SAND2013-6471C

Probing the Consequences of Antenna Truncation in Cyanobacteria

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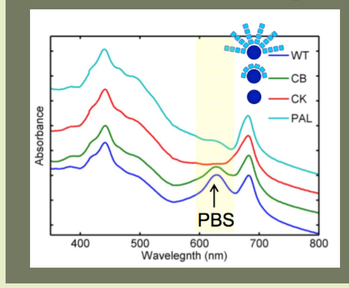
Introduction

Photosynthetic antenna systems, such as those found in cyanobacteria, function in the process of converting sunlight into cellular fuel. In the cyanobacterium *Synechocystis* sp. PCC 6803, light harvesting is accomplished by a combination of membrane intrinsic pigment-proteins and large extrinsic phycobilisome complexes. The phycobilisomes associate with the cytoplasmic surface of thylakoid membranes, so that thylakoid layers are typically separated by a space sufficient for a double row of phycobilisomes. Studies have shown that modulation of phycobilisome antenna size results in changes to the membrane spacing (1). We are using a combination of approaches to explore the consequences of antenna modification in terms of physiology and membrane morphology and dynamics in wild type *Synechocystis* 6803 and a series of mutants with varying degrees of phycobilisome truncation.

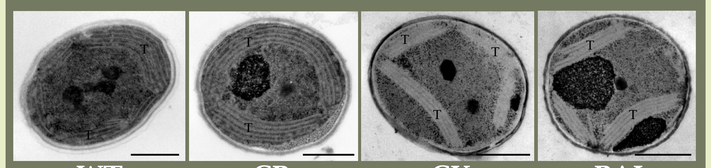
Synechocystis 6803 phycobilisome mutants

Comparison of phycobilisomes in wild type (WT) and mutant cells. The mutants are CB (phycobilisomes contain only one phycocyanin (PC) hexamer per rod), CK (phycobilisomes lacking phycocyanin rods but retaining the allophycocyanin (APC) core), and PAL (completely lacking assembled phycobilisomes) (2, 3). These strains were a kind gift of Ghada Ajlani.



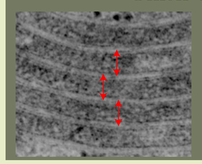
Whole-cell absorption spectra of WT and antenna mutants. Mutants show the characteristic decrease in absorbance by phycocyanin at 625 nm (4).

Thylakoid membrane organization is altered in antenna mutants



WT ~55 nm | **CB ~48 nm** | **CK ~47 nm** | **PAL ~34 nm**

Center-to-center thylakoid membrane distances

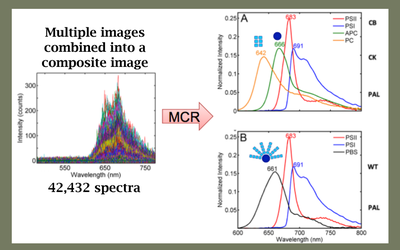


Phycobilisome truncation results in decreased distance between thylakoid membrane layers and less membrane curvature.

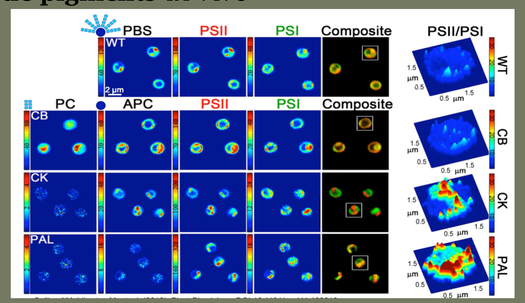
T, thylakoid membranes. Bar = 500 nm

Antenna modification results in changes in photosynthetic pigment localization

Hyperspectral confocal fluorescence microscopy (HCFM) and multivariate curve resolution (MCR) can differentiate multiple overlapping photosynthetic pigments *in vivo*

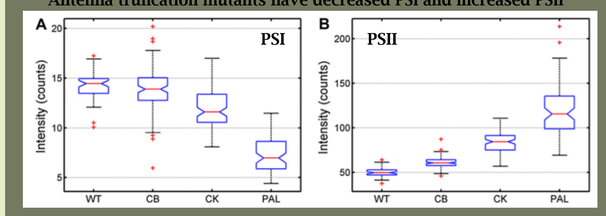


MCR analysis of composite image was used to identify pure spectra. Separate MRC models were developed to describe the fluorescence signatures from WT (A) and mutant cells (B) (4).



MCR derived concentration maps of photosynthetic pigments in WT and mutants. RGB composite image shows the spatial segregation of PSI (green) and PSII (red). The PSII/PSI ratio shows changes in the photosystem concentration with increased antenna modification (4).

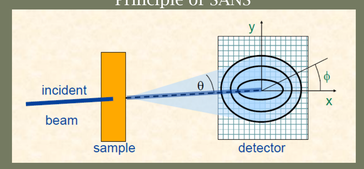
Antenna truncation mutants have decreased PSI and increased PSII



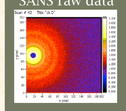
Statistical box plot of the mean intensities for PSI (A) and PSII (B) for each strain. The red center line designates the median values, and the 95% confidence band is denoted by notches. The bottom and top of the box represent the 25th and 75th percentiles, respectively. The red crosses represent statistical outliers. n = 332 cells. (4).

Small angle neutron scattering (SANS)

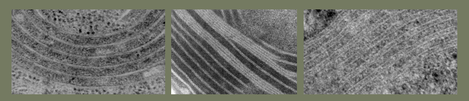
Principle of SANS

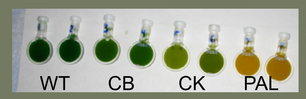


SANS raw data



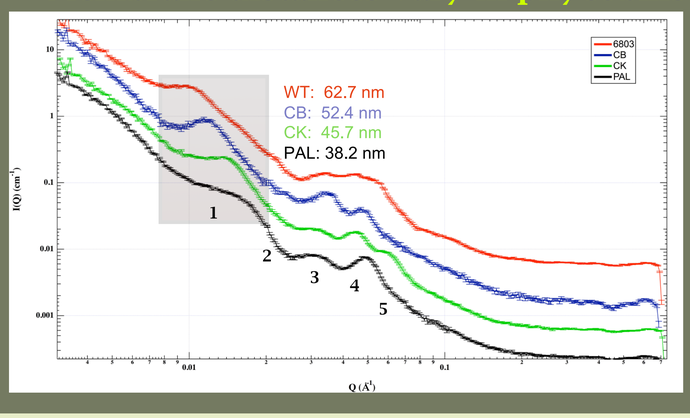
Thylakoid membranes in cyanobacteria have regular repeating distances that can be analyzed by SANS





Samples are live cells in a controlled environment

Membrane distances vary in phycobilisome mutants



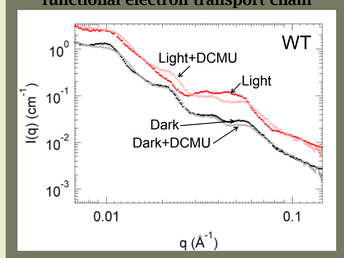
SANS data of *Synechocystis* 6803 wild type and phycobilisome mutants shows a number of peaks for each strain. Distances were calculated for these peaks to attempt to correlate them to specific cellular distances. We found that the peaks centered around Q positions 0.01-0.02 corresponded appoximately to the center-to-center distances between thylakoid membrane layers. These distances decreased as phycobilisome complexes were truncated in the mutant strains, so that the smallest distances were observed in the PAL mutant that lacks phycobilisomes (5).

Summary

• *Synechocystis* 6803 wild type and phycobilisome mutants were probed by a combination of strategies to understand the consequences of antenna modification.
• Phycobilisome truncation resulted in modified thylakoid membrane morphology and changes in pigment localization.
• Repeat distances between thylakoid membranes measured by SANS were correlated to TEM data, and corresponded to the degree of phycobilisome truncation.
• SANS data showed that thylakoid membranes have a high degree of structural flexibility: changes were rapid and reversible upon illumination, and depended upon a functional electron transport chain.

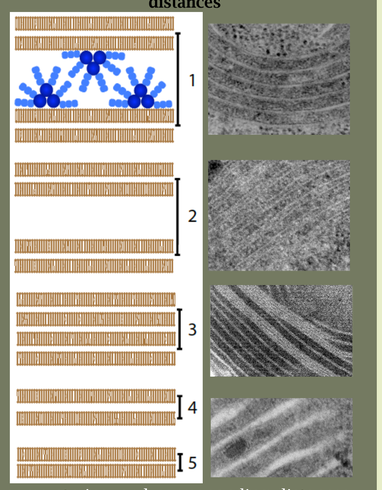
Structural flexibility of the thylakoid membrane system

Membrane changes depend on a functional electron transport chain



Cells were incubated with DCMU, an inhibitor of electron transport from photosystem II. DCMU treatment did not perturb the dark-adapted structure. However, SANS measurements from these samples after 30 min of illumination showed little change from the dark state (5).

Assignment of SANS peaks to cellular distances



Up to 5 distinct peaks were detected in some strains, and corresponding distances were correlated with TEM data and assigned to specific membrane distances as follows: Peak 1, center-to-center repeat distance between thylakoid membrane pairs with phycobilisomes; 2, repeat distance between thylakoid membrane pairs insufficient for phycobilisomes; 3, repeat distance originating from closely appressed thylakoid membrane pairs; 4 and 5, repeat distance originating from a single thylakoid membrane layer with lumen of varying size (5).

References
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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.
Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.
The BioSANS instrument is a resource of the Center for Structural Molecular Biology at Oak Ridge National Laboratory that is supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research Project ERKP291. Bio-SANS is located at the Oak Ridge National Laboratory's High Flux Isotope Reactor. The neutron source is sponsored by the Scientific User Facilities Division, Office of Basic Energy Sciences, U.S. Department of Energy.