APPLICATION NOTE

Cryo-electron tomography for microbiology

What tricks do bacterial invaders play to infect and survive inside their hosts?

Microbiology incorporates many interdisciplinary aspects, from cell biology and immunology to genetics and evolution. Cryo-electron tomography (cryo-ET) has enabled the *in situ* visualization of many aspects of prokaryotic cell evolution from development of cellular membranes and "cytoskeletons" to subcellular organization, flagellar rotor complexes for motility and mechanisms of pathogenesis.

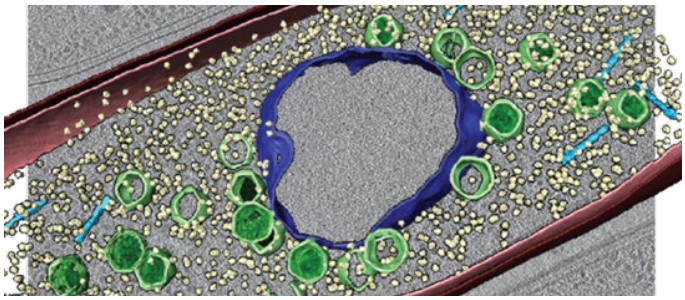
Due to technological imaging advances, bacteria and archaea can no longer be viewed as mainly undifferentiated sacs of jumbled enzymes. These advances have led to an broadened view of the intricate processes within prokaryotic cells. Finer details of microbial cell biology reveal organized assemblies of macromolecular machines that are optimized to travel through and interact with complex and dynamic environments. These fascinating observations raise a number of unanswered questions and shed new light on uncharted areas of discovery.

Why use cryo-ET?

Cryo-ET combines optimal structure preservation with nanometer-scale resolution. This method acquires 3D snapshots of the cellular interior and visualizes protein complexes within their crowded physiological environments. Such high-resolution 3D images of the interior of cells provide new insights into cellular function and shed light on the arrangement and structure of native protein complexes. This technique can bridge the gap between light microscopy and near atomic-resolution techniques such as single particle electron microscopy.

Research highlights

Chaikeeratisak V., Nguyen K., Khanna K., Brilot A.F., Erb M.L., Coker J.K.C., Vavilina A., Newton G.L., Buschauer R., Pogliano K., Villa E., Agard D.A., Pogliano, J. 2017 Assembly of a nucleus-like structure during viral replication in bacteria. Science 355: 194-197. doi: 10.1126/science.aal2130



Cryo-electron tomography shows how the bacterial cell is reorganized to resemble a more complicated plant or animal cell with a nucleus-like compartment (blue) and ribosomes (small yellow structures). The reproducing viruses appear with green heads and blue tails. Image courtesy of Villa Lab.

Chaikeeratisak V., Khanna K., Nguyen KT., Sugie J., Egan ME., Erb ML., Vavilina A., Nonejuie P., Nieweglowska E., Pogliano K., Agard DA., Villa E., Pogliano J. 2019 Viral capsid trafficking along treadmilling tubulin filaments in bacteria. Cell 177: 1771-1780. doi: 10.1016/j.cell.2019.05.032

Bacteria…not so simple

For the first time, biologists have documented how very large viruses reprogram the cellular machinery of bacteria during infection to more closely resemble an animal or human cell. A previous study found that several Pseudomonas phages assemble a bipolar nucleus-like spindle composed of a phage tubulin-like protein (PhuZ) that encloses phage DNA (image above). In a following study, using time-lapse light microscopy and cryo-electron tomography, Chaikeeratisak et al. observed that capsids traffic along a viral encoded tubulin filament and this treadmilling of the filament provides the mechanism of capsid movement through the cell. The spindle rotates the phage

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nucleus, which the authors hypothesize distributes capsids for efficient DNA packaging. The discovery demonstrates that bacteria have more in common with sophisticated human cells than previously believed and are part of a renewed interest due to their potential of using them for phage therapy.

Review. Oikonomou C.M., Chang Y., Jensen G.J. 2016 Cryo-Electron Tomography: can it reveal the molecular sociology of cells in atomic detail? Trends in Cell Biology 26(11): 825-837. doi: 10.1016/j.tcb.2016.08.006

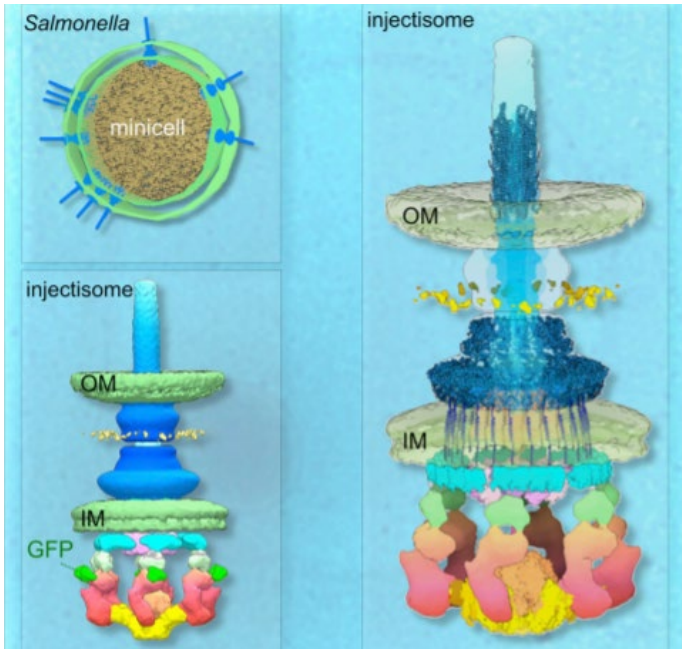
Cryo-ET enables entire cells or parts of cells to be viewed with macromolecular resolution in 3D. A tomogram (image below) of a *Bdellovibrio bacteriovorus* cell reveals the flagellum, flagellar motor, chemoreceptor array, secretion pores, ribosomes, tubular structure, nucleoid, inner membrane, outer membrane and pilus. This is just one example of how cryo-ET has provided structural and mechanistic insights into such bacteria. More examples are discussed in this review.

Bdellovibrio bacteriovorus. Image © 2016 Elsevier Inc.

Hu B., Lara-Tejero M., Kong Q., Galán J.E., and Liu J. 2017 In Situ Molecular Architecture of the Salmonella Type III Secretion Machine. Cell 168, 1065-1074.e10. doi: 10.1016/j.cell.2017.02.022

Bacterial delivery of proteins

Salmonella and many other bacterial pathogens use a nano syringe-like device to deliver toxic proteins into target human cells. "The device is like a stinger and injects ready-made bacterial proteins into mammalian cells to commandeer them for the benefit of the pathogen," said Jorge Galan, the Lucille P. Markey Professor of Microbial Pathogenesis and co-senior author of the paper. Knowledge of the structure could help researchers devise new anti-infective strategies against a variety of bacterial pathogens such as *Salmonella, Pseudomonas, Escherichia coli, Yersinia pestis,* and *Chlamydia*. Source: Yale University Reference: Hu et al., Cell 168, 1065, 2017.



Complete structure of the Salmonella type III secretion machinery explains how bacteria deliver proteins into eukaryotic cells. Image © 2017 Elsevier Inc.

Further reading

Bock D., Medeiros J.M., Tsao H.-F., et al. 2017 *In situ* architecture, function, and evolution of a contractile injection system. Science 357, 713–717. doi: 10.1126/science.aan7904

Chetrit D., Hu B., Christie P.J., Roy C.R., et al. 2018. A unique cytoplasmic ATPase complex defines the Legionella pneumophila type IV secretion channel. Nature Microbiology 3, 678–686. doi: 10.1038/s41564-018-0165-z

Cohen E.J., Ferreira J.L., Ladinsky M.S., et al. 2017 Nanoscale-length control of the flagellar driveshaft requires hitting the tethered outer membrane. Science 356, 197–200. doi: 10.1126/science.aam6512

Depelteau, J., Brenzinger, S., Briegel, A. 2019 Bacterial and archaeal cell structure. Chapter in Encyclopedia of Microbiology. doi: 10.1016/B978-0-12-809633-8.20679-1.

Wang J., Brackmann M., Castaño-Díez D., et al. 2017 Cryo-EM structure of the extended type VI secretion system sheath–tube complex. Nature Microbiology 2, 1507–1512. doi: 10.1016/j.cell.2015.01.037

Review. Wang, W., Briegel, A. 2020. Diversity of bacterial chemosensory arrays. Trends in Microbiology 28, 68-80. doi: 10.1016/j.cell.2019.05.032

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