SND2013-6408C

Photosystem Segregation in Cyanobacterial Thylakoids

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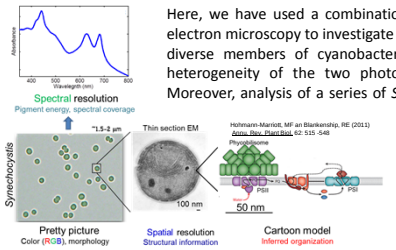
Highlights

- Global pigmentation in cyanobacteria responds dynamically to light quality
- *Synechocystsis* antenna mutants have altered thylakoid morphology and pigmentation
- Hyperspectral confocal fluorescence microscopy and multivariate analysis reveal unique photosystem I profiles among various cyanobacteria

Introduction

Cyanobacteria are oxygenic photosynthetic prokaryotes that are the progenitors of the chloroplasts of algae and plants. Light harvesting is realized by a combination of membrane extrinsic and intrinsic pigment-protein complexes to harness light excitation over most of the visible spectral region. The structure and function of the individual constituents of the light-harvesting antenna and two photosystems are well known for some strains however, how these complexes are organized and distributed in to the thylakoid membrane is not known in detail.



Here, we have used a combination of hyperspectral confocal fluorescence microscopy and electron microscopy to investigate the organisms-wide distribution of pigments among several diverse members of cyanobacteria. Our results indicate a reoccurring theme of lateral heterogeneity of the two photosystems observed for most of the organisms studied. Moreover, analysis of a series of *Synechocystis* mutants where the phycobilisome antenna is progressively truncated revealed an adaptive strategy to balance the absorption capabilities of Photosystem I and II under light-limiting conditions. These results demonstrate the noteworthy modulation and plasticity of cyanobacterial thylakoids not observed previously.

Hyperspectral Fluorescence Microscope / Image Analysis

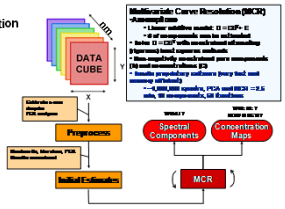
Hyperspectral confocal fluorescence microscopy (HCFM)

- Custom built
- 488 nm laser excitation
- 60x Oil objective
- Lateral res. = 250 nm
- Axial res. = ~0.6 µm
- Spectral range 490-800 nm
- Spectral res. = 1-3 nm
- Acquisition rate = 4150 spec/s

Multivariate Curve Resolution (MCR)

Discover & quantify all emitting species in a sample simultaneously with no *a priori* knowledge

Mathematical isolation of pure spectral components, independent concentration maps

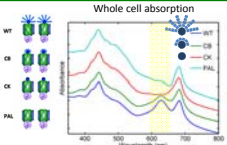


Sinclair, MB., et al. (2006) Appl. Opt. 45, 3283-3291.

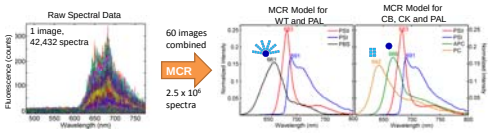
Jones et al., (2008) J Chemom. 22:482-490 and references therein

Light-limited remodeling of thylakoid membranes

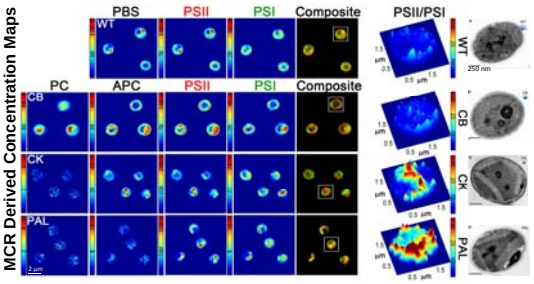
We explored the effect of antenna modification in a series of *Synechocystis* mutants with progressively truncated phycobilisomes.



- 24+ cells imaged from each of 2-3 biological replicates for each cell type
- Multivariate curve resolution (MCR) analysis of composite image set to identify pure spectra
- Weighted classical least squares (wtCLS) prediction to determine relative concentrations of pigments

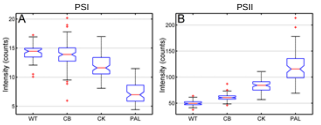


Two MCR models were developed to delineate fluorescence from all cell types.



MCR derived concentrations maps for PC, APC, PBS, PSII and PSI within each cell type. A red-green composite image was constructed to highlight spatial segregation between PSII and PSI. PSII intensity was colored red and PSI colored green. The per-pixel ratio of PSII:PSI displays significant PSII segregation with decreasing antenna size.

Statistical box plots of the per-cell mean intensities for PSI and PSII. The red center line represents the median of these values, and the 95% confidence band is denoted by notches. The bottom and top of the box represent the 25th and 75th percentiles, respectively. The dotted extended lines indicate 62.7s (99.3% data coverage), and the red crosses represent statistical outliers

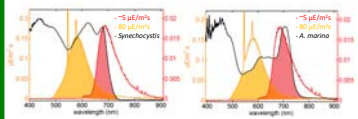


n = 332 cells

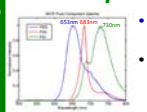
Global Pigment Dynamics in Response to Light Quality

Synechocystis PCC. 6803
Cyanothece 51124
Acaryochloris marina (MBIC11017)
Spirulina platensis

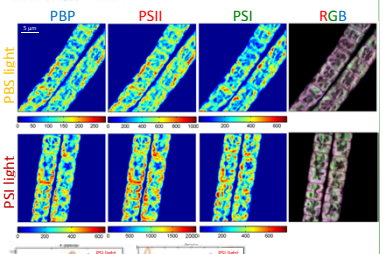
- Grown under yellow filtered or red filtered light to selectively excite PBS or PSI
- Interrogated with HCFM and MCR analysis

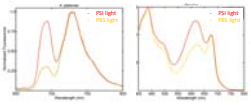


Spirulina platensis



- PBS and PSII increase drastically under PSI light
- PBS reorganize from mainly interior thylakoid under yellow light to periphery under red light.

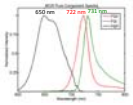




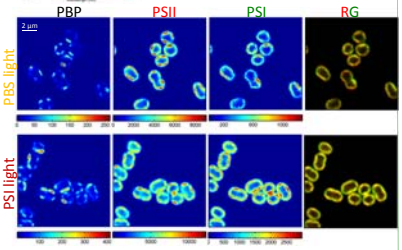
Bulk measurements confirm relative changes in PBS, PSII and PSI

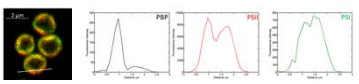
Acaryochloris marina

Contains Chl *d* and minor amounts of Chl *a*



- PBS and PSI increases drastically in red light
- PSII increase slightly



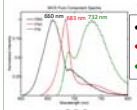


Line plot profiles highlight spatial heterogeneity of photosynthetic complexes

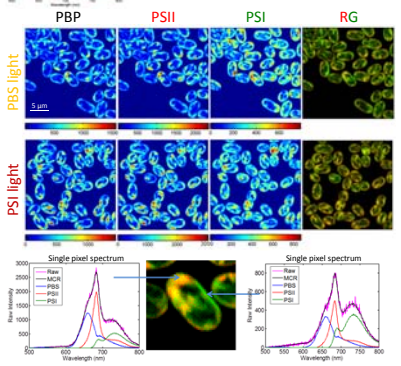
- PSI more uniformed distributed in thylakoids
- PSII enriched domains
- PBP distribution restricted; not always colocalized with PSII

Cyanothece

Nitrogen fixing, unicellular cyanobacterium



- Unique PSI emission profile
- PSII increases in red light
- PSI levels unchanged with light



Single pixel spectra show emission collected from 250 x 250 x 600 µm volume inside of the cell

- Large domains of PSII and PBS enrichment
- Abundance of PSI more constant throughout thylakoids

Per-cell statistics

Individual cells were masked from 2D images and were indexed. Appressed celled were manually segmented and non-cell artifacts were removed. Each spatial pixel was mapped to the spectral domain. Per-cell mean intensities for PSB, PSII and PSI were calculated by summing the component intensity across all pixels in the mask and then dividing by the total number of pixels that comprise the mask.

Relative Change from PBS to PSI light				
	A. marina	*Cyanothece*	*S. platensis*	*Synechocystis*
ΔPBS	+116%	+18%	+127%	+39%
ΔPSII	+39%	+52%	+129%	+90%
ΔPSI	+154%	-1%	+31%	+7%
n (cells)	141	229	25 (images)	195

Conclusions

- Each species of cyanobacteria responded dynamically to limitations in light quality.
- Mutants with truncated phycobilisomes aggregate PSI and PSII in increase absorption cross-sections
- *Spirulina* and *Cyanothece* possess unique PSI emission profiles
- All species adjust their PSI and PSII content in response to PBS and PSI light

  → Ensure linear electron transport between PSII and PSI

Acknowledgements

This material is based upon work supported as part of the Photosynthetic Antenna Research Center (PARC), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Award Number DE-SC 0001035 and by Sandia National Laboratory Directed Research and Development (LDRD) funds.

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