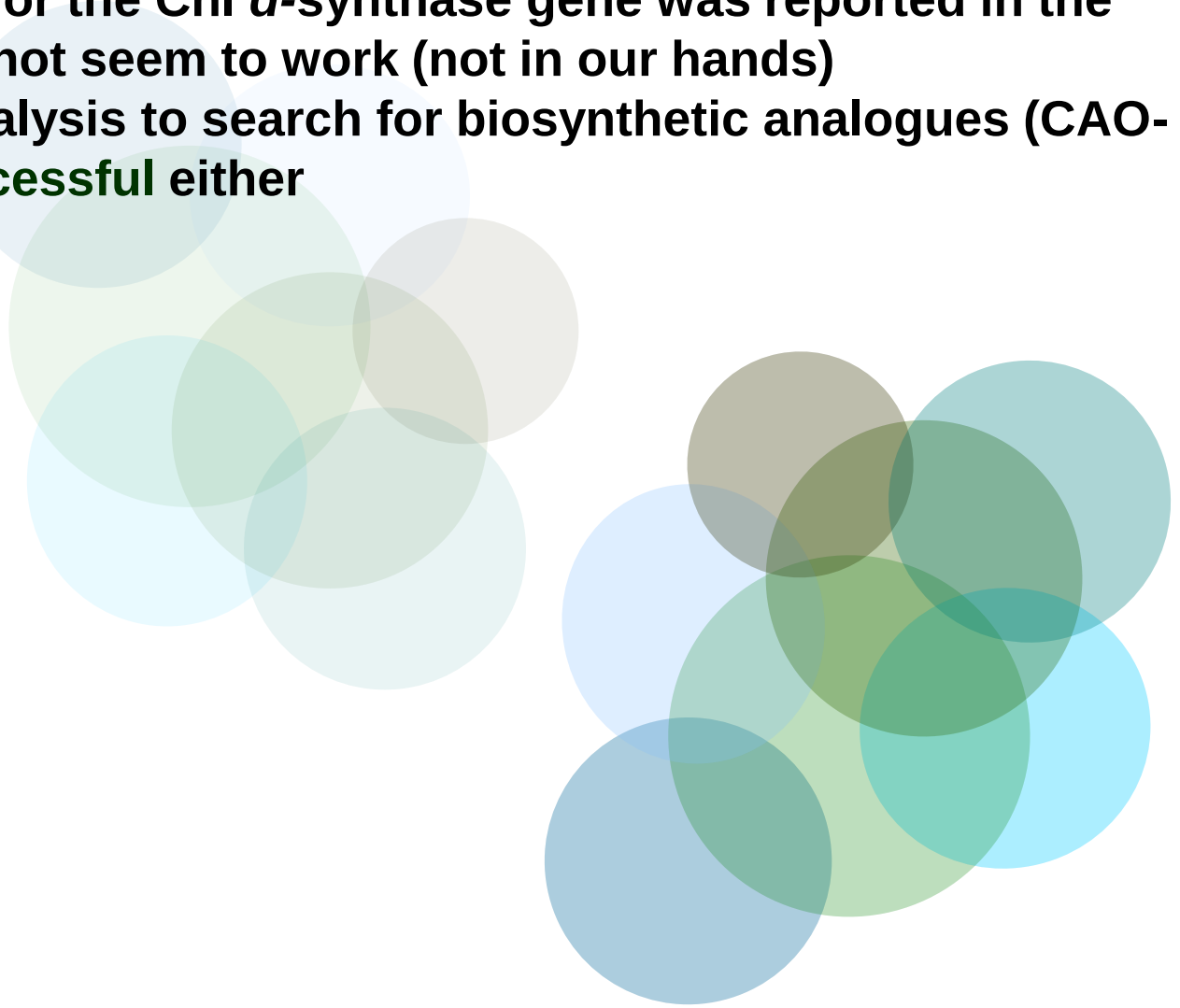


Results on “light harvesting tailoring”

- **An assignment for the Chl *d*-synthase gene was reported in the literature: does not seem to work (not in our hands)**

Results on “light harvesting tailoring”

- **An assignment for the Chl *d*-synthase gene was reported in the literature: does not seem to work (not in our hands)**
- **Bioinfomatic analysis to search for biosynthetic analogues (CAO-family): not successful either**



Results on “light harvesting tailoring”

- **An assignment for the Chl *d*-synthase gene was reported in the literature: does not seem to work (not in our hands)**
- **Bioinfomatic analysis to search for biosynthetic analogues (CAO-family): not successful either**
- **Attempt at generating Chl *d-KO* by UV-irradiation: unsuccessful (essential or internal DNA repair overcomes the mutations)**

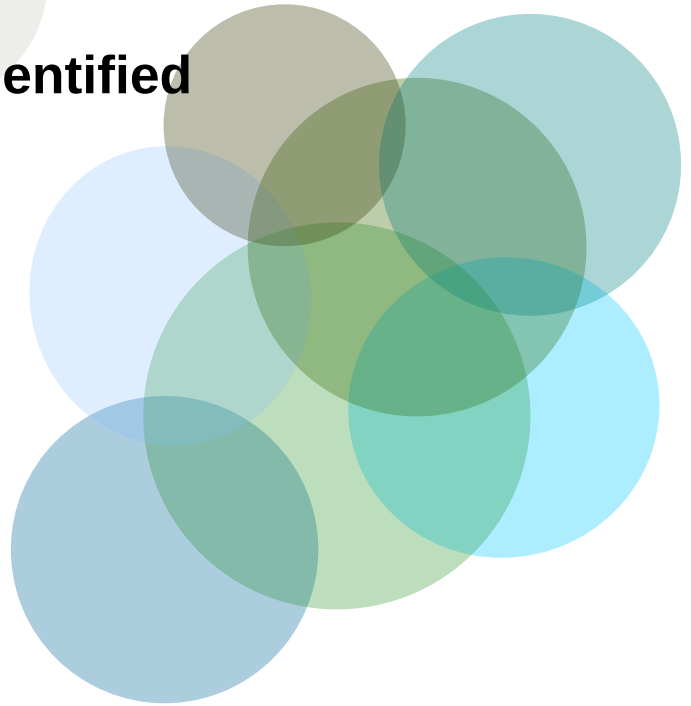
- **Chl d-synthase still need to be *really* identified**

Results on “light harvesting tailoring”

- **An assignment for the Chl *d*-synthase gene was reported in the literature: does not seem to work (not in our hands)**
- **Bioinfomatic analysis to search for biosynthetic analogues (CAO-family): not successful either**
- **Attempt at generating Chl *d-KO* by UV-irradiation: unsuccessful (essential or internal DNA repair overcomes the mutations)**

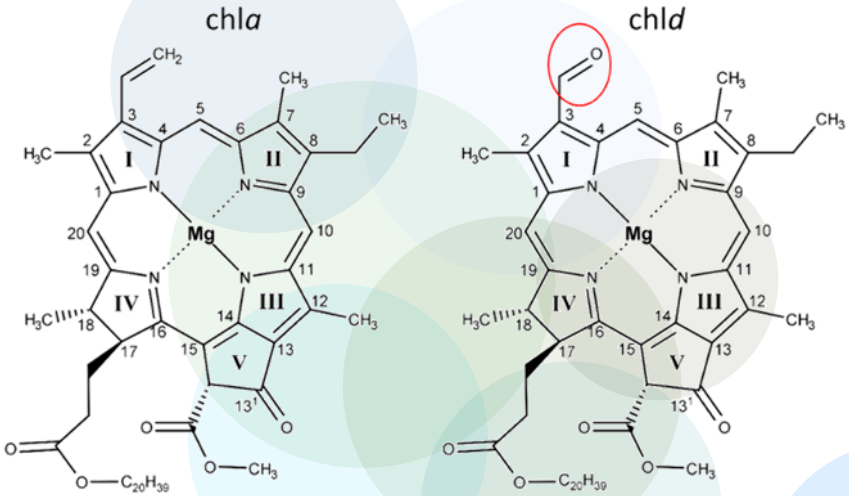
- **Chl d-synthase still need to be *really* identified**

- **Alternative strategies**



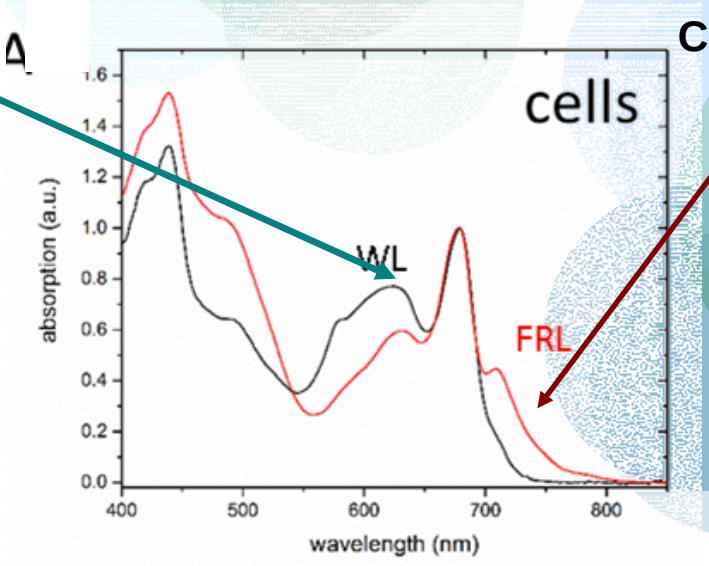
Results on “light harvesting tailoring”: alternative strategies

- **Nature already does what we want: FaRLIP response**



Chlorophyll *a*

White Light Grown



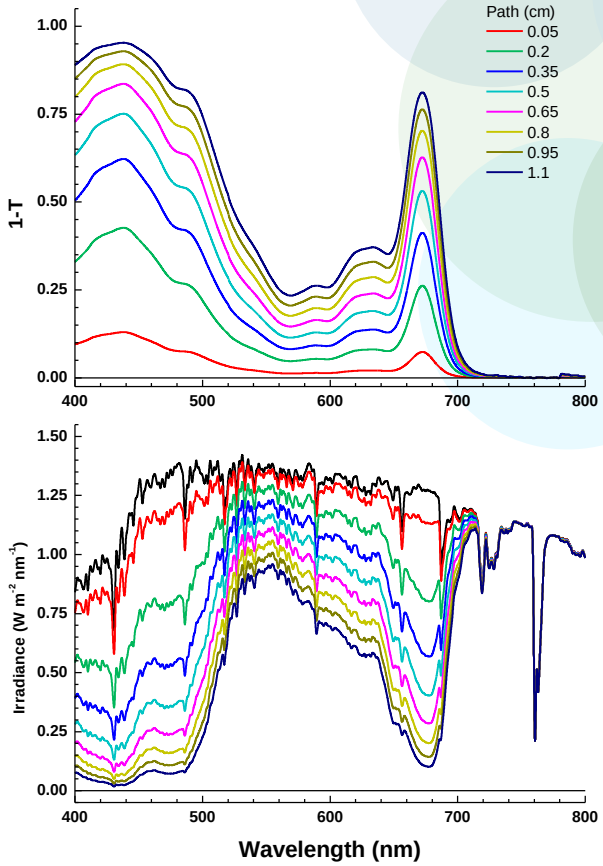
Chlorophyll *f*

Far-Red (750 nm) Grown

This was very preliminar at the start of the project, now many more info. **(Paola Talk)**

Results on “light harvesting tailoring”: alternative strategies

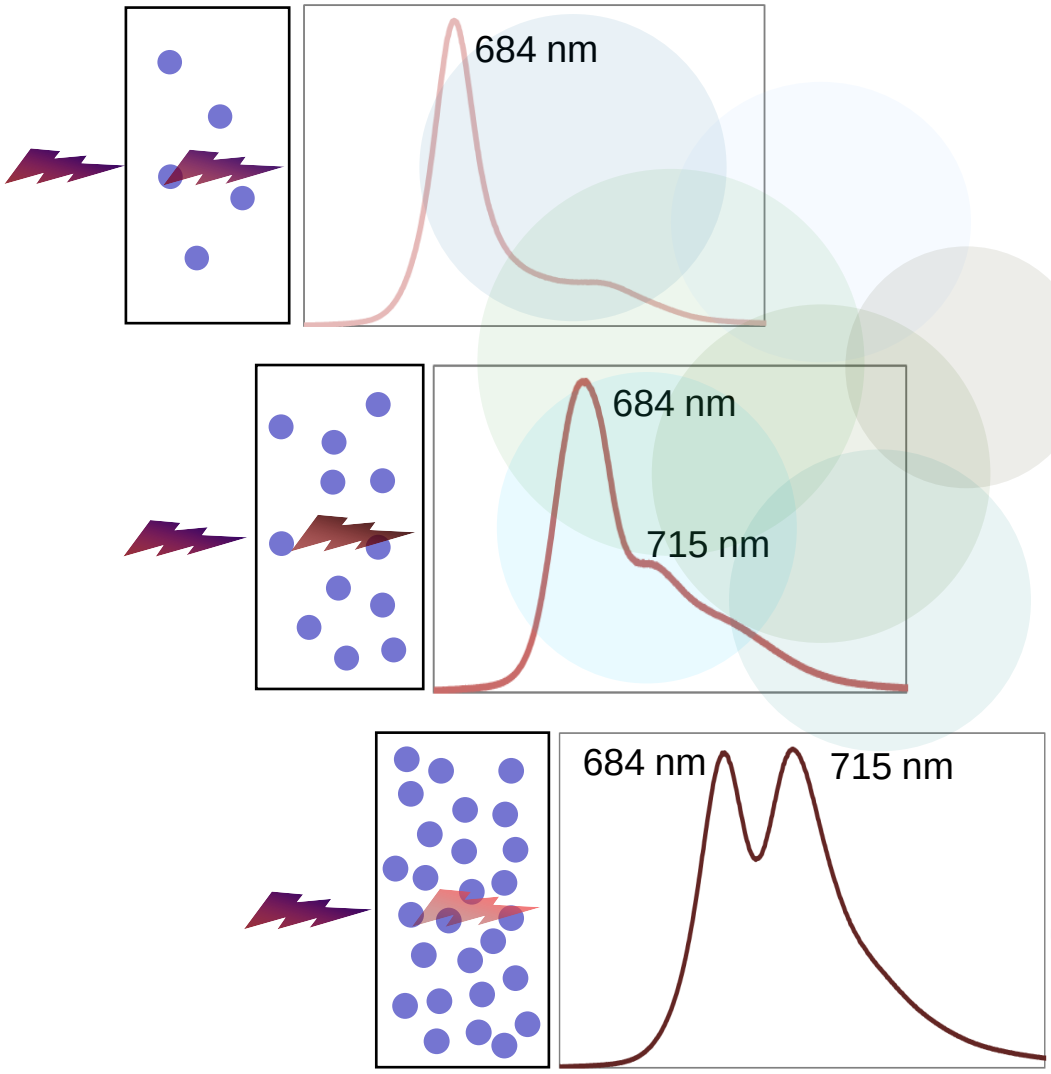
- **Other lessons’ from Nature: long-wavelength light harvesting in red algae (LHC-tuning)**



Self-Shading in *P. tricornutum*

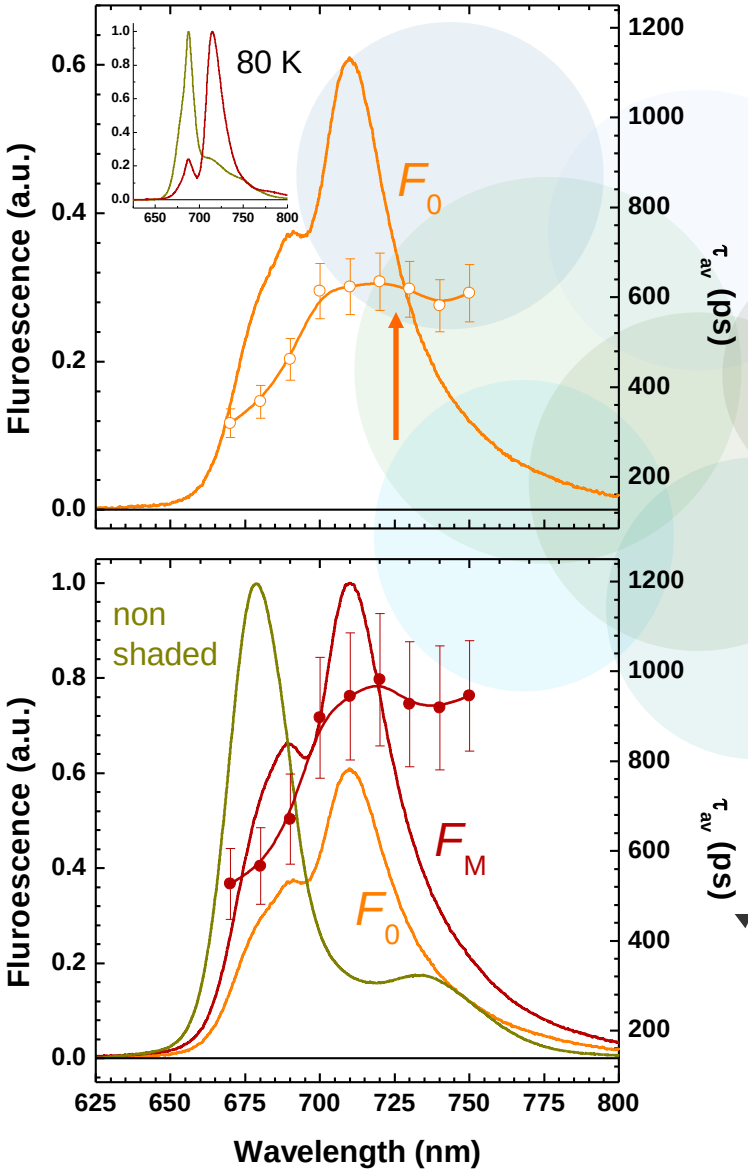
Exactly the same issue, in the Far Red-Near IR as for other unicellular algae (true also for any vegetation layer)

Limit to light penetration: responses (diatoms)



- Diatoms show a long-wavelength adaptation too
- Appearence of long wavelenght emission (at RT) coupled to PSII
- Due to the synthesis of an LHC isoform, not to the synthesis of different Chls
- Chl *a* red-states are typically associated to PSI only: do red states decrease the quantum efficiency of PSII?

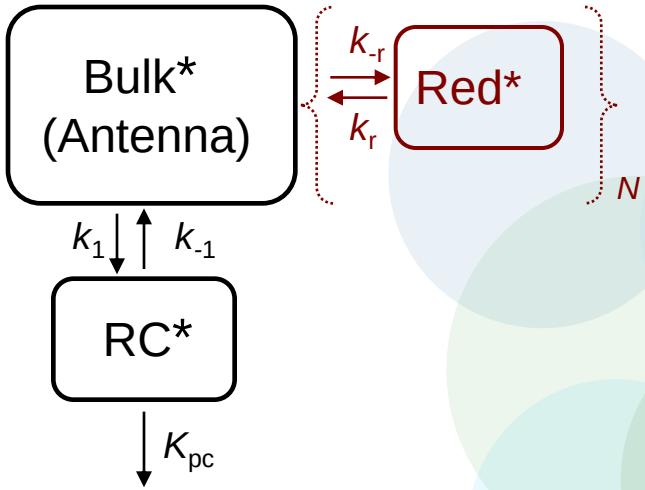
Red-Shifted PSII antenna in Diatoms



- Diatoms show a long-wavelength adaptation too
- Appearence of long wavelenght emission (at RT) coupled to PSII
- Due to the synthesis of an LHC isoform, not to the synthesis of different Chls
- Chl *a* red-states are typically associated to PSI only: do red states decrease the quantum efficiency of PSII?
- probably, they do (in progress)

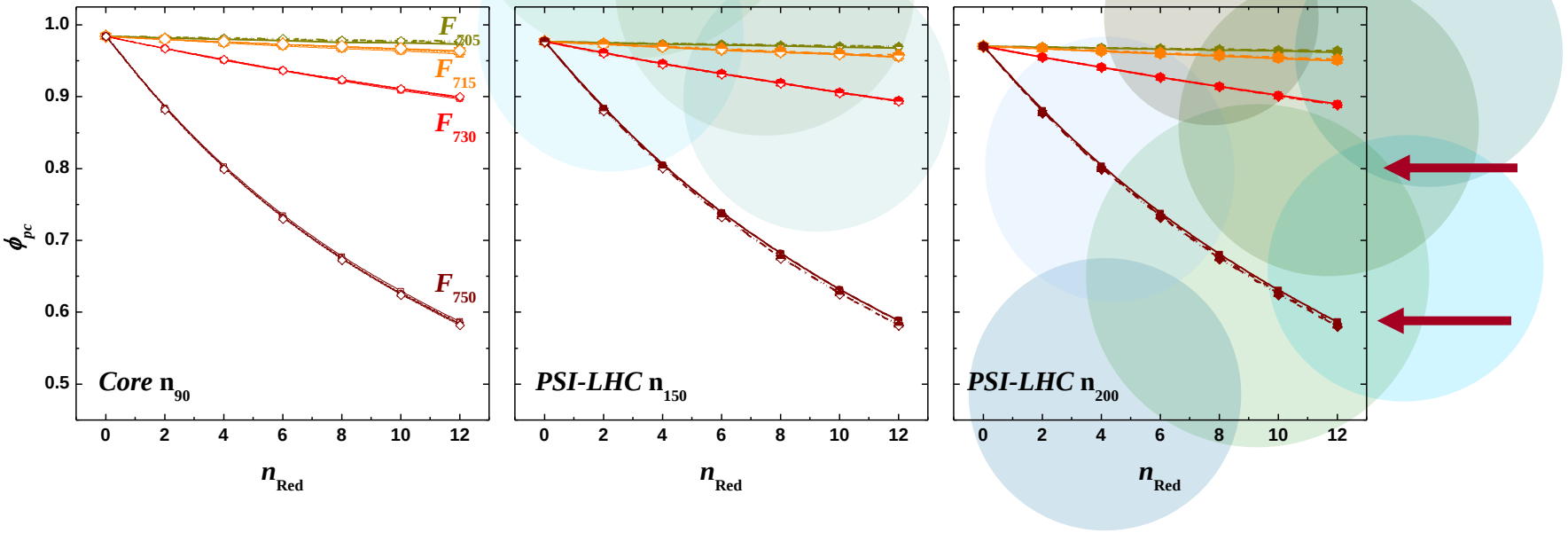
This parameter is (inversely) proportional to the Quantum yield of photon-conversion

Red-Shifted antenna (in general)



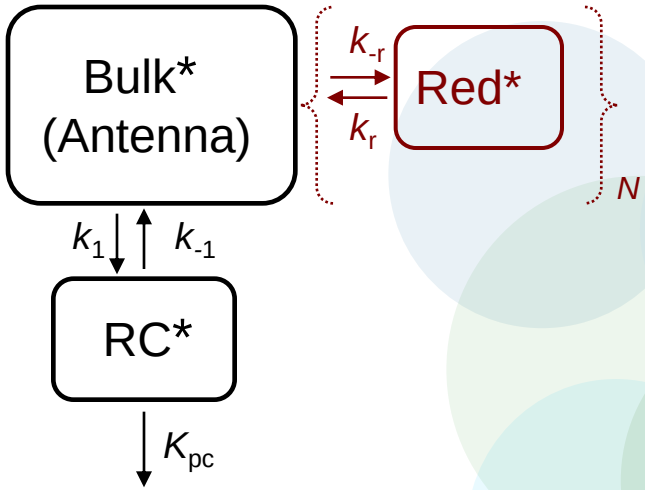
What' s the balance? From predictions

- **PSI is quite robust, but emission forms centred at 750 nm start to be limiting**



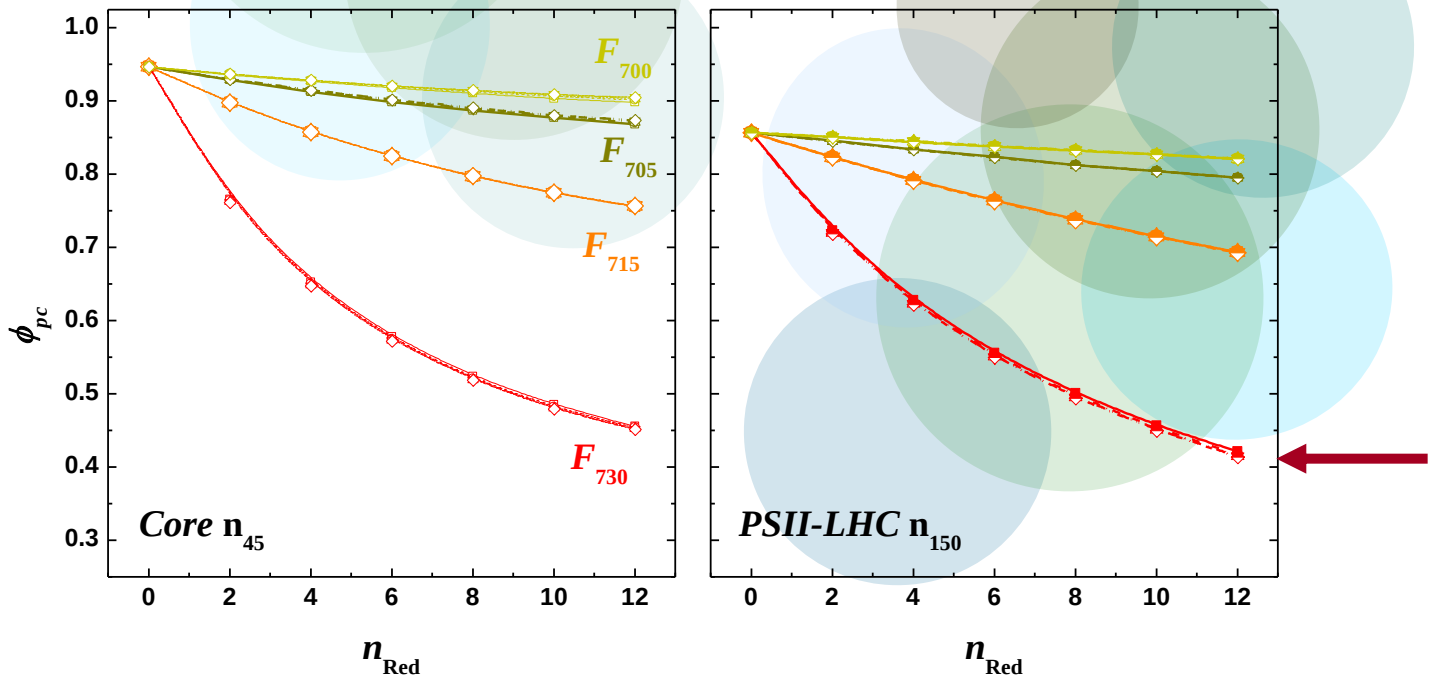
Noteworthy: 750 nm is about the red-most in Nature (*Spirulina platensis*)

Red-Shifted antenna (in general)

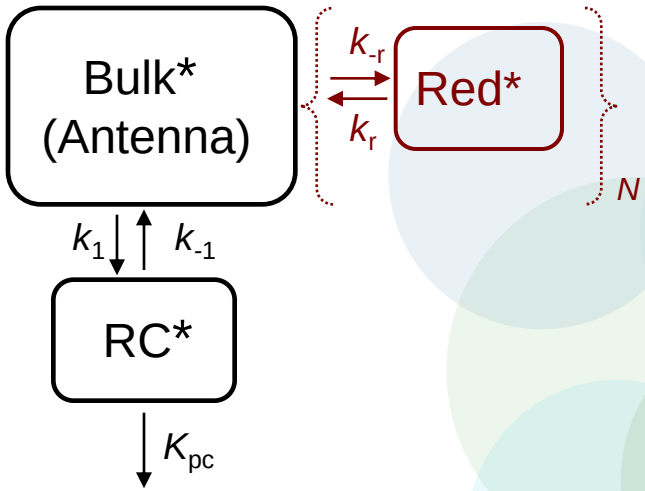


What' s the balance? From predictions

- **PSI is quite robust, but emission forms centred at 750 nm start to be limiting**
- **PSII is more sensitive, emission forms centred at 730 nm are predicted to decrease efficiency largely**



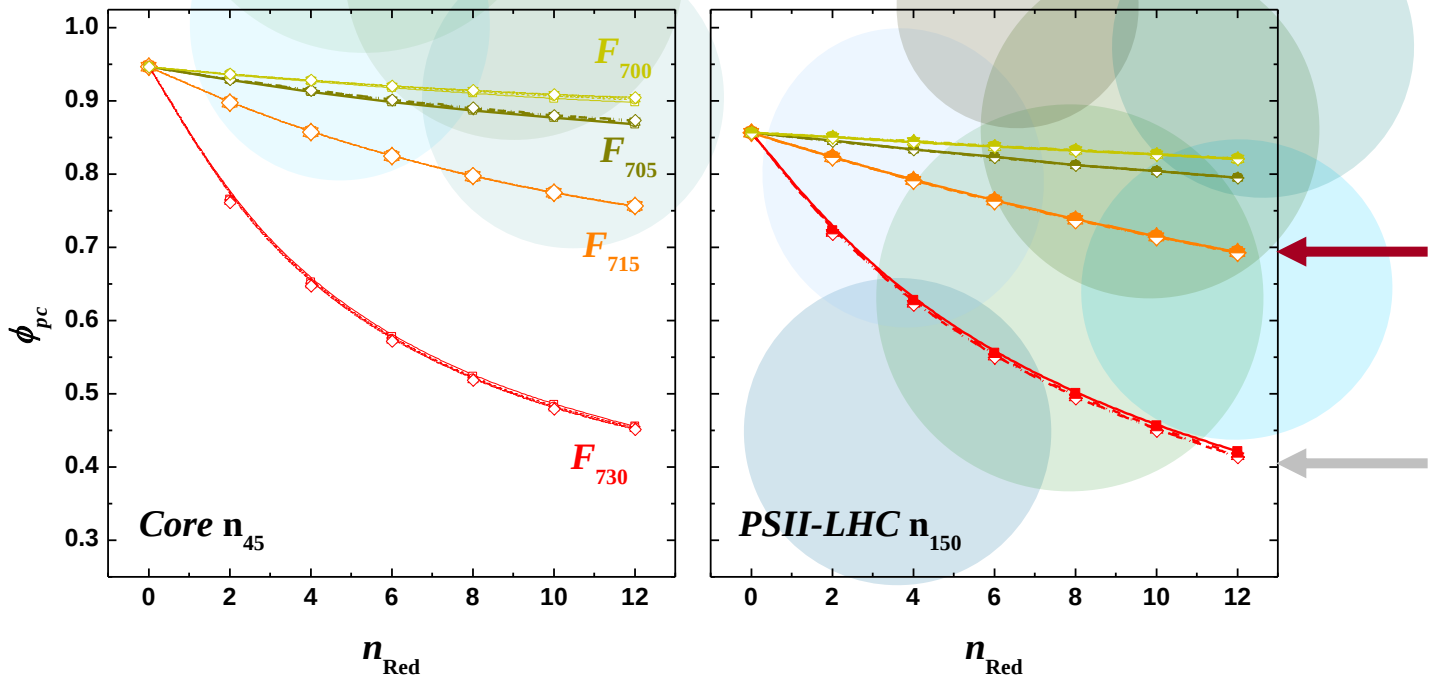
Red-Shifted antenna (in general)



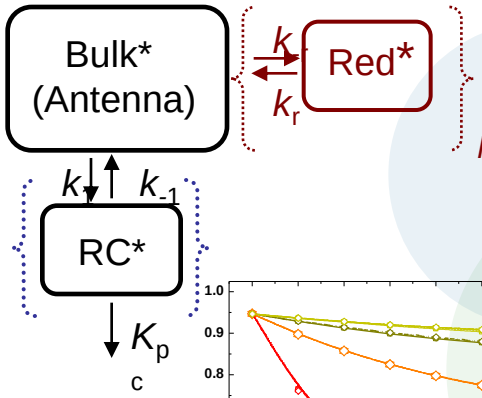
What' s the balance? (From predictions)

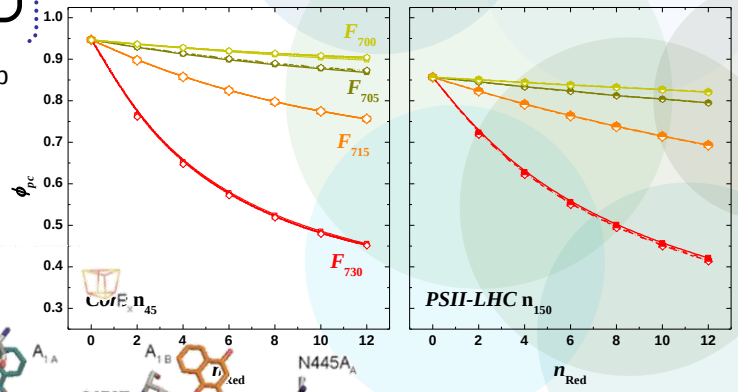
•PSII is more sensitive, emission forms centred at 730 are predicted to decrease efficiency largely

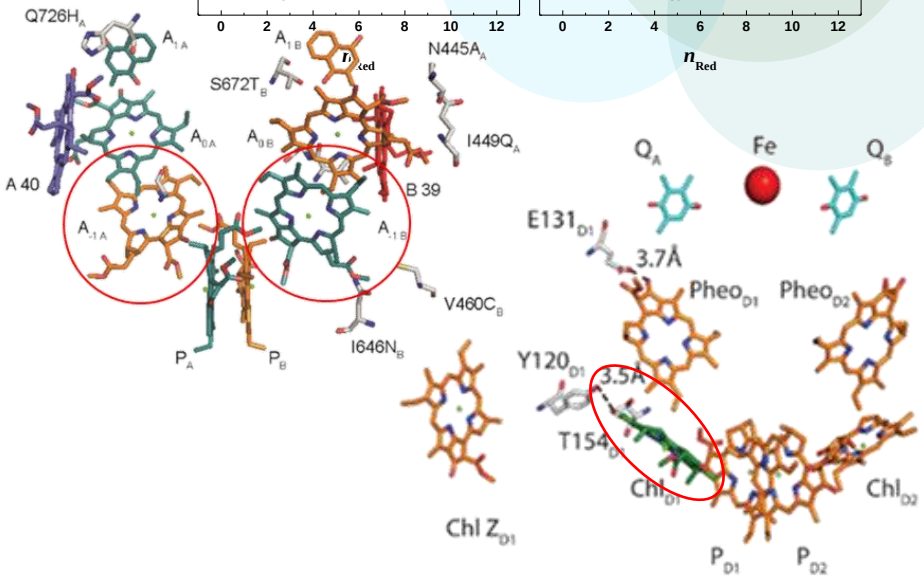
• The low-loss limit is predicted to be at around 715 nm: as found in nature!



What's the "real" balance?



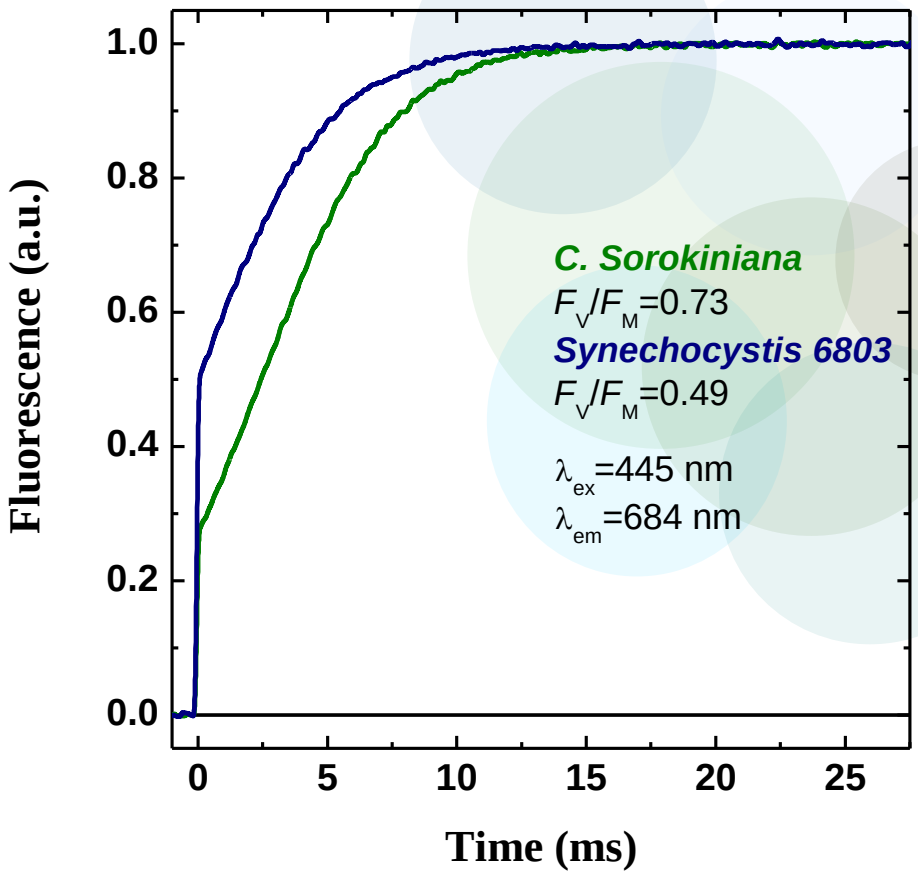




- **How red can the antenna be pushed before quantum efficiency will drop significantly?**
- **Can that be compensated by shifting the energy of RC?**
- **If so *how much energy loss* can be afforded to sustain electron transfer?**

A little bit of extra…

F_V/F_M: maximal yield of PSII in cyanobacteria



- Comparison with green algae:
- lower F_V/F_M (down to 0.2-0.3)
- also from literature data

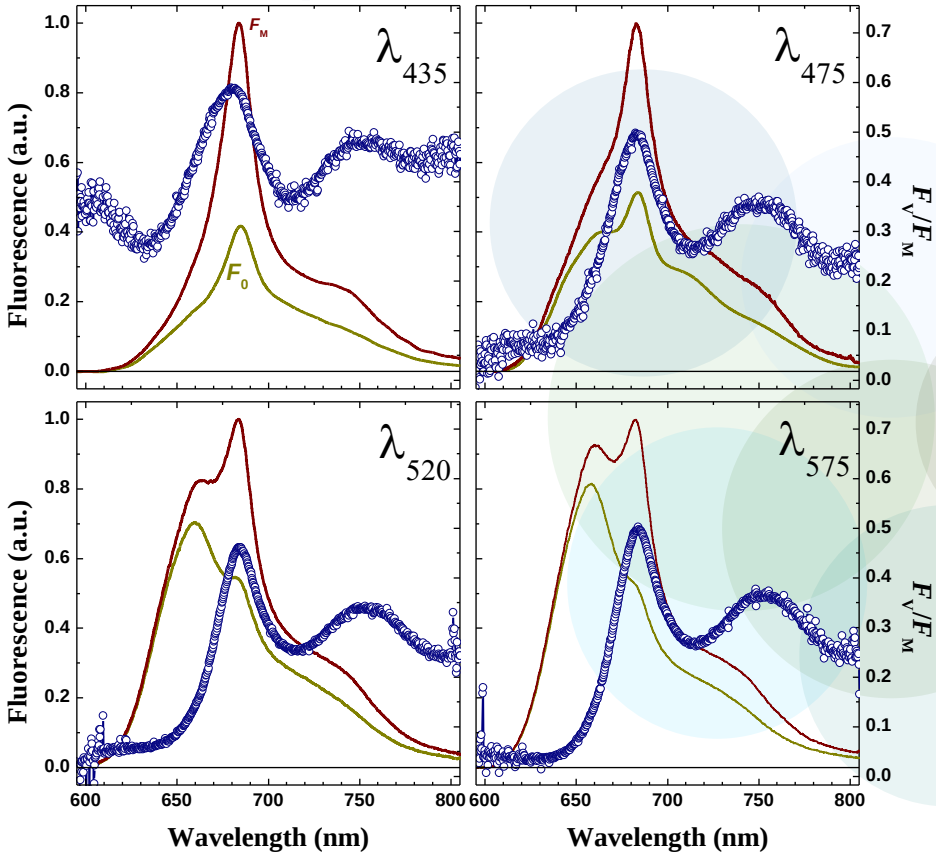
How comes?

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

Is it not valid?

F_V/F_M spectral dependence: emission/excitation

Synechocystis sp. PCC6803

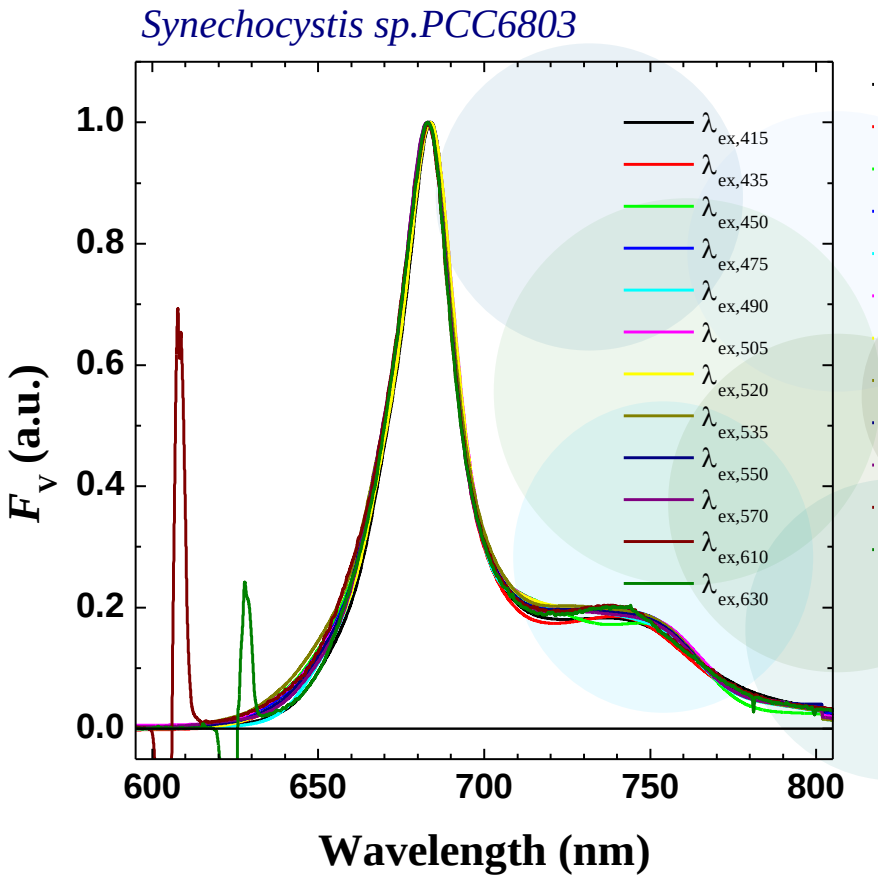


In *Synechocytis sp.* PCC6803

- **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 wavelengths**

Same in Synechococcus PCC7942

F_V/F_M spectral dependence: emission/excitation



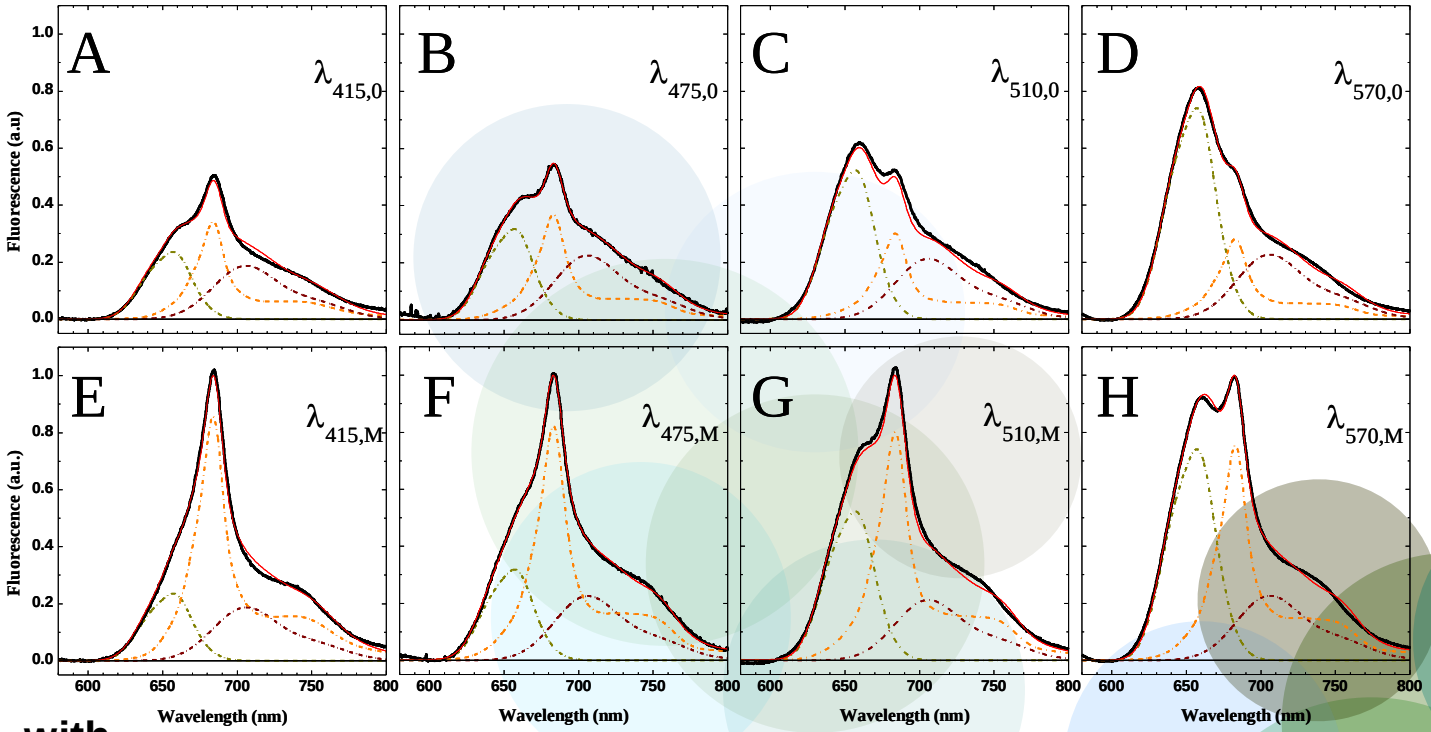
In *Synechocytis sp.* PCC6803

- 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 wavelengths

but

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

Decomposition of spectra into components (cyanobacteria)



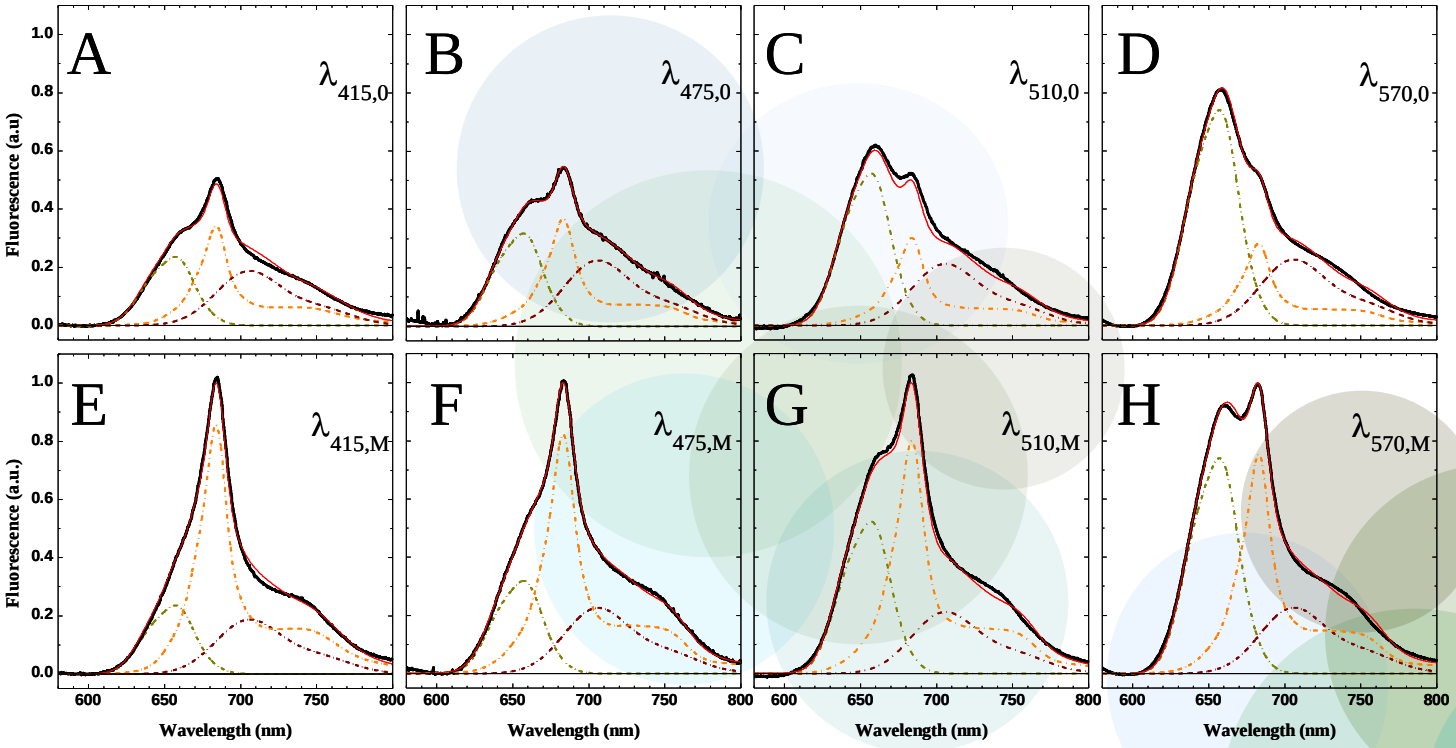
with

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

Then

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

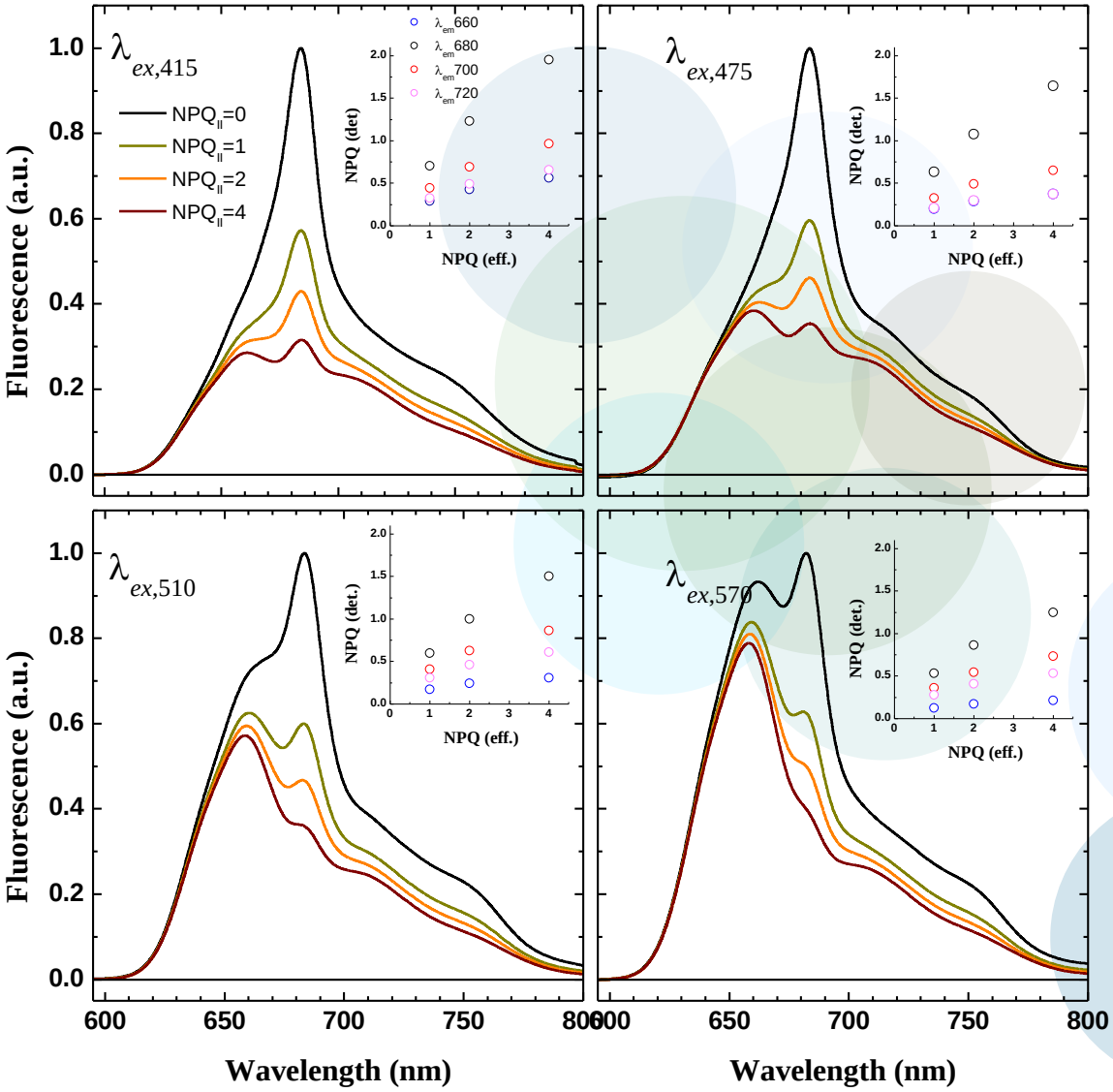
Decomposition of spectra into components (cyanobacteria)



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

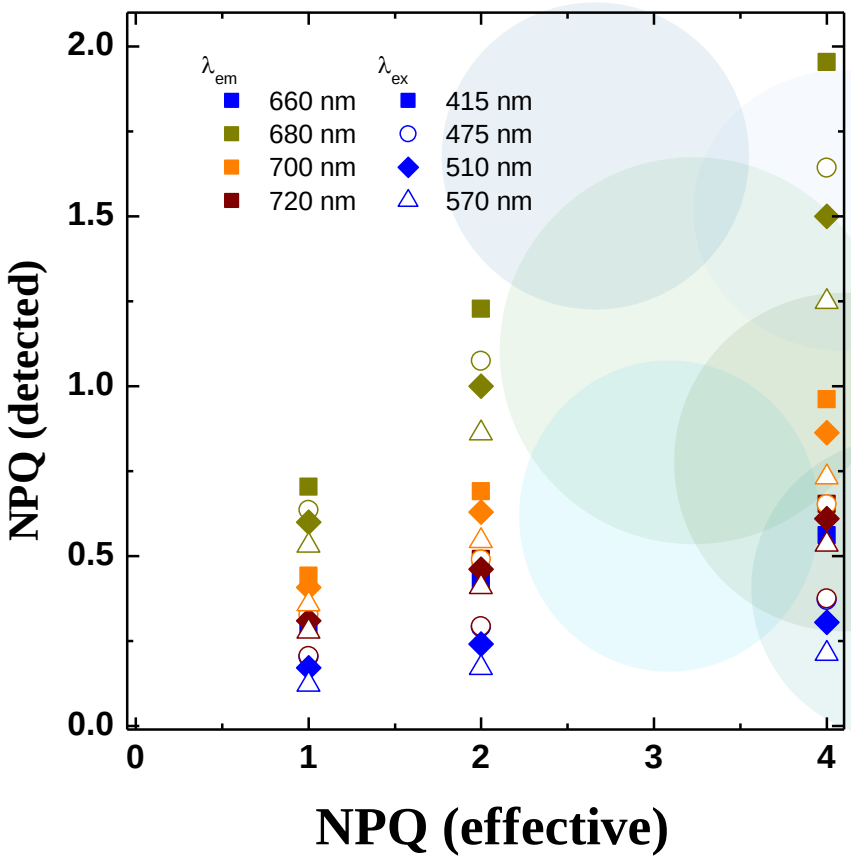
- highlights the different contributions of PSII, PSI and uncoupled PBS at each set of excitation/emission wavelengths

Impact on other parameters: NPQ (cyanobacteria)



- 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 filters
- largely underestimated!

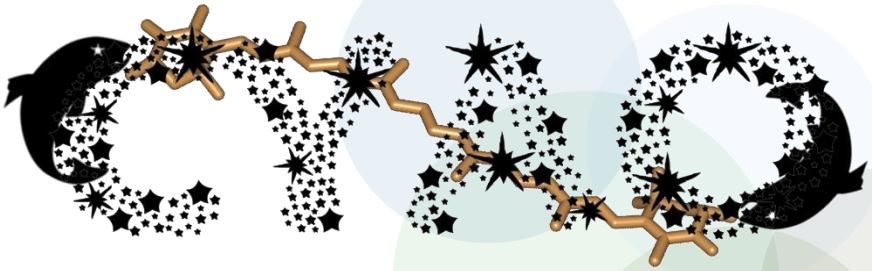
Impact on other parameters estimation: NPQ



- larger values (50% underestimated) for PSII max detection/Soret Excitation
- lower values (>80% underestimated) for PBS detection/PBS Excitation

- can be corrected, knowing the super-complexes absorption/emission cross-sections
- can be useful to distinguish different mechanisms/sites of quenching

Thank You and..



!

Impossible without the generous support from:

Fondazione CARIPLO

And the efforts of all researches involved