Cryo-Electron Tomography of Chromatophore in *Rhodobacter Sphaeroides*

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*Rhodobacter Sphaeroides* is a widely studied photosynthetic bacterium. Scientific inquiries have focused primarily on chromatophore vesicles, which facilitate light harvesting and subsequent energy transfer to generate ATP within the bacteria. Despite the plethora of literature on the chromatophores of *R. Sphaeroides*, the spatial arrangement of vesicle proteins is not completely understood [1]. The current structural models derived from atomic force microscopy, electron microscopy, and optical fluorescence data [1-3], do not provide true 3D volumetric information on individual chromatophores. Structural identification of both internal and external membrane components will help elucidate the vesicle’s architecture and photosynthetic function. While the 3D structure of chromatophores close to a bacterium’s membrane can be directly studied using cryo-electron tomography [Ref], imaging of vesicles in its interior is hampered by the size of the bacterial cell. Here, we use cryo-focused ion milling (cryo-FIB) to gain access to the chromatophore networks inside *R. Sphaeroides*.

Vitrified lamellae of *R. Sphaeroides* were prepared using cryo-FIB milling. FIB uses a focused Ga⁺ ion beam to create thin, site-specific cross-sections of biological materials at cryogenic temperature for observation in cryo-TEM. This allows for observation of the previously inaccessible internal structure of chromatophores. Thin, artifact-free lamellae were fabricated for microscopic analysis. High-resolution projection images of the sample were acquired in a tilt series and reconstructed into 3D tomograms using computational, iterative reconstruction algorithms. Implanted Ga⁺ ions provided the alignment fiduciaries.

Tomographic reconstructions are ~110 nm thick, allowing for structural analysis of chromatophore in 3D space (Fig.1). The chromatophore in fresh *R. Sphaeroides* bacteria exist primarily as isolated vesicles that perpetuate through the sample volume with diameters 58.7±2.3 nm. In fresh bacteria, the chromatophore sometimes form invaginations with the bacteria double membrane. More often, the chromatophore form a reticulum of several neighboring vesicles (Fig.2).

Fused chromatophore are uncommonly present in young *R. Sphaeroides* bacteria. The prevalence of irregular, fused chromatophore increases with bacterial age. Fig.3 demonstrates the stark difference in structure between young bacteria and old bacteria. The fusion and degradation of chromatophore is possibly a consequence of the aging process. The diameters of these fused vesicles varied from 76 to 156 nm, indicating the fusion of up to several chromatophore.

Interior visualization of individual chromatophore cross-sections reveals a double-membrane structure, a feature lacking in most chromatophore models (Fig.4). Small clusters of proteins ~3 nm in diameter appear inside the chromatophores (Fig. 4). These proteins are entirely housed within the vesicle and would not be visible from its exterior without FIB milling. The size, location, and spatial
distribution of these proteins suggest that they may be $c_2$ cytochrome proteins shuttling between the reaction centers distributed along the vesicle membrane.