

# Structure and Function of Photosynthetic Complexes in Cyanobacteria Revealed by Hyperspectral Confocal Fluorescence & Electron Microscopy

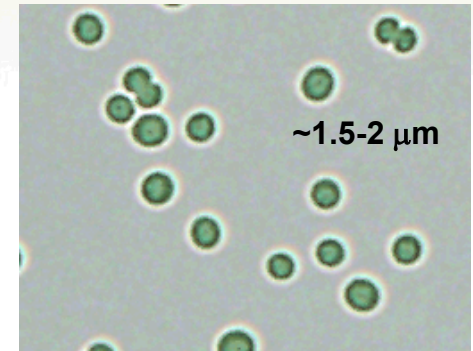
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- Motivation/Introduction
- Methodology
- Comparison of photosynthetic antennae mutants
  - Intracellular structural organization by transmission electron microscopy
  - Intracellular pigment distribution by hyperspectral confocal fluorescence microscopy
- Conclusions/Future Work

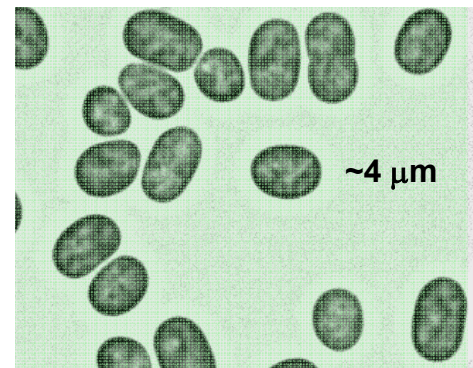
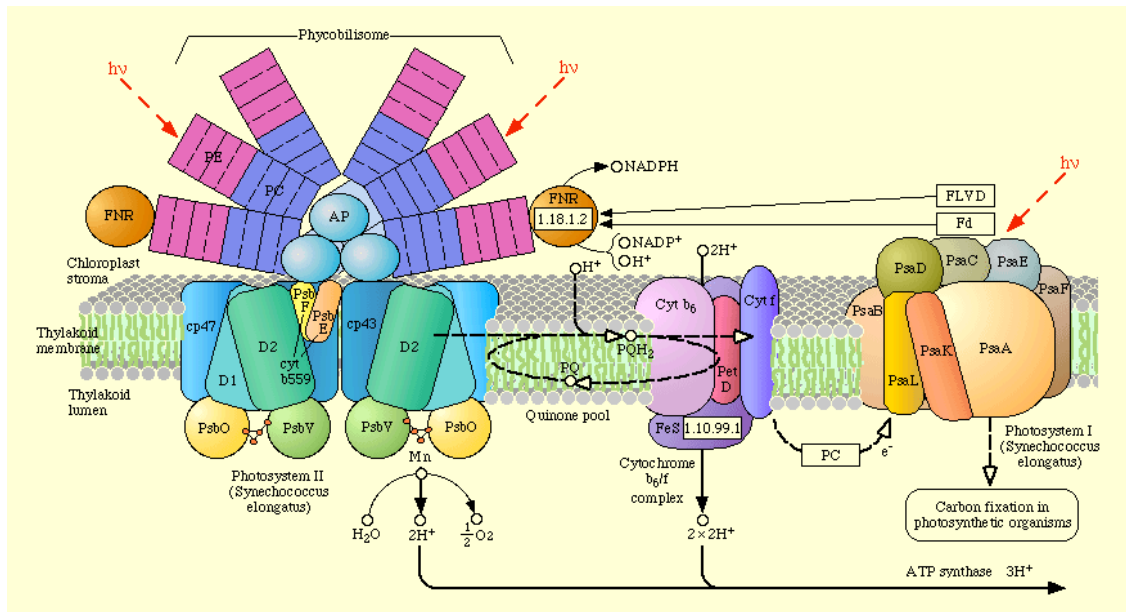
# Cyanobacteria are a diverse group of oxygenic photosynthetic prokaryotes

- Morphologically diverse and metabolically complex
- Photosynthetic machinery resides in the thylakoid membrane system
- Thylakoid membrane structure and function are tightly related



~1.5-2  $\mu\text{m}$

*Synechocystis* 6803



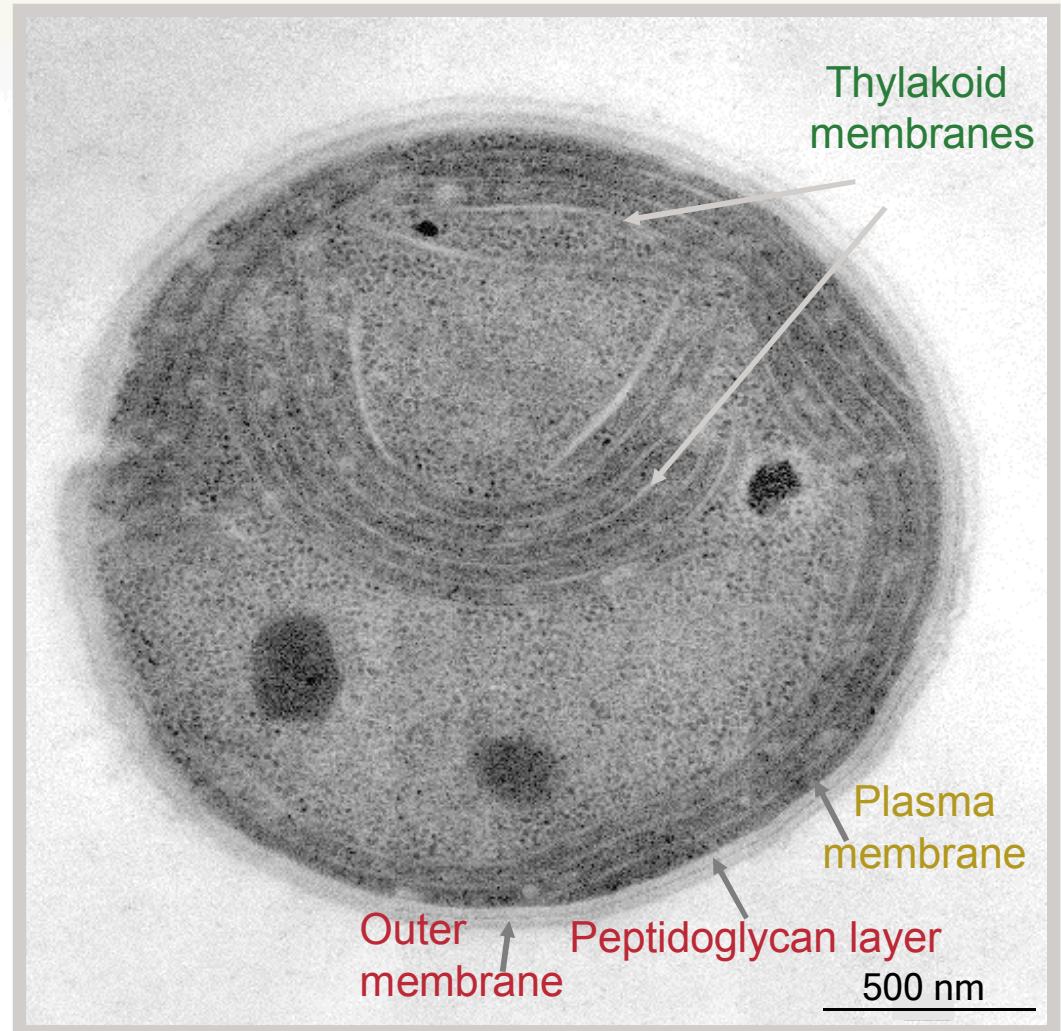
~4  $\mu\text{m}$

*Cyanothece* 51142



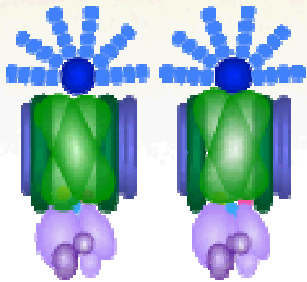
# *Synechocystis* sp. PCC 6803

- First photosynthetic organism with sequenced genome
- Unicellular, non-diazotrophic
- Photoautotrophic and heterotrophic growth, naturally competent

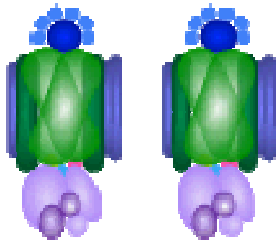


# Phycobilisome antenna mutants in *Synechocystis*

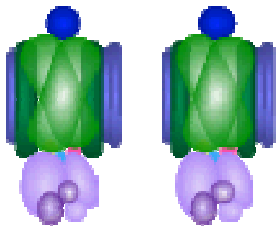
**WT**



**CB**



**CK**



**PAL**

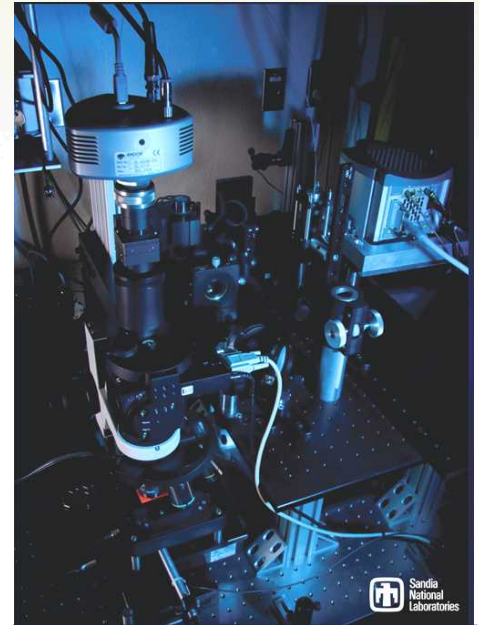


- WT: phycobilisomes contain four phycocyanin hexamers per rod
- CB: phycobilisomes contain only one phycocyanin hexamer per rod
- CK: phycobilisomes lacking phycocyanin rods but retaining allophycocyanin core
- PAL: completely lacking assembled phycobilisomes

# Methodology

## Hyperspectral Confocal Fluorescence Microscopy

- 20+ cells imaged from each of 3 biological replicates for WT and PAL
- Custom microscope (Fig. at right)
  - 60x objective (NA 1.4)
  - 4150 spectra/sec
  - 488 nm excitation
- Multivariate curve resolution (MCR) analysis of composite image set to identify pure spectra (Vermaas PNAS 2008, Jones J Chemom. 2008)
- Weighted classical least squares (CLS) prediction to determine relative concentrations of pigments



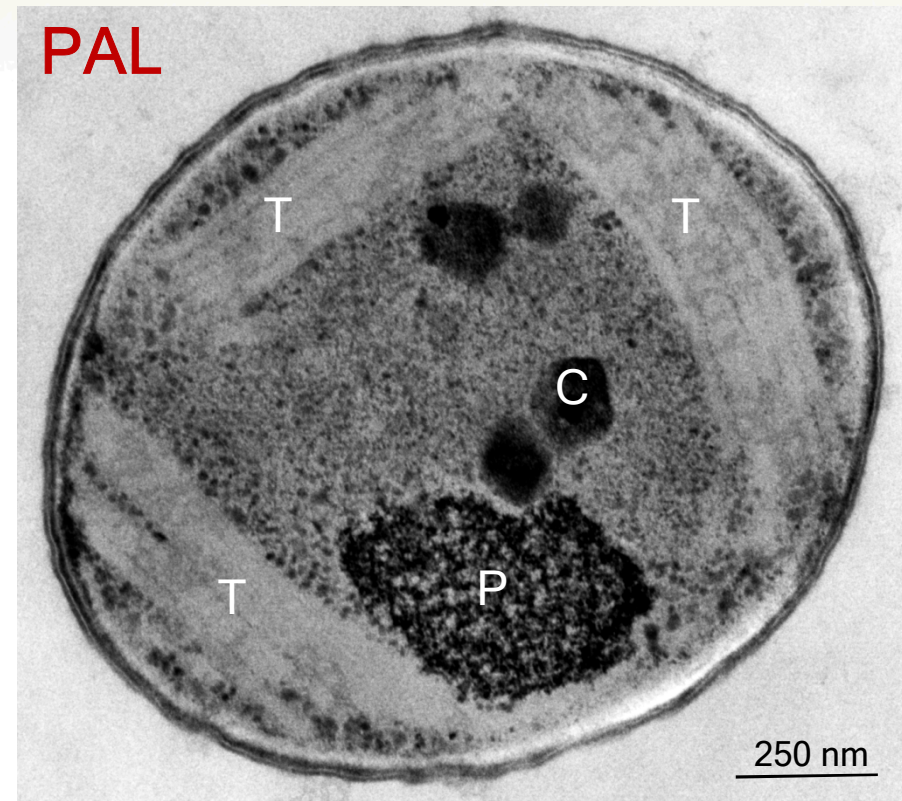
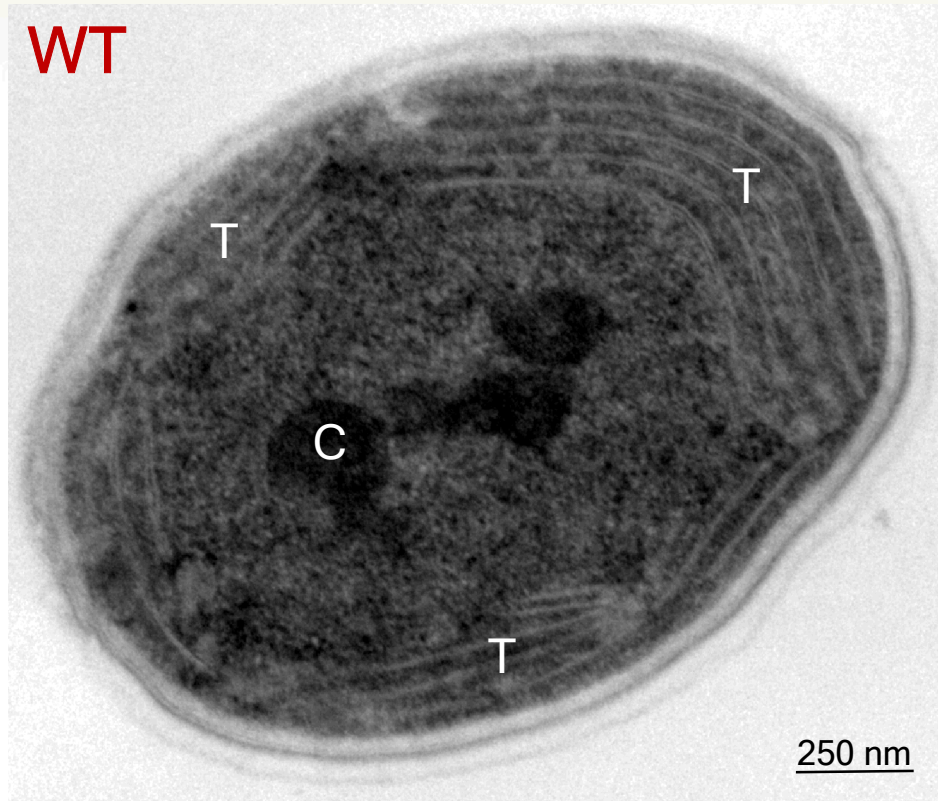
Sinclair, et. al. "Hyperspectral confocal microscope," *Applied Optics*, 45, 6283-6291 (2006).

## Transmission Electron Microscopy

- High pressure freezing, resin embedded
- Thin sections (80nm)
- LEO 912 transmission electron microscope equipped with a ProScan digital camera



# Intracellular organization in *Synechocystis*

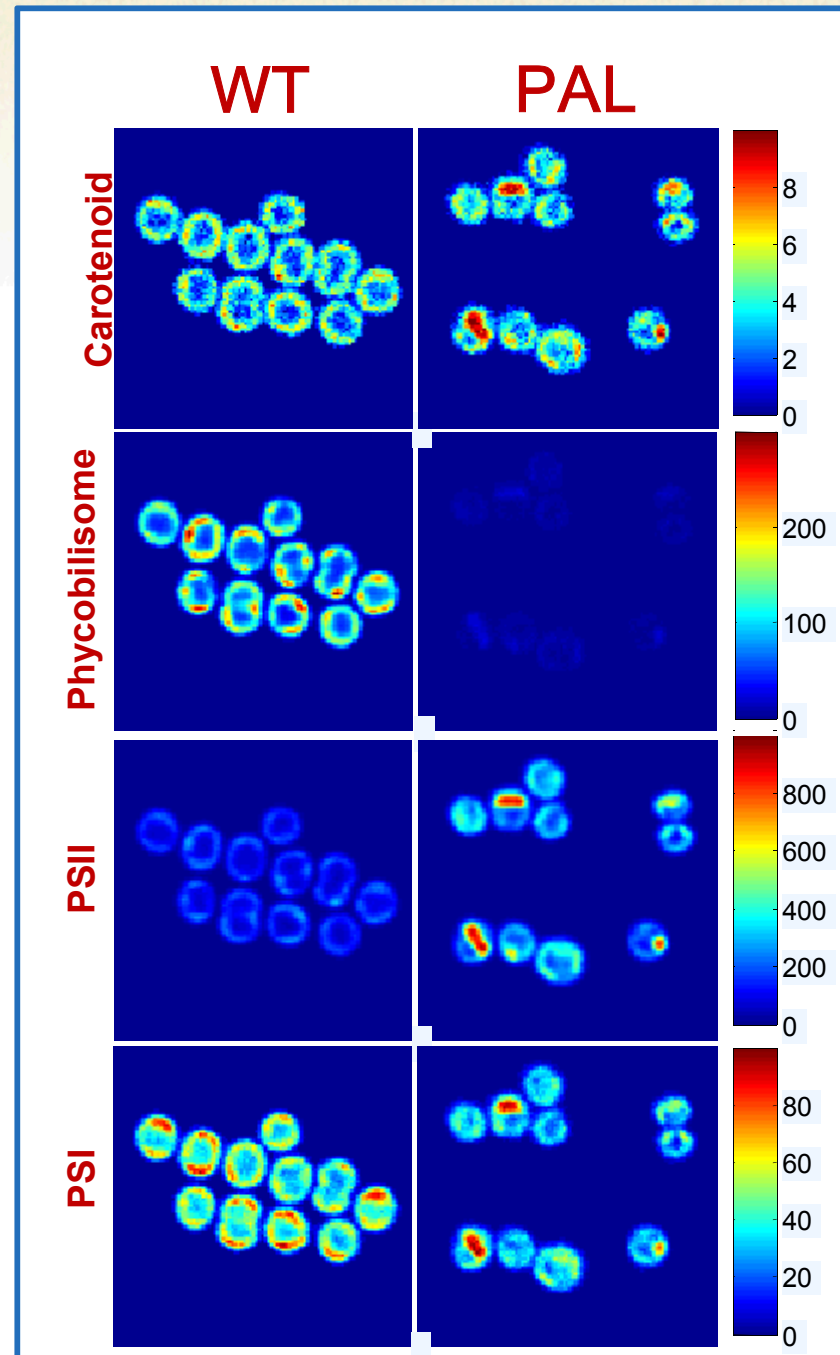
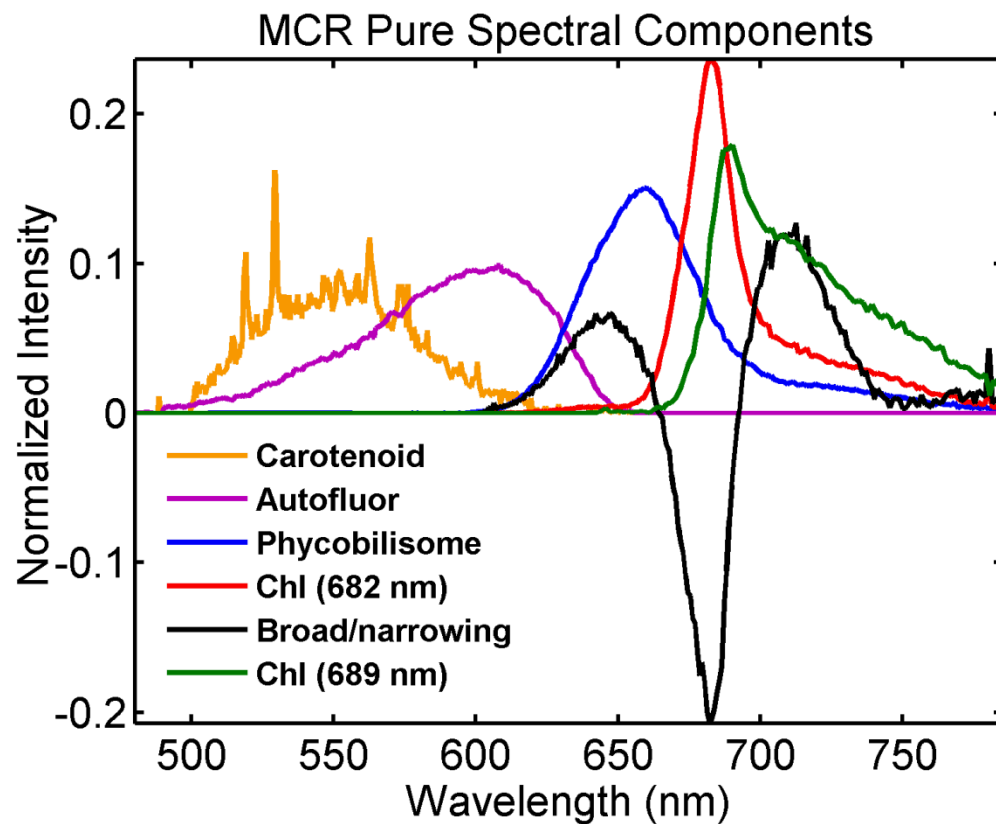


T, thylakoid membrane; C, carboxysome; P, polyphosphate body

## Thylakoid membrane morphology is altered in the PAL mutant

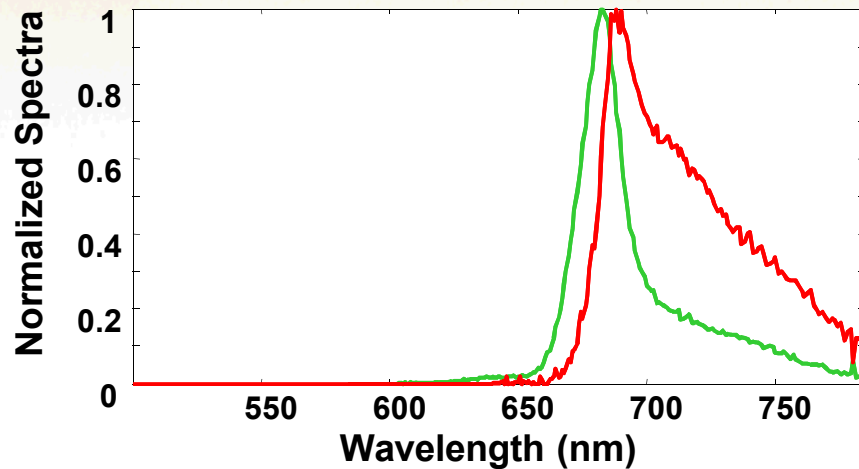
- Decreased curvature
- Similar thylakoid thickness
- 2X increase in cytoplasmic space between thylakoid bilayer

# Photosynthetic Pigment Organization



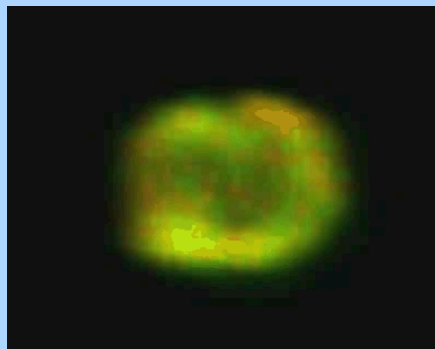


# Differential Organization of PSI & PSII

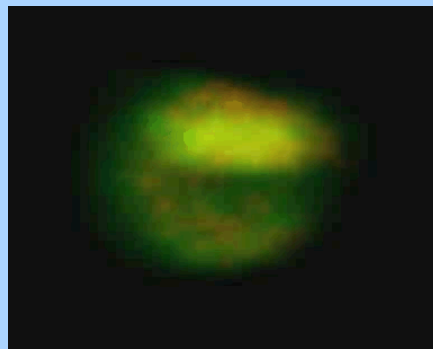


Heterogeneous pigment organization

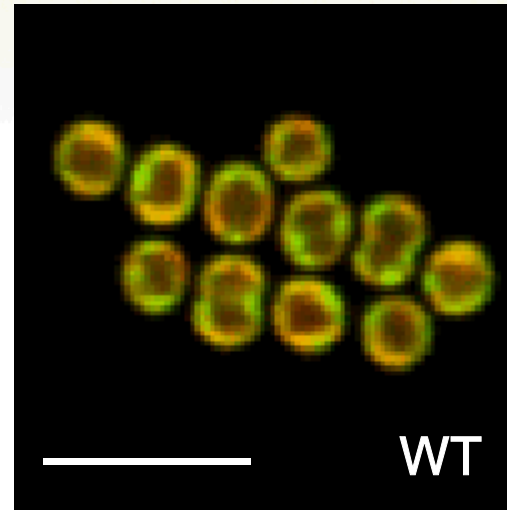
- PSI, PSII
- Single loci containing PSI, PSII, carotenoid



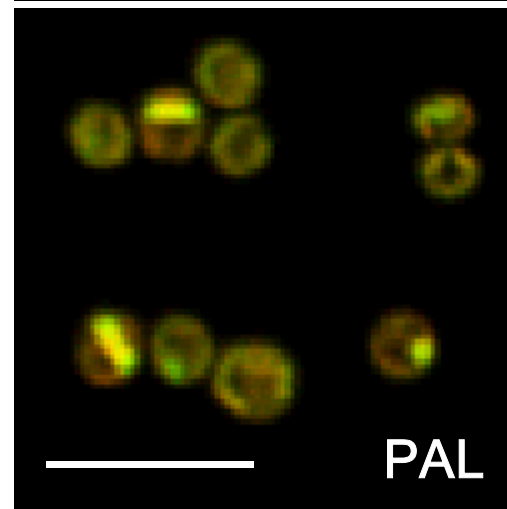
WT



PAL



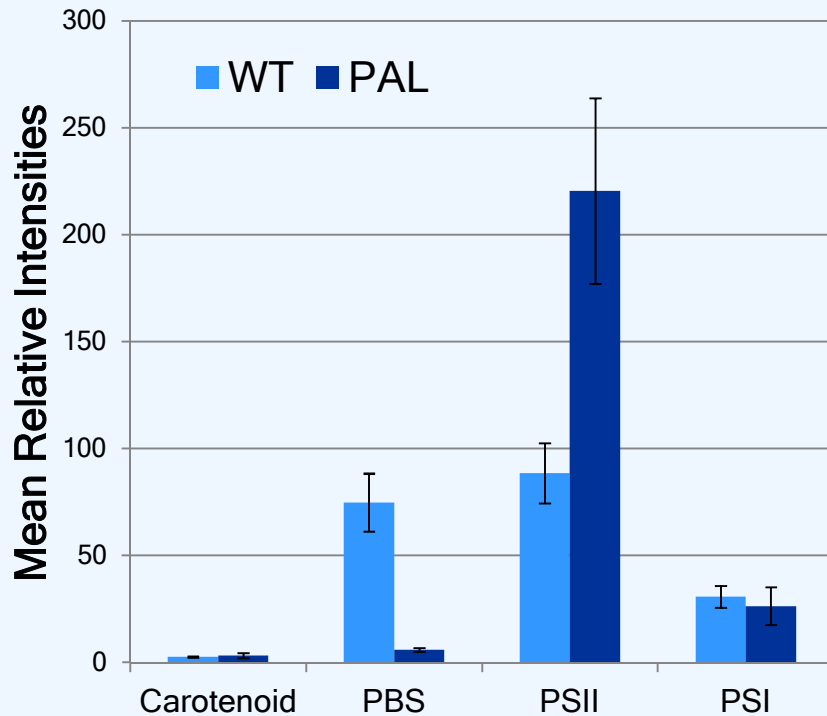
WT



PAL

Channel intensities scaled  
independently.  
Scale bar = 5  $\mu\text{m}$

# Quantitative Single Cell Analysis



Mean values were calculated from images of 114 WT cells and 131 PAL cells (including 3 biological replicates per cell type)

ANOVA analysis confirms significant differences between the WT and the PAL mutant cells for

- Phycobilisomes
- PSII
- Shift/broadening (not shown).

PAL cells have comparable amounts of PSII and carotenoid as the WT cells but 2.5X the PSII and insignificant amounts of PBS

Shift/broadening component is + in WT and - in PAL

→ Narrowing and blue shift in PAL cells compared to WT

→ Smaller distribution of chlorophyll states in PAL cells



# Conclusions and Future Work

- Thylakoid membrane morphology is altered in the PAL mutant
  - Lower degree of thylakoid curvature
  - Increased stacking density
- Pigment distribution is altered in PAL mutant
  - Cells compensate by increasing PSII
  - PSI & PSII segregation w/ large, singular foci
- Investigate CB & CK antennae mutants
- Quantify the changes in thylakoid membranes



# Acknowledgements

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