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High Resolution Electron and Ion Microscopy of Photosynthetic Complexes

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Cyanobacteria are able to carry out photosynthesis for conversion of light energy into chemical energy to fuel the organisms' activities. They are the only photosynthetic bacteria that possess phycobilisomes for highly efficient light capture machines. Attached to thylakoid membrane, phycobilisomes act as light harvesting antennae for the photosystems.

The organization of protein complexes in the thylakoid membranes of photosynthetic organisms is closely linked to the functioning and efficiency of energy conversion processes. However, there is currently a lack of detailed knowledge concerning the organization of protein complexes in cyanobacterial thylakoid membranes, We applied a combination of high resolution microscopy imaging techniques to this topic to enable characterization of molecular machines such as phycobillisome antenna and photosynthetic complexes.

Using synthetic biology approaches, *Synechocystis* sp. PCC 6803 strains with progressively truncated phycobillisome antenna complexes have been generated and studied using high resolution imaging [1]. We are investigating the topology of isolated thylakoid membranes from wild type and mutant strains with or without attached phycobillisomes.

The transmission electron microscopy, the technique of negative staining is being used for high resolution characterization of isolated phycobillisomes and protein complexes in membranes in 2d projections, as well as the 3d surface topology by the helium ion microscope.

The Zeiss Orion helium ion microscope (HeIM) is a new type of scanning microscope that uses helium ions for surface imaging and analysis. Its functionality is similar to a scanning electron microscope, but it uses a focused beam of helium ions instead of electrons. Since helium ions can be focused into a smaller probe size and provide a much smaller interaction volume at the sample surface compared to electrons, the helium ion microscope generates higher resolution images with better material contrast and improved depth of focus. Therefore, the helium ion microscope offers a significant advantage over traditional SEM technology.

Focused ion beam / scanning electron microscopy (FIB/SEM) tomography is a novel powerful approach for three-dimensional (3D) imaging of biological samples. Furthermore, by appropriate selection, we can sequentially cross-section to create a series of ‘slices’ at specific intervals. 3D reconstruction software can then be used to volume-render information from the 2D slices, enabling us to immediately see the spatial relationships between microstructural components. For FIB/SEM tomography, a thin slice is removed with the ion beam and the newly exposed face is imaged with the electron beam, usually by recording the backscattered electrons. The process, also called “slice and view,” is repeated until the desired volume is imaged.

Additionally, the phycobillisomes ultrastructure in their native association within the thylakoid membranes was studied by using high resolution TEM tomography, to obtain as 3d information on the phycobillisomes components orientation.

References:

[1] Arteni, A.A., Ajlani, G., and Boekema, E.J., BBA – Bioenergetics **1787** (2009), p. 272.

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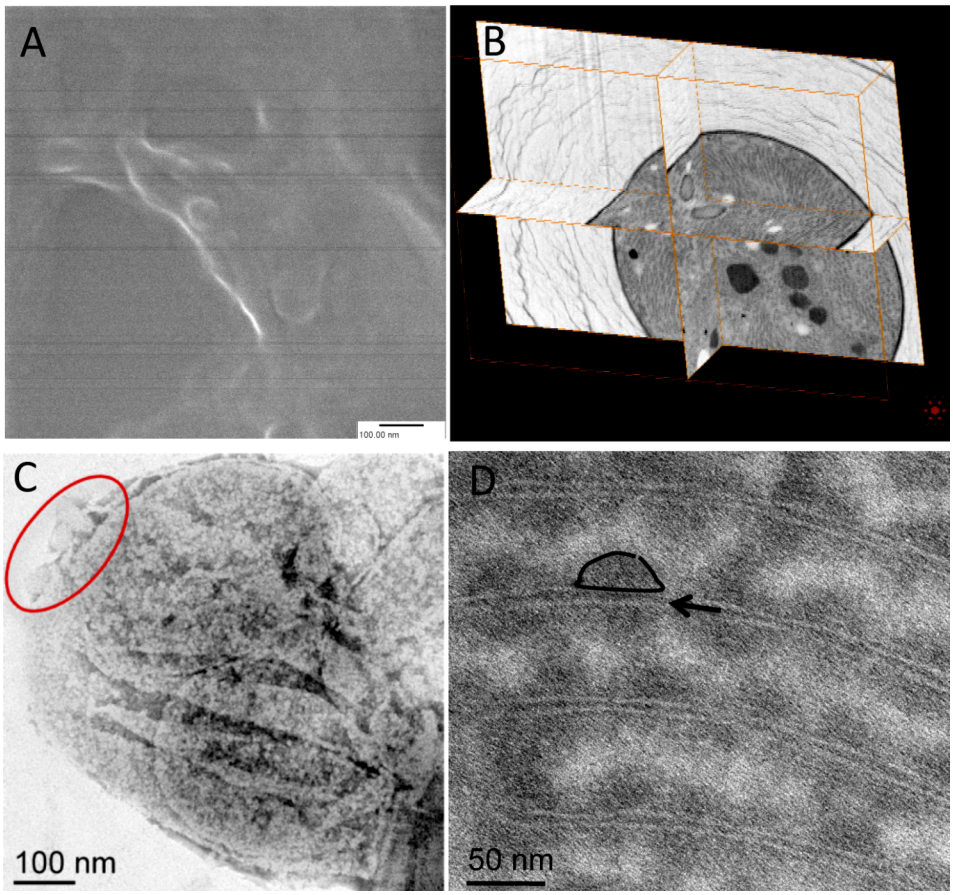

Figure 1. HeIM Image of isolated membrane fractions with phycobillisomes (A), and reconstructed volume of a cyanobacterial cell obtained by the Slice and View method (B). TEM whole mount of membrane fractions (C), and TEM of an ultra-thin section of phycobillisomes in their native association with thylakoid membranes (D).

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