Cryoelectron tomography of bacteria and their
macromolecular machines

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Dein Reich komme, Dein Wille geschehe
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Abstract

Cryoelectron tomography (CET) fills a glaring gap in the imaging capabilities of biology by reconstructing cells to medium resolution. The technique was applied in three areas to understand biology’s macromolecular machines: (1) the quaternary structure of the octahedrally-cored *E. coli* pyruvate dehydrogenase (PDHC) and 2-oxoglutarate dehydrogenase (OGDHC) complexes *in vitro*; (2) the ultrastructure of the spirochete *Treponema primitia*; and (3) the structure of the *in situ* flagellar motors from *T. primitia*, *Hylemonella gracilis*, *Caulobacter crescentus*, and *Vibrio cholerae*. Whereas the complexes PDHC and OGDHC were thought to have their subunit proteins E1 and E3 bound directly to the octahedral E2 core—the so-called face/edge model—it was discovered that the subunits are flexibly tethered 11 nm from the corners of the core. Several novel structures were discovered in the spirochete *T. primitia*. Spirochetes are spiral-shaped cells that propel themselves with periplasmic, not external, flagella. Bowl-shaped structures dot its surface and hook-like appendages that form arcades stripe the length of the cell. Fibrils extend from its cell tips that might help attach the cells to surfaces. Inside the periplasm, porous, cone-shaped structures reside at each cell tip and a second periplasmic layer undergirds its outer membrane, which might prevent the periplasmic flagella from rupturing the cell. Previous imaging of the flagellar motor produced either high-resolution reconstructions of the purified basal body removed from its context or low-resolution images of the *in situ* motor. Our *in situ* 3-D reconstructions described for the first time the structure of the stators, the membrane embedded component that spins the rotor. Novel shapes were discovered that indicate there are various attachments and versions of the flagellar motor that were never expected.
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