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

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Cyanobacteria are abundant organisms that play vital roles in the freshwater, marine, and terrestrial ecosystems. Cyanobacteria convert light energy to chemical energy through photosynthesis that occurs in highly organized complexes arranged in an internal organelle known as the thylakoid membrane. Though much is known about the photosynthetic machinery in cyanobacteria, there are also many unanswered questions, especially as to the subcellular structure and function of these components. In this work we will present insights into the subcellular arrangement of the thylakoid membranes and photosynthetic antenna pigments in the model cyanobacterium *Synechocystis* sp. PCC 6803 at the single cell level using a combination of hyperspectral confocal fluorescence microscopy and transmission electron microscopy. Previously we have demonstrated the power of the hyperspectral confocal fluorescence microscopy method to identify and localize highly overlapped photosynthetic pigments in living *Synechocystis* 6803 cells [1]. Currently, we compare and contrast *Synechocystis* 6803 WT with the mutant strain PAL which lacks the light harvesting pigments phycocyanin and allophycocyanin and therefore cannot assemble functional phycobilisome antenna complexes [2]. The hyperspectral confocal microscopy provides excellent chemical specificity and thus photosynthetic complex function through its ability to extract overlapping fluorescent emissions, while the electron microscopy provides critical ultrastructural information.

A minimum of 20 cells from each of three separate biological replicates of *Synechocystis* 6803 WT and PAL cells were imaged using a custom hyperspectral confocal fluorescence microscope [3] at 60x magnification. The complete set of hyperspectral imaging were concatenated into one data set and analyzed using custom multivariate curve resolution software to extract the underlying spectral components and relative concentrations [4]. For electron microscopy, *Synechocystis* 6803 WT and PAL cells were prepared by high pressure freezing followed by freeze substitution and embedding in resin. Thin sections (~80 nm) were cut and images were viewed and collected using a LEO 912 transmission electron microscope and a ProScan digital camera.

The hyperspectral confocal fluorescence microscopy confirms the PAL mutant exhibits an increased PSII/PSI ratio compared to WT; however, unlike previous reports which rely only on bulk fluorescence measurements; the spatial and spectral information available with the hyperspectral microscopy technique permits localization of the PSI, PSII, and carotenoid components independent of one another within a single-cell. Figure 1 highlights the observed differences in relative concentrations and spatial localization of these three pigment components in WT and the PAL mutant. We are working to pair this pigment localization information with ultrastructural electron microscopy data to identify structural differences in the thylakoid membrane architecture and relate those to the photosynthetic antenna function.

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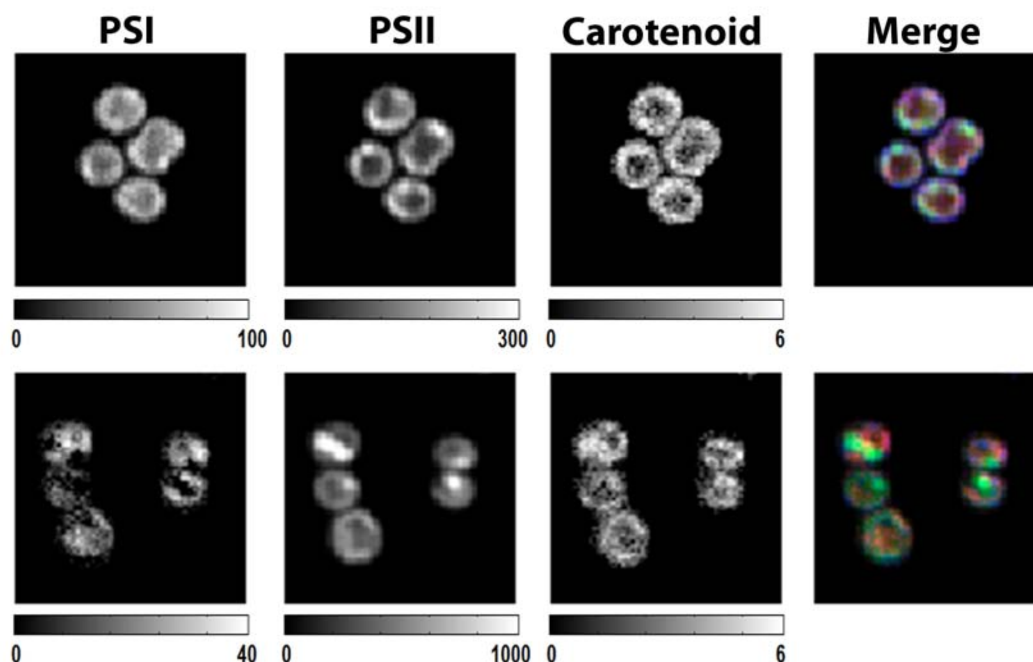

FIG. 1. Hyperspectral confocal fluorescence microscopy results in independent images of PSI, PSII, and carotenoid localization in *Syncehocystis* sp. PCC 6803 (top row) and PAL cells (bottom row). The RGB composite image was created by scaling each color independently from minimum to maximum intensity. Red corresponds to PSI, Green to PSII, and Blue to carotenoid. Images are 8.6 µm across.