Cryogenic electron tomography and 3D reconstructions of the intracellular extracellular nanowire interface of *Geobacter sulfurreducens*

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Background

- *Geobacter sulfurreducens* are bacteria that can convert organic substrates into electrical current through cellular respiration using **extracellular electron transfer (EET),** in which electrons are transported to an external electron acceptor via conductive filaments called **microbial nanowires**.[1]
- Much remains to be elucidated about *G. sulfurreducens* **nanowire circuitry**, including the nanowire anchoring system, method of excretion, and mechanism of trans-membrane electron transfer.
- **Cryo-electron tomography (cryo-ET)** is a novel microscopy technique that captures high resolution tilt series of the internal fluid architecture of whole cells fixed in vitreous ice, imaging their volume in its near-native state.[2]

Goals

1. Obtain cryo-ETs of the *G. sulfurreducens* nanowire membrane interface to elucidate information about its structural connection and nanowire circuitry.
2. Construct 3D models from cryo-ETs to obtain spatial information and 360˚ visualization of the *G. sulfurreducens* nanowire-membrane interface.

Materials and Methods

Fig 1. Direct culturing of *G. sulfurreducens* on cryo-EM grids

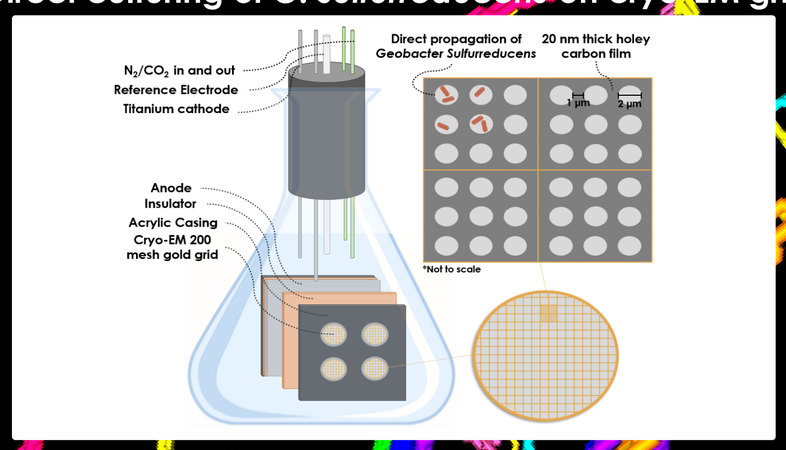
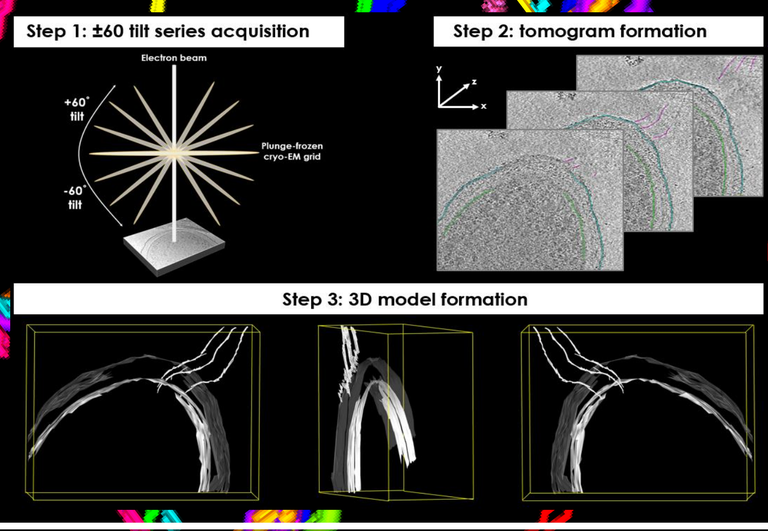


Fig 2. Cryo-ET acquisition and 3D modeling



Results

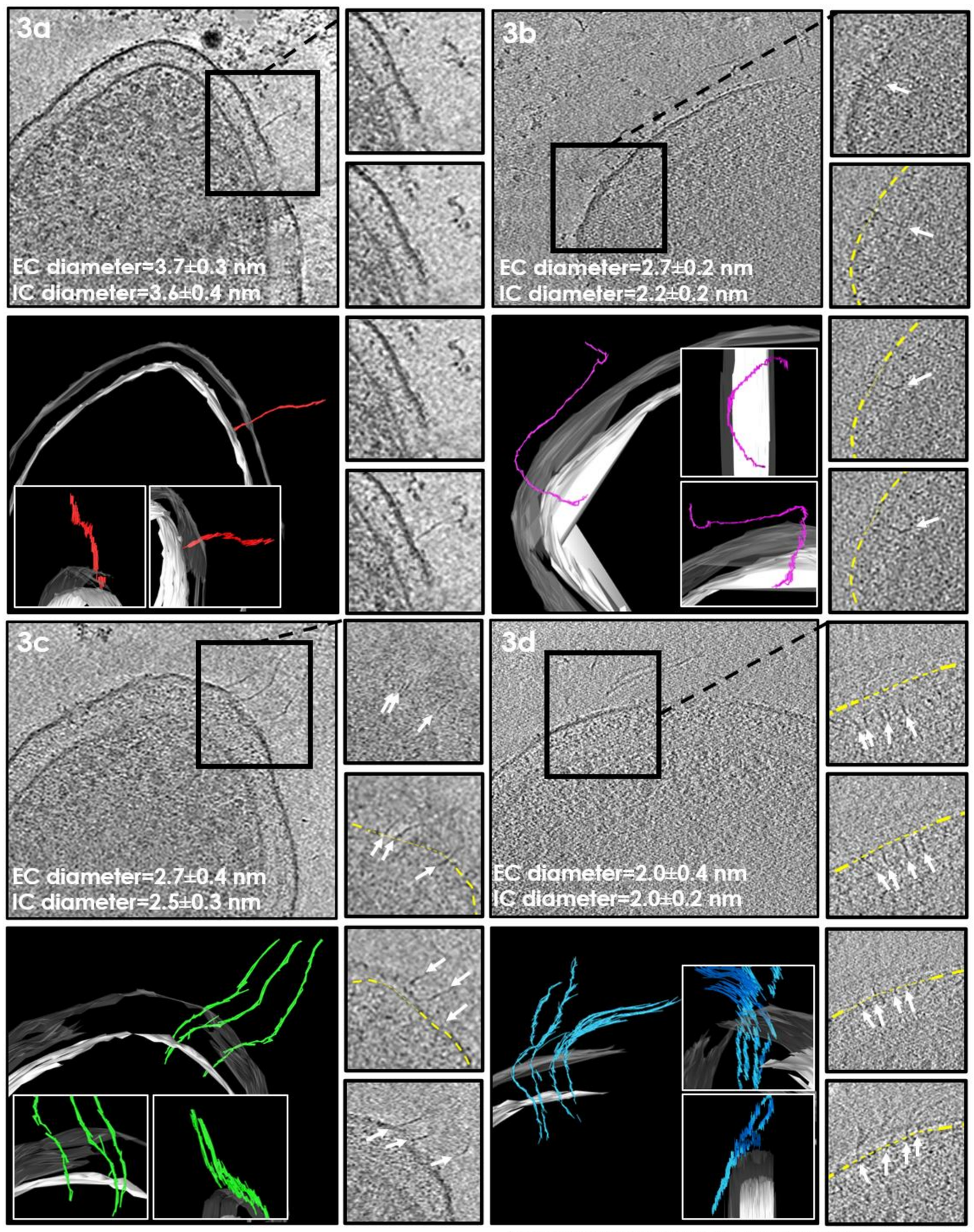
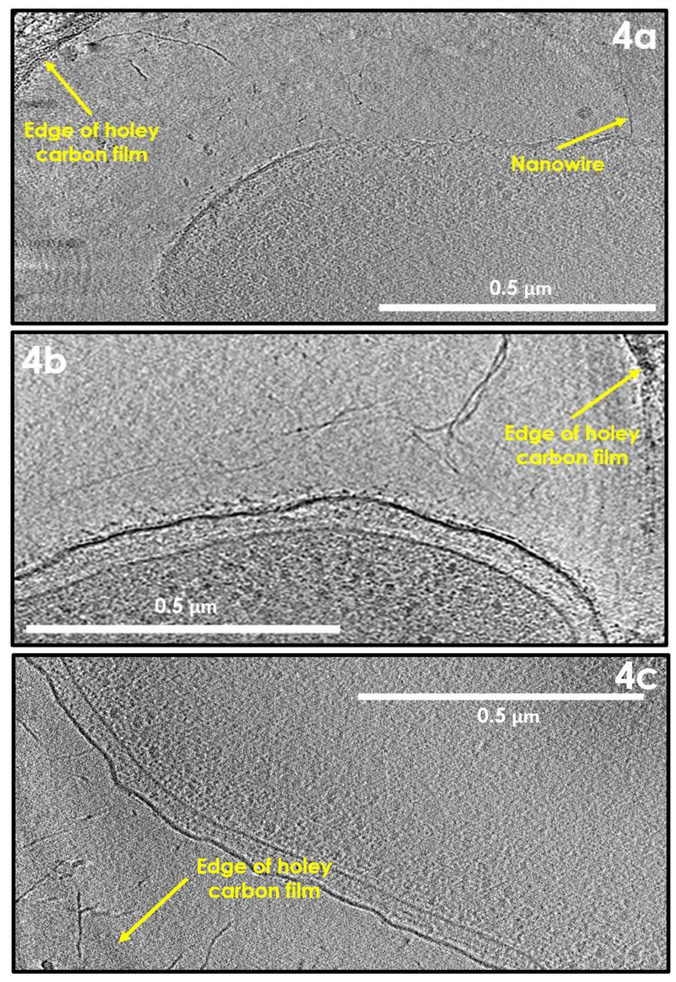


Fig 3. Filament diameter is maintained across the outer membrane. (a-d) Raw cryo-ET Z slices of microbial nanowires appearing to cross the outer membrane accompanied by sequences of Z slices showing nanowire development over the z-axis and corresponding 3D models. Intracellular (IC) and extracellular (EC) diameters taken in IMOD are consistent across the outer membrane. Yellow dotted lines approximate outer membrane location and white arrows emphasize nanowire location.

Fig 4. Nanowires extend toward the anode and appear around cell periphery. (a-c) Raw cryo-ET Z-slices show that microbial nanowires extend toward the anodically-active holey carbon film of cryo-EM grids, and that nanowires are not location-specific, emanating from both the sides and tips of *G. sulfurreducens* cells.



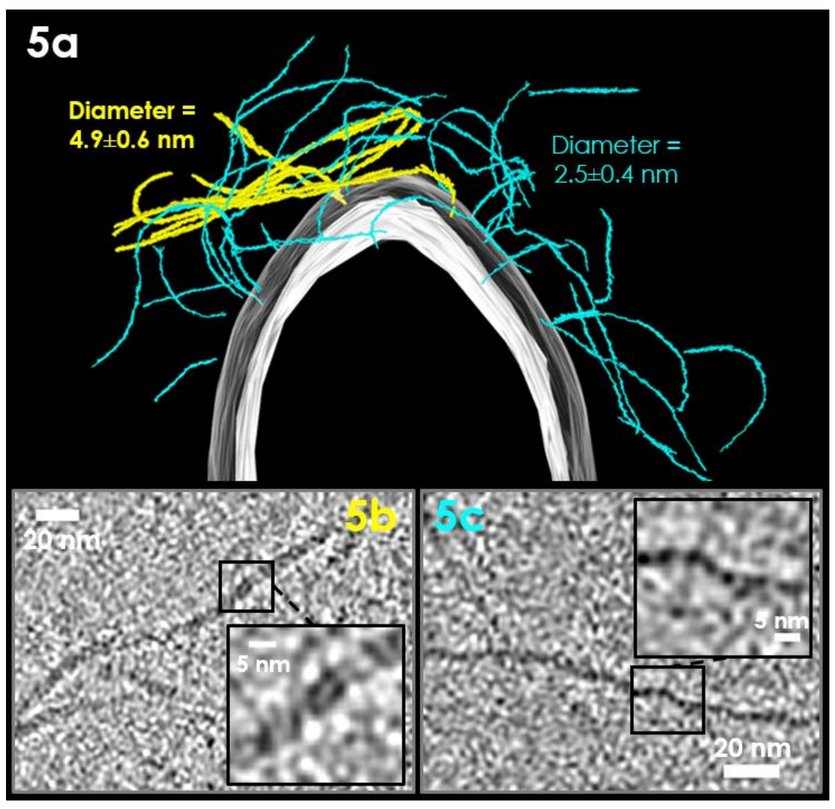


Fig 5. Individual *G. sulfurreducens* cells present filaments of multiple thicknesses. (a) 3D model of individual cell with thicker filaments (~5 nm) in yellow and thinner (~2.5 nm) in cyan. **(b-c)** Raw tomograms of thicker and thinner filaments, displaying diameters and configurations characteristic of OmcS and OmcZ nanowires, respectively.

Conclusions and Future Work

Important observations that result from cryo-ET and 3D modeling of the *G. sulfurreducens* nanowire-membrane interface are: (1) microbial nanowires appear to maintain their diameter across the outer membrane, (2) microbial nanowires are not site-specific and appear around both the tips and sides of the cell, (3) microbial nanowires grow in the direction of the anode, and (4) individual *G. sulfurreducens* cells present filaments of multiple thicknesses Future efforts will entail performing subtomogram averaging on cryo-ETs, an image processing technique that will improve the resolution of the interface, the location where the nanowires meet the cell membrane.

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References. 1) Reguera, G. et al., *Adv. Microb. Physiol.* 2019, *74*, 1-96. **2)** Zhang, P., *Curr. Opin. Struct. Biol.* 2019, *58*, 249–258.

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