The Atlas of Bacterial & Archaeal Cell Structure

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by Catherine M. Oikonomou & Grant J. Jensen

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Introduction

"It is very easy to answer many of these fundamental biological questions; you just look at the thing!*"*
- Richard Feynman [1]

Introduction

In the 1960s, electron microscopes were opening a new window in biology, allowing scientists to look not just *at* cells, but *into* them. This revealed a rich world of ultrastructures too small to resolve with light microscopes, including organelles inside eukaryotic cells. To share this new vista with scientists and medical students who did not have microscopes to look for themselves, authors like Don Fawcett [2] and John Dodge [3] created atlases of electron microscopy images that remain valuable resources for biological and medical novices, as well as experts.

More than fifty years later, we are once again enjoying an expanded view of biology, thanks to another great advance in electron microscopy. The development of cryogenic electron microscopy, or cryo-EM, allows us to look inside cells in their native state. This has opened up even the smallest cells for examination, and revealed some surprising things. In particular, bacteria and archaea, orders of magnitude smaller than eukaryotic cells and lacking prominent organelles, previously seemed to be relatively unstructured bags of nucleic acids and protein. In the last decade, cryo-EM has challenged this idea, revealing a startling degree of structure in these tiny cells. Understanding this intricate molecular machinery is a fascinating pursuit and might enable us to engineer new biological tools in the future. And so, inspired by the atlases of eukaryotic cell structure from the 1960s, here we offer an atlas of bacterial and archaeal cell structure, highlighting many of the molecular machines we have discovered so far. We hope it will be a useful tool for microbiology courses, serving as a quick introduction to the cells and what they contain before students go on to study aspects of biochemistry or medical importance.

If you are new to cryo-EM, we suggest starting with Chapter 1, which describes the methods used in structural biology, particularly cryo-EM. If you are already an expert, or pressed for time, go straight to the cells in Chapter 2.

As Charles Darwin wrote in 1837, "I shall always feel respect for every one who has written a book, let it be what it may, for I had no idea of the trouble which trying to write common English could cost one" [4]. The task was made immeasurably easier for us by the help of many minds and hands. For a partial list, see the acknowledgments on the next page.

Acknowledgments

We are grateful to Rob Phillips, our colleague at Caltech, for impressing Grant with the lasting value of Fawcett's Atlas *and encouraging him to create another. Readers of early drafts, including Lydia Jensen and Natalie Jensen, gave us useful feedback to improve this experience. We particularly thank Grigorios Oikonomou and past and present Jensen Lab members for advice and feedback. We are grateful to Ashley Jensen and Tony Kukavica for help with research, and to Travis Alvarez, Camille Ogilvie, Natalie Jensen and Aditee Prabhutendolkar, who created most of the videos. Animations illustrating methods in Chapter 1 are the work of a team of talented animators, led by Daniel Villanueva Avalos and including Mackenzie Taylor and Uberto De La Rosa Arguello. We are most grateful of all to our colleagues whose work at the microscope filled these pages. Click on their names throughout the book to learn a little bit more about them.*

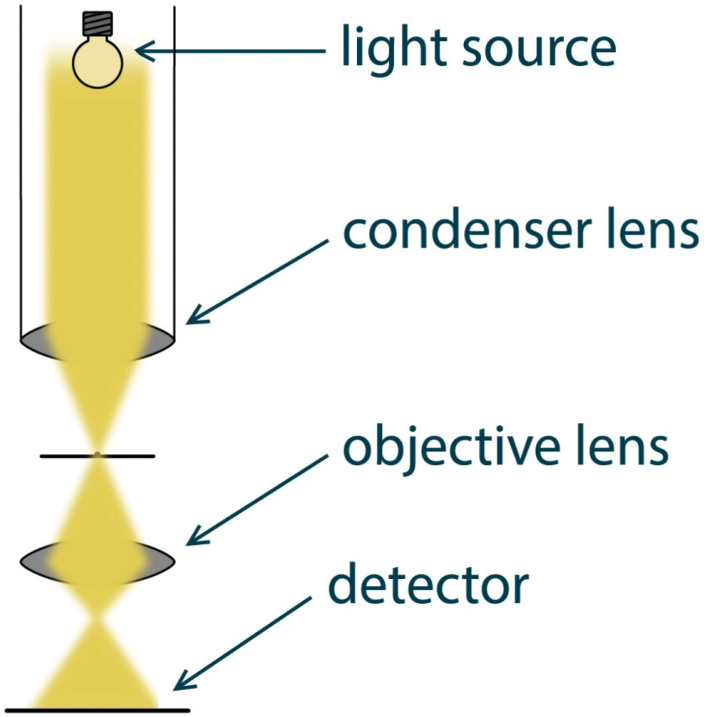
All of the images in this book were acquired in the course of research projects. Major funding for these projects in the Jensen Lab has come from the National Institutes of Health (NIH), Howard Hughes Medical Institute (HHMI), Beckman Institute, Gordon and Betty Moore Foundation, Agouron Institute, and John Templeton Foundation. Cryo-electron microscopy was performed in the Beckman Institute Resource Center for Transmission Electron Microscopy at Caltech and the HHMI Janelia Farm CryoEM Facility. Most of these projects were also collaborative, and we thank the researchers who provided the cells we imaged, from the groups of Gladys Alexandre, Yannick Bomble, Sean Crosson, Mike Dyall-Smith, Moh El-Naggar, Robert Gunsalus, Alan Hauser, Chris Hayes, Bill Hickey, Matthias Horn, Jack Johnson, Marina Kalyuzhnaya, Arash Komeili, Jared Leadbetter, Eric Matson, Sarkis Mazmanian, John Mekalanos, Dianne Newman, Victoria Orphan, Tracy Palmer, Kit Pogliano, Eric Reynolds, Carrie Shaffer, Nicholas Shikuma, Liz Sockett, Lotte Sogaard-Andersen, David Stahl, Ronald Taylor, Martin Thanbichler, Kasthuri Venkateswaran, Joseph Vogel, Matthew Waldor, Kylie Watts, Douglas Weibel, and Patricia Zambryski.

We used the IMOD software package (developed by David Mastronarde, Rick Gaudette, Sue Held, Jim Kremer, Quanren Xiong, John Heumann and others at the University of Colorado with support from the NIH) to create and visualize tomographic datasets, and we are grateful to David Mastronarde for his tireless support of the software, including improving a function to help us make these videos. We used UCSF Chimera (developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH grant P41 GM103311) to create the visualizations of atomic models from the Worldwide Protein Data Bank (wwPDB). We generated and visualized the phylogenetic tree with phyloT and iTOL [5]. We thank Jane Ding, Andrew Jewett, Yi-Wei Chang, Ariane Briegel and Min Xu for 3D segmentations. High-resolution structures of proteins and complexes are the work of many labs; see references for full details. Likewise, our understanding of the functions of cellular structures derives from the enormous body of work of generations of scientists.

The Caltech Library, including Kristin Briney, Robert Doiel, Donna Wrublewski and Gail Clement, supported and enabled our vision of open access publishing and we are enormously grateful for their work and ingenuity in creating a platform tailored to the content and our shared vision of open accessibility. We are particularly grateful to Thomas Morrell for coordinating the technical infrastructure. We thank Vicki Chiu for design advice. Last but certainly not least, we are deeply grateful to the talented web developer Kian Badie who created the digital interface.

1
Methods

"No doubt, man will continue to weigh and to measure, watch himself grow, and his Universe around him and with him, according to the ever growing powers of his tools."
- Albert Claude [6]


light source
condenser lens
objective lens
detector

1. Light Microscopy

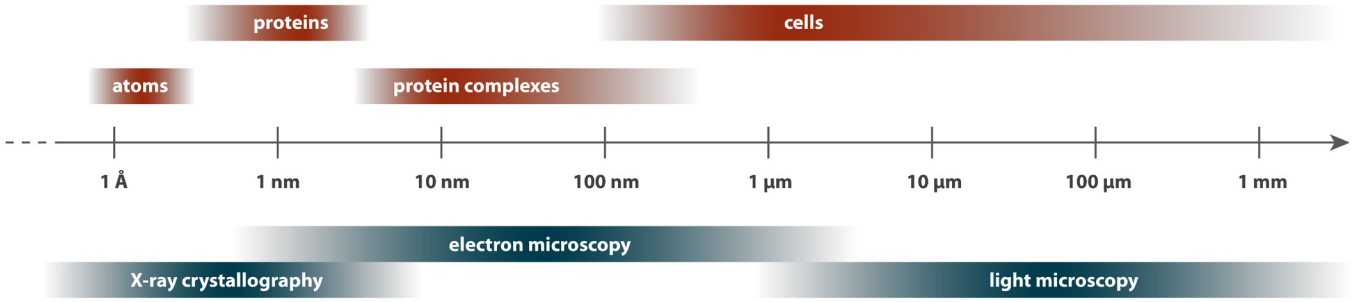
To understand a picture, it helps to know how it was made. So before we start looking at the structures of cells, let us quickly cover some of the techniques we use to see them.

Cell biology occurs over a vast scale, from angstrom-level (0.1 nm) rearrangements of molecules inside cells to millimeter-level (1,000,000 nm) interactions between cells. Different tools of structural biology cover different ranges of this scale, complementing one another to provide a more complete view (*Learn More* on the next page). To discuss these tools, we will start at the "big" end of the scale and work our way down, from light microscopy to electron microscopy to X-ray crystallography. Our guide will be a cellular structure called the flagellum, which bacteria use to swim (you will see how it works in Chapter 6).

Bacteria and archaea are, with very few exceptions, invisible to our eyes. As you can see with these *Staphylococcus aureus* being chased through a field of human blood cells by an immune cell, they are orders of magnitude smaller even than eukaryotic cells. As a consequence, they were unknown to us until about 350 years ago, in the seventeenth century, when Antonie van Leeuwenhoek created the first microscopes capable of revealing such tiny cells. The simplest microscope is a magnifying glass which, combined with the lens of your eye, produces a magnified image on your retina. A compound microscope has two or more lenses, whose magnifications multiply, and this is what we commonly mean when we refer to a "light microscope."

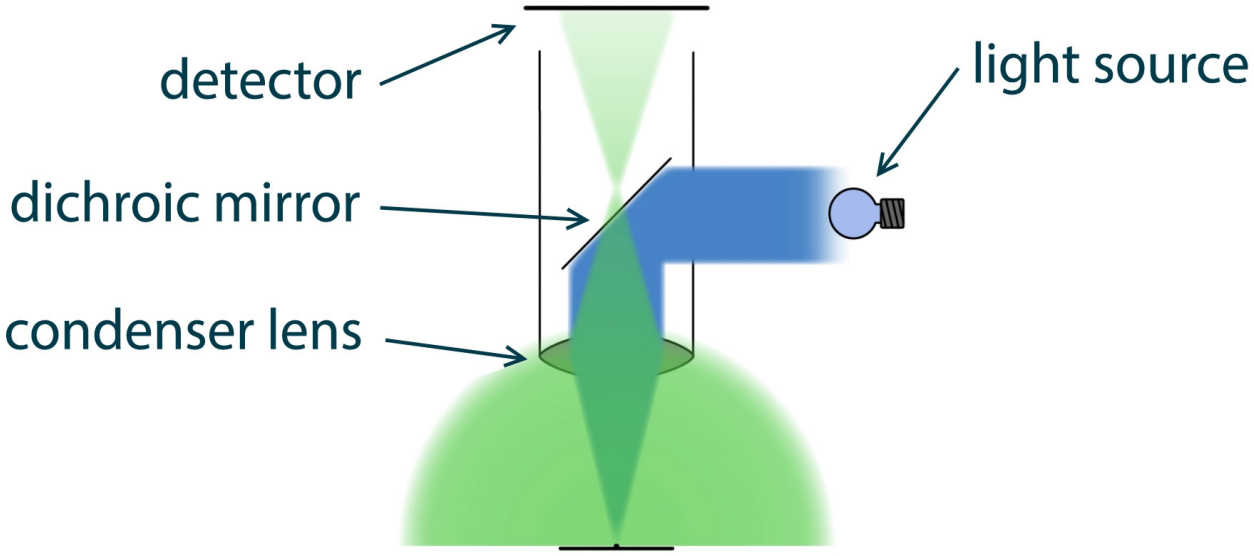
The cells here were imaged by **light microscopy**, captured on film by David Rogers in the 1950s [7]. They illustrate how light microscopy can reveal general properties, such as the shape, of cells. The video also illustrates how light microscopy can capture not just single snapshots of cells, but how they grow, divide and move over time.

Staphylococcus aureus Collected by: David Rogers DOI: 10.22002/D1.1463


proteins
cells
atoms
protein complexes
1 Å
1 nm
10 nm
100 nm
1 µm
10 µm
100 µm
1 mm
electron microscopy
X-ray crystallography
light microscopy

Learn More: Toolkit

Cell biology occurs on a length scale that spans seven orders of magnitude. Different visualization techniques cover various sections of this span, as shown here with a few examples. Keep in mind that there are more techniques than these; we touch only on those you will see in the coming pages.

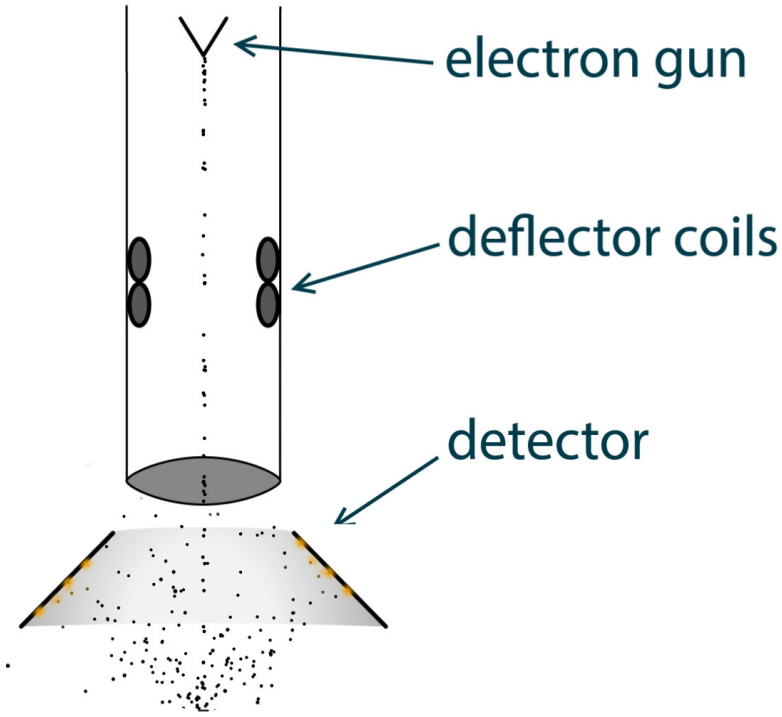

detector
light source
dichroic mirror
condenser lens

2. Fluorescence Light Microscopy

The addition of **fluorescence** to light microscopy allows us to look not just *at* cells, but *for* things inside them. Specific cellular components can be fluorescently labeled, with a stain or antibody that binds a particular molecule. Alternatively, a protein of interest can be genetically linked to a fluorescent protein such as Green Fluorescent Protein (**GFP**, isolated from a bioluminescent jellyfish off the Pacific coast in the 1970s and adapted as a revolutionary molecular biology tool in the 1990s). While tagging a protein can sometimes change its properties (e.g. affecting its function or altering its localization), this technique often enables us to identify where in the cell a protein is found, and what it might be doing there.

As an example, this movie from Howard Berg's lab [8] [9] shows *Escherichia coli* cells stained by a fluorescent dye that binds to and highlights their flagella – long, thin appendages that propel them through their environment. (We will discuss this and other ways cells move in Chapter 6.)

Escherichia coli Collected by: Howard Berg Lab DOI: 10.22002/D1.1464


electron gun
deflector coils
detector

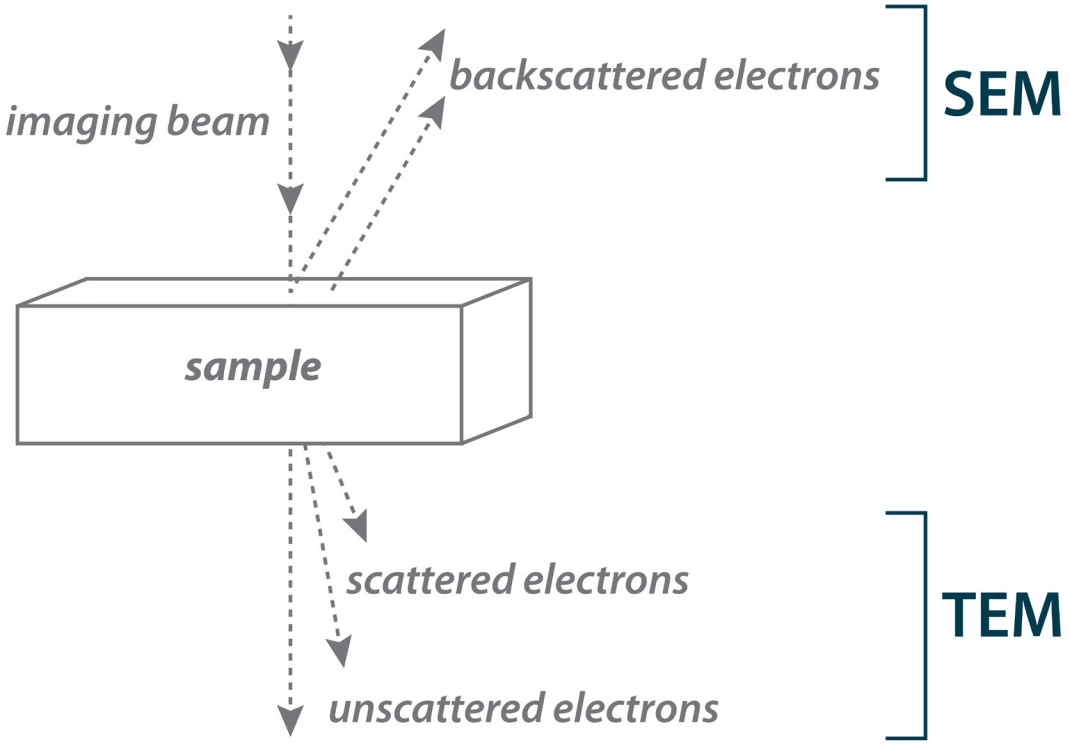
3. Scanning Electron Microscopy

While fluorescence allows us to highlight subcellular structures, we still cannot resolve much of their detail. This is because the resolution of microscopy is limited by the wavelength of the imaging beam. For light microscopy, the wavelength of the photons limits the resolution to a few hundred nanometers (more powerful for higher-energy blue light and less for lower-energy red). This resolving power is on the order of the width of many bacterial and archaeal cells. There are some technical "super-resolution" tricks to more finely pinpoint fluorescent molecules, but the overall subcellular details of bacteria and archaea are beyond the resolution of light microscopy.

One way to get around the resolution barrier is to use an imaging beam of higher-energy/shorter-wavelength particles. The discovery in the early 1900s that electrons have wave-like properties, and the subsequent realization that they can be focused by a "lens" consisting of a toroidal magnetic field, led to the development of **electron microscopy (EM)** in the 1930s.

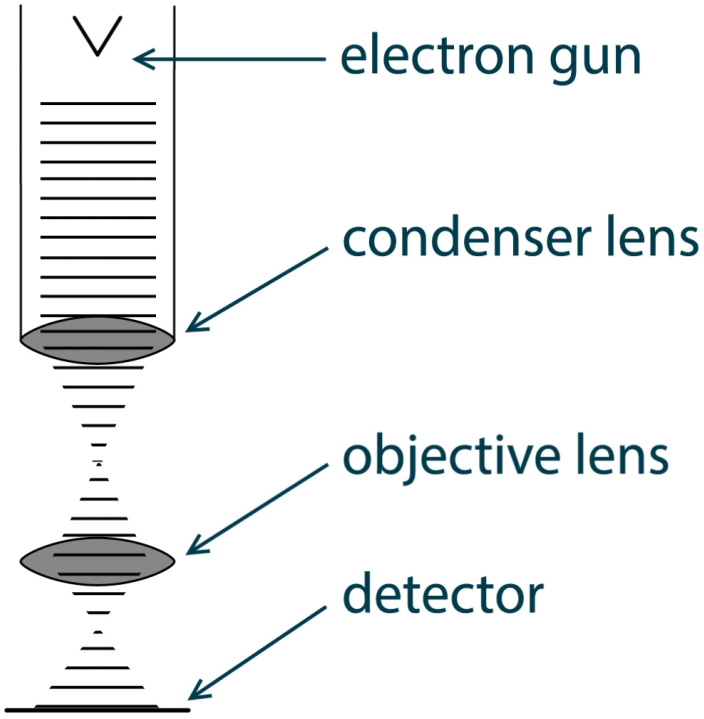
There are two main electron microscopy approaches (*Learn More* on the next page). In **Scanning Electron Microscopy (SEM)**, we detect electrons that are scattered backward from the sample, producing an image of the surface of the sample. The *Shewanella oneidensis* cells you see here were imaged by SEM. Note the magnified details of the cells' shape and flagella compared to the light microscopy images you just saw.

Shewanella oneidensis Collected by: Sahand Pirbadian DOI: 10.22002/D1.1465


imaging beam
backscattered electrons
SEM
sample
scattered electrons
unscattered electrons
TEM

Learn More: EM Modes

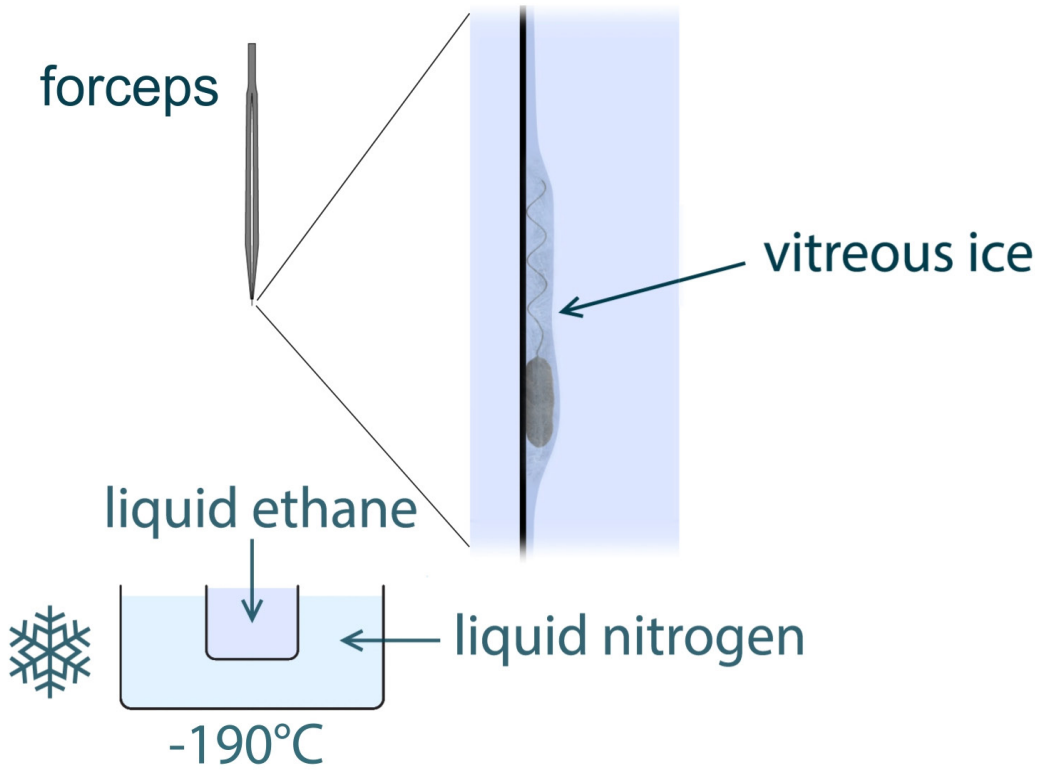
In electron microscopy, interactions of the imaging beam with the sample result in scattering events. Different imaging modes detect different types of scattering. Here we focus on two modes: SEM, which detects backscattered electrons and therefore maps the surface of the sample; and TEM, which detects transmitted electrons and therefore projects information about the full sample volume. We will discuss TEM on the next page.


electron gun
condenser lens
objective lens
detector

4. Transmission Electron Microscopy

In **Transmission Electron Microscopy (TEM)**, we detect electrons that have interacted with atoms in the sample as they passed through it, producing a **"projection" image** of the 3D object onto a 2D plane, similar to a medical X-ray image. This shows details throughout the cell, not just on the surface.

Electron microscopy, whether SEM or TEM, relies on the interactions of electrons with biological material to create an image. These interactions, however, also present some problems. First, they damage the sample, so exposure has to be limited, which in turn limits the contrast of images, or how much signal we see relative to noise. Second, electrons interact not just with the sample, but also with anything else in their path, so imaging must be conducted in a vacuum. This is problematic for biological material, which is mostly water that instantly boils away in a vacuum. To circumvent these problems, we can dehydrate samples to remove the water (changing the structure in the process) and coat them with metal to increase electron dose tolerance and contrast. The *Shewanella oneidensis* cells you saw on the last page were coated with platinum before imaging. In TEM, samples are often coated with a "**negative stain**" such as uranyl acetate; the electron-dense (dark in an image) metal pools around the sample, leaving the interior lighter and thereby creating a negative image. This *S. oneidensis* cell was negative-stained this way before imaging. The resulting projection image again shows the shape of the cell and its flagellum, but not many internal details.


forceps
vitreous ice
liquid ethane
liquid nitrogen
-190°C

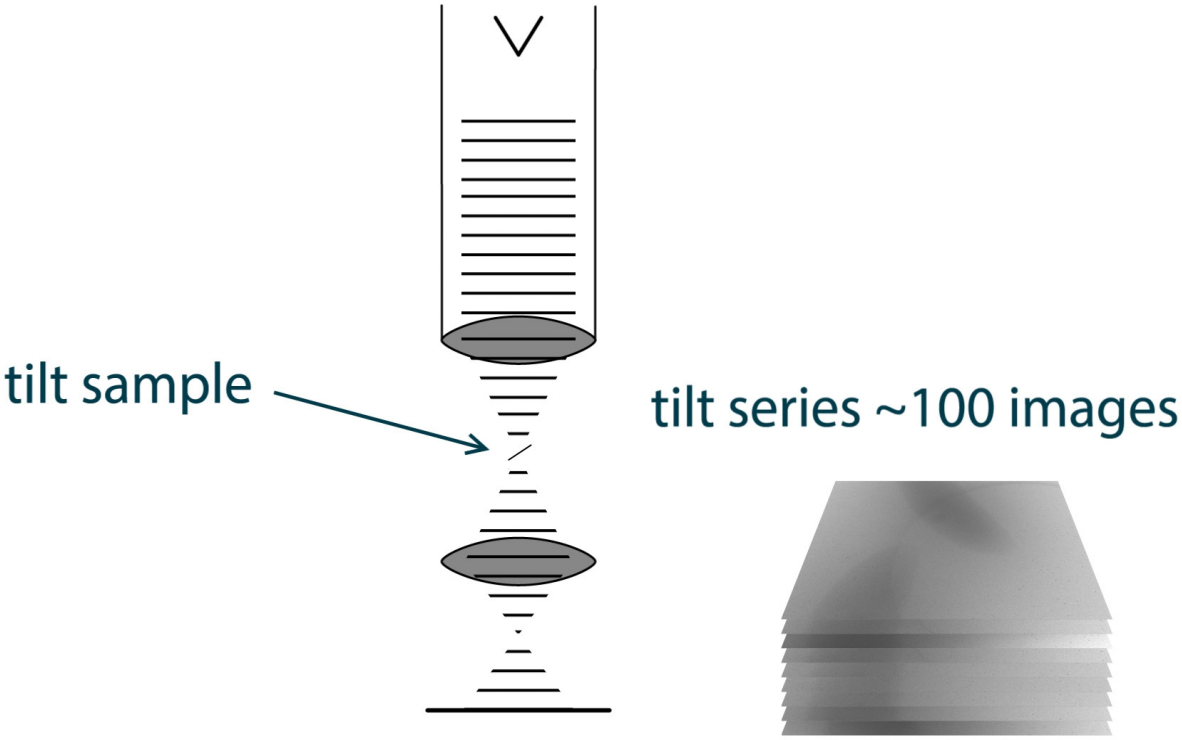
5. Cryogenic Electron Microscopy

We are able to visualize the native structure of bacterial and archaeal cells thanks to a breakthrough in TEM sample preparation. Instead of getting rid of the water, why not just freeze it, since ice sublimates very slowly in a vacuum? The problem is that water expands as it freezes into crystalline ice, damaging the cell in the process. But in the 1980s scientists discovered that freezing a sample quickly enough (done by rapidly plunging a small volume into a very efficient cryogen like liquid ethane) creates a very different kind of ice. The water molecules are immobilized so abruptly that they do not have a chance to find binding partners to form a crystal. The result, called "vitreous" ice for its glass-like properties, preserves cells in their native, fully-hydrated state. The frozen sample can then be inserted directly into the vacuum of the TEM without needing additional treatment or staining. This technique is called **cryogenic electron microscopy**, or **cryo-EM**.

Here you see a projection image of a *Caulobacter crescentus* cell imaged by cryo-EM. Let us quickly go through how it was prepared. First a drop of culture was placed onto an EM grid. Instead of the glass slides used to support samples in a light microscope, EM sample supports are small circular grids of metal, ~3 mm across, overlaid by a thin mesh of carbon with 2 μm-wide holes. Excess liquid was then blotted away with paper, leaving a thin film of sample across the grid. The grid was then plunged into a cryogen, and the frozen sample was imaged in a special TEM that kept the sample cold (near the temperature of liquid nitrogen, approximately -190°C) so that the vitreous ice did not warm and transition to a more damaging (and opaque) crystalline state. In this image, you can see the edge of one of the holes in the carbon mesh of the grid; note the slightly increased clarity in the hole where there is nothing but culture media compared to the region covered by carbon. Whenever possible, we choose to image cells lying at least partially in holes. You will also notice many small dark circles – these are gold beads that were added to the sample; you will see why on the next page.

With cryo-EM, we can start to see the true structure of cells. Compared to the images you have already seen, note the added level of detail visible here, including the cell's multi-layered boundary, and the braided texture of its flagellum.

Caulobacter crescentus Collected by: Steven Wang DOI: 10.22002/D1.1467


tilt sample
tilt series ~100 images

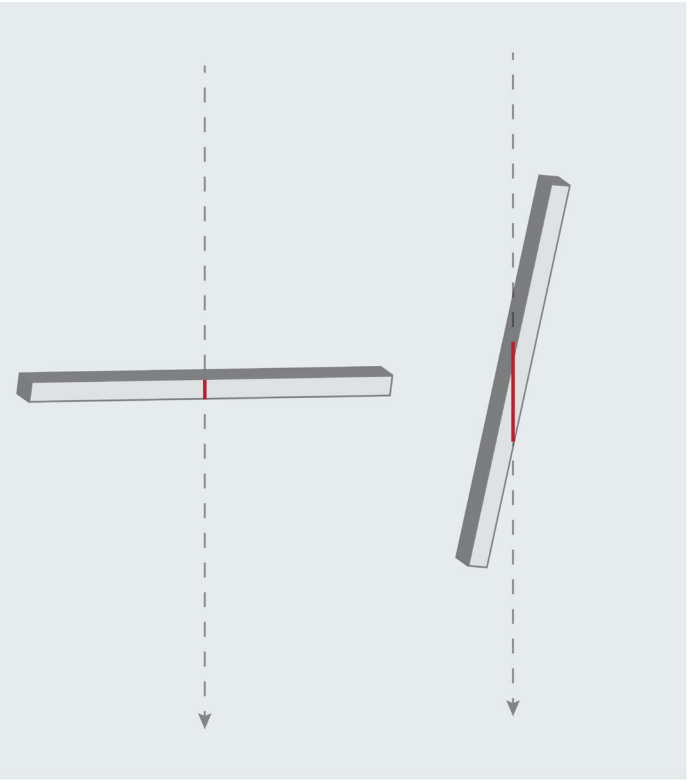
6. Cryogenic Electron Tomography

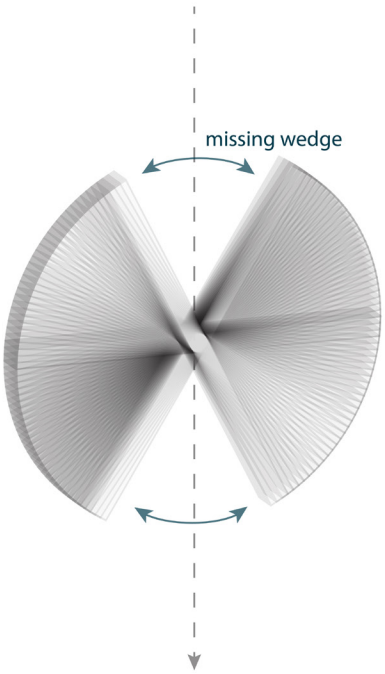
To truly understand a three-dimensional object, we need to be able to visualize it in three dimensions. To do that, we can use **tomography** (from the Greek for "writing slices"). The process may be familiar from medical Computed Tomography, or CT, scans. Simply, the object is imaged from different angles (in a CT scan, the camera moves around the patient; in our case we keep the imaging path constant and simply rotate the small sample). This produces a "**tilt-series**" of projection images that can be digitally processed into a 3D reconstruction of the object: a **tomogram**. To compute the reconstruction, we need to be able to precisely align the images, which is difficult because of the low contrast from cryo-EM samples (unstained and sensitive to electron dose). This is where the gold beads come in. As you saw on the previous page, they provide clear markers in the images to guide the alignment.

Here you see a tilt-series and resulting tomogram of a *Caulobacter crescentus* cell. In this and following videos, we view the tomogram as a series of slices scanning (or "writing") from bottom to top. Note the further level of detail that this technique provides, separating the cell's structures into their three-dimensional locations. We can also rotate the tomogram and slice along a different axis to view structures from different angles, although not all features are visible at all angles (*Learn More* on the next page).

Since the early 2000s, cryo-ET has transformed our understanding of microbial cells, revealing their structural richness and diversity, as you will see in the chapters that follow.

Caulobacter crescentus Collected by: Steven Wang DOI: 10.22002/D1.1468

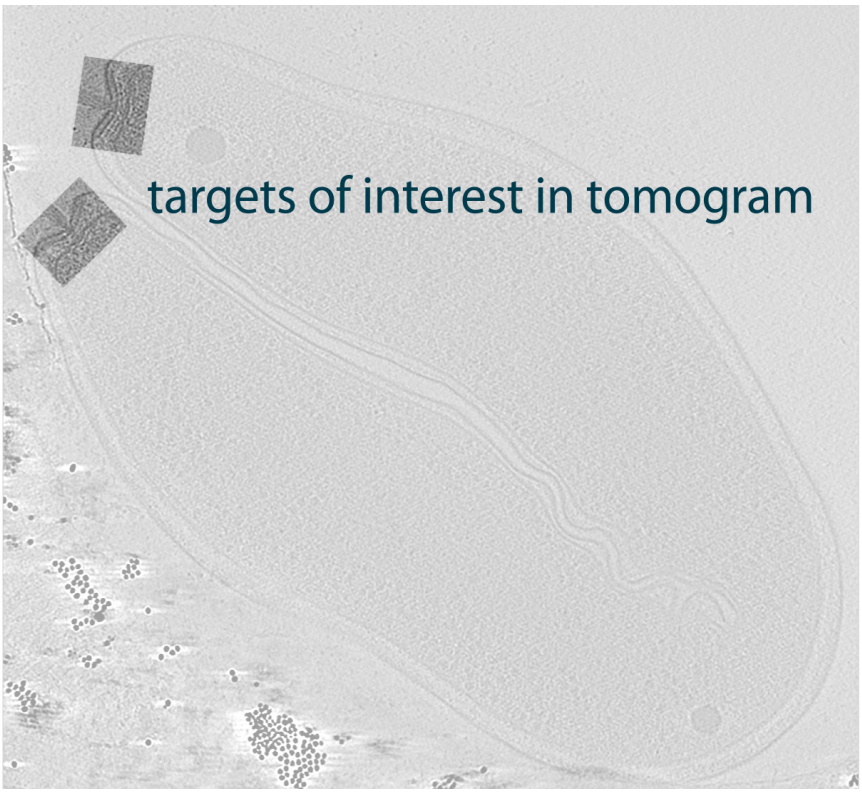



missing wedge

Learn More: Missing Wedge

Think again about how a tilt-series is made: by taking images from different angles. If we could tilt the sample all the way to 90°, we would have information from every angle. But the sample gets effectively thicker as we tilt it, since the beam has to pass through more and more of the surrounding material. This effect, illustrated on the left, usually becomes prohibitive beyond 60°, so a typical tilt-series spans only ~2/3 of the possible angles, leaving a "missing wedge" of information corresponding to those high tilt angles, as you can see on the right. The missing wedge blurs densities in the direction of the imaging beam. In practice it means that if we look at a cross-section of a cell, we cannot trace thin features like membranes all the way around. You will see an example of this effect in Chapter 5.10.

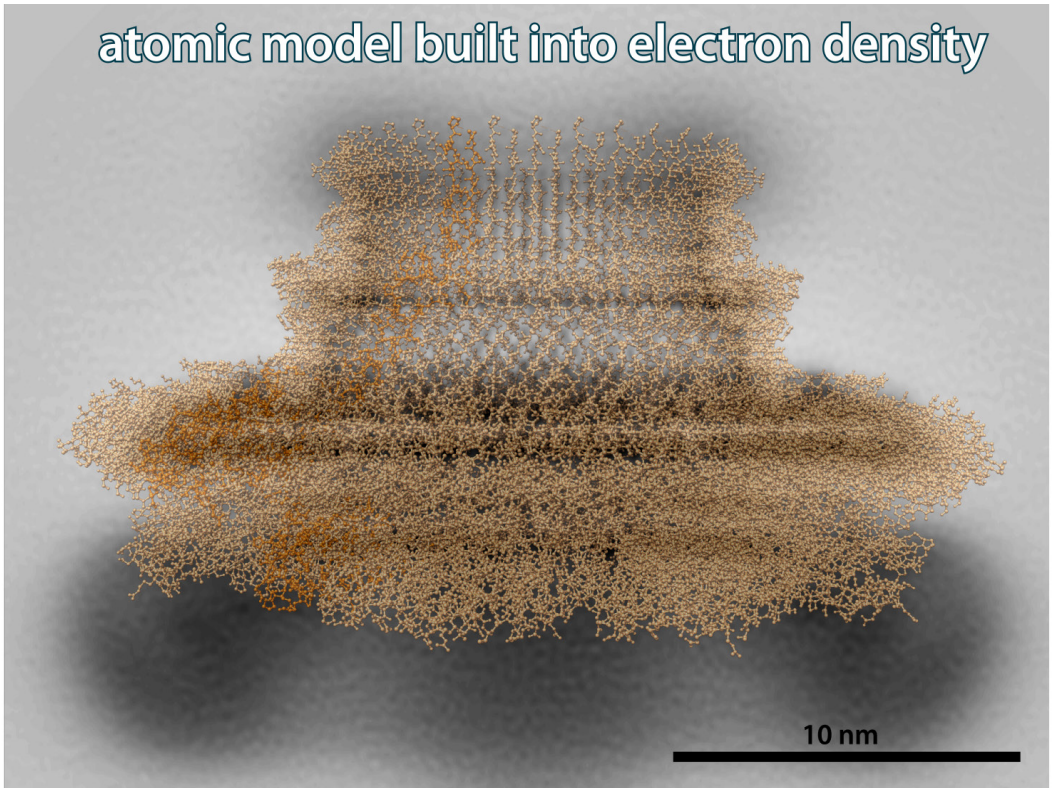
Collected by: *DOI:*

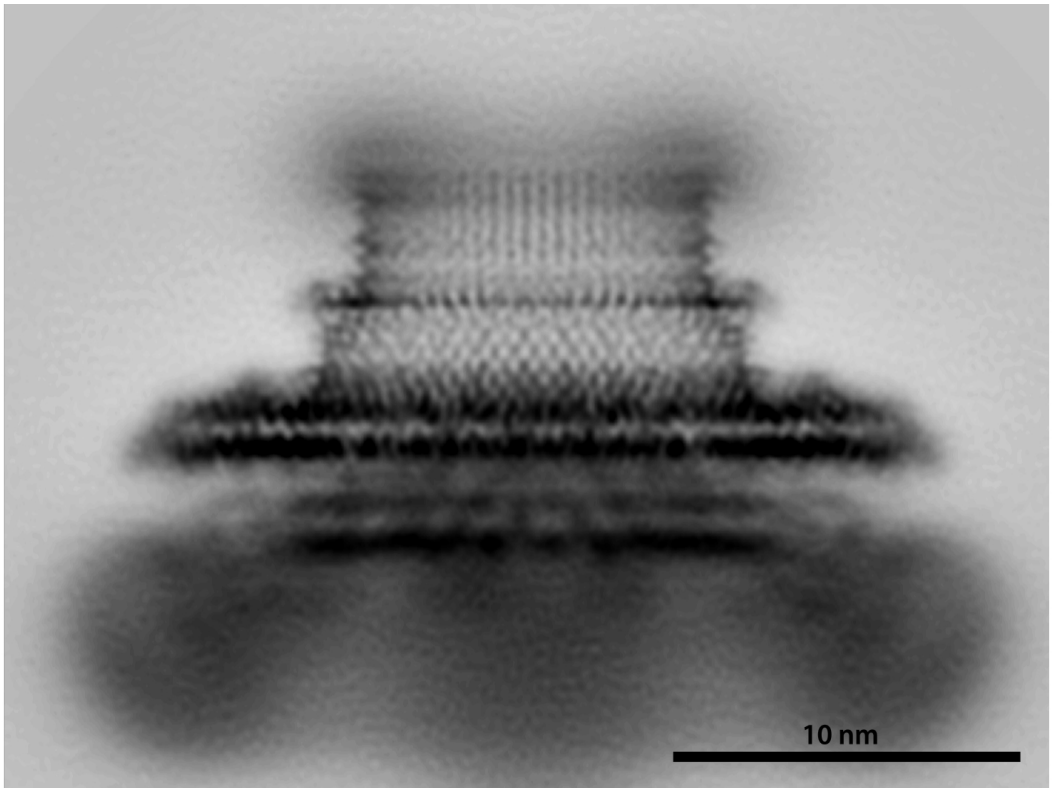

targets of interest in tomogram

7. Sub-Tomogram Averaging

What if we want to zoom in further to examine a particular structure more closely? While in theory electron microscopy can resolve atoms, in practice the resolution is limited by many factors including the radiation sensitivity and thickness of the sample. For relatively thick samples like the (small) bacterium on the last page, we can achieve ~5 nm resolution, enough to see the shapes and arrangement of large macromolecular complexes. To boost our resolving power further, we can gather strength in numbers. By averaging multiple copies of a structure, either from the same tomogram, or from multiple cells in different tomograms, we can build up the signal relative to the noise. Here you see an example of this approach, called **sub-tomogram averaging**, applied to the motor that spins the flagellum. Hundreds of *Campylobacter jejuni* cells were imaged by cryo-ET and their individual flagellar motors were digitally extracted from the resulting tomograms and averaged to produce a higher-resolution view [10]. Note how densities that vary between motors, indicating that they are not stably associated with the structure, get washed out, while densities that appear in the same place in each motor reinforce one another. This approach only works for structures, and parts of structures, that are fairly rigid, but it can be a powerful tool to study structures inside the cell.

Structure: EMD-3150


atomic model built into electron density
10 nm

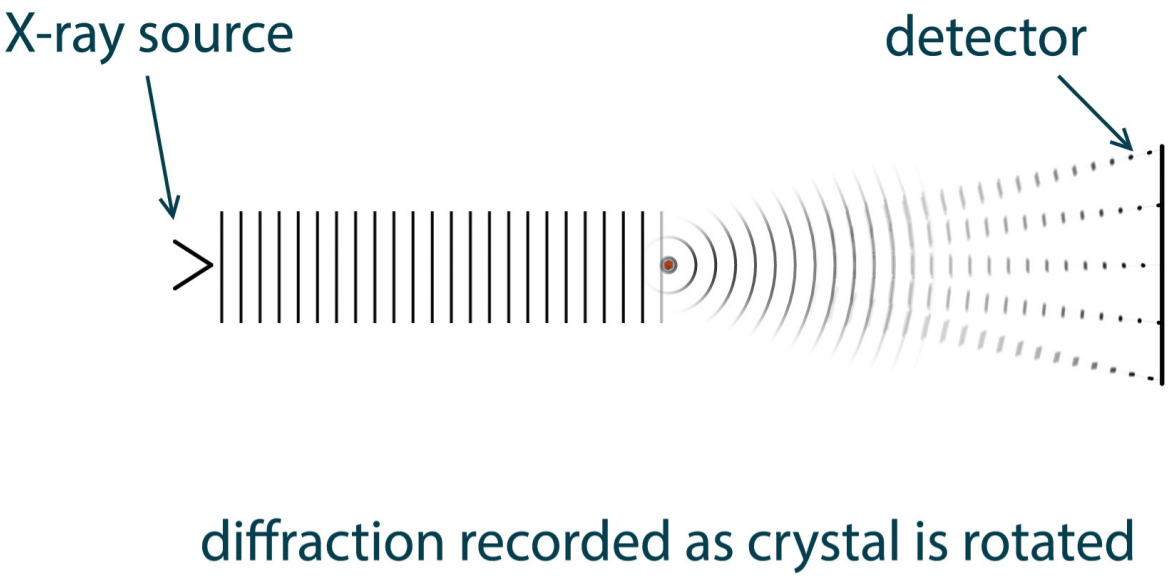

10 nm

8. Single Particle Reconstruction

If a structure can be purified out of the cell, we can visualize even more details. For a large, complex machine like the flagellar motor you just saw, particularly if it is embedded in the cell's membranes, the complete structure can only be studied in situ, inside the cell. But parts of it can be purified out of the cell and visualized by **cryo-EM single particle reconstruction**. This technique is conceptually similar to sub-tomogram averaging, with images of many identical copies of a structure of interest averaged to produce a higher-resolution view. Instead of using tomography to see different angles, we take advantage of the fact that a random snapshot will capture individual copies in different orientations to provide all the views we need from just one or a few projection images. Usually tens of thousands of copies are averaged, often yielding resolutions of a few angstroms, sufficient to see the placement of each amino acid so that we can construct an atomic model of the structure like you see here. This structure is a ring complex embedded in the membrane that functions as part of the rotor of the flagellar motor [11]. It comprises more than two dozen copies of a protein called FliF; a single monomer is highlighted here in dark blue.

You will see many examples of high-resolution structures solved by this technique in the pages to come. It is mainly applied to large proteins and complexes, since most of the individual proteins in the cell are too small to be accurately aligned for reconstruction.

Structures: EMD-10143; PDB: 6SCN


X-ray source
detector
diffraction recorded as crystal is rotated

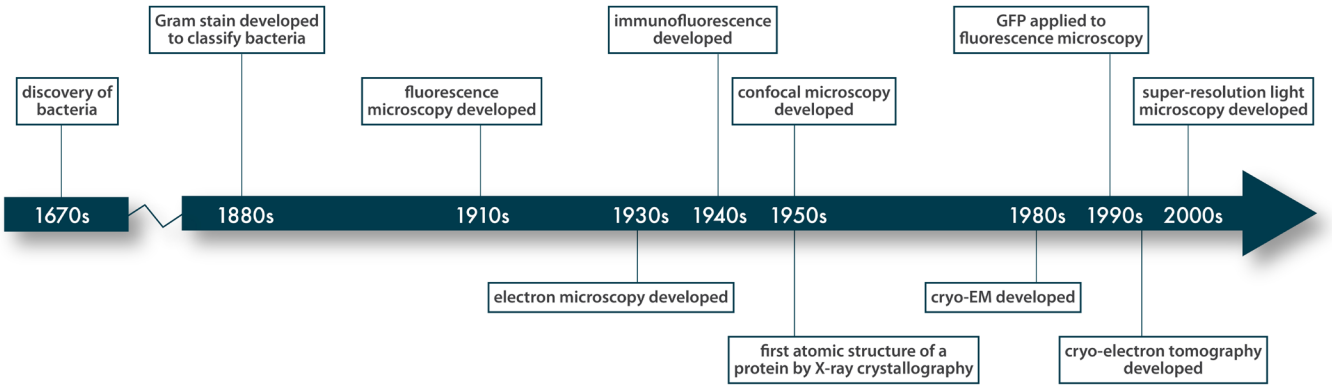
9. X-Ray Crystallography

For smaller protein complexes and individual proteins or pieces of proteins, we can use a different technique, based on photons produced by electron interactions: X-rays. This technique, too, relies on a kind of averaging of many particles, in this case identical molecules that have been purified from the cell and crystallized. The wavelength of X-rays (~0.01 - 10 nm) is on the order of the distances between atoms in the crystal, which means that the atoms' electrons scatter the X-rays into a **diffraction pattern** that can be used to deduce the precise arrangement of each atom in the crystal. Beginning in the 1950s, **X-ray crystallography** has been enormously successful in revealing the structure of biological macromolecules like proteins and, famously, deoxyribonucleic acid (DNA) polymers (*Learn More* on the next page).

Not every protein can be induced to form large, well-ordered crystals, but many can, and as of 2020, X-ray crystallography had been used to solve nearly 150,000 structures, including the one you see here, which shows the interaction between the tail of the protein you saw on the last page, FliF, and the head of another protein, FliG, that helps form the other ring complex of the rotor [12]. This interaction locks the two rotor components together.

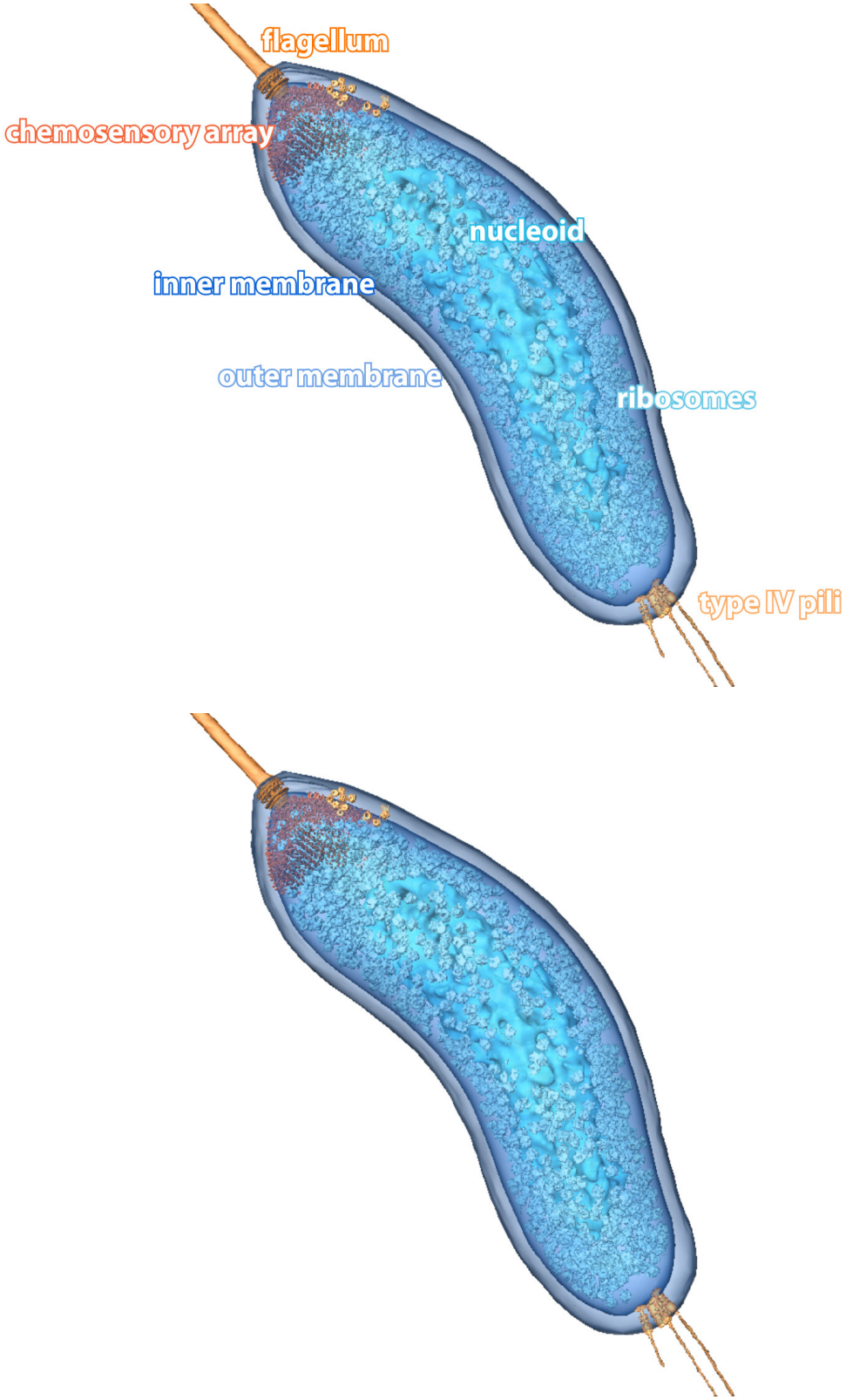
Structure: PDB 5WUJ

Collected by: DOI:


discovery of bacteria
Gram stain developed to classify bacteria
fluorescence microscopy developed
immunofluorescence developed
confocal microscopy developed
GFP applied to fluorescence microscopy
super-resolution light microscopy developed
1670s
1880s
1910s
1930s
1940s
1950s
1980s
1990s
2000s
electron microscopy developed
first atomic structure of a protein by X-ray crystallography
cryo-EM developed
cryo-electron tomography developed

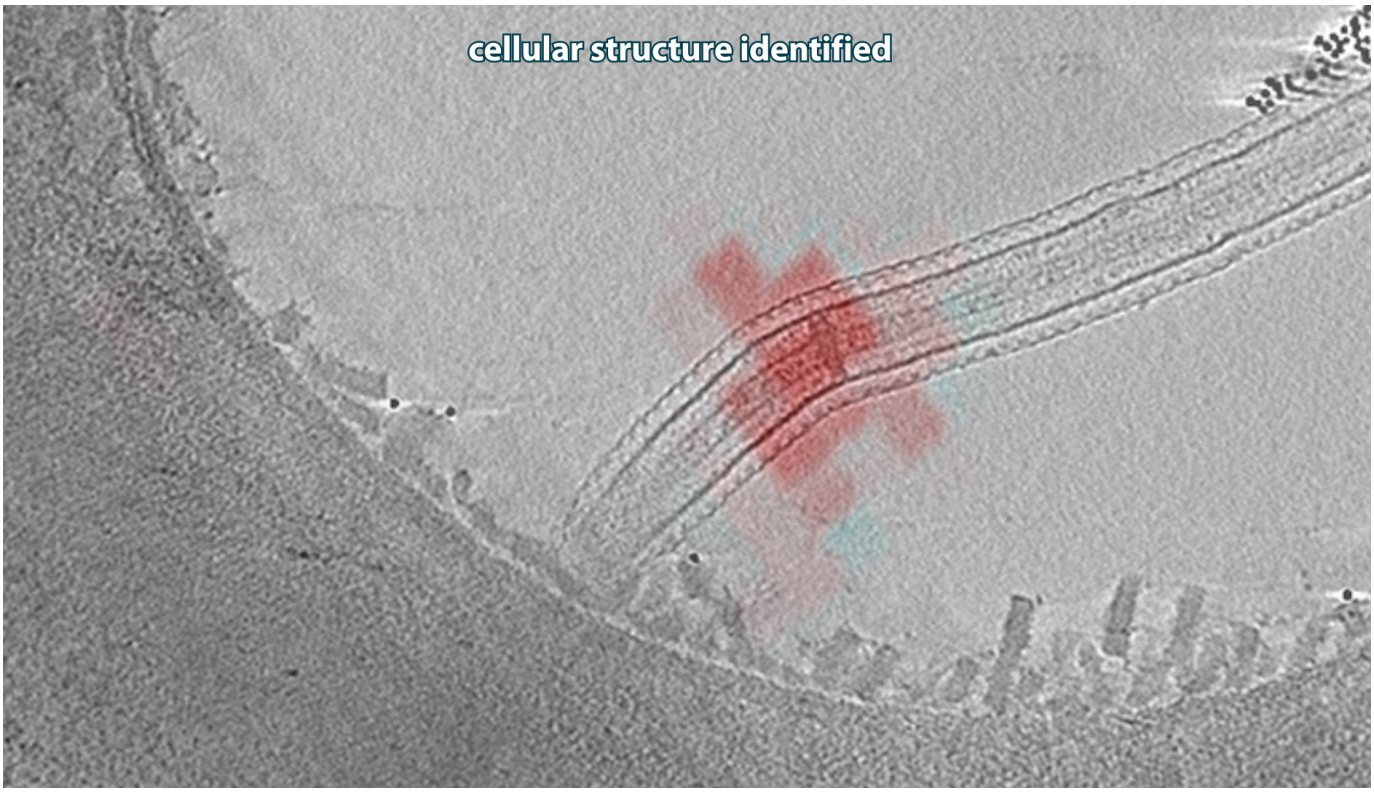
Learn More: Timeline

This chart gives a rough idea of when key techniques in structural biology were developed.


flagellum
chemosensory array
nucleoid
inner membrane
outer membrane
ribosomes
type IV pili

10. Putting It All Together

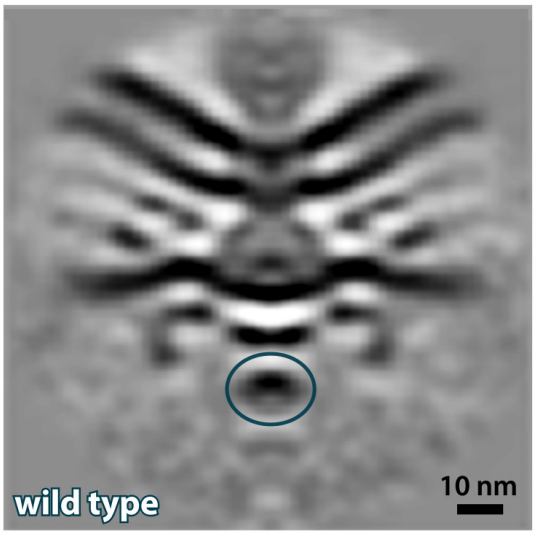
Science benefits from collaboration, and structural biology is no exception. This extends to our tools. To understand cells across their full length scale, we need to combine what we learn from different techniques. Consider this *Bdellovibrio bacteriovorus* cell. To visualize its overall structure, we can use cryo-ET. To identify a particular structure in the cell, we can alter its abundance (by genetically deleting it or overexpressing it) or use a fluorescent tag (*Learn More* on the next page). Once we have identified a structure, we can get a higher-resolution view of it by sub-tomogram averaging. Then we can again use genetics to locate the positions of individual pieces in the structure (*Learn More* on following pages). Combining this information with clues from other biochemistry methods, we can place high-resolution structures of components solved by X-ray crystallography and single particle reconstruction into their correct context. In this way, we can begin to build up a full picture, from individual atoms to entire cells. We still have a long way to go, but someday we hope to be able to map the location and interactions of every protein in a bacterium or archaeon, creating a true, molecular atlas of the cell.

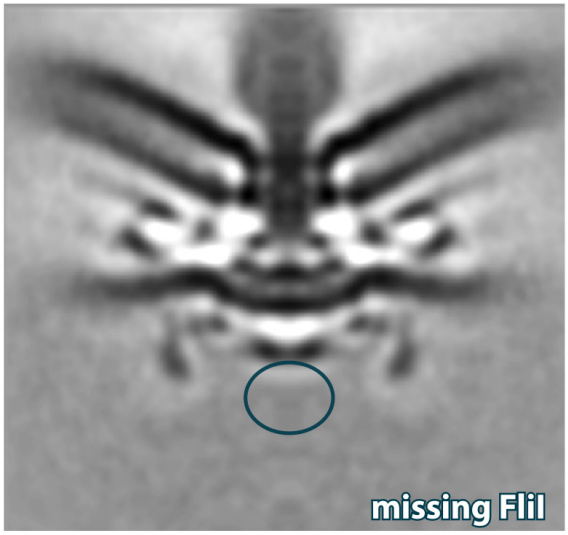

cellular structure identified

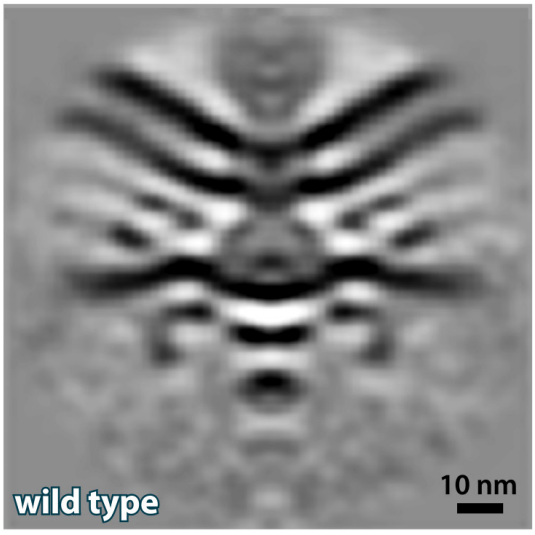
Learn More: CLEM

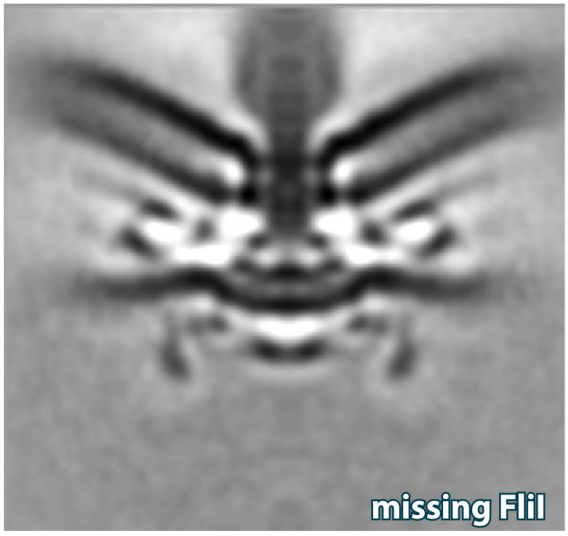
To identify what a macromolecular complex looks like in the cell, we can use ***Correlated Light and Electron Microscopy****, or* ***CLEM****, as demonstrated by this example in* Caulobacter crescentus *[13]. A structure of interest, in this case a "stalk band" in a cellular appendage (more on that in Chapter 4), is genetically tagged with a fluorescent label. Cells are plunge-frozen on an EM grid and first imaged by light microscopy, to locate the tagged structure within the cell. The sample is then transferred to the TEM and landmarks such as large fluorescent beads are used to find the same location. Then we can zoom in and image that location by cryo-ET to reveal the structure in detail.*

Source: Schlimpert et al. (2012)


wild type
10 nm


missing FliI


wild type
10 nm

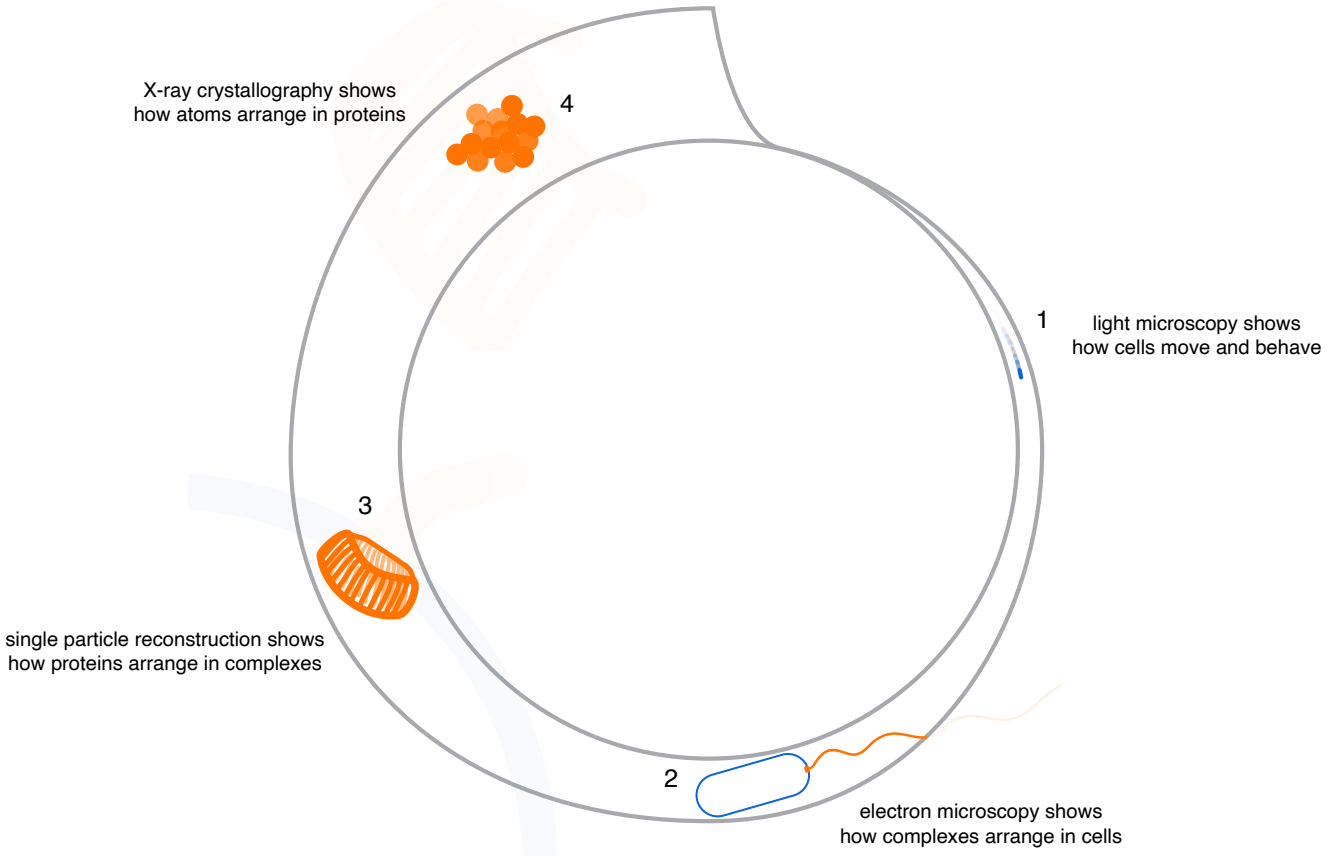

missing FliI

Learn More: Mapping

To identify the location of a component within a large macromolecular complex, ***difference mapping*** *can be helpful. In this approach, the gene corresponding to that component is either knocked out or a tag, like GFP, is added that will make the protein larger. A sub-tomogram average of the complex is produced and compared to a sub-tomogram average of the complex from wild-type (unmodified) cells. Often, a difference in the structure is visible, corresponding to the missing or altered component. Here you see an example of how this was used to locate a component of the flagellar motor, a protein called FliI, in* Campylobacter jejuni *[14].*

Structures: EMD-5300; EMD-10457

Campylobacter jejuni *Collected by: Morgan Beeby* *DOI: 10.22002/D1.1473*


X-ray crystallography shows
how atoms arrange in proteins
4
1
light microscopy shows
how cells move and behave
3
single particle reconstruction shows
how proteins arrange in complexes
2
electron microscopy shows
how complexes arrange in cells

Summary

Concept Check Questions

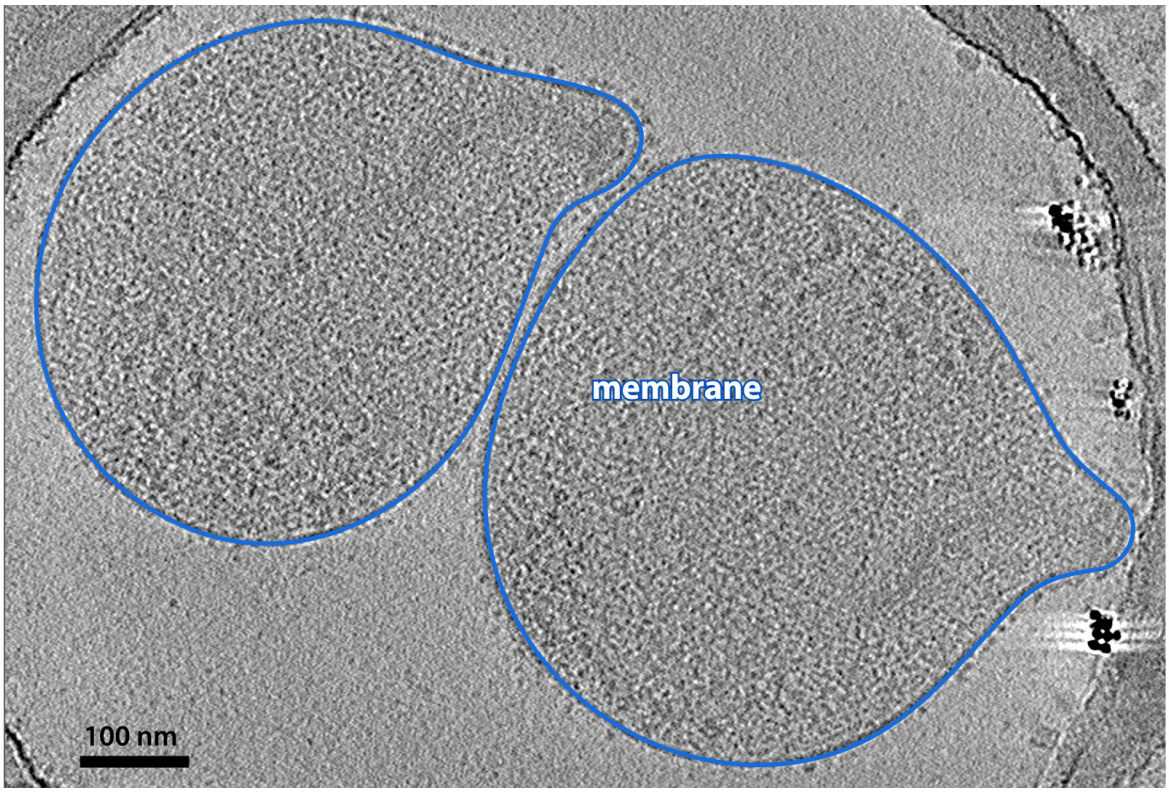
- How can you locate a protein of interest by light microscopy?
- What happens to liquid water in a vacuum?
- What are gold beads used for in cryo-ET?
- Which technique can reveal the atomic structure of a small protein?

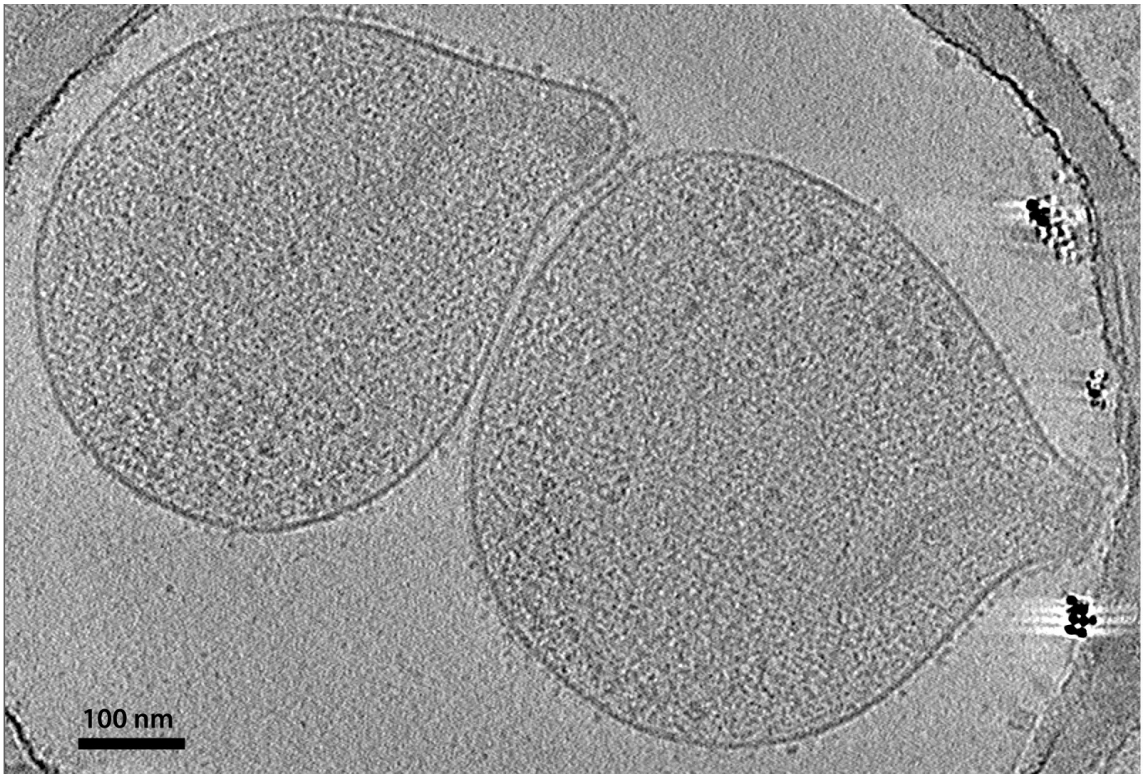
Further Reading

- Jensen. *Getting Started in Cryo-EM* Video Lectures [15].
- Oikonomou and Jensen (2017). *Cellular electron cryotomography: toward structural biology in situ* [16].
- Ruska (1987). *Nobel Lecture: The development of the electron microscope and of electron microscopy* [17].
- Tsien (2005). *Breeding molecules to spy on cells* [18].

2
Cells

"It is not a simple life to be a single cell, although I have no right to say so, having been a single cell so long ago myself that I have no memory at all of that stage of my life."
- Lewis Thomas [19]


membrane
100 nm


100 nm

1. Membrane

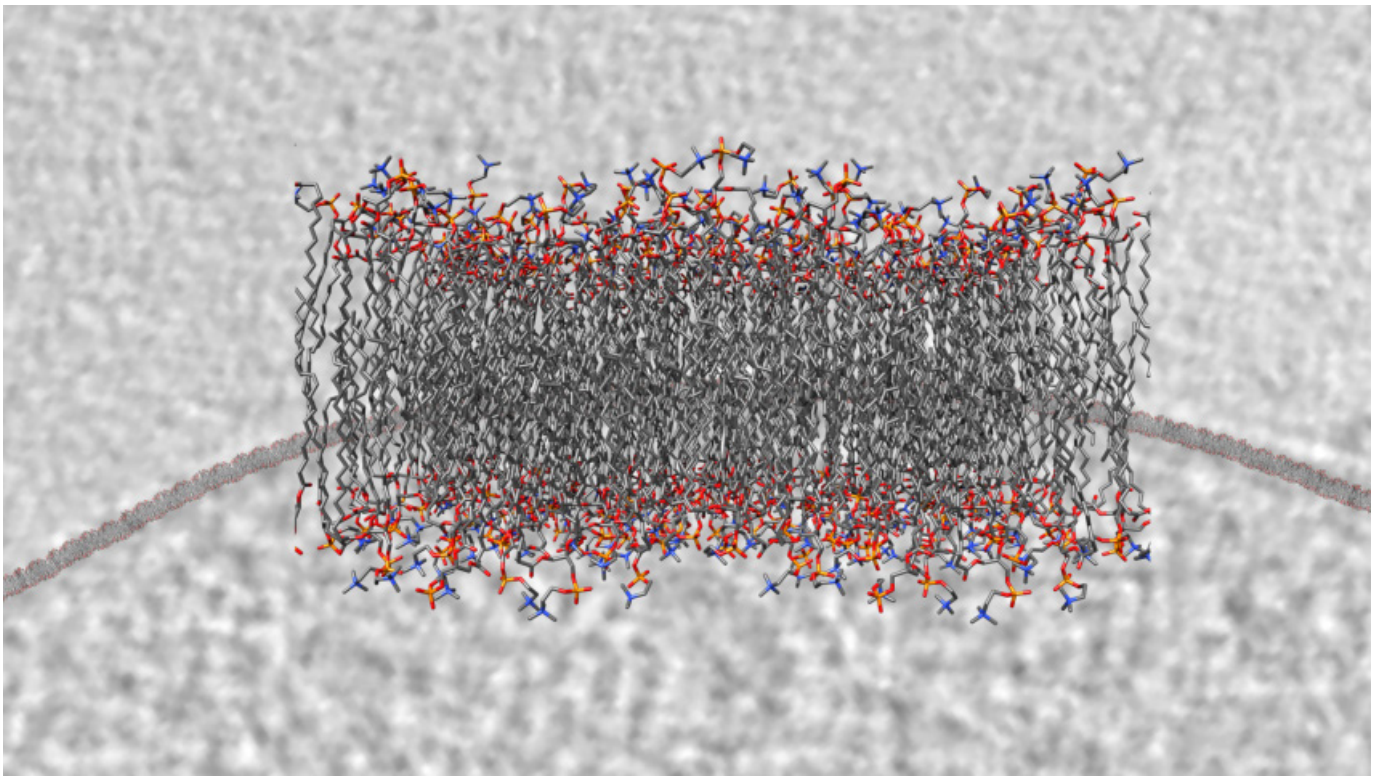
The fundamental unit of life is the **cell**–a contained self-replicating assembly. For many species, including all bacteria and archaea, the organism consists of a single cell. And for nearly all species, no matter how many cells an organism eventually contains (probably around 10 trillion in your case), it started life as a single cell (an egg, in your case). The details vary, but every cell on Earth is the same at heart–a DNA-based replicating machine built from just four macromolecules: nucleic acids, proteins, lipids and carbohydrates. In the environment, molecules interact rarely and randomly. Bringing them together enables the reproducible reactions required for life. So no matter what the first self-replicating molecules were (likely ribonucleic acid, or RNA), they were not a cell until they acquired a container.

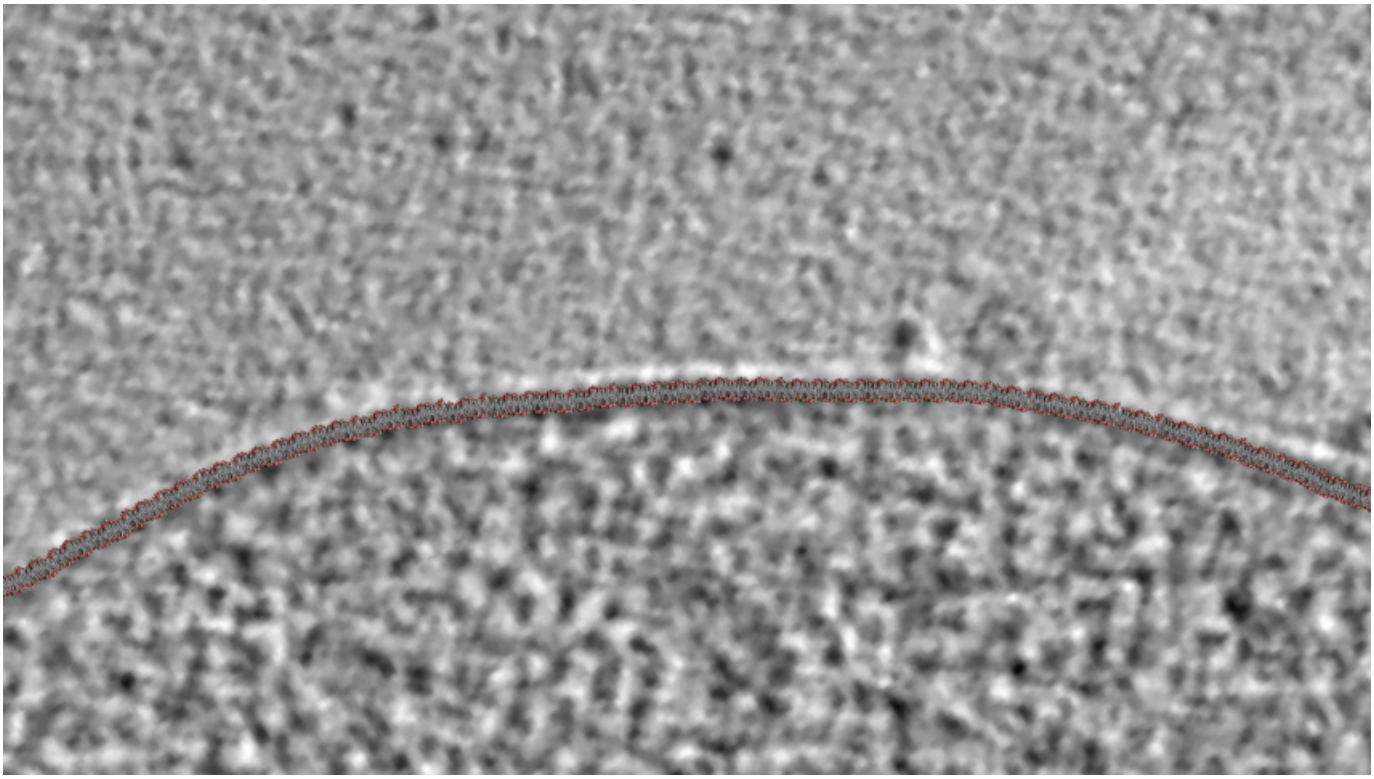
How would you build a container for a cell? You would probably want a porous material that allowed you to sort specific molecules from the environment. Evolution agrees. All cells are enclosed by a selectively permeable **membrane**, made of phospholipids and proteins (*Learn More* on the next page), that allows them to separate their contents from the environment. The chemical properties of phospholipids make membranes impermeable to ions and large or hydrophilic molecules (but not to water). This property is a critical feature for the life of the cell (Learn More on following pages).

With a membrane, your cell now has a clearly delineated exterior and interior. The interior is called the cytoplasm ("cell substance," from the Latin for something molded, in this case by the membrane). Almost all archaea and many bacteria, like these *Mycoplasma genitalium* cells, are **monoderms** ("single skin"). This means that their cytoplasm is enclosed by a single membrane. At this resolution, the membrane looks like a single dark line, but remember that it is really a bilayer, as you will be able to see in some later examples. The cytoplasm contains the many macromolecules that carry out the various functions of the cell's metabolism. The most prominent are the ribosomes, which produce new proteins (*Learn More* on following pages).

Other structures you see in this cell function in motility and will be explained in Chapter 6. Since tomograms show cells in their entirety, the example we choose to illustrate one feature will likely highlight others as well. For now, focus on the feature being discussed. Later, when you have learned about other features, you may want to use the *Feature Index* to find additional examples of them. To help orient you, feature labels are color-coded according to the chapter in which they are discussed.

Mycoplasma genitalium Collected by: Gregory Henderson DOI: 10.22002/D1.1350



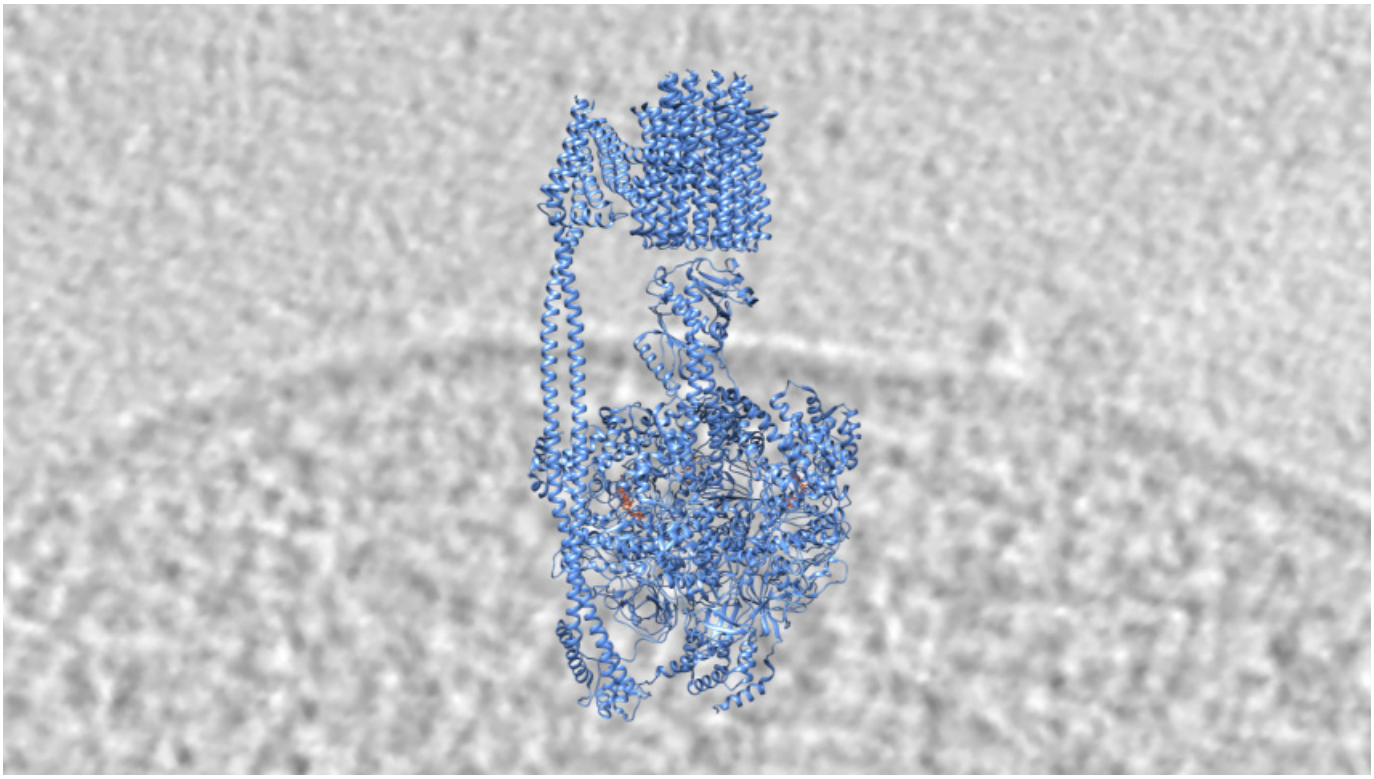


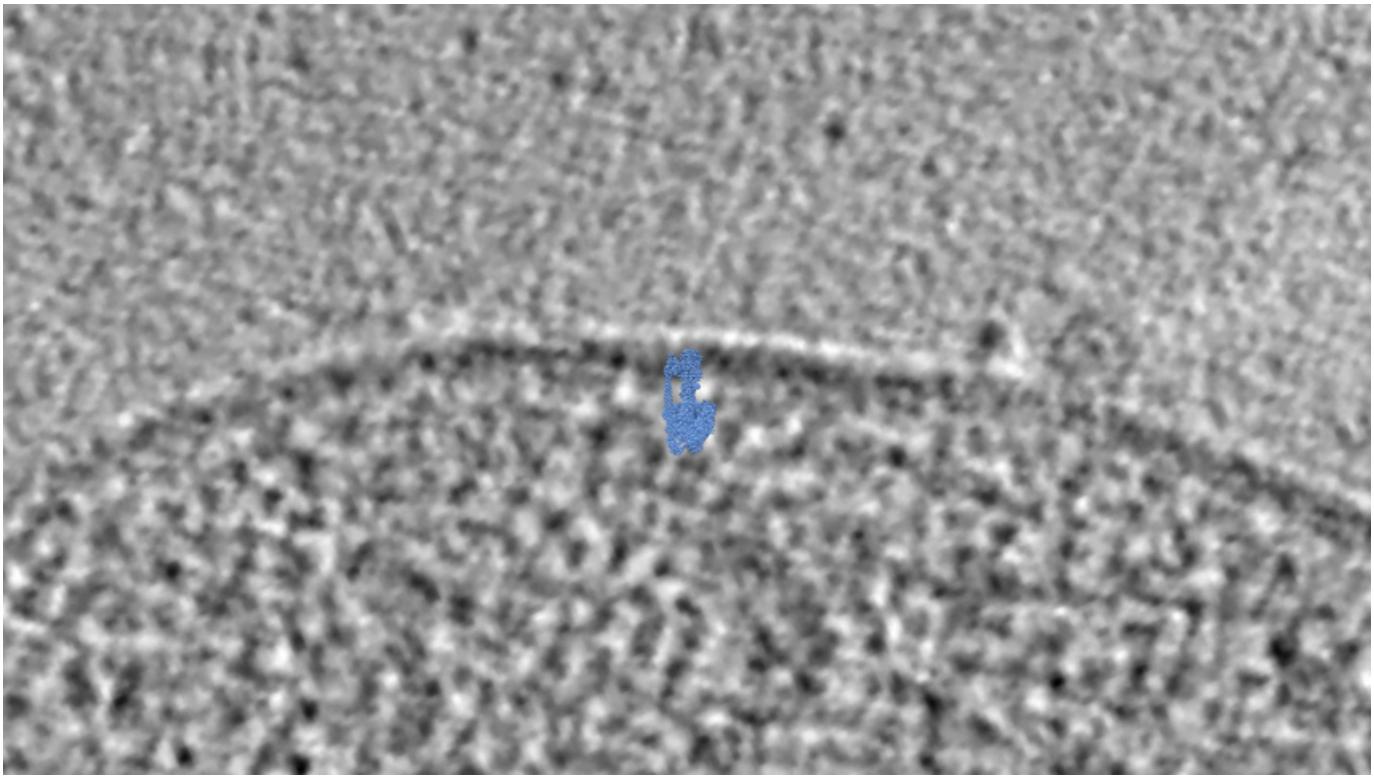
Learn More: Membrane

Phospholipids have a hydrophilic head (colored here) and hydrophobic tails (grey); in water they spontaneously pack side-by-side and tail-to-tail to shield their tails from unfavorable interactions with water. This results in closed double-layered bags: membranes. One key difference between archaea and bacteria (and with them, eukaryotes) is the kind of lipid in their membranes. Hybrid membranes containing both these lipid types can be made artificially, and it is possible that the last common ancestor of all cells on Earth contained both types, with specialization occurring later.

Membranes also contain many proteins. Some have hydrophobic regions that embed them into the lipid bilayer. Other proteins are fused, or tethered, to the lipids. In fact, cells' "lipid" membranes are made up of roughly equal parts phospholipids and proteins.

Source: Lam Nguyen



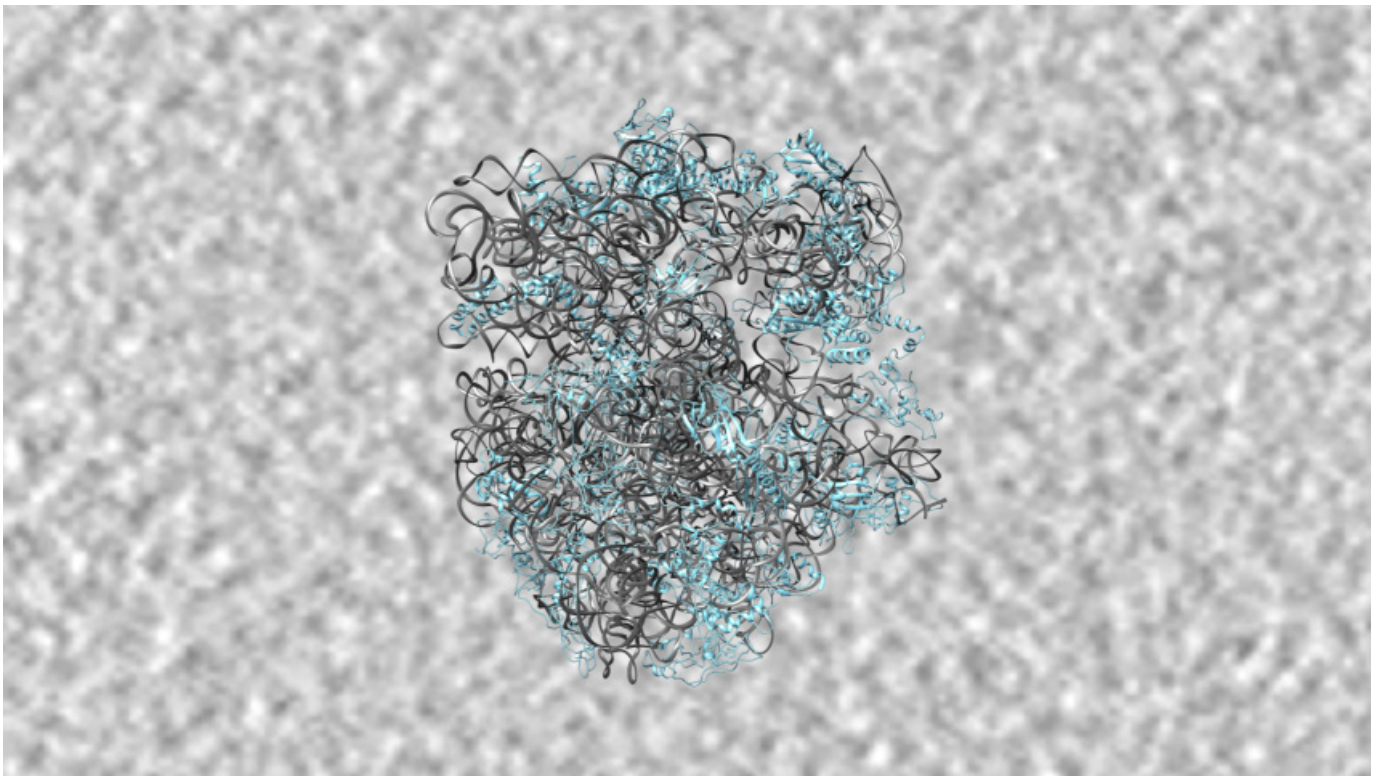


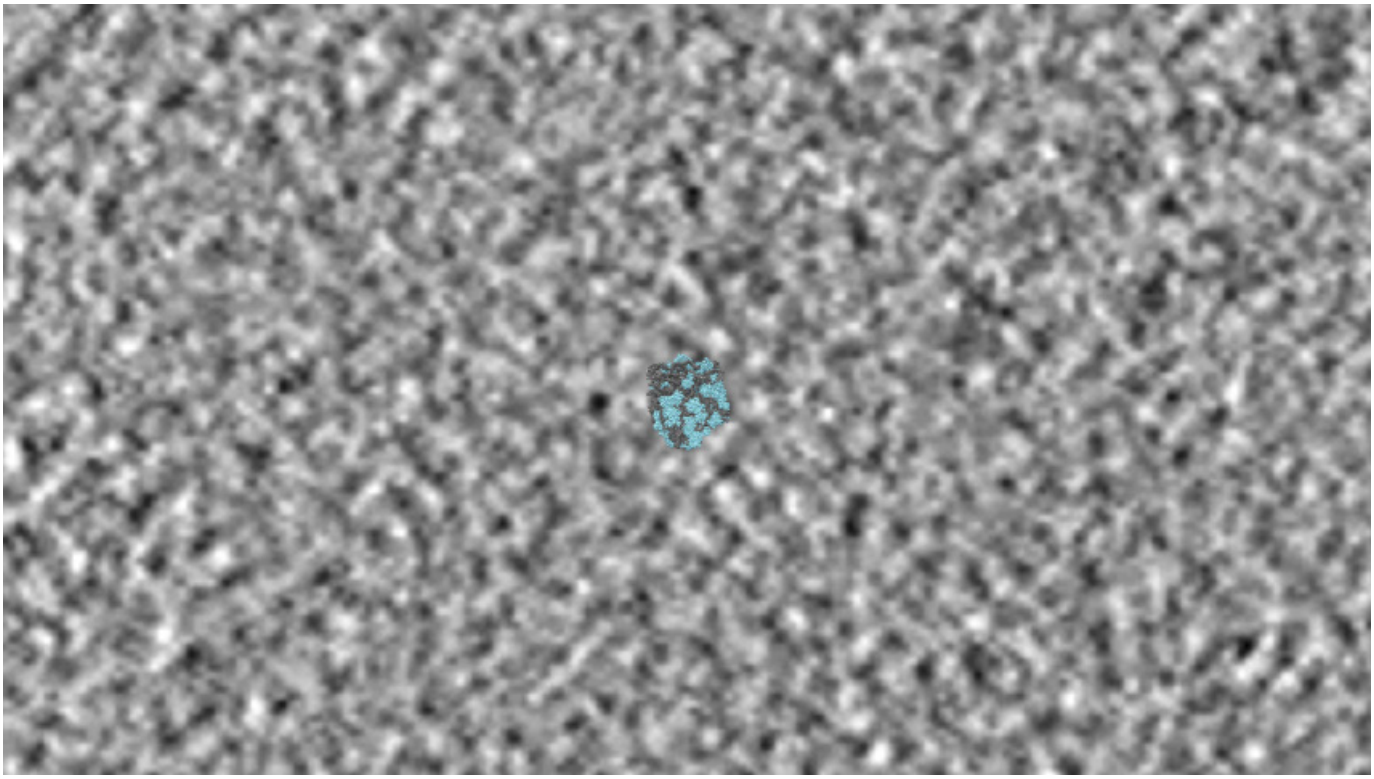
Learn More: ATP Synthase

Cells take advantage of the phospholipid bilayer's impermeability to charged molecules to establish an ion gradient across the membrane, using a chain of electron-carrying proteins in the membrane to pump protons out of the cell. Protein complexes in the membrane called ATP synthases, like this one from Escherichia coli *[20], use the resulting ion potential to generate energy. The portion of the complex in the membrane provides a conduit for protons to flow down their potential, producing a "proton-motive force" that spins the rotor, generating energy that is chemically stored in Adenosine Triphosphate (ATP), the energetic currency of the cell. (You can watch an animation illustrating this process on YouTube: https://www.youtube.com/watch?v=kXpzp4RDGJI.)*

For this reason, we say that the membrane is "energized." Holes in the membrane allow the ion gradient to equilibrate, destroying the cell's means of generating energy, and thus its life.

Source: Sobti et al. (2016) *Structure: PDB 5T4O*

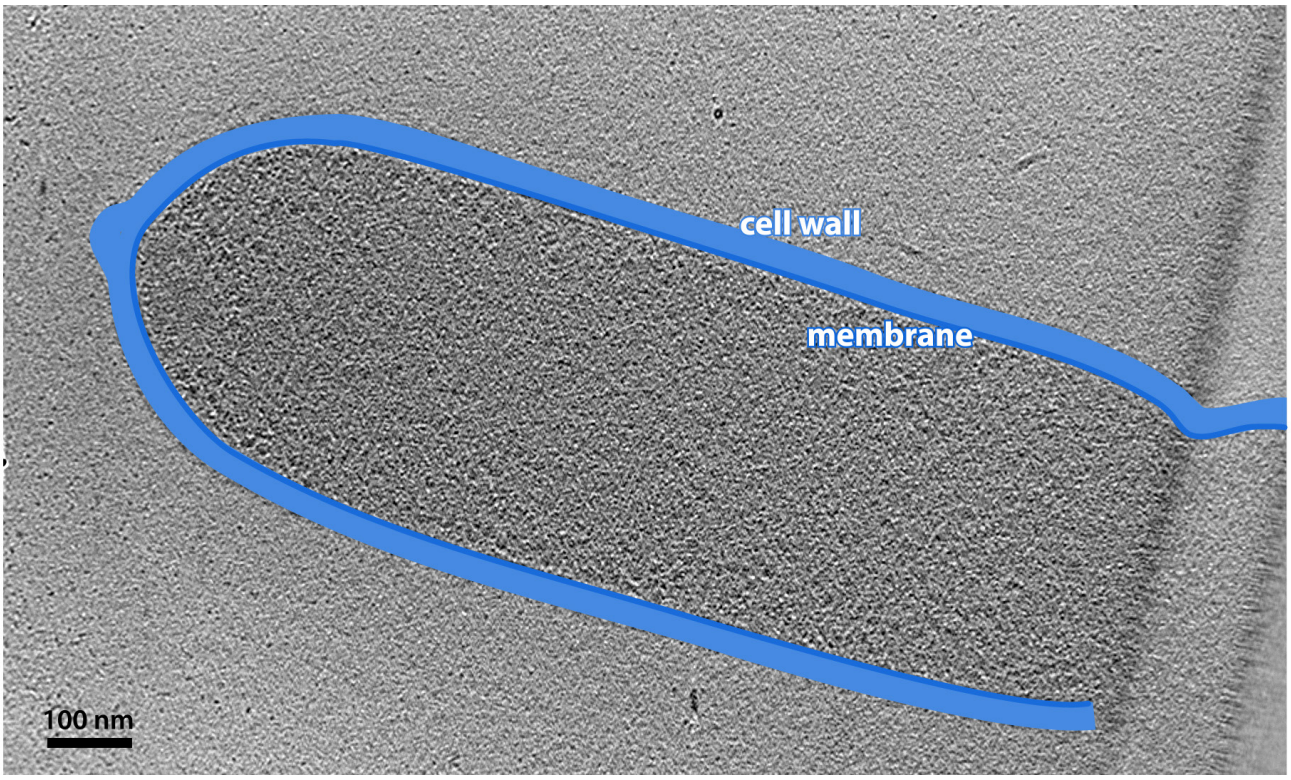


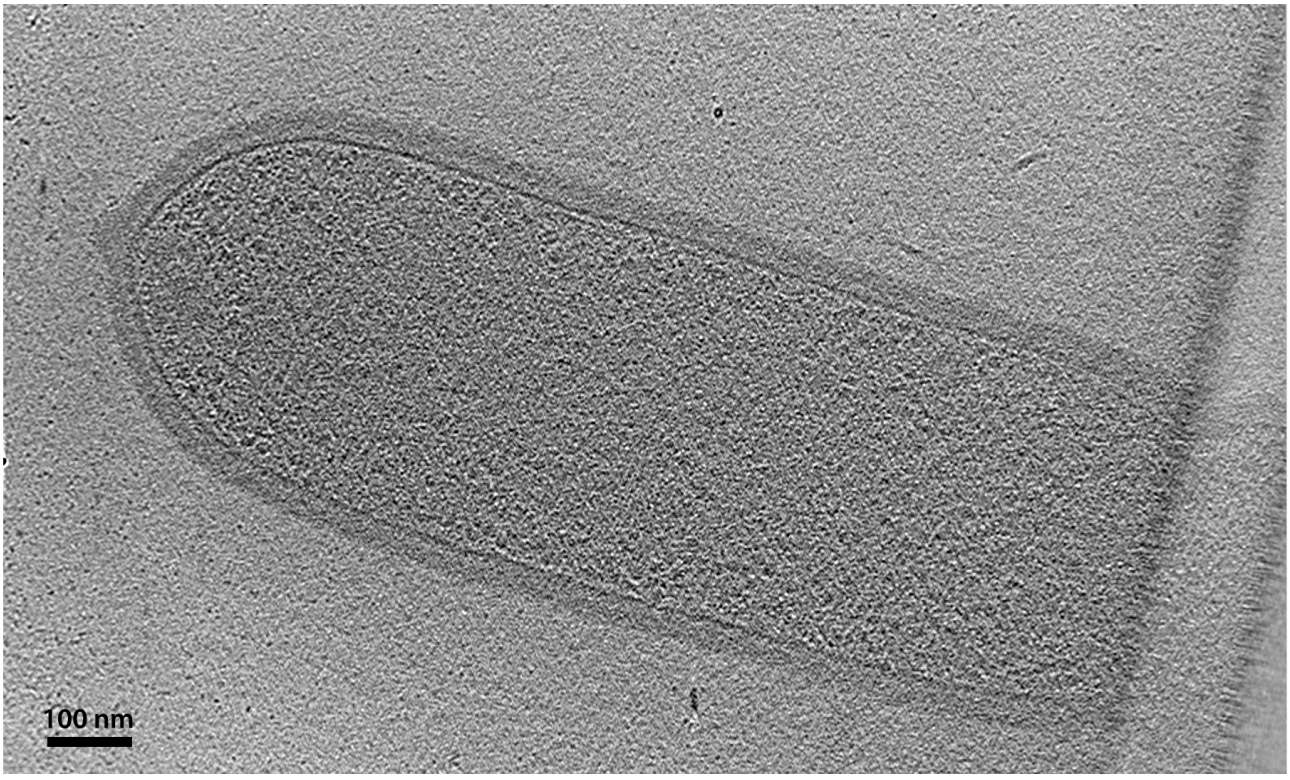


Learn More: Ribosome

Most of the metabolic work of the cell is performed by proteins. The translation of messenger RNA into new proteins, however, is performed by a hybrid complex containing both proteins and RNA molecules. (You can watch an animation illustrating the process on YouTube: https://www.youtube.com/watch?v=q_n0Ij3K_Ho.) This ribosome from Escherichia coli *contains ~50 different protein molecules (shown in cyan) and 3 RNA molecules (in grey) [21]. Electrons interact more strongly with RNA than with protein, making ribosomes stand out darker than surrounding proteins in cryo-EM images. You will also notice their abundance; each cell employs up to tens of thousands of ribosomes to churn out its protein workforce.*

Source: Kaledhonkar et al. (2019) *Sructure: PDB 6O9J*


cell wall
membrane
100 nm


100 nm

2. Cell Wall

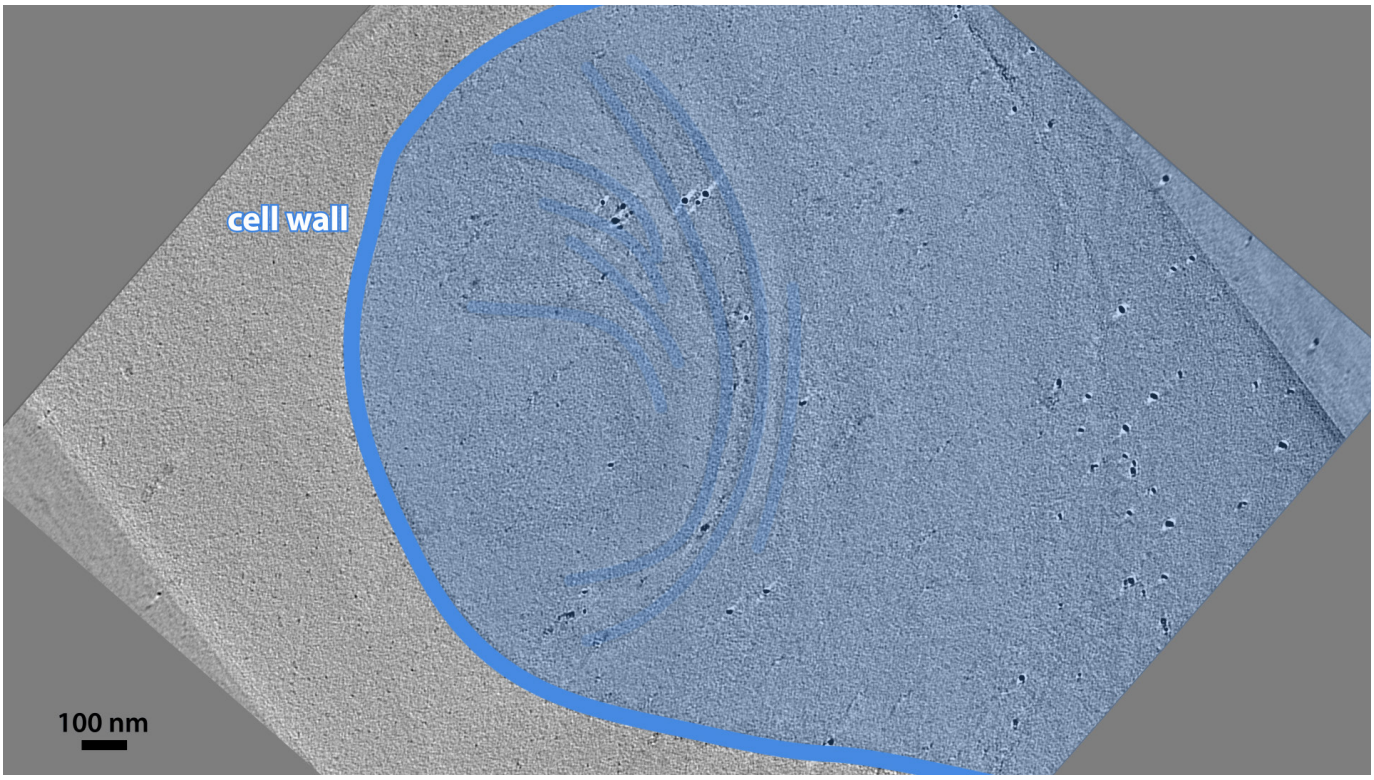
Being able to selectively move things into your cell enables it to do some powerful things. It also poses a structural problem. Remember that water can pass freely through the membrane, which means that increasing the solute concentration inside relative to the environment outside will cause water to rush in as well, introducing a pressure (known as **turgor pressure**) on the membrane. Lipid bilayers, unfortunately, are unable to withstand much pressure. If your cell lives exclusively in a constant, and fairly high-osmolarity, environment (like our bodies, in the case of the pathogenic *Mycoplasma genitalium* you just saw), it can balance internal and external osmolarity to minimize turgor pressure on its membrane. But most cells experience more variable, and dilute, environments. How could you keep your cell from bursting in such conditions?

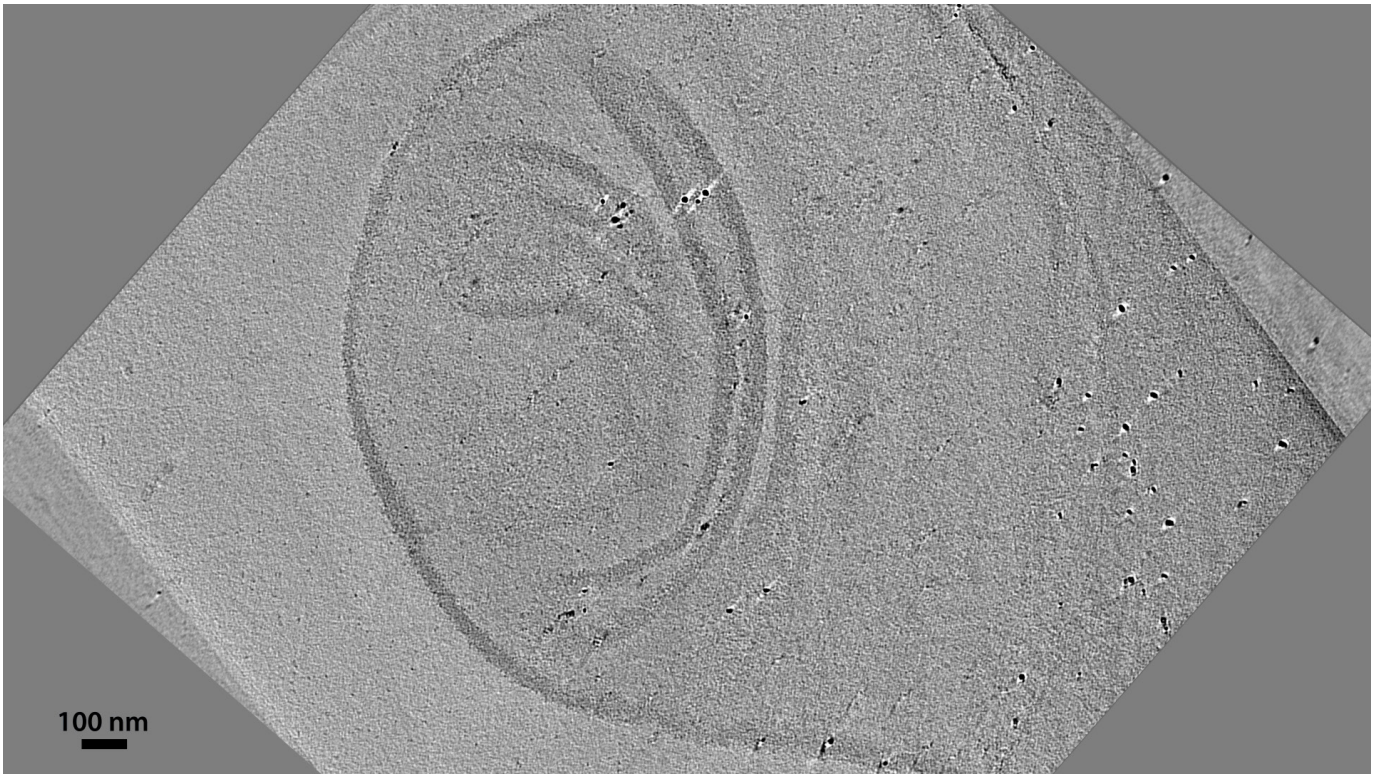
You might want to add some rigid scaffolding outside the membrane to buttress it against turgor pressure. Nearly all bacteria do this with a material called **peptidoglycan**: long stiff polymers of glycan sugars crosslinked by short peptides into a chain-mail-like mesh. The full scaffold of this material surrounding the cell is called its **cell wall**. In monoderm bacteria like this *Listeria monocytogenes*, the cell wall is significantly thicker than the membrane. It comprises several layers of peptidoglycan, which are indistinguishable at this resolution, so the cell wall appears as a uniformly textured layer (*Learn More* on the next page). It remains a mystery how large molecules can pass through this dense layer on their way to and from the cell.

Some archaea also have cell walls, made of a molecule similar to peptidoglycan (*Learn More* on following pages). Most archaea, though, rely on a different structure for support, which you will see in a few pages.

A wall can be a boon to your cell, but it can also prove a liability in certain conditions. For instance, many antibiotics target the cell wall. Some bacteria have developed the ability to jettison their walls in such conditions, taking on an amorphous, so-called "L-form" (named for the Lister Institute where it was discovered).

Listeria monocytogenes Collected by: Ariane Briegel DOI: 10.22002/D1.1351

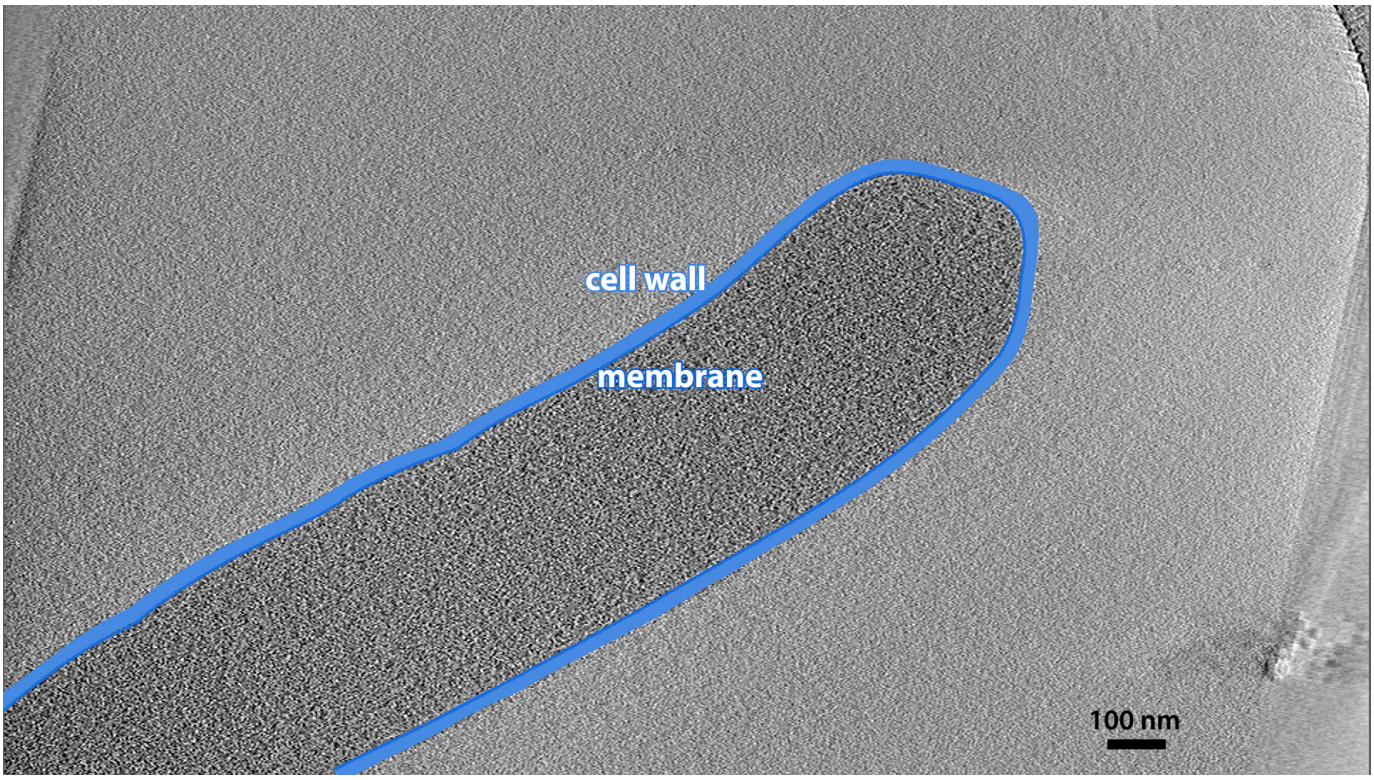

cell wall
100 nm

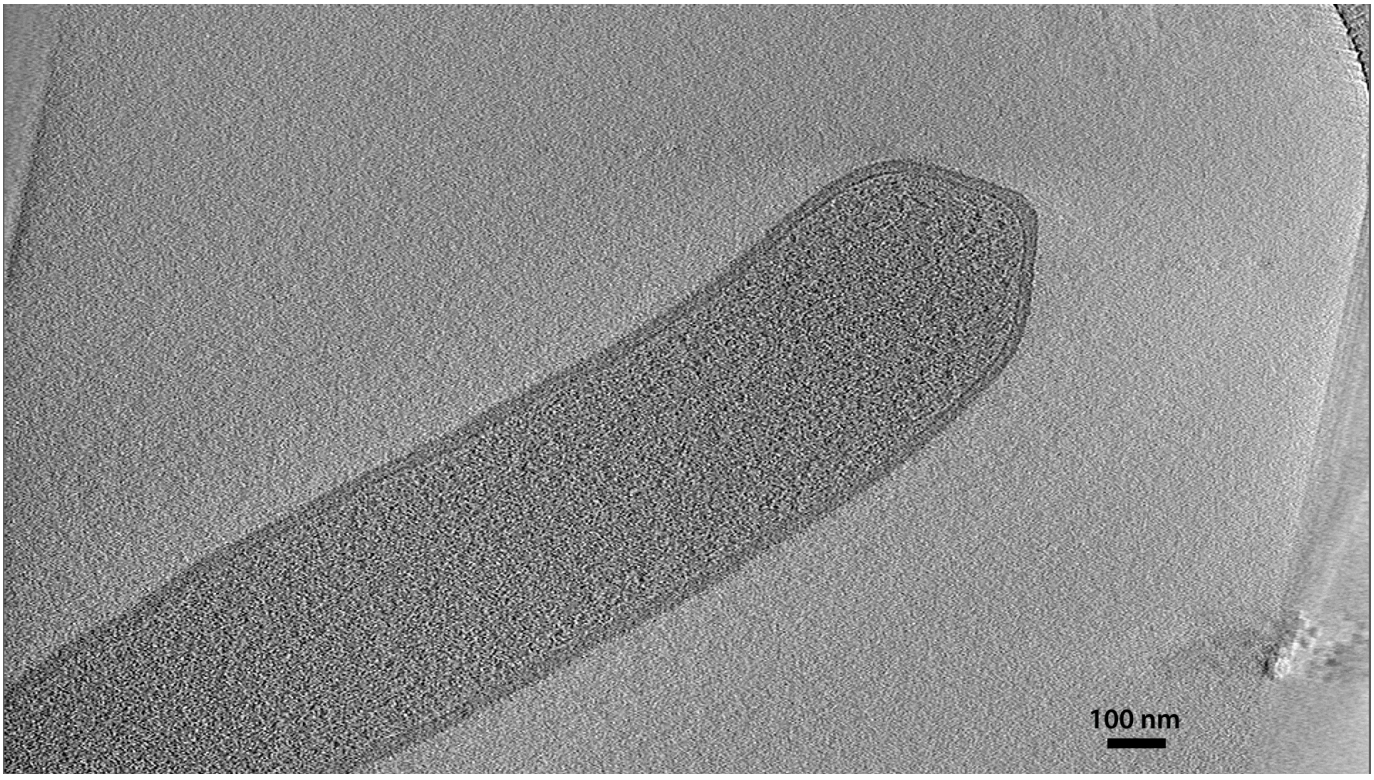

100 nm

Learn More: Wall Architecture

The cell wall is so robust that it persists even after cells are lysed and their other components digested. This cell wall isolated from a Bacillus subtilis *cell has retained its shape, simply flattening with the release of contents and pressure from inside. The way the cell wall crumples is one clue to its architecture: the long glycan strands form hoops parallel to the short axis of the cylindrical cell and the short peptide crosslinks are more or less aligned with the long axis.*

Bacillus subtilis *Collected by: Morgan Beeby* *DOI: 10.22002/D1.1360*

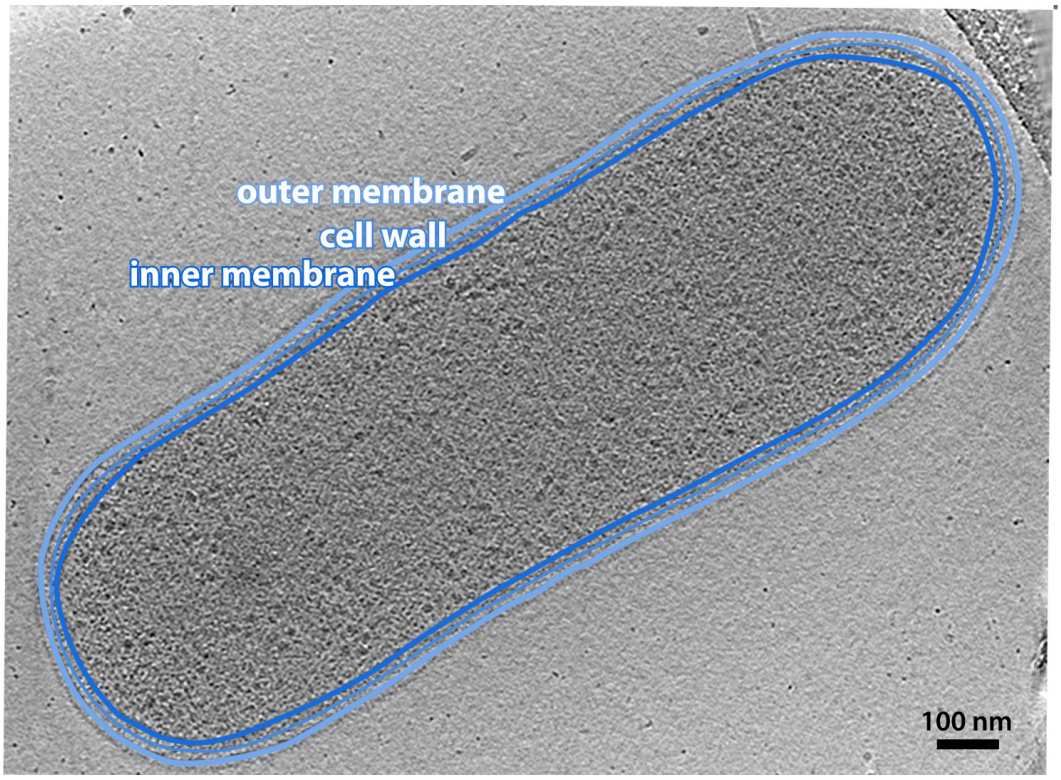

cell wall
membrane
100 nm

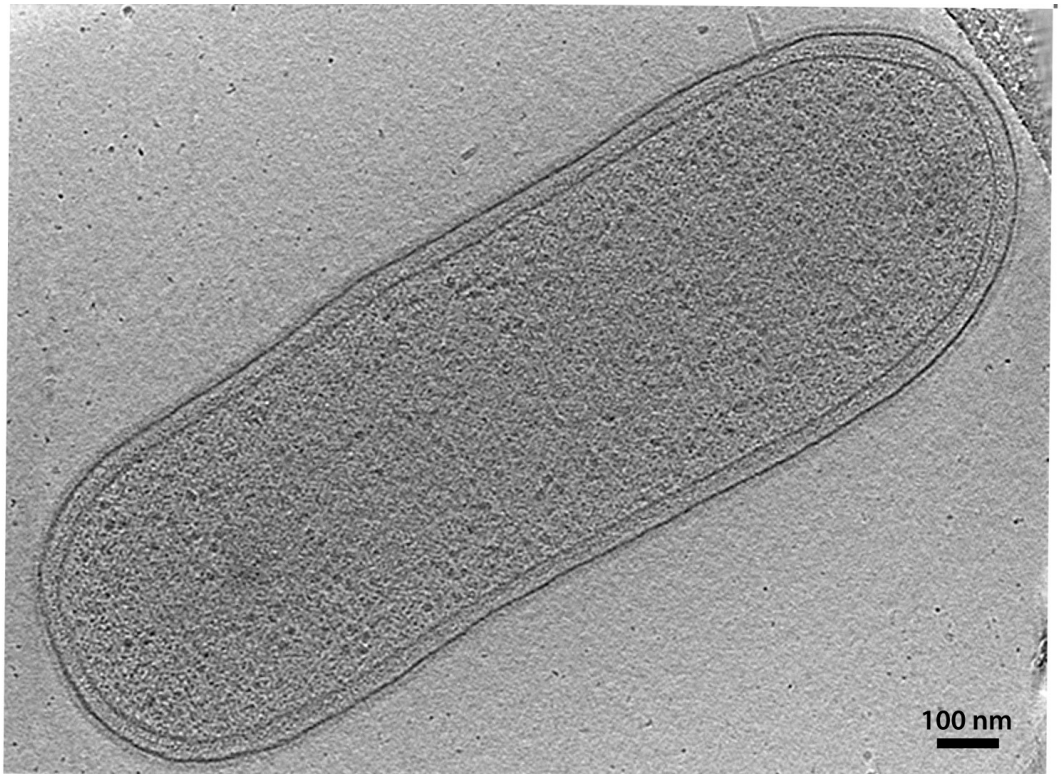

100 nm

Learn More: Archaeal Walls

Some archaea, like this Methanobacterium formicicum*, build cell walls from "pseudo-peptidoglycan," which has a slightly different chemical composition than peptidoglycan. As you can see, though, the overall architecture of the wall is the same.*

Methanobacterium formicicum *Collected by: Elitza Tocheva* *DOI: 10.22002/D1.1361*


outer membrane
cell wall
inner membrane
100 nm


100 nm

3. Outer Membrane

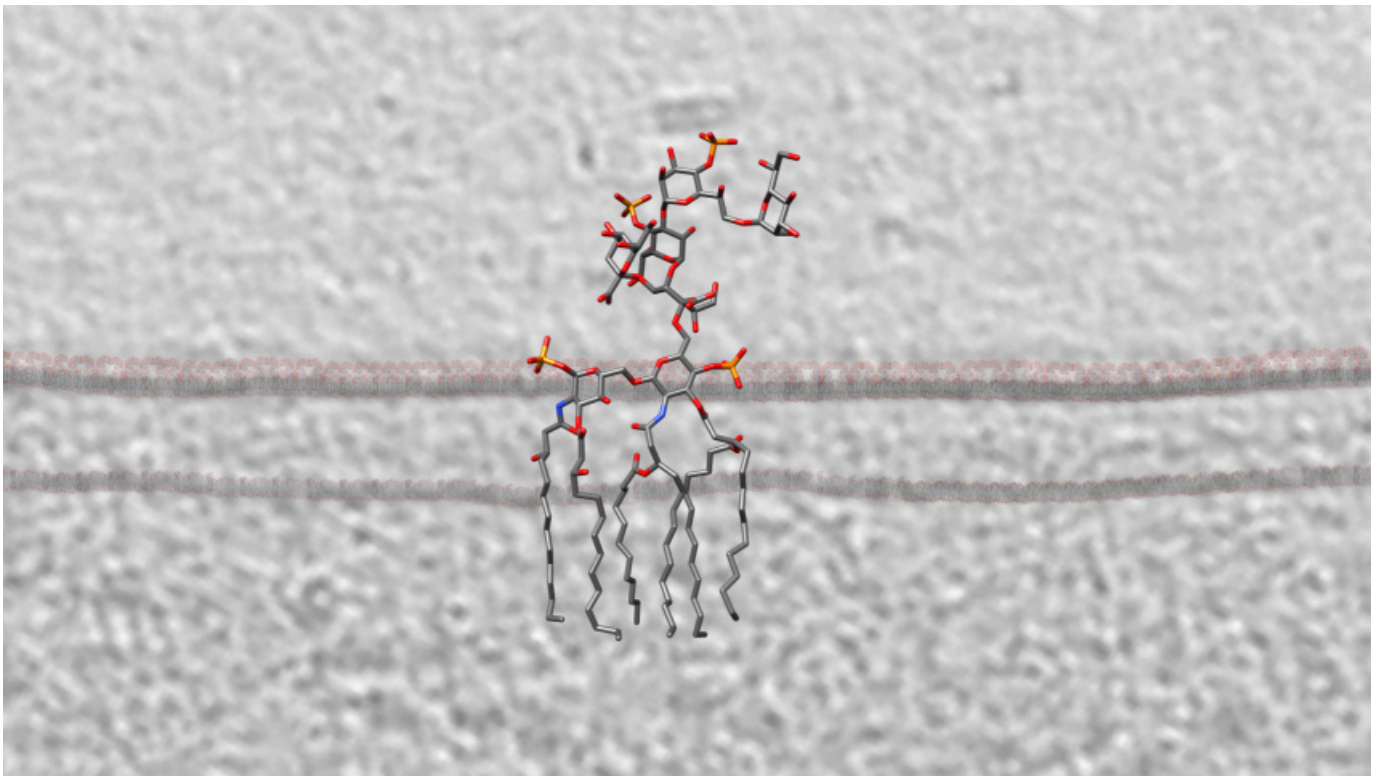
Why stop at one membrane? Eukaryotic cells use internal membranes to form specialized compartments like the nucleus and mitochondria. While bacteria lack such organelles, many create an additional compartment *outside* the cell with a second, **outer membrane**. Such bacteria, like the *Hydrogenovibrio crunogenus* cell you see here, are called **diderms** ("double skin"). The extra compartment between their membranes is known as the **periplasm** ("substance between"). This antechamber contains a unique subset of proteins, many of which function in escorting things into and out of the main cell.

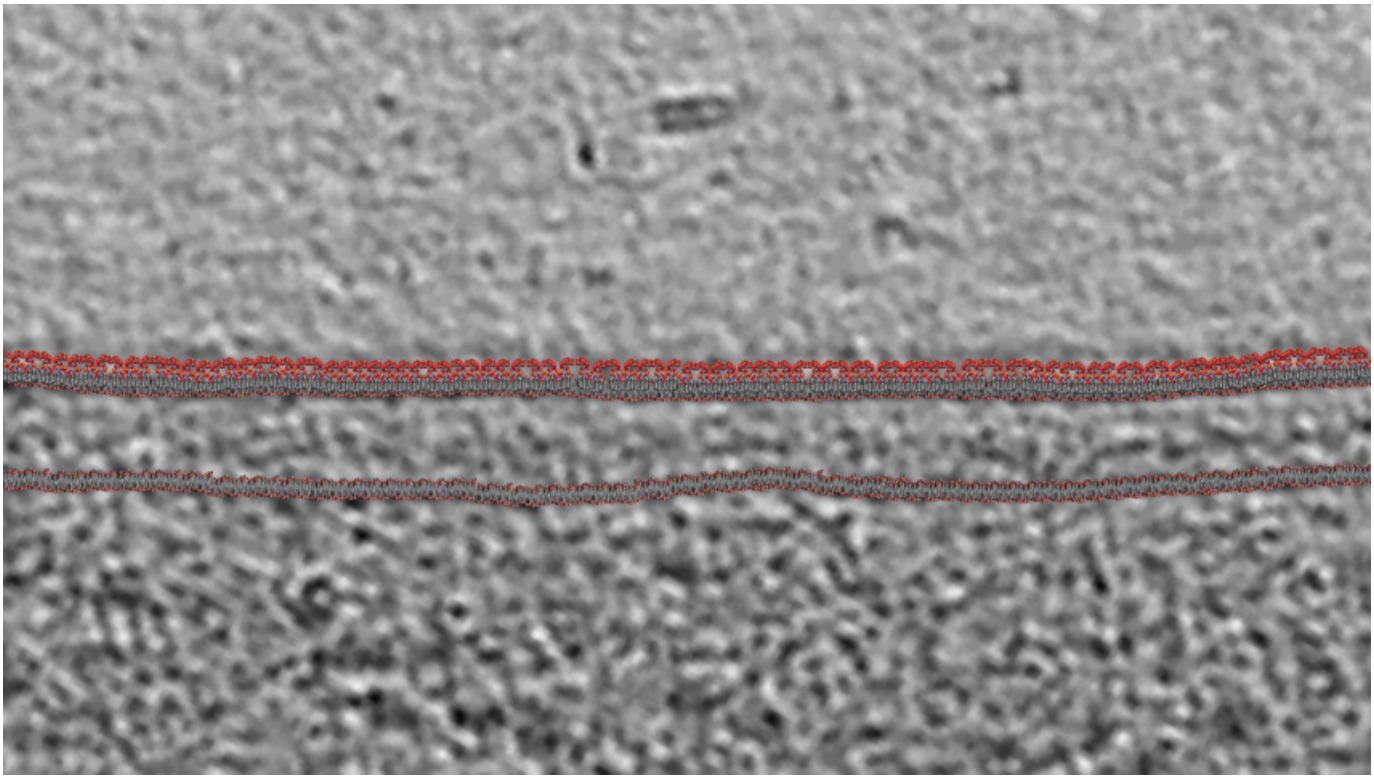
Compared to the inner membrane, the outer membrane has some unique properties. It is more permeable and not proton-tight (so it cannot be used to generate ATP). It is also usually asymmetric, with very different molecules in the outer leaflet than in the inner (*Learn More* on the next page). A few species of bacteria, particularly pathogens, have labile outer membranes (you will see an example in Chapter 8.2). Most, however, firmly anchor their outer membrane to the cell wall (*Learn More* on following pages).

Rather than containing many layers of peptidoglycan like in the *Listeria monocytogenes* you just saw, the cell wall of diderms is usually composed of a single layer of peptidoglycan mesh (*Learn More* on following pages), which is visible here as a thin line in the periplasm. This single-layered cell wall presents a considerable challenge for growth: how can you remodel it to grow bigger without letting turgor pressure burst the cell in the process? The answer, as we are beginning to figure out, is very carefully (*Learn More* on following pages).

The difference in thickness of monoderm and diderm cell walls enables a well-known classification system: the Gram stain, which binds peptidoglycan. Gram-positive bacteria, typically monoderm, contain much more peptidoglycan than Gram-negative bacteria, which are typically diderm.

Hydrogenovibrio crunogenus Collected by: Cristina Iancu DOI: 10.22002/D1.1352

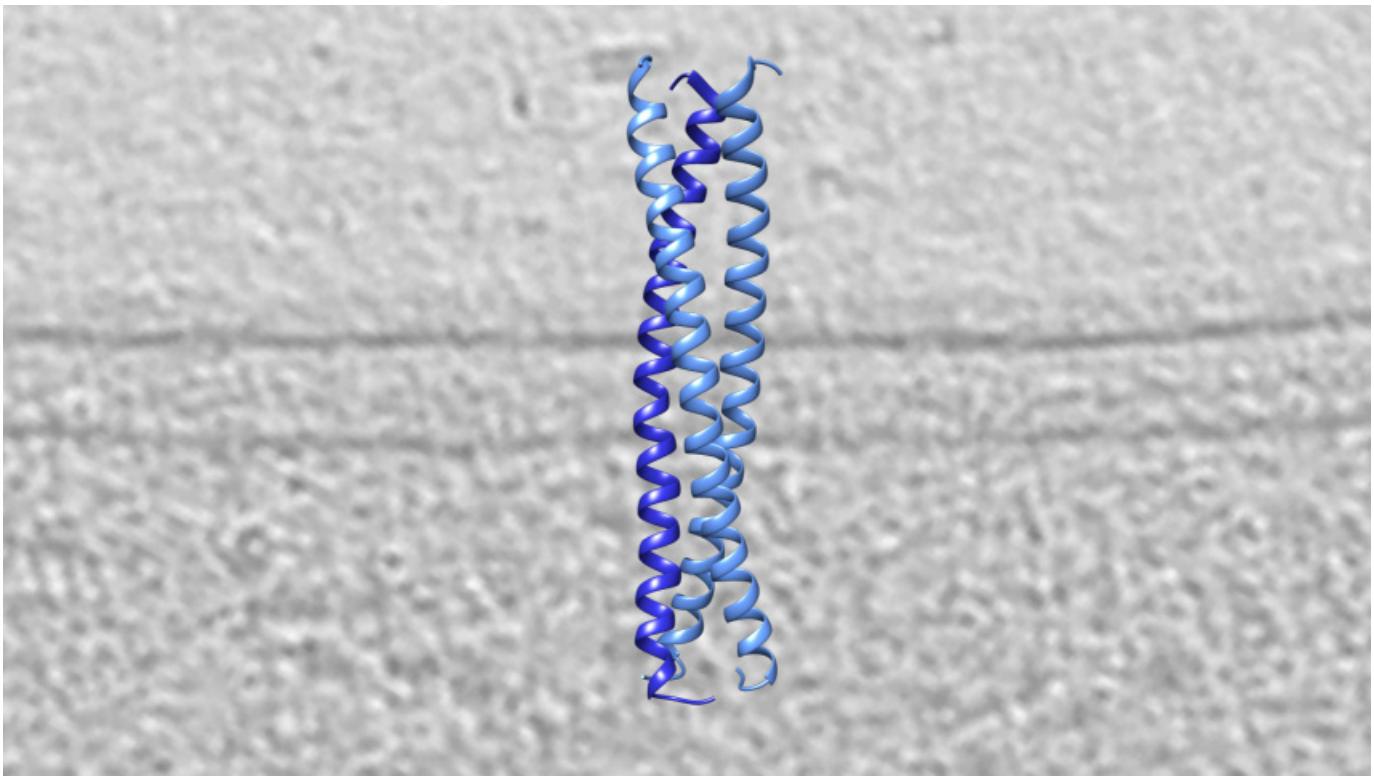


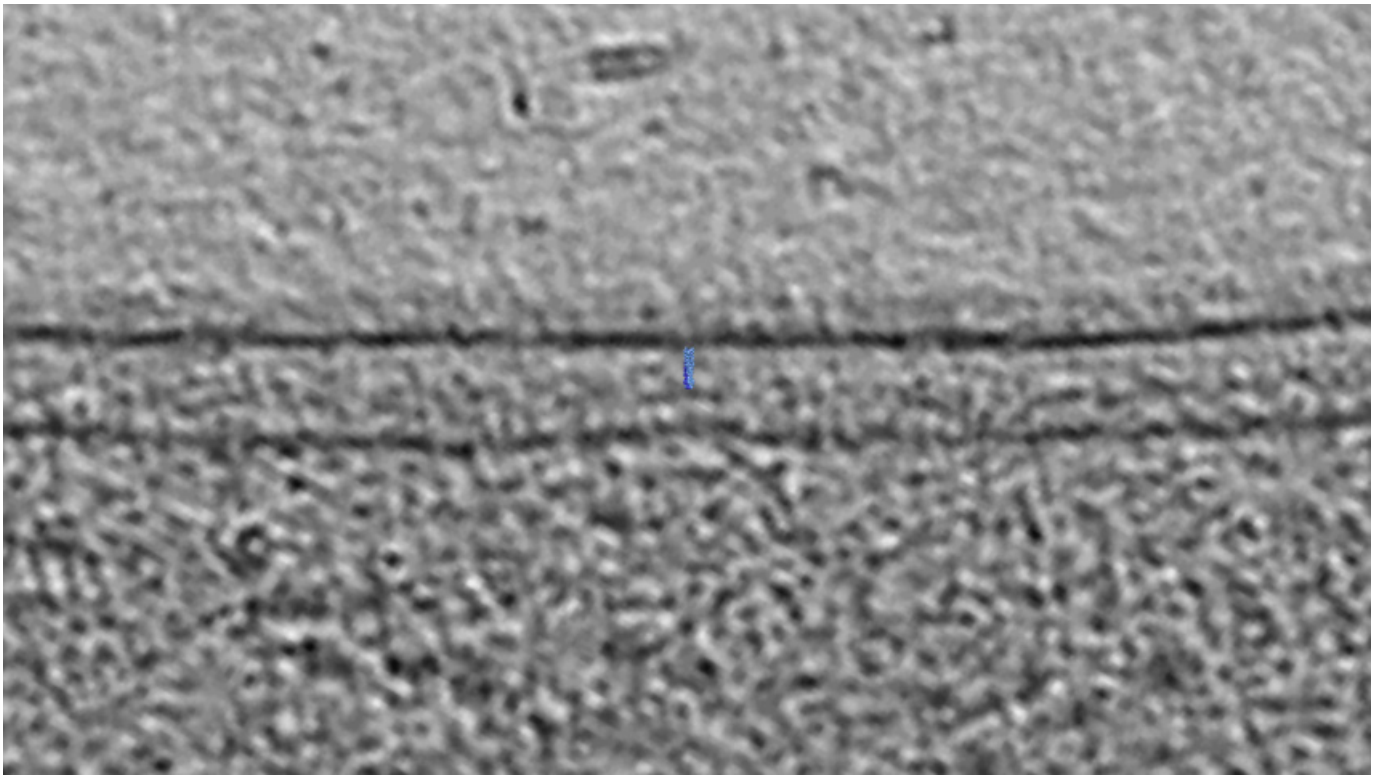


Learn More: LPS

Unlike the symmetric bilayer of the inner membrane, the two leaflets of the outer membrane are different. The inner leaflet consists of familiar phospholipids. In most diderms, though, the outer leaflet is composed mainly of ***lipopolysaccharide****, or* ***LPS****, molecules. These molecules have lipid portions which interact with the lipids of the inner leaflet and again form a barrier against hydrophilic molecules. They also have sugar portions, which extend out from the cell and present an additional barrier to hydrophobic molecules [22]. The dense LPS layer protects the cell from things like antibiotics and, for pathogens, the defenses of the host. On the flip side, our immune systems have evolved to detect and strongly react to LPS molecules.*

Source: Tang et al. (2019) *Structure: PDB 6S8H*



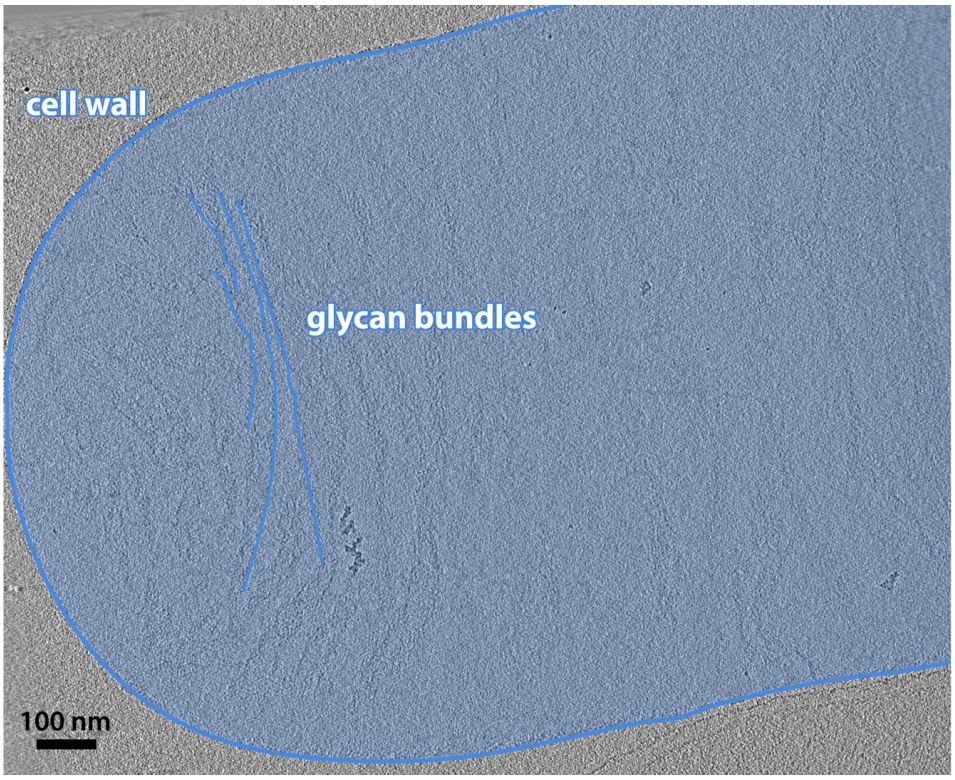


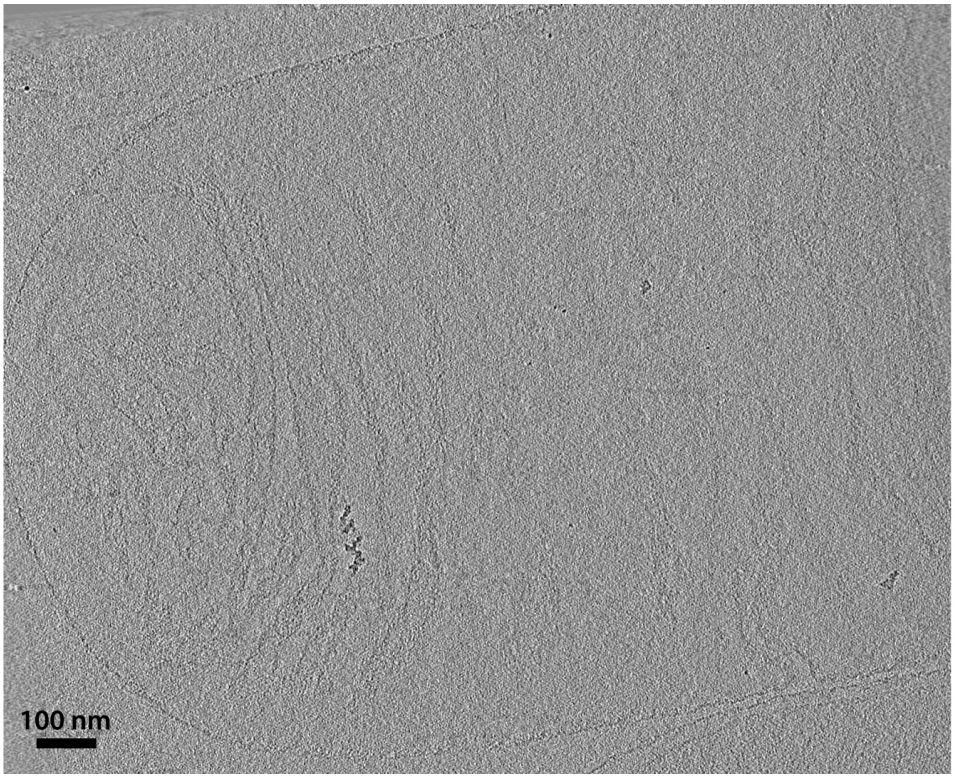
Learn More: LPP

Lipoproteins are hybrid molecules, formed from covalently-linked lipid and protein pieces. The lipid allows them to embed into a membrane, tethering the attached protein to function nearby. Braun's lipoprotein, or ***Lpp*** *[23], is one of the most abundant molecules in the outer membrane of cells like* Escherichia coli. *The top of the trimeric protein "coiled-coil" shown here contains the lipid tether. The bottom binds the peptidoglycan, creating a rigid link between the outer membrane and the cell wall that sets the distance between the two layers. A typical* E. coli *cell may contain 100,000 such links. Some other species use different proteins for the same purpose.*

Source: Shu et al. (2000)

Structure: PDB 1EQ7

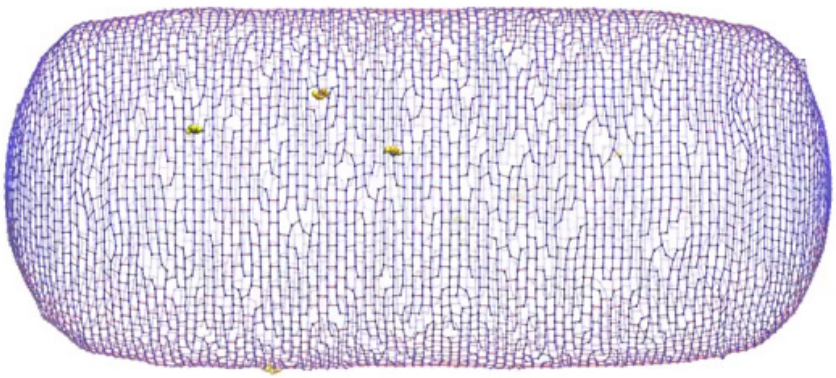

cell wall
glycan bundles
100 nm


100 nm

Learn More: Architecture

Compare this diderm cell wall purified from Escherichia coli *to the monoderm cell wall on the last page. Since this one is thinner, we can make out more details, like the bundles of glycan strands running around the circumference of the cell. The main difference between the two types of walls seems to be whether they have largely a single layer of peptidoglycan (diderm) or many layers (monoderm). So even though the two look different, their architecture is fundamentally the same. In some circumstances, as you will see in Chapter 8, cells can even switch between the two forms.*

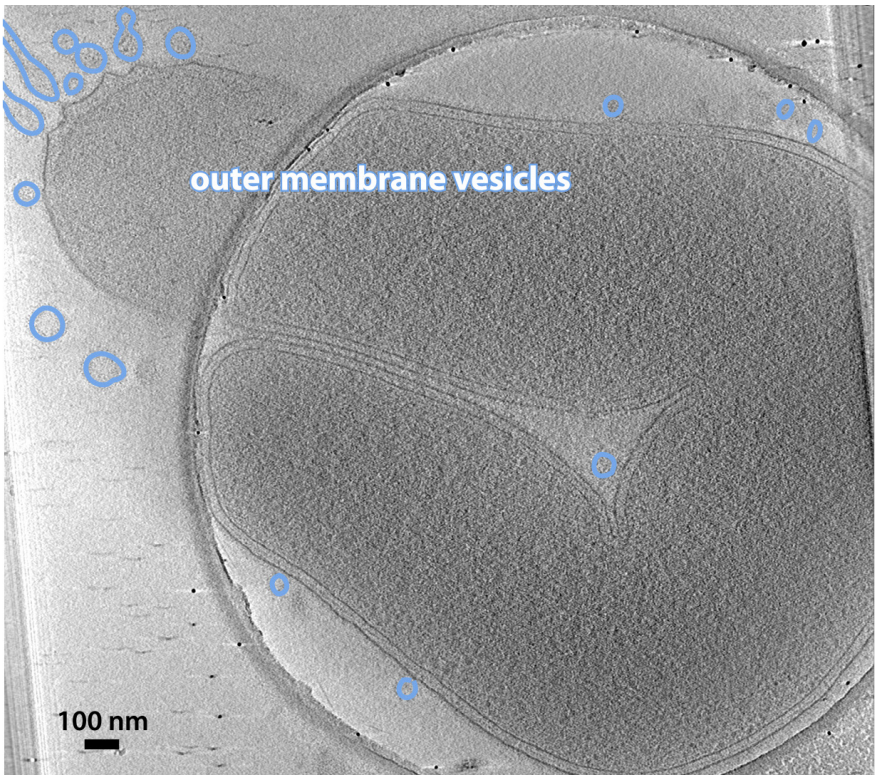
Escherichia coli *Collected by: Lu Gan* *DOI: 10.22002/D1.1362*

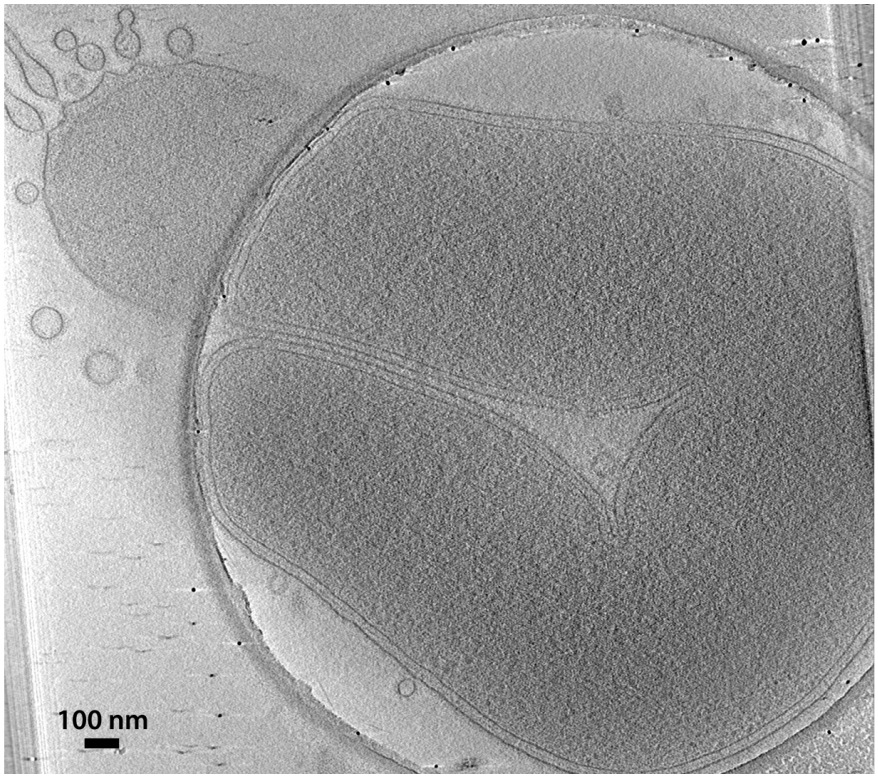


Learn More: Growth

Encasing your cell in a rigid scaffold presents a problem: how can it grow? It is easy to make membranes larger simply by adding more lipids. But to add more peptidoglycan strands, they must be linked into the existing network, which means breaking existing links to accommodate them. To do this, cells use three tools: an enzyme that links glycan sugars into strands, an enzyme that links glycan strands together with peptide bonds, and an enzyme that cuts these peptide links to allow new strands to be incorporated. Remember, though, that your cell, with its solute-rich interior, has a turgor pressure pushing outward with a force of perhaps 3 atmospheres, equivalent to what we would feel at a depth of 20 meters in the ocean. This is more than enough pressure to lyse an exposed membrane, so the tools must be wielded with care or the cell would burst. We are still figuring out how this works, with help from computer simulations like this one by Lam Nguyen. Here you see a model of an Escherichia coli *cell wall being enlarged using the three enzyme tools we just described (the colored balls). This simulation was run to test whether just having the tools function in a complex rather than separately might provide enough coordination for safe growth [24]. (The answer was yes. You can watch the full movie describing this research on YouTube: https://youtu.be/_5Ov3vp6Qyg.)*

Source: Nguyen et al. (2015)


outer membrane vesicles
100 nm

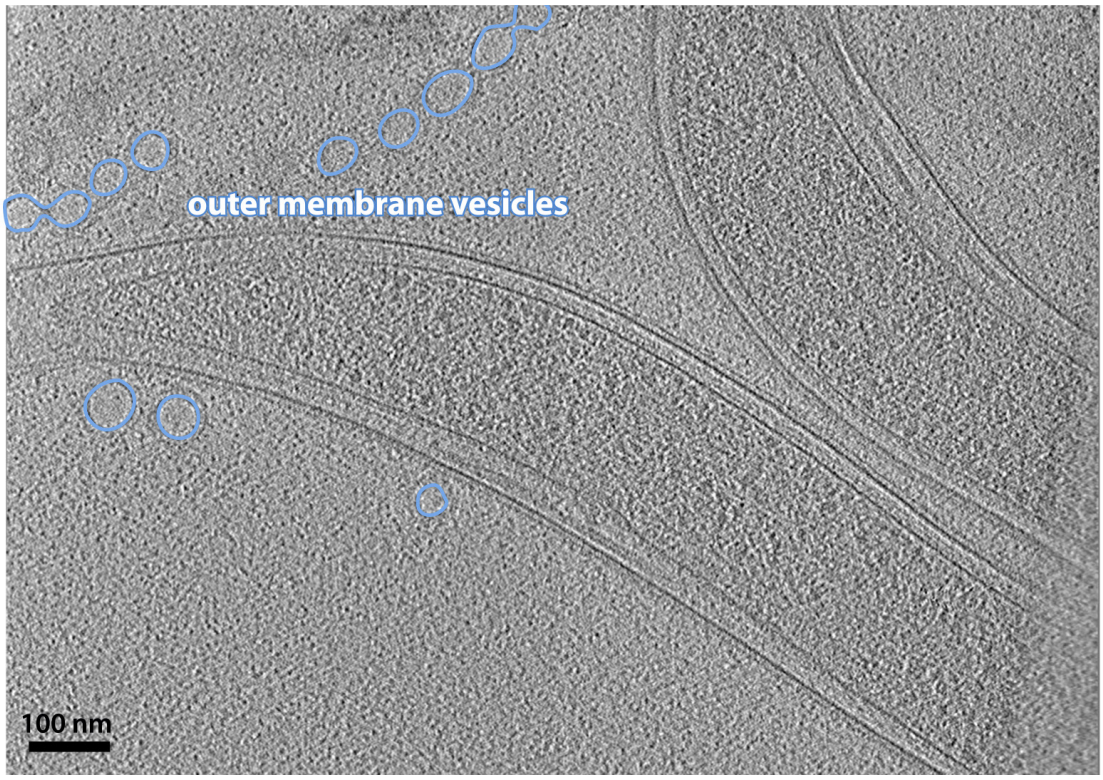

100 nm

4. Vesicles

What else can your cell do with an extra membrane? Since membranes make such excellent containers for molecules, why not get into the shipping business? In the coming chapters (especially Chapter 9), you will see some of the ways that cells interact with each other and their environment. For diderm bacteria, many of these interactions are made possible by outer membrane **vesicles** ("little bladders")–self-contained pockets budded off the membrane. The vesicles may carry cargo of antibiotics to inhibit competitors' growth, or toxins to lyse neighboring cells, or enzymes to digest those lysed remains into nutrients that your cell can use. Alternatively, they may carry emergency kits, first aid and survival factors for other members of a biofilm community. The appearance of these vesicles can vary as much as their contents (*Learn More* on the next page). They are usually spherical, though, of a fairly consistent size, and often come off the cell at one or a few sites, forming chains, as you can see in this *Myxococcus xanthus*.

Not all diderms produce outer membrane vesicles, and even for those that do, we still do not know exactly how they do it. Maybe it happens spontaneously due to the physics of lipids and proteins in a certain configuration. Or maybe there is a dedicated protein machine in the membrane, blowing bubbles. Vesicles can also bud from the cytoplasmic or (for diderms) inner membrane into the cytoplasm or periplasm (*Learn More* on following pages). This seems to be a less regulated process than outer membrane vesicle formation, and we see it in many species when they are stressed by low nutrients or high cell density. Cells shrink in harsh conditions (more on that in Chapter 8), so cytoplasmic or periplasmic vesicles may simply offer a place to put the extra membrane until the time comes to grow again. Just as with outer membrane vesicles, the appearance of cytoplasmic vesicles varies widely (*Learn More* on following pages). Archaea also produce membrane vesicles (*Learn More* on following pages). They have been studied less than their bacterial counterparts, but likely serve similar roles in metabolism and community interactions.

Myxococcus xanthus Collected by: Yi-Wei Chang DOI: 10.22002/D1.1474

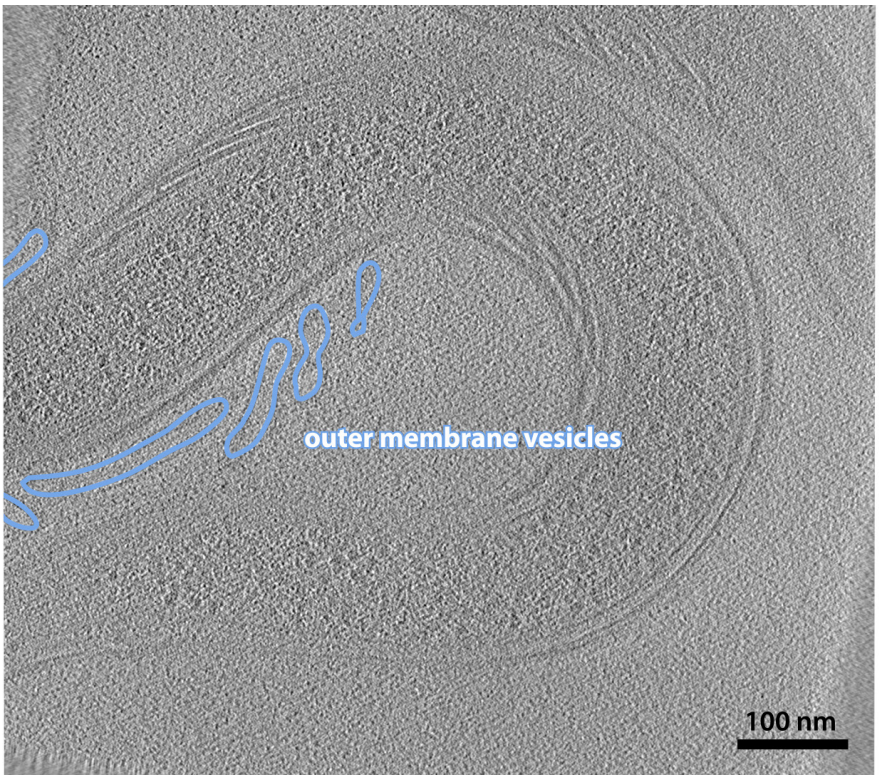

outer membrane vesicles
100 nm

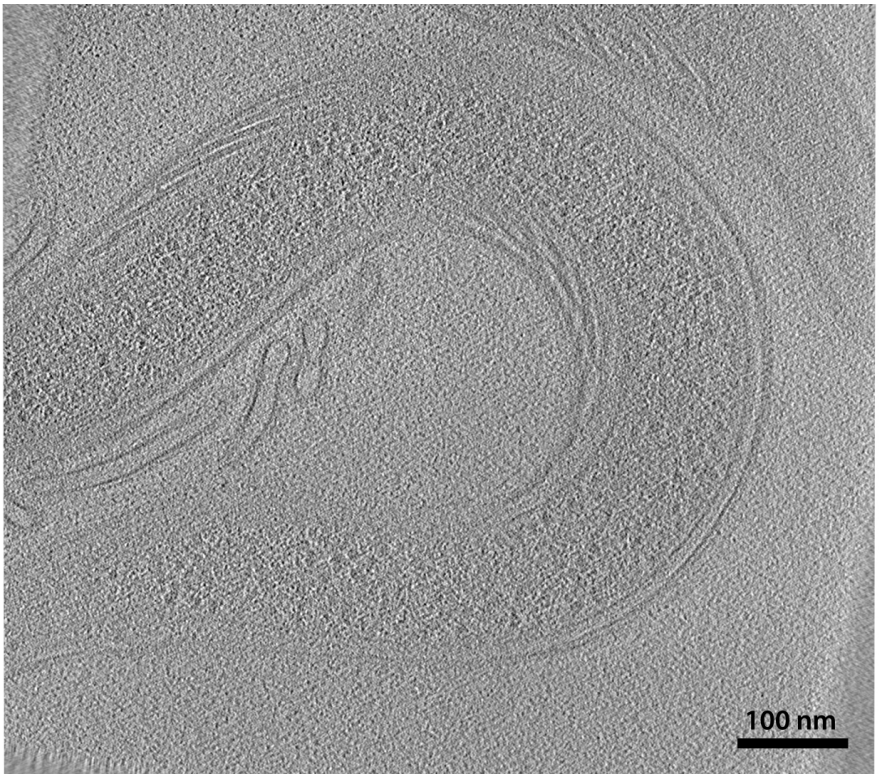
100 nm

Learn More: Pearled

Different species can produce outer membrane vesicles that look very different. Even vesicles from the same species can look very different. Sometimes they come off the cell as a chain of spheres; sometimes the spheres remain connected, like a string of pearls, as in this Borrelia burgdorferi. *Sometimes vesicles form long tubes instead (*Learn More *on the next page). Sometimes the same chain can be tubular in one section (usually at the base, connected to the cell), and a string of spheres in another.*

Borrelia burgdorferi *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1363*

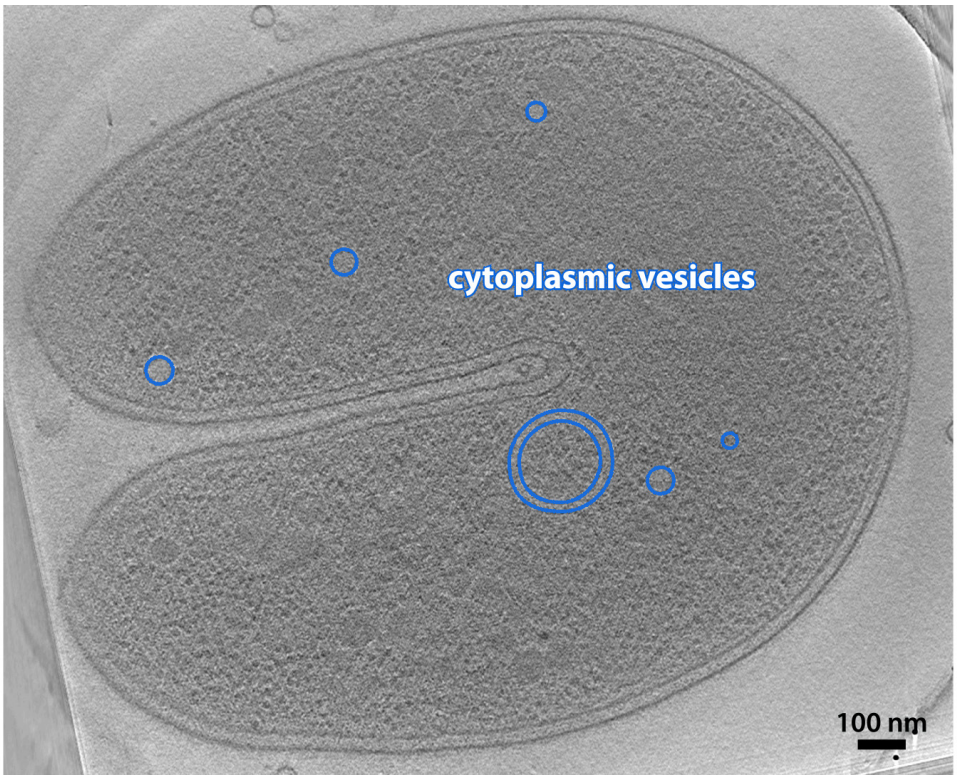

outer membrane vesicles
100 nm

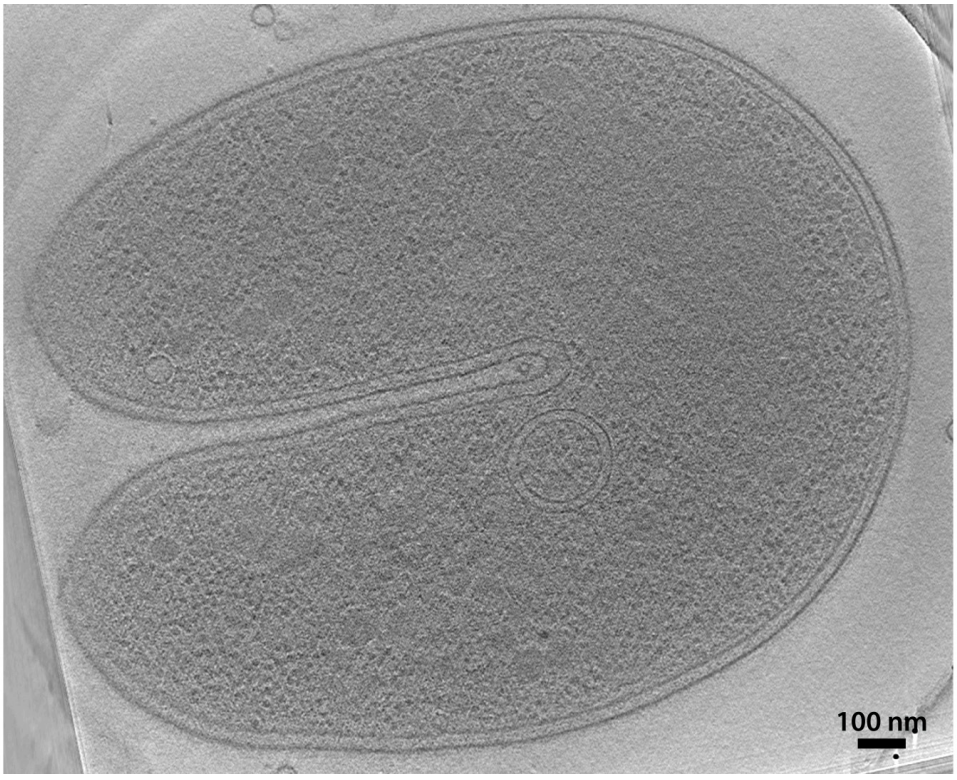

100 nm

Learn More: Tubular

Here, again from a Borrelia burgdorferi *cell, you can see extended, tubular outer membrane vesicles.*

Borrelia burgdorferi *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1353*

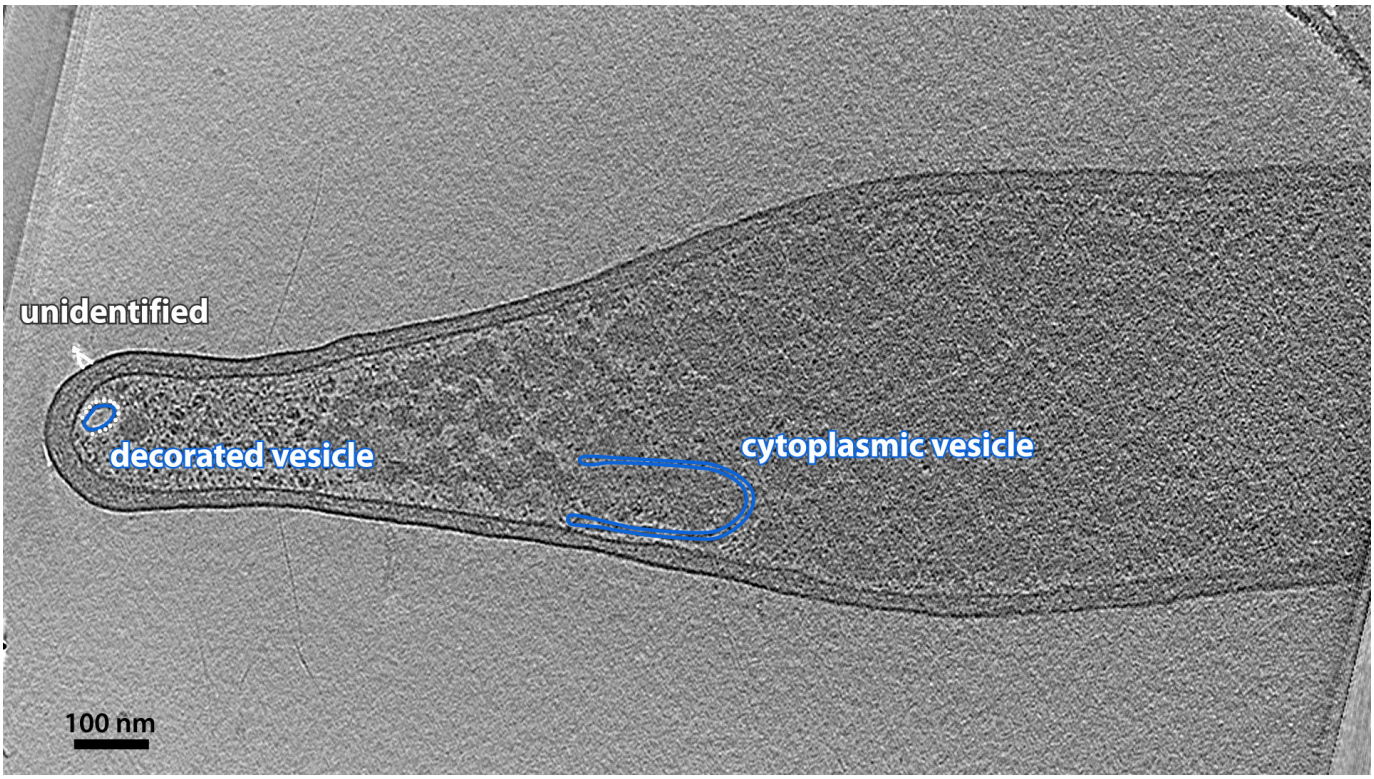

cytoplasmic vesicles
100 nm

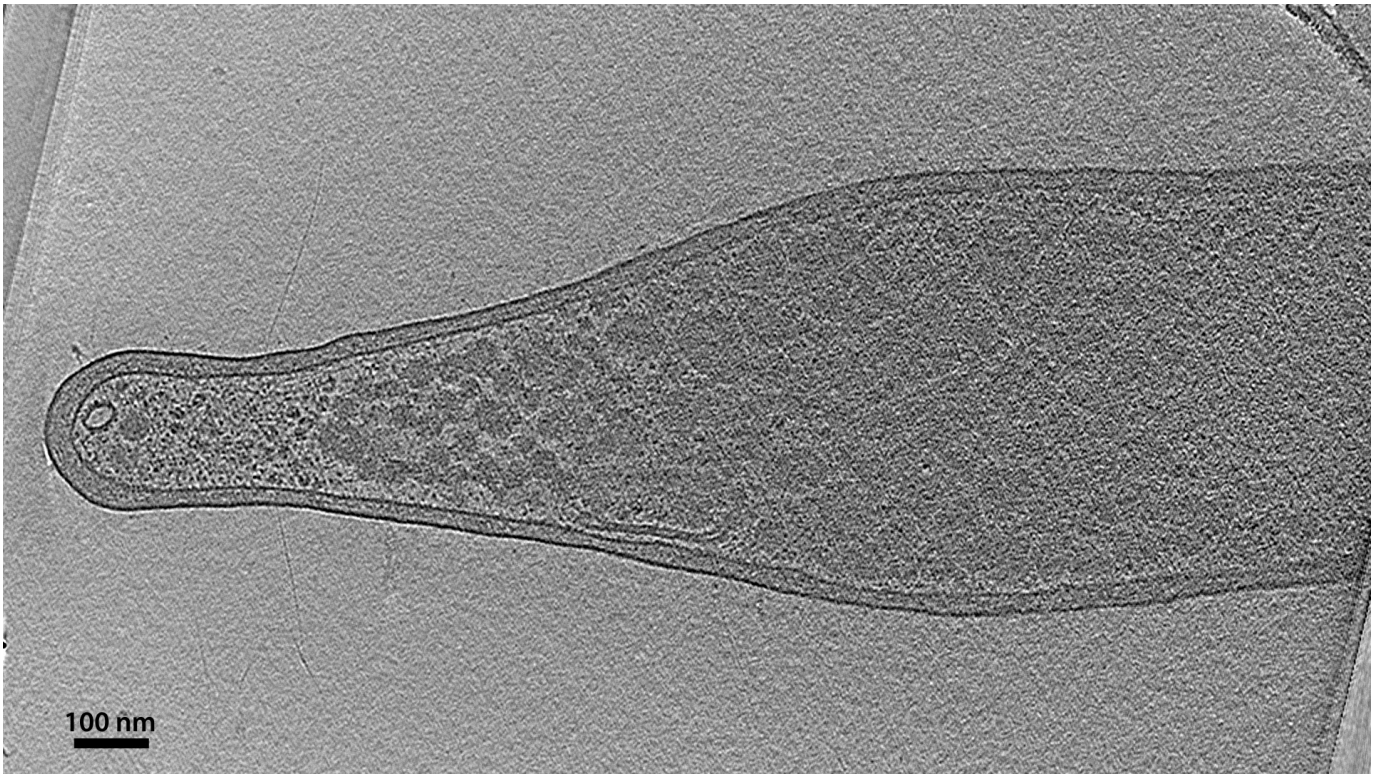

100 nm

Learn More: Cytoplasmic

Not all vesicles come from the outer membrane. The cytoplasmic or inner membrane can also form vesicles that are released into the cytoplasm, as in this Myxococcus xanthus *cell, or into the periplasm.*

Myxococcus xanthus *Collected by: Matthew Swulius* *DOI: 10.22002/D1.1364*


unidentified
decorated vesicle
cytoplasmic vesicle
100 nm

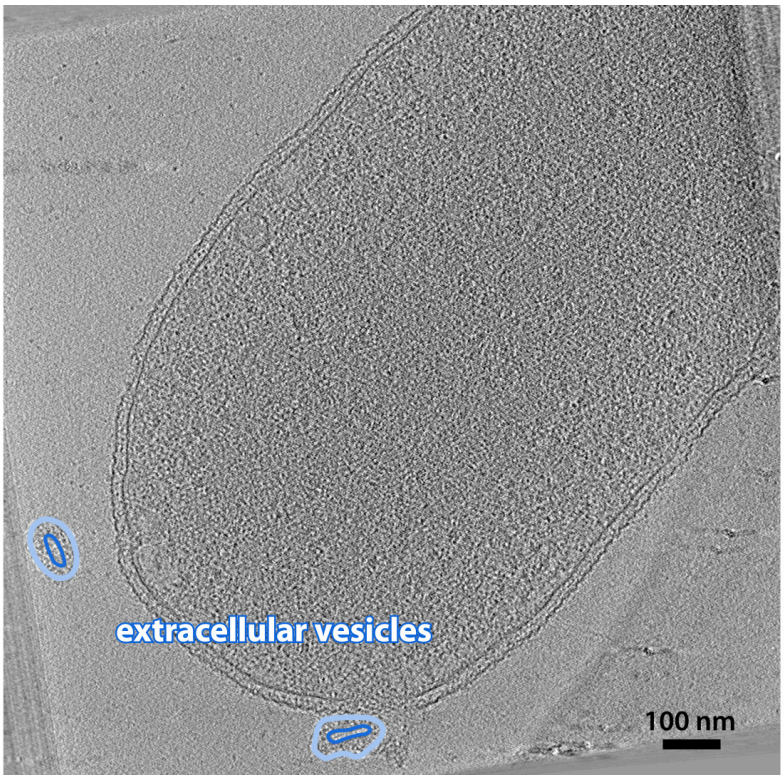

100 nm

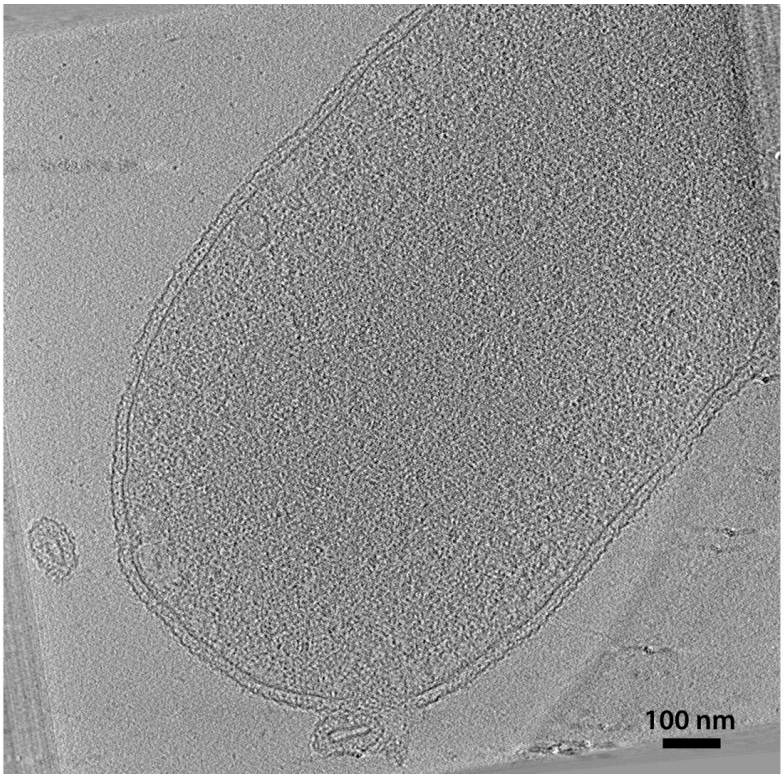
Learn More: Variety

Cytoplasmic vesicles exhibit a variety of sizes and shapes. Some are nested, with vesicles inside vesicles. In this Prosthecobacter debontii *cell, you can see two other morphologies. One resembles a flattened horseshoe. Another is a more typical spherical shape, but is decorated with what look like protein complexes.*

This cell also has unusual structures on its surface that have yet to be identified.

Prosthecobacter debontii *Collected by: Martin Pilhofer* *DOI: 10.22002/D1.1365*

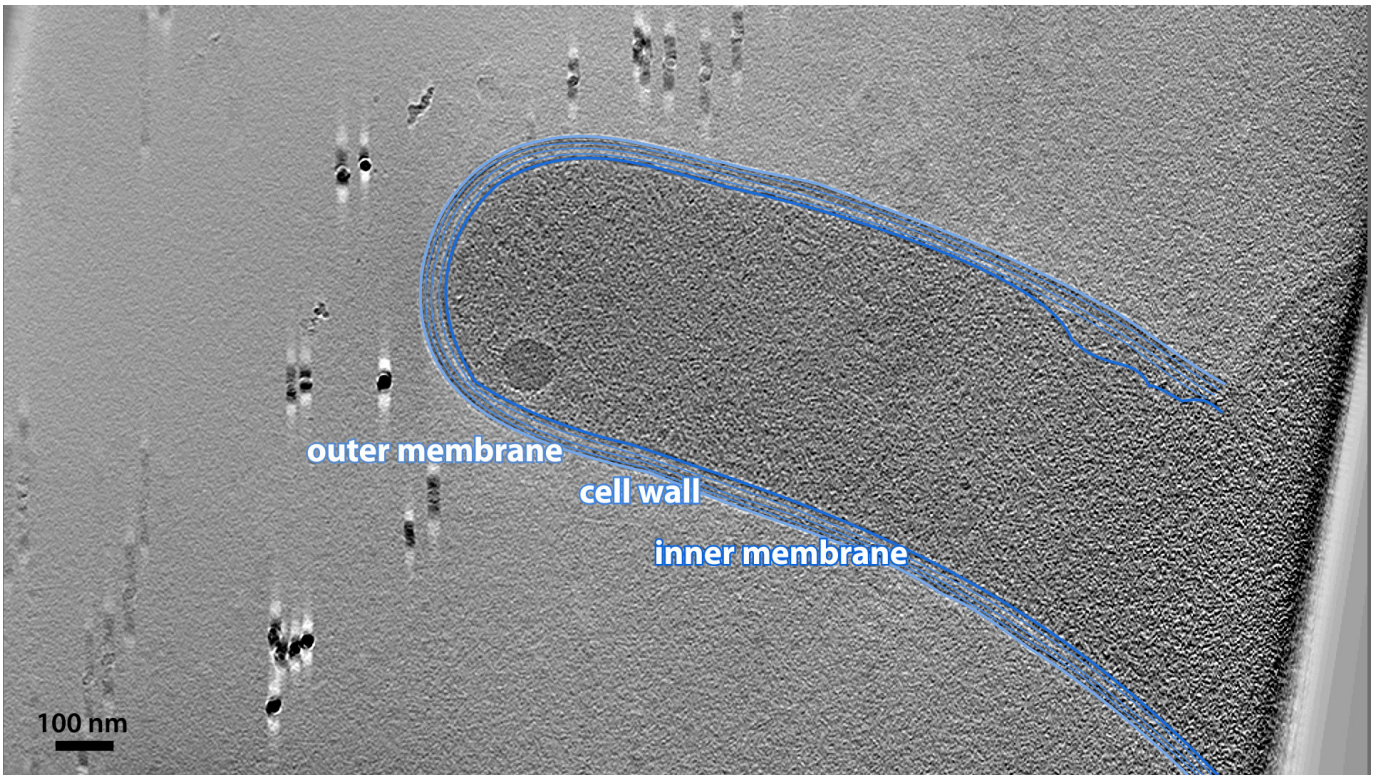

extracellular vesicles
100 nm

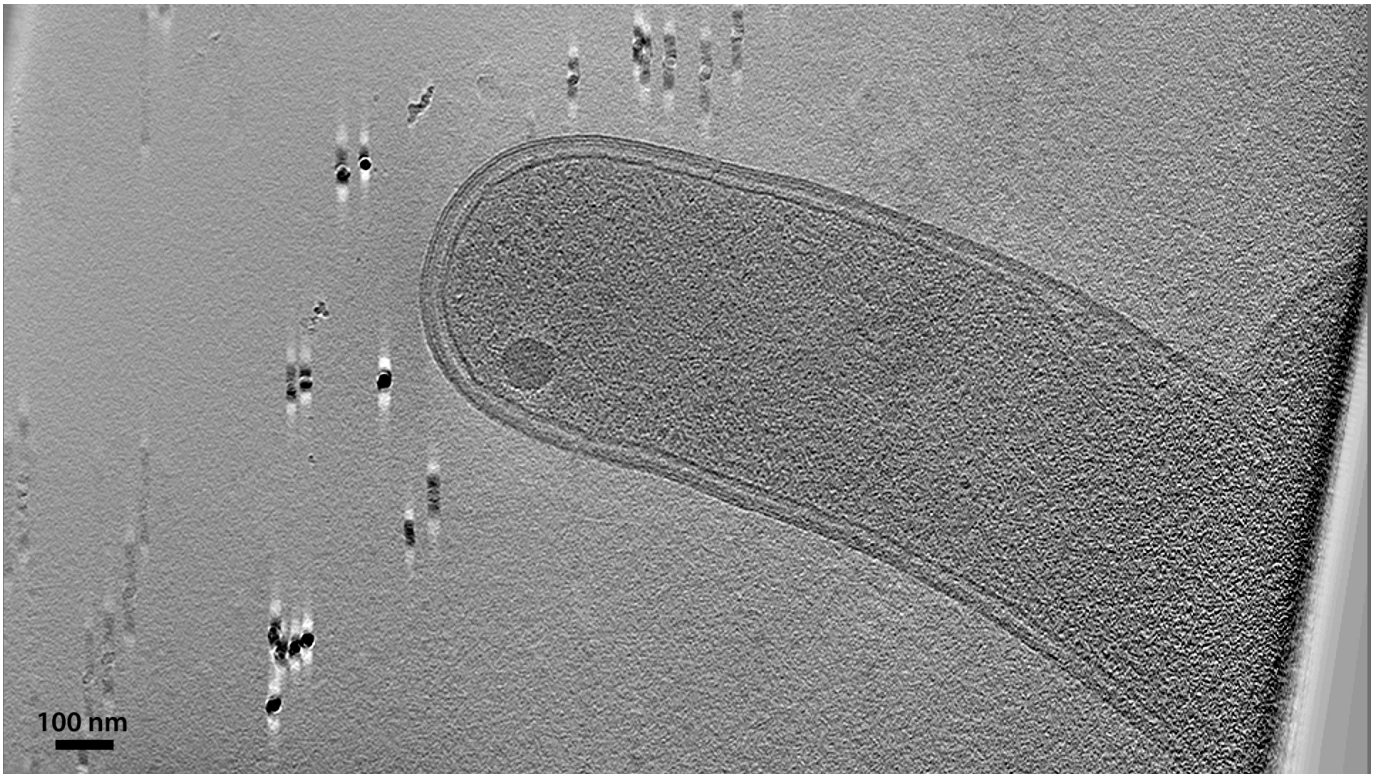

100 nm

Learn More: Archaeal

Just like bacteria, archaea also produce vesicles, as you can see in this Halomicrobium mukohataei.

Halomicrobium mukohataei *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1475*


outer membrane
cell wall
inner membrane
100 nm

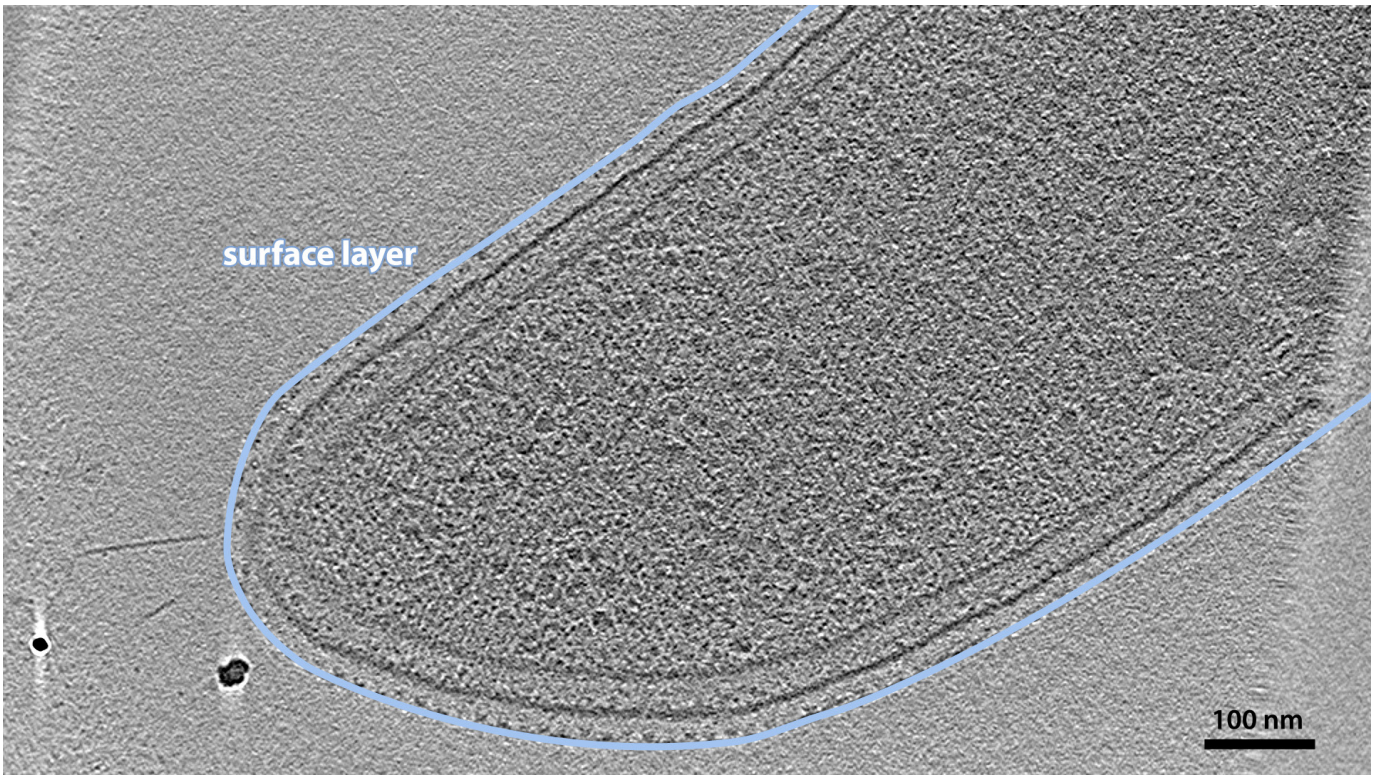

100 nm

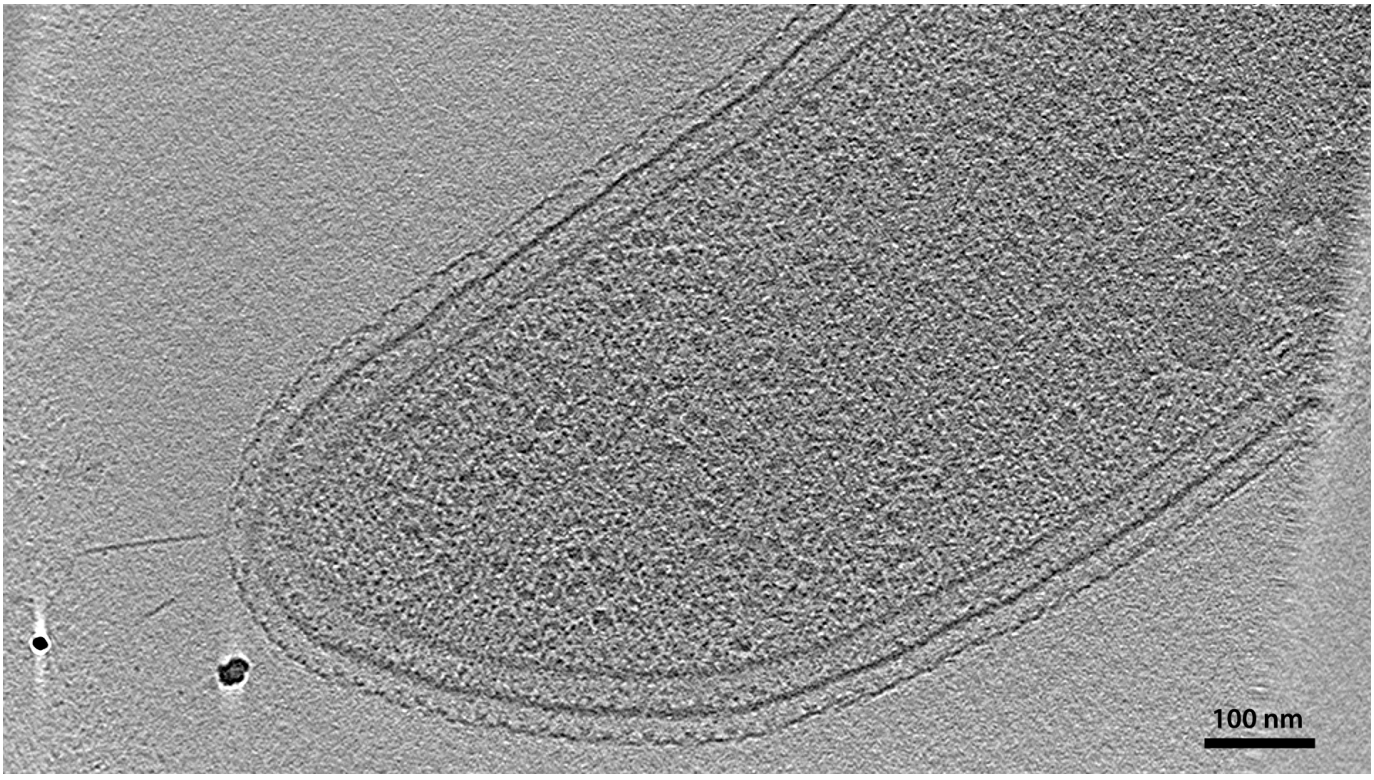
5. Classification Exceptions

Evolution is endlessly creative, presenting exceptions to nearly every classification rule. We just described a neat breakdown of bacteria into monoderms (one membrane, thick cell wall, positive Gram stain) and diderms (two membranes, thin cell wall, negative Gram stain). But some cells, like these *Mycobacterium marinum*, defy such easy classification. Mycobacteria are diderm, with an inner and an outer membrane, and a cell wall. But they have unique molecules (named mycolic acids in their honor) in the outer membrane. These acids interfere with Gram staining, yielding an intermediate result between positive and negative. And their cell wall comprises three layers of sugars, each with a unique molecular composition. The middle layer is the familiar peptidoglycan.

These cells illustrate another important point: we cannot always see everything that is there. In this case, we cannot see an additional layer outside the outer membrane called the **capsule**. The capsule, present in many bacteria (mostly diderm, but also some monoderm), is formed by an "extracellular polymeric substance," or EPS: long chains of sugars, sometimes linked to the outer membrane and sometimes free-floating. These sugars help the cell attach to surfaces and offer an extra layer of protection, trapping water to prevent desiccation and making it more difficult for hydrophobic molecules like detergents to get through to disrupt the membrane(s). It also makes it more difficult for viruses to reach the cell, and for eukaryotic predators like macrophages to eat it. The capule is unstable and therefore often lost during sample preparation for cryo-ET.

Mycobacterium marinum Collected by: Elitza Tocheva DOI: 10.22002/D1.1354


surface layer
100 nm

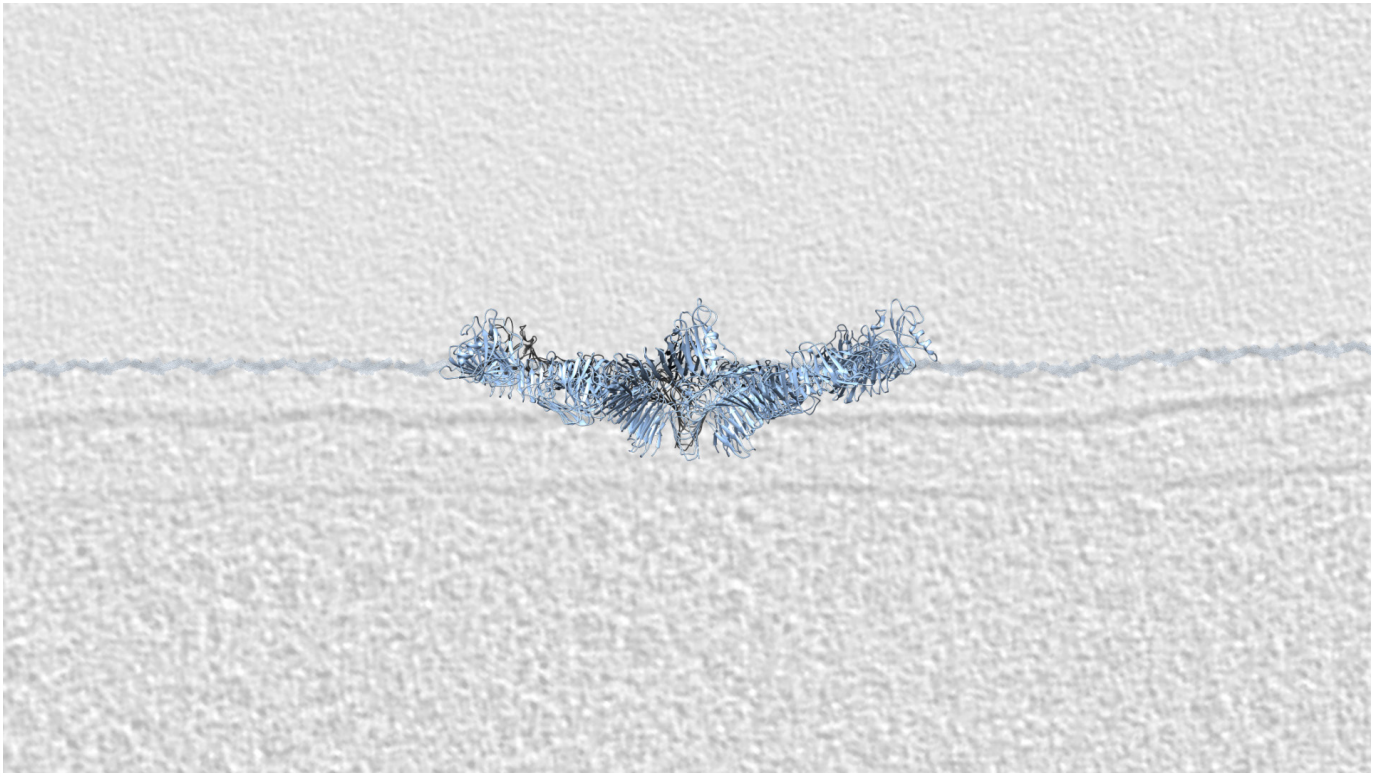

100 nm

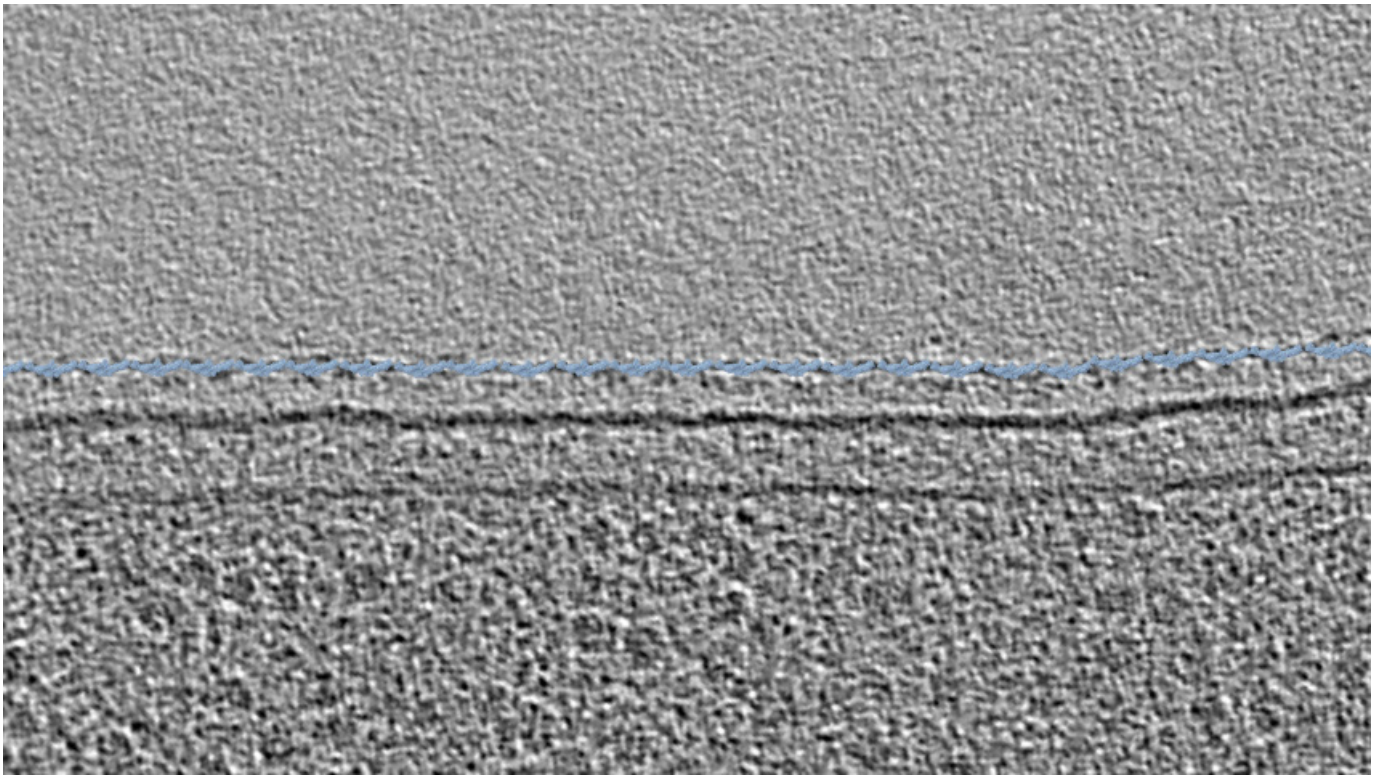
6. Surface Layer

How else can you protect your cell from the rigors of a harsh world? What about encasing it in an armored shell a la the armadillo? Many bacteria (both monoderms and diderms) and archaea use modular proteins for this purpose, interlocking Lego-block-like pieces into a shell called the **surface layer**. This must offer a significant evolutionary advantage since up to 15% of the total protein in the cell can be found in the structure. In fact, surface layers play many roles for cells, but one of their main functions for bacteria is as a gatekeeper, preventing large things like viruses from reaching the membrane.

Surface layers are crystalline lattices, and they can be striking in appearance, as on this *Caulobacter crescentus* cell. Amazingly, the lattice is made from (many copies of) a single protein (*Learn More* on the next page). The pinwheel-like subunits interact laterally, leaving pores large enough for nutrients to pass through, but too small for viruses. The modular nature of the lattice means that units can be popped in as the cell grows, or popped out to allow a cell appendage to poke through. They even accommodate vesicles (*Learn More* on following pages).

Caulobacter crescentus Collected by: Ariane Briegel DOI: 10.22002/D1.1355

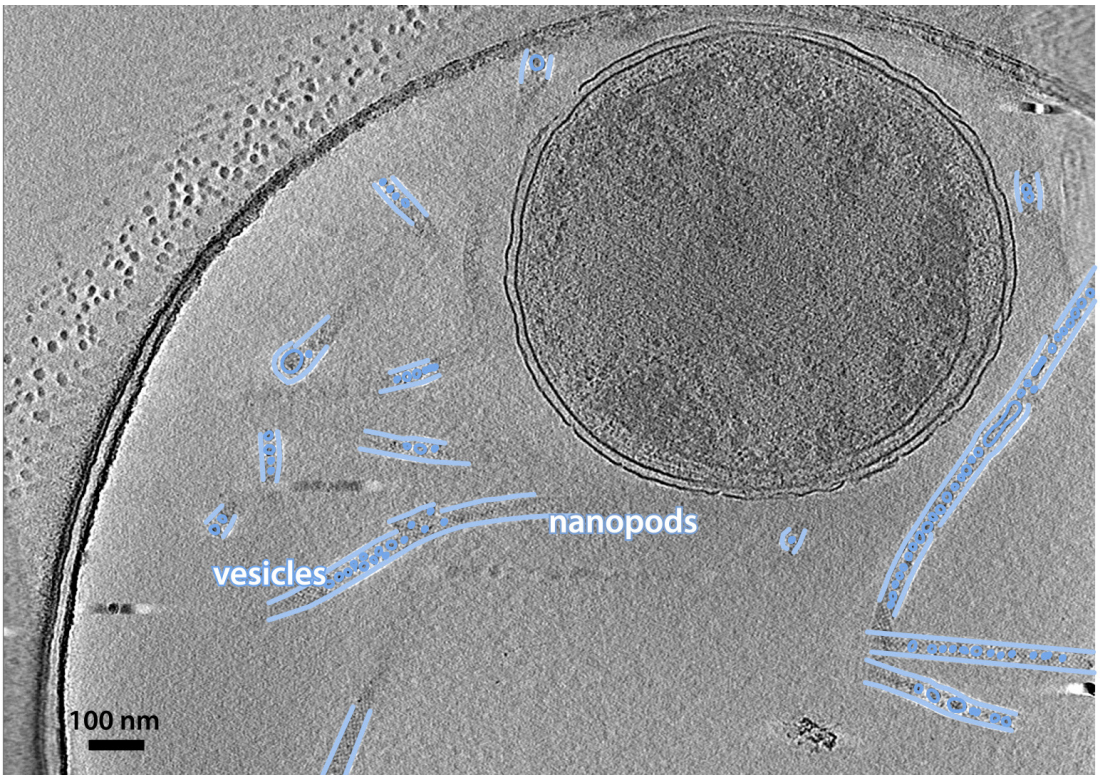


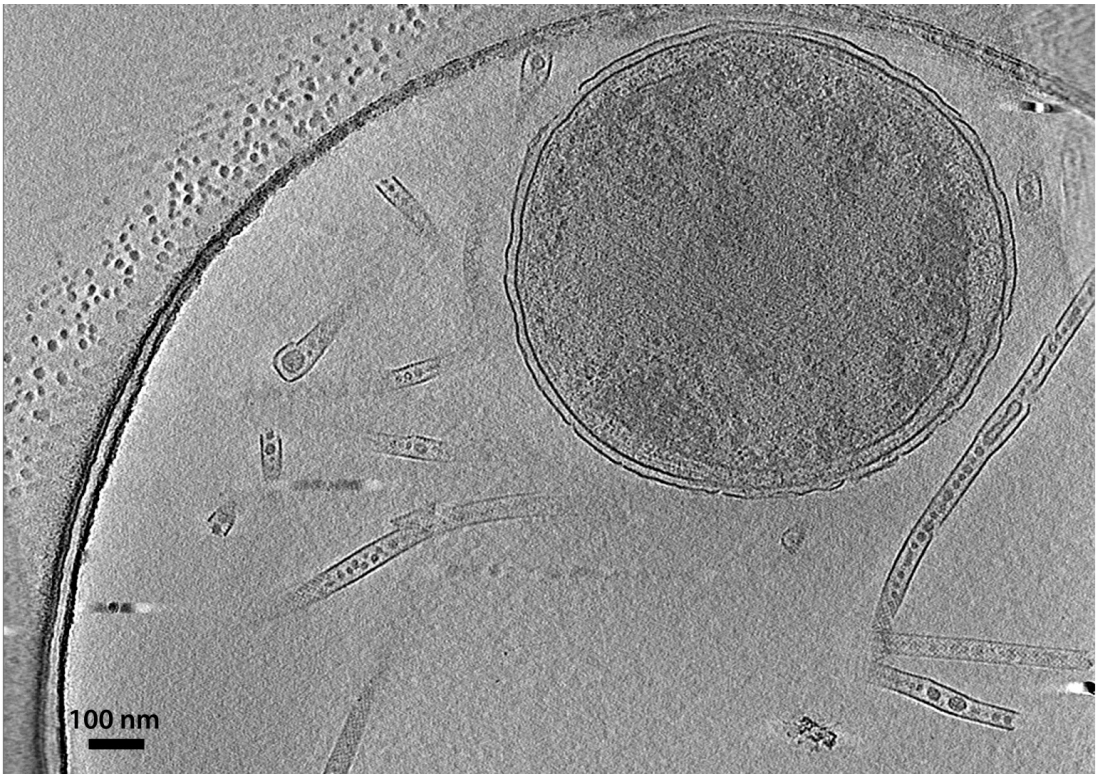


Learn More: Architecture

A single protein forms the surface layer you just saw in Caulobacter crescentus. *The protein has two domains. The bottom domain anchors it to lipoproteins attached to the outer membrane. The top domain, seen here, forms the canopy of the surface layer, organizing hierarchically into hexameric rosettes which in turn pack into a larger hexameric lattice [25]. This lattice is flexible enough to curve around even narrow regions of the cell like the stalk you will see in Chapter 4.1.*

Source: Bharat et al. (2017) *Structure: PDB 5N97*

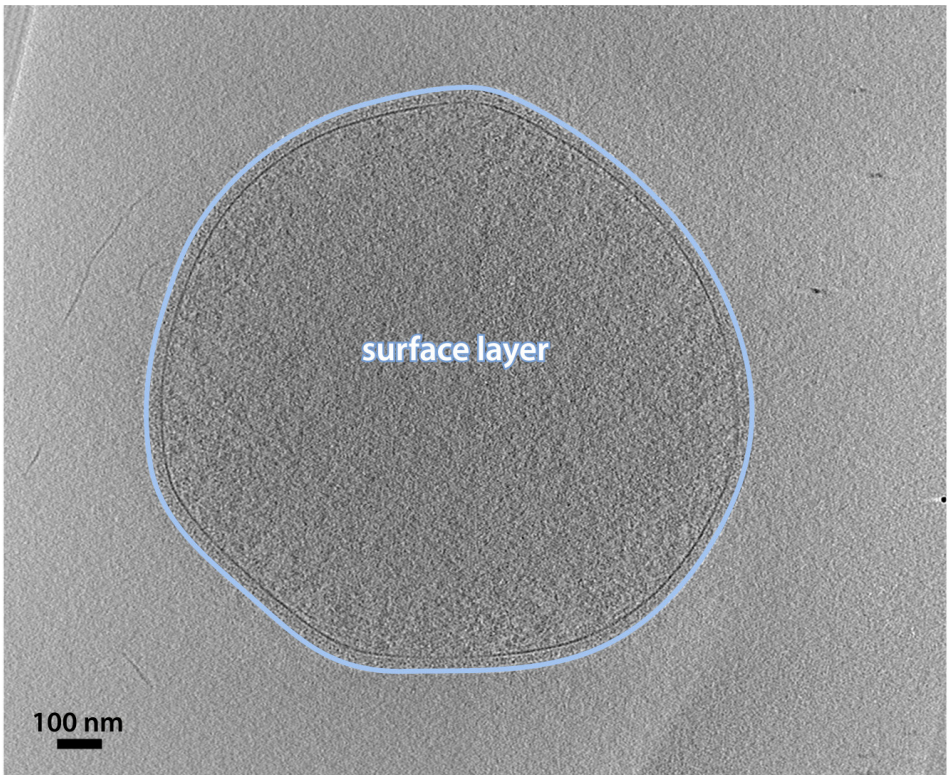

nanopods
vesicles
100 nm

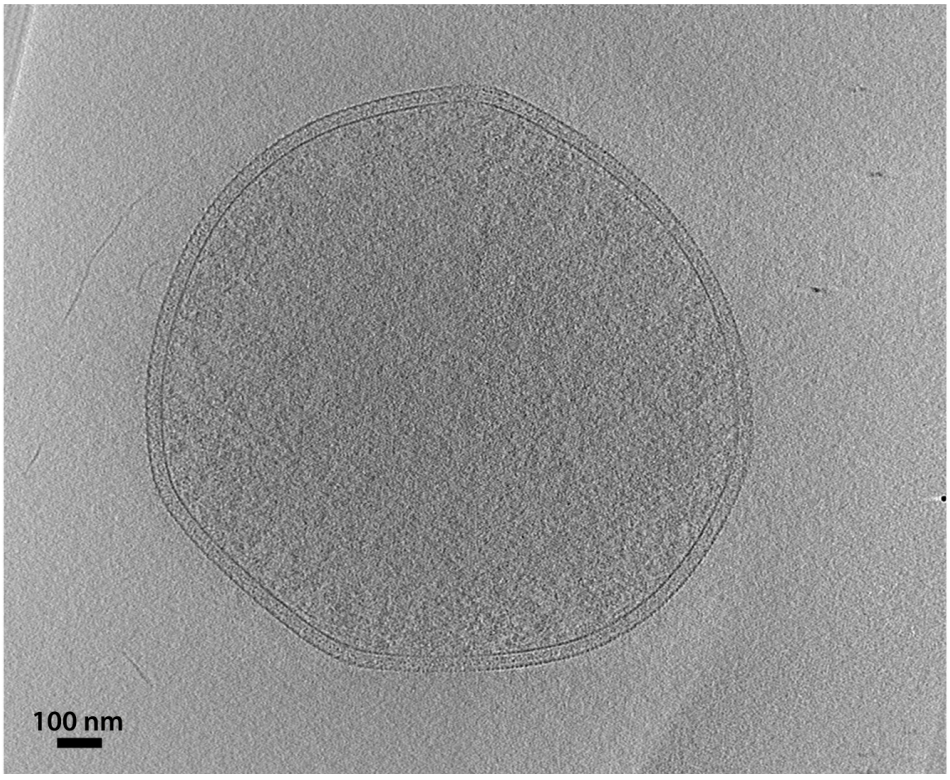

100 nm

Learn More: Nanopods

Archaea and bacteria with surface layers produce characteristic outer membrane vesicles: they bud off with the surface layer attached. You will see plenty of examples in the coming pages. Delftia acidovorans *like this produce so-called nanopods: chains of outer membrane vesicles ensheathed in surface layer.*

Delftia acidovorans *Collected by: Elitza Tocheva* *DOI: 10.22002/D1.1366*


surface layer
100 nm

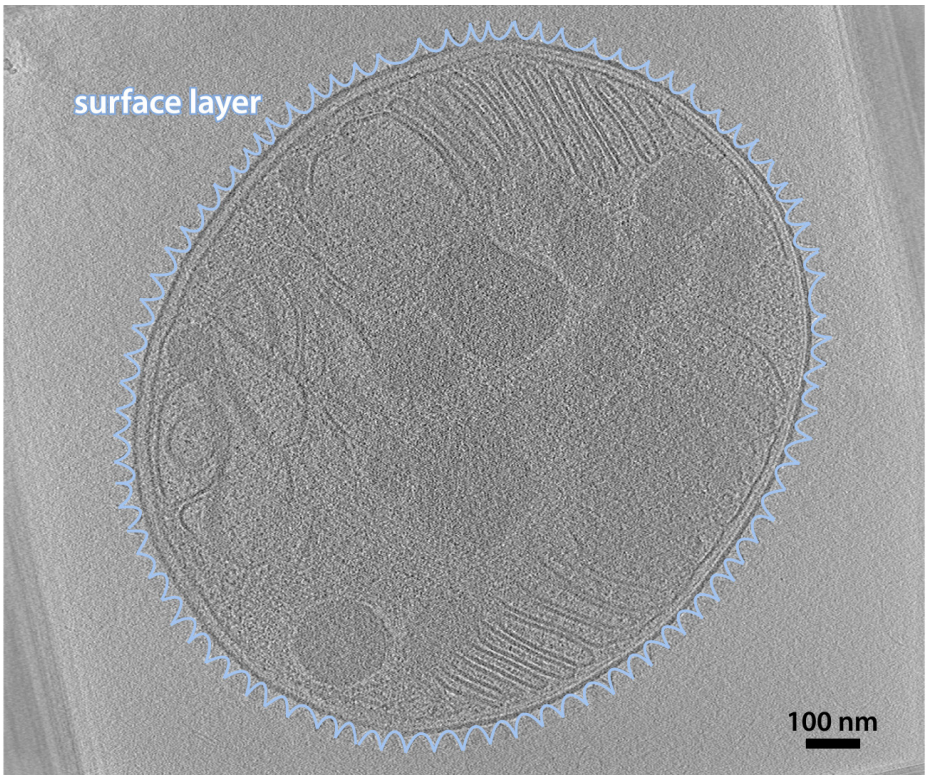

100 nm

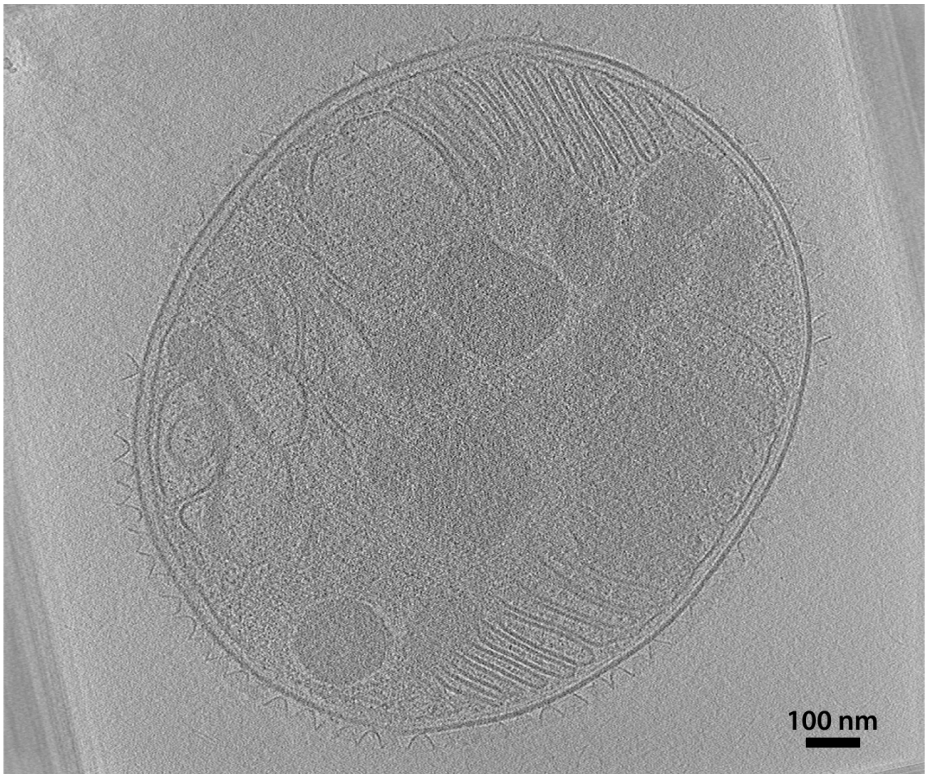
7. Surface Layer Variety

One of the most striking features of the surface layer is how different it can look in different species. For instance, compare this archaeal *Sulfolobus solfataricus* cell to the bacterium on the last page, or to other diderm (*Learn More* on the next page) or monoderm (*Learn More* on following pages) bacteria, or archaea (*Learn More* on following pages). All surface layers are crystalline lattices of a single–or in a few cases, two–proteins, but the particular pattern of the lattice depends on the shape of this building block and how it multimerizes into a higher-order structure. The shape of the building block varies considerably; there is almost no sequence homology between surface layer proteins from different species. And shapes come together in different ways, forming repeating units of one, two, three (as on this cell), four, or six blocks.

Surface layers are very common in archaea. Most archaea lack cell walls, but their surface layer plays the same role, functioning as external scaffolding. Remember, too, that nearly all archaea are monoderms, lacking the extra periplasmic compartment that diderms have. Here again the surface layer serves a similar function, making a canopy above the cell's membrane that encloses a **pseudo-periplasmic space**. Just as with the bacterial periplasm, this space serves as an antechamber for the cell, restricting access by large molecules. In some cases, the pseudo-periplasmic space also contains proteins that function in metabolism.

Sulfolobus solfataricus Collected by: Lu Gan DOI: 10.22002/D1.1356

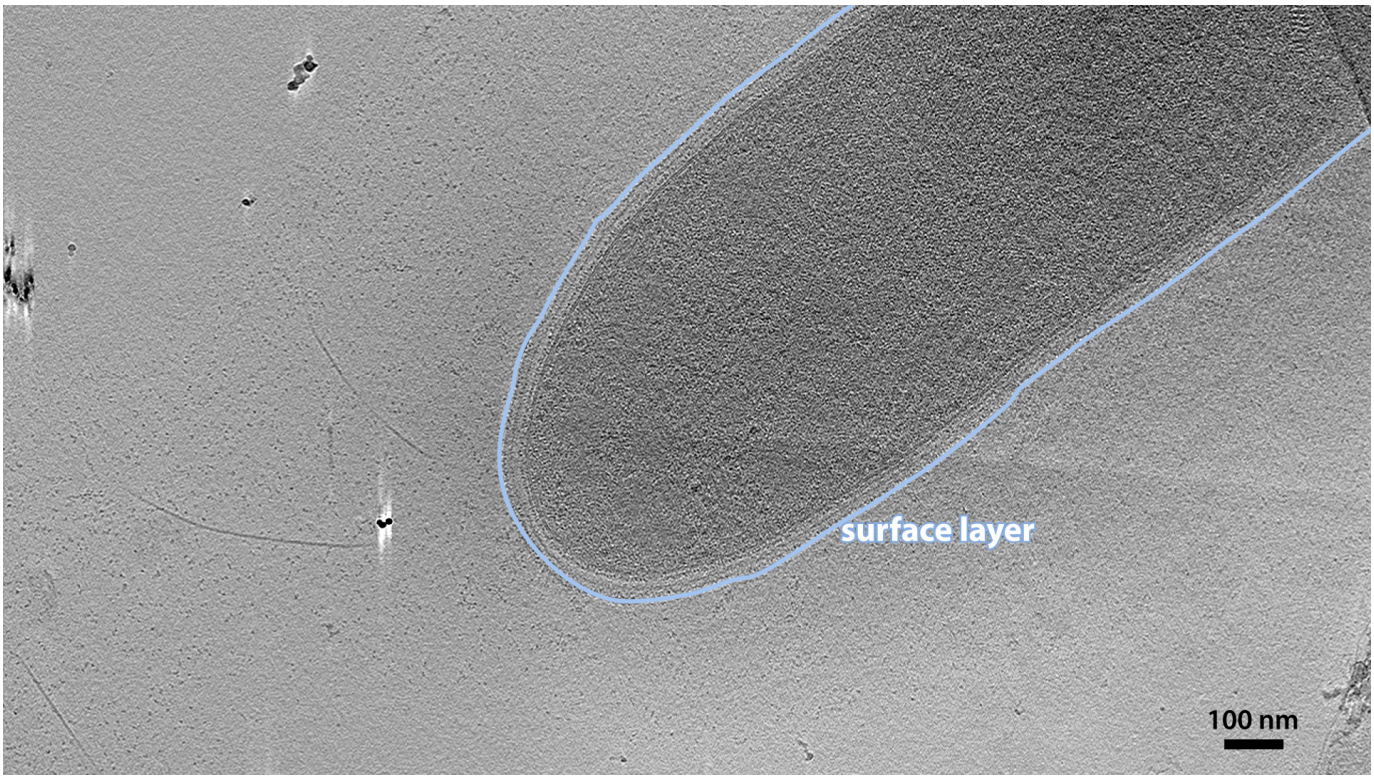

surface layer
100 nm

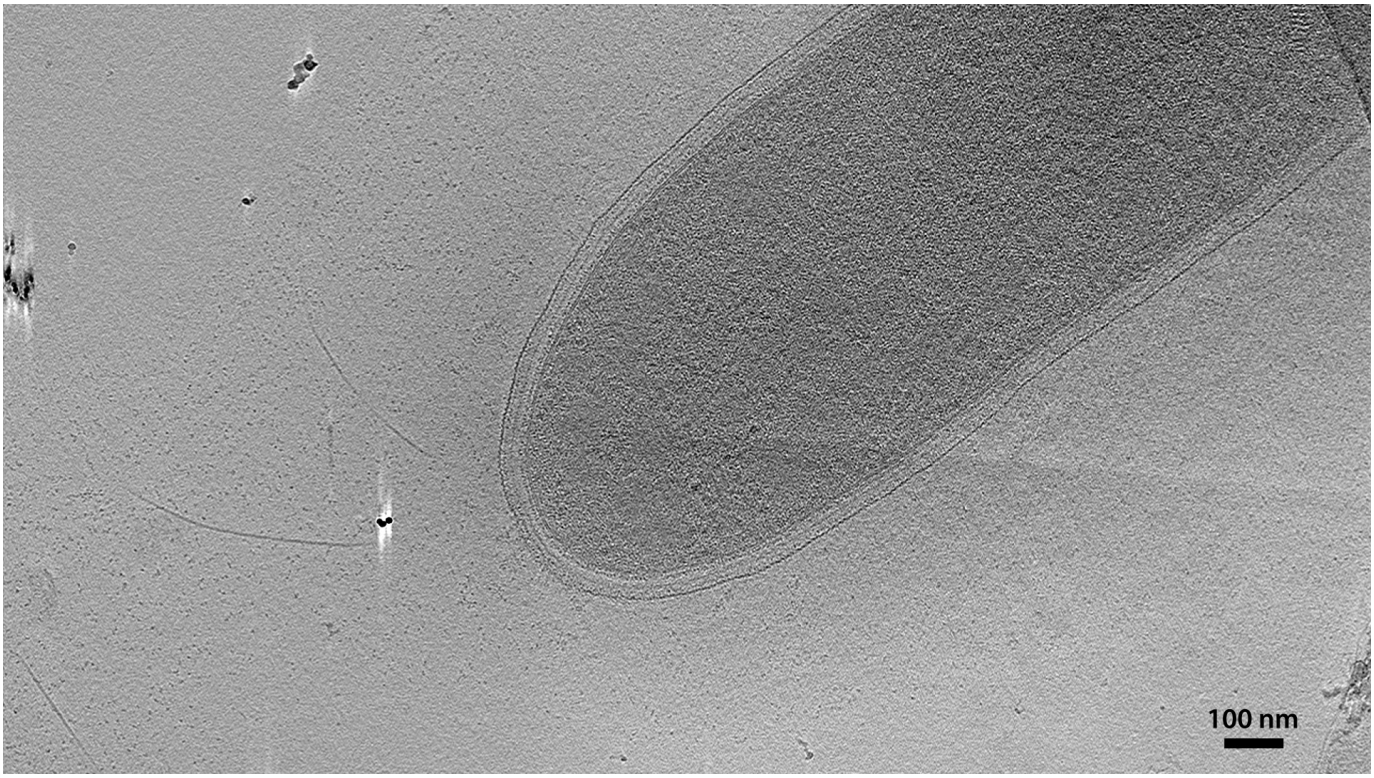

100 nm

Learn More: Diderm 2

In Methylomicrobium alcaliphilum*, V-shaped surface layer proteins come together to form cups that pack into a hexagonal pattern.*

Methylomicrobium alcaliphilum *Collected by: Songye Chen* *DOI: 10.22002/D1.1367*

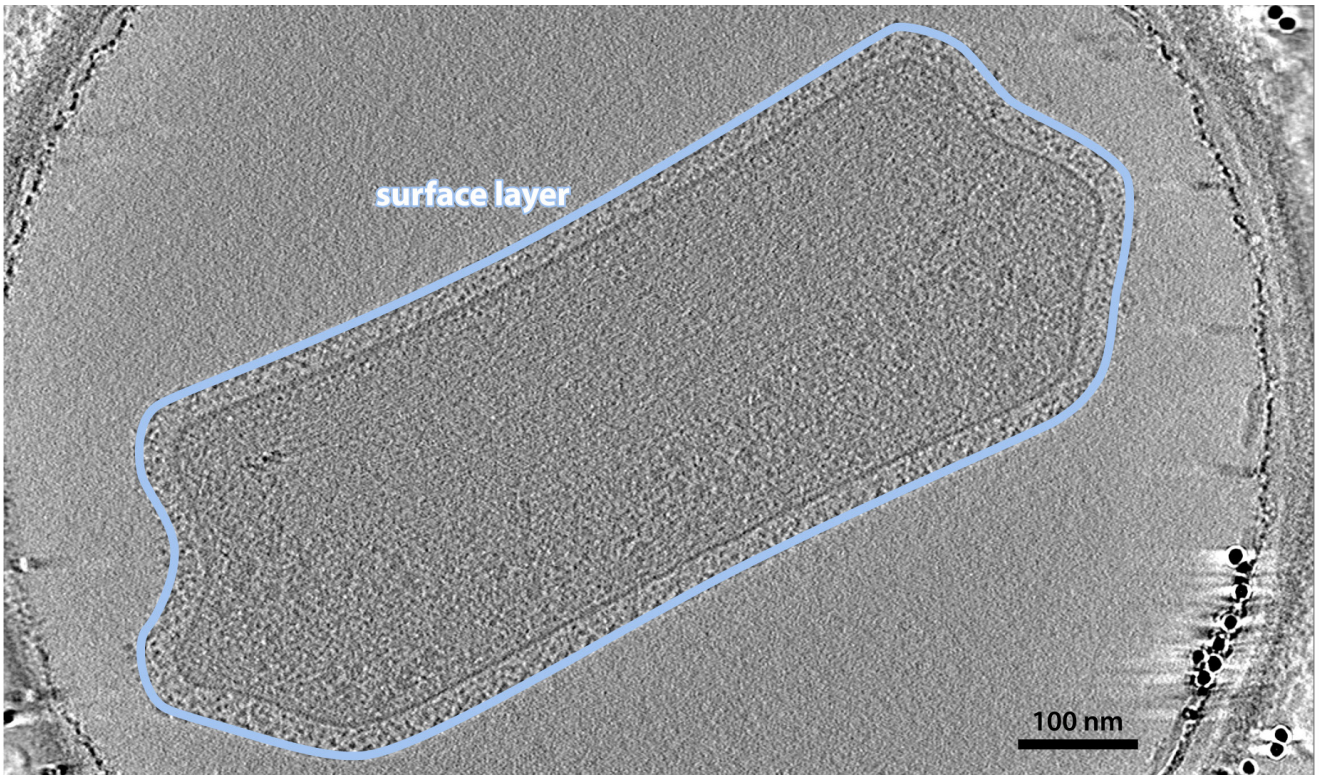

surface layer
100 nm

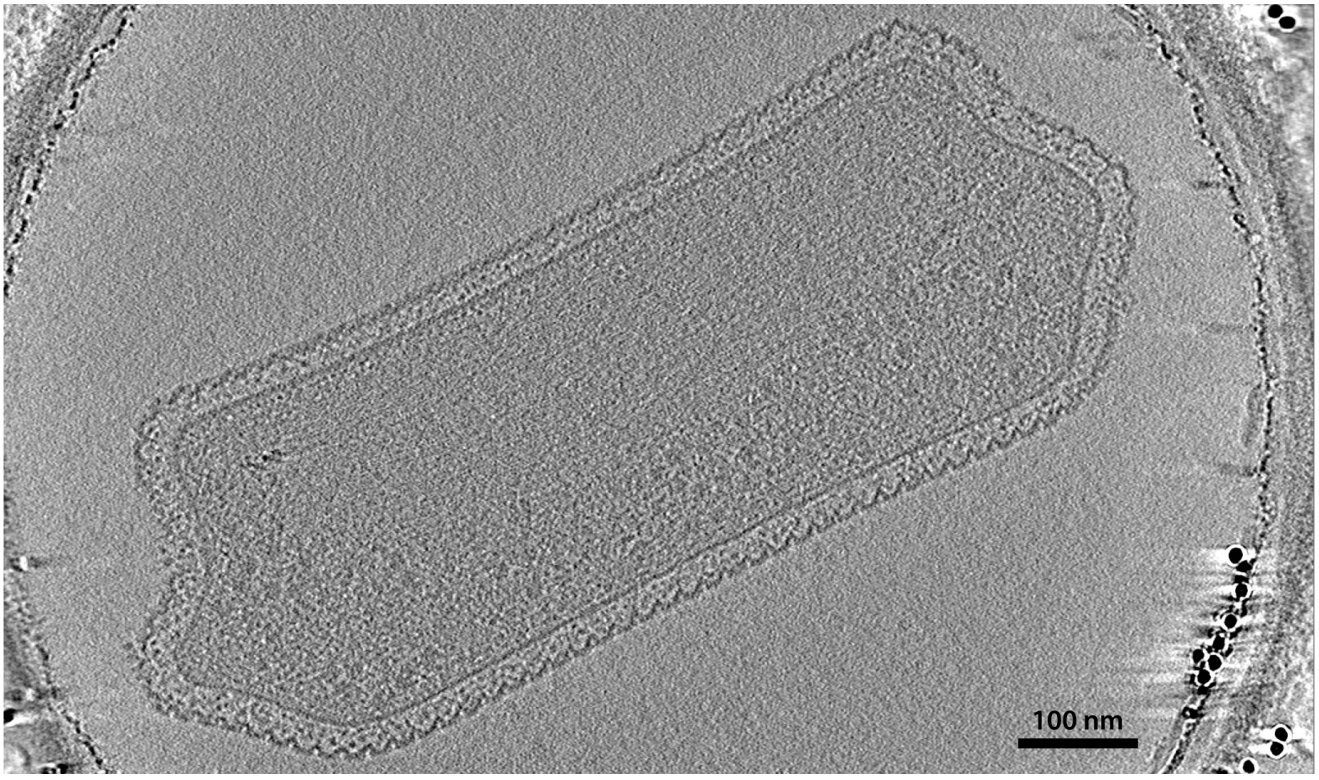

100 nm

Learn More: Monoderm

Not only diderm bacteria have surface layers, as you can see on this monoderm Clostridium thermocellum *cell. The main difference is that the proteins are anchored to the cell wall, rather than to the outer membrane (or associated lipoproteins).*

Clostridium thermocellum *Collected by: William Nicolas* *DOI: 10.22002/D1.1476*

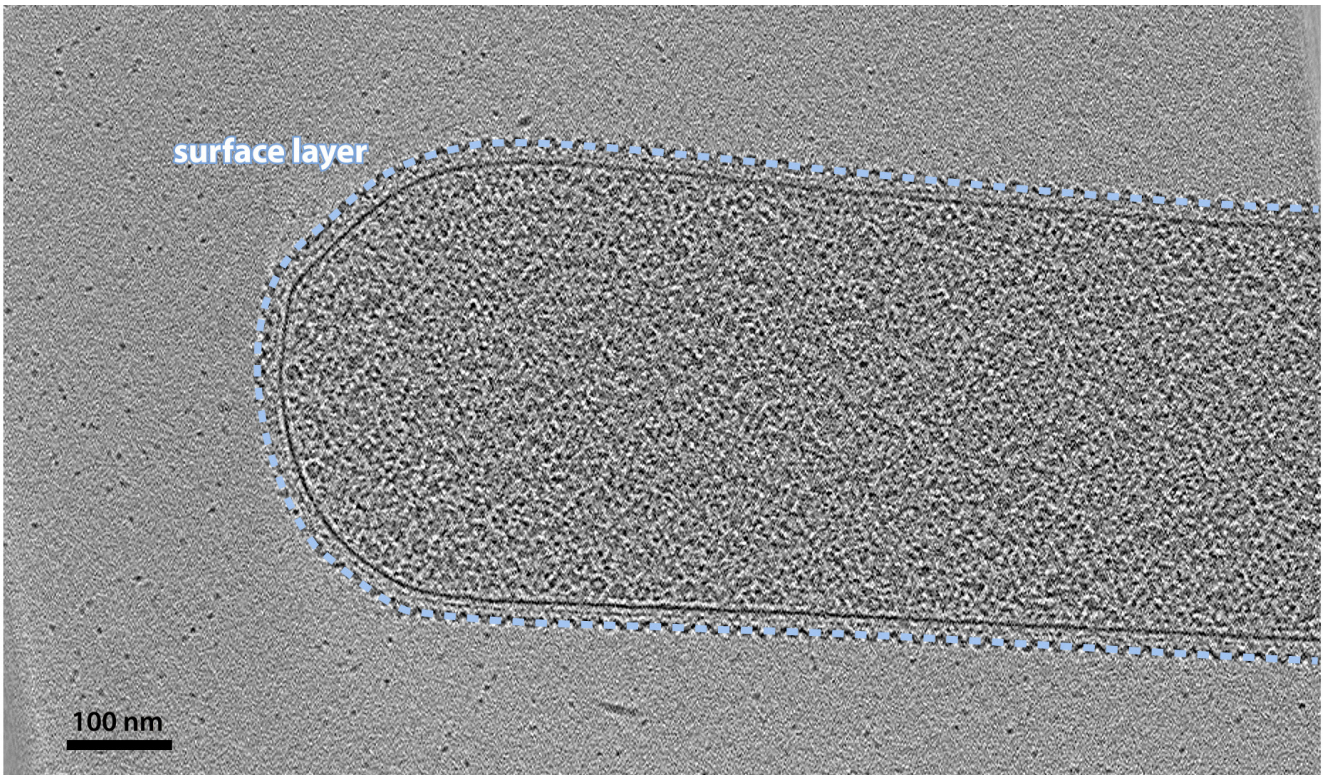

surface layer
100 nm

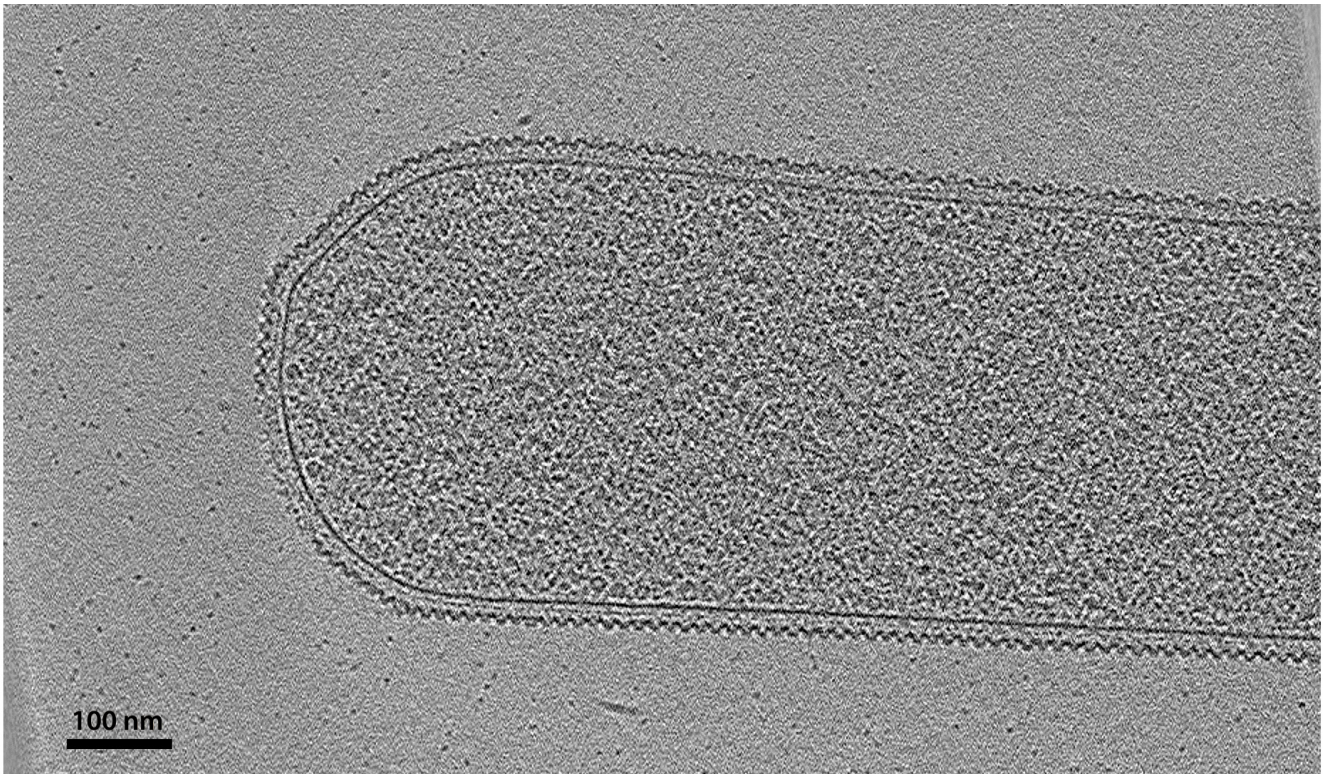

100 nm

Learn More: Archaeal 2

In Nitrosopumilus maritimus*, surface layer proteins form hexagonal rosettes that in turn pack into a hexagonal lattice.*

Nitrosopumilis maritimus *Collected by: Zhiheng Yu* *DOI: 10.22002/D1.1368*

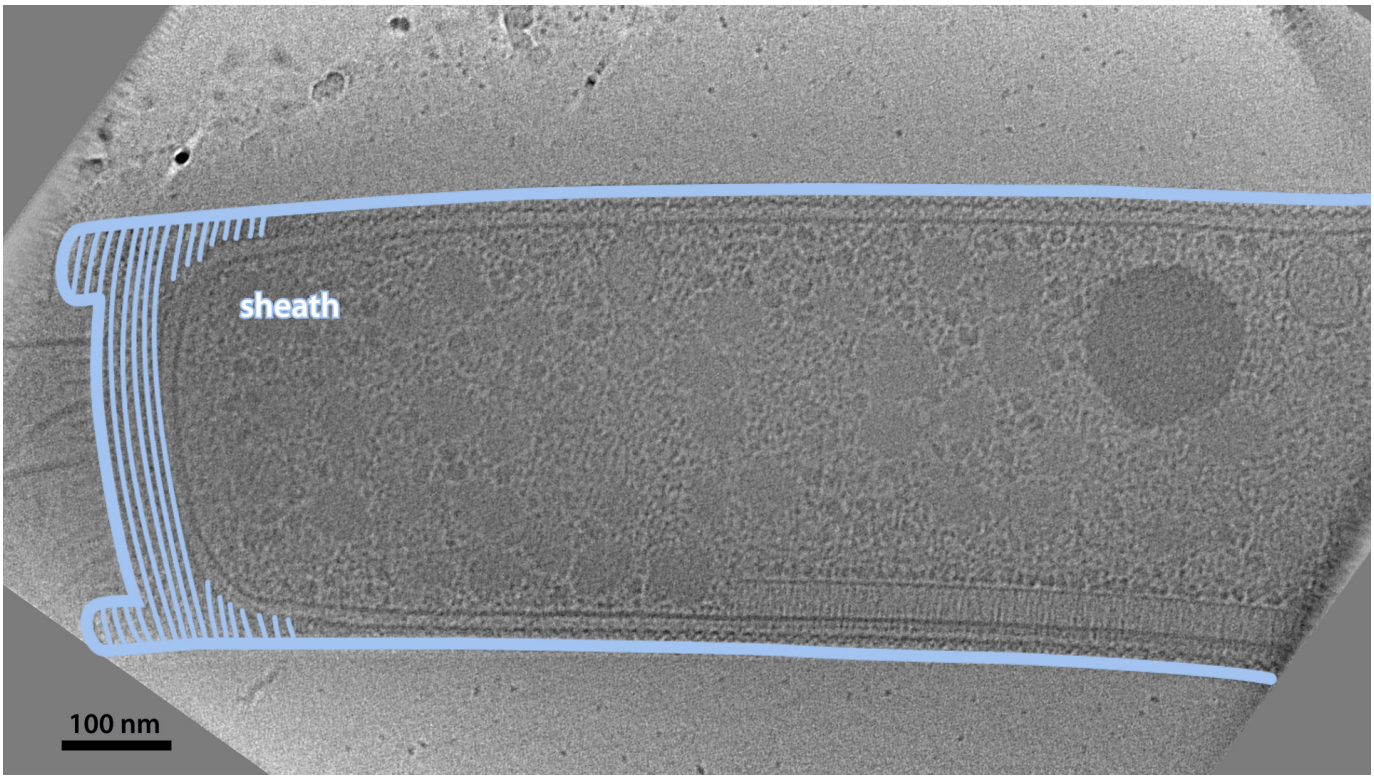

surface layer
100 nm

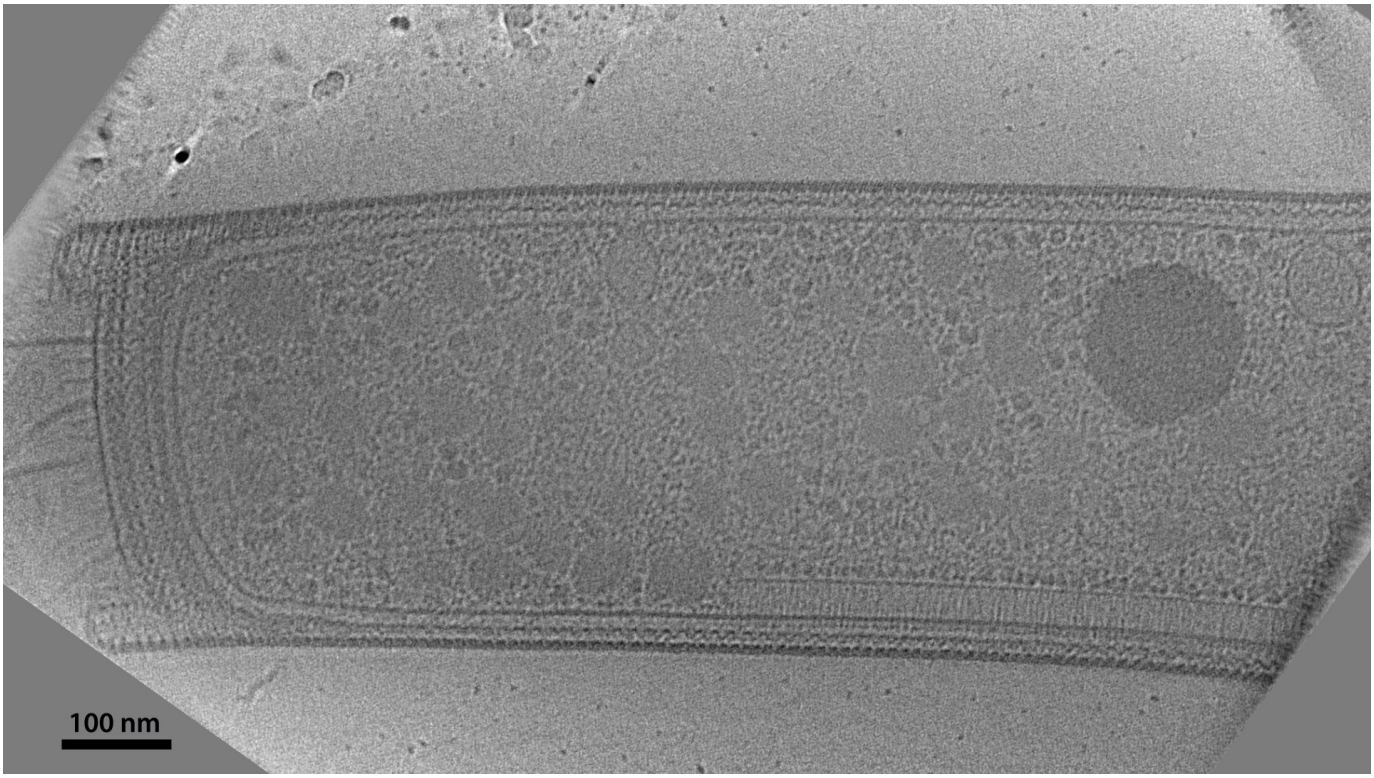

100 nm

Learn More: Archaeal 3

The surface layer of Methanoregula formicica *is a hexagonal lattice of small, nearly circular subunits.*

Methanoregula formicica *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1369*


sheath
100 nm

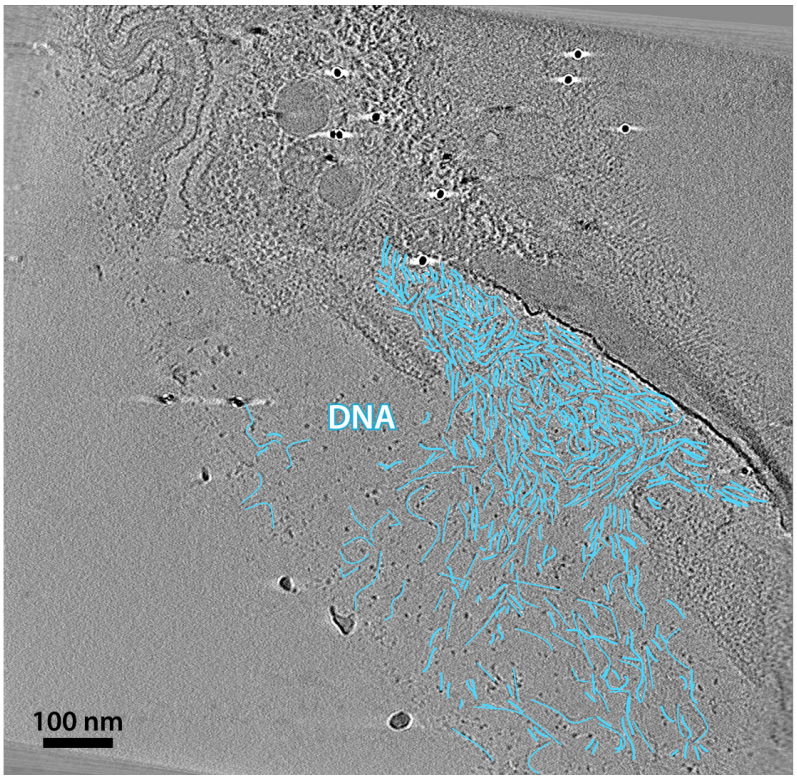

100 nm

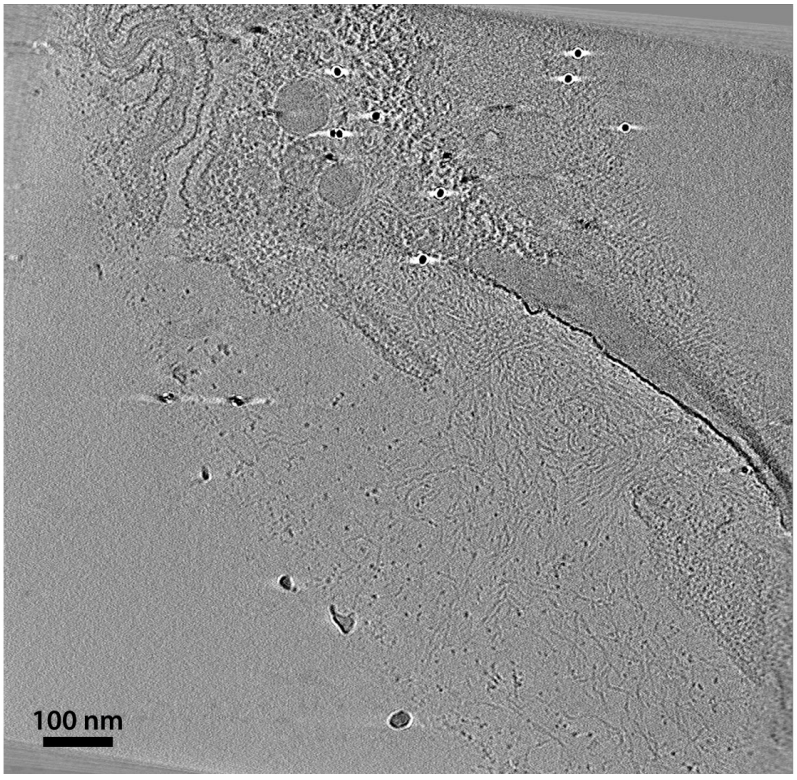
8. Sheath

Why stop at a single layer of protein? For proof that nature is endlessly inventive, consider this *Methanospirillum hungatei* cell. These archaea encase themselves in a surface layer, as well as an additional protein layer that forms a highly impermeable **sheath**. The sheath is also very resistant to pressure, which could be important in these cells' line of work. *M. hungatei* were discovered in sewage, where they break down organic waste, producing methane. One theory is that the sheath acts as a pressure regulator; when enough methane builds up inside the cell, the pressure expands the sheath, opening its pores wide enough to allow the methane to dissipate and new metabolic substrates like hydrogen and carbon dioxide to enter.

The rules of architecture remain the same, though. Just as in the bacterial cell wall, sheath polymers are arranged as hoops perpendicular to the long axis of the rod-shaped cell. At the ends of the sheath, multiple protein layers stack into a thick plug. Cells divide within the sheath, and long chains of cells in a continuous sheath are often observed.

Methanospirillum hungatei Collected by: Ariane Briegel DOI: 10.22002/D1.1357


DNA
100 nm

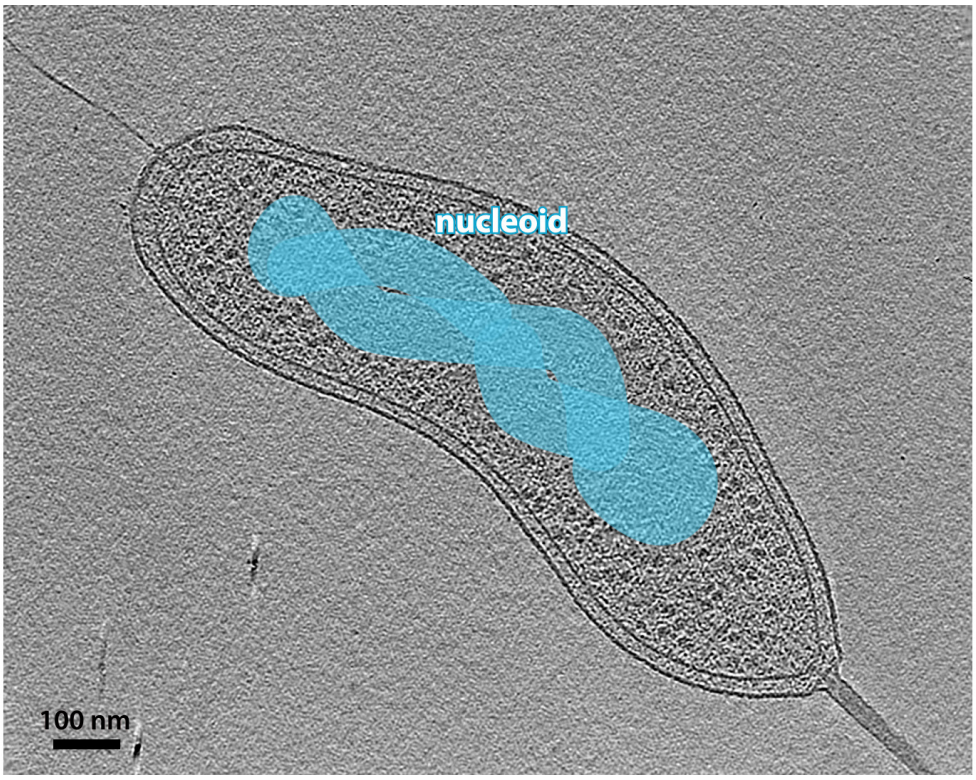

100 nm

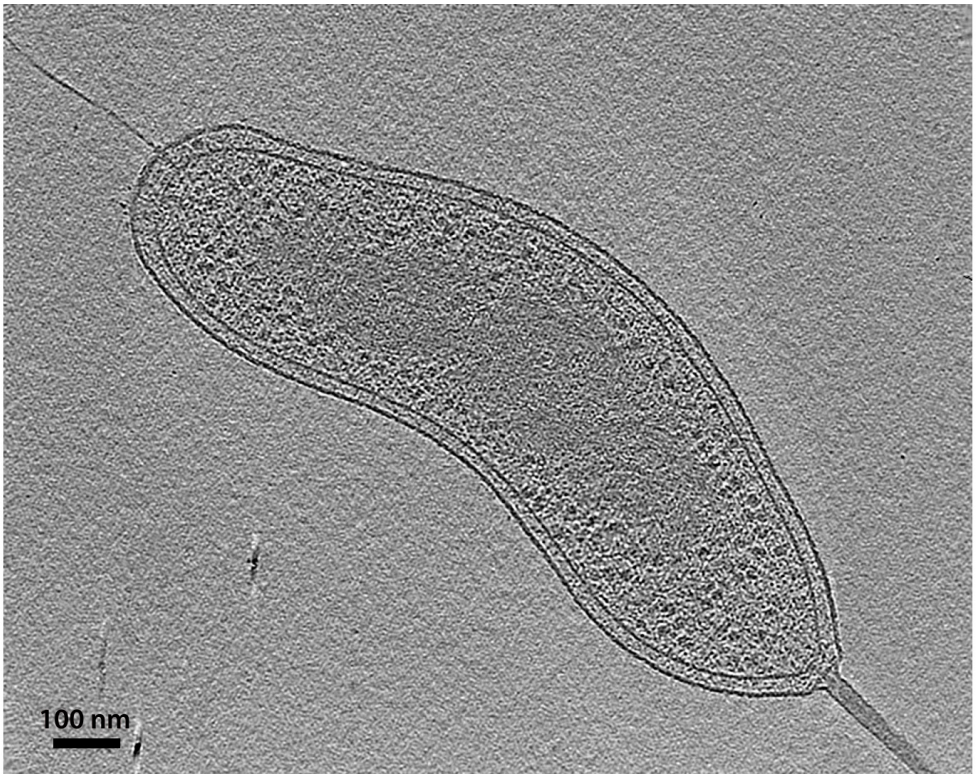
9. DNA

All the layers we just discussed collectively make up the container, or **envelope**, of a cell. As you have seen, different species use different combinations of these components to form their envelopes; the only constant is the cytoplasmic (or inner, for diderms) membrane.

Now consider what these envelopes contain. In addition to water and small molecules, you have already seen some large protein complexes like motility machines. You have also seen many ribosomes–the protein/RNA complexes responsible for translating RNA into proteins. But you might have been surprised not to see something else: deoxyribonucleic acid, or **DNA**. The molecule containing the instructions for the life of the cell is of paramount importance, yet often invisible by microscopy. But not always. Thin filaments of DNA, only about 2 nm (two billionths of a meter) wide, blend in with the dense cytoplasm of the cell. When a cell lyses, though, its cytoplasm diffuses into the environment and the DNA filaments stand out against the now-much-reduced background. You can get an idea of the sheer abundance of DNA inside a cell from this *Haloarcula argentinensis* whose envelope has ruptured and contents are spilling out.

Haloarcula argentinensis Collected by: Ariane Briegel DOI: 10.22002/D1.1358


nucleoid
100 nm

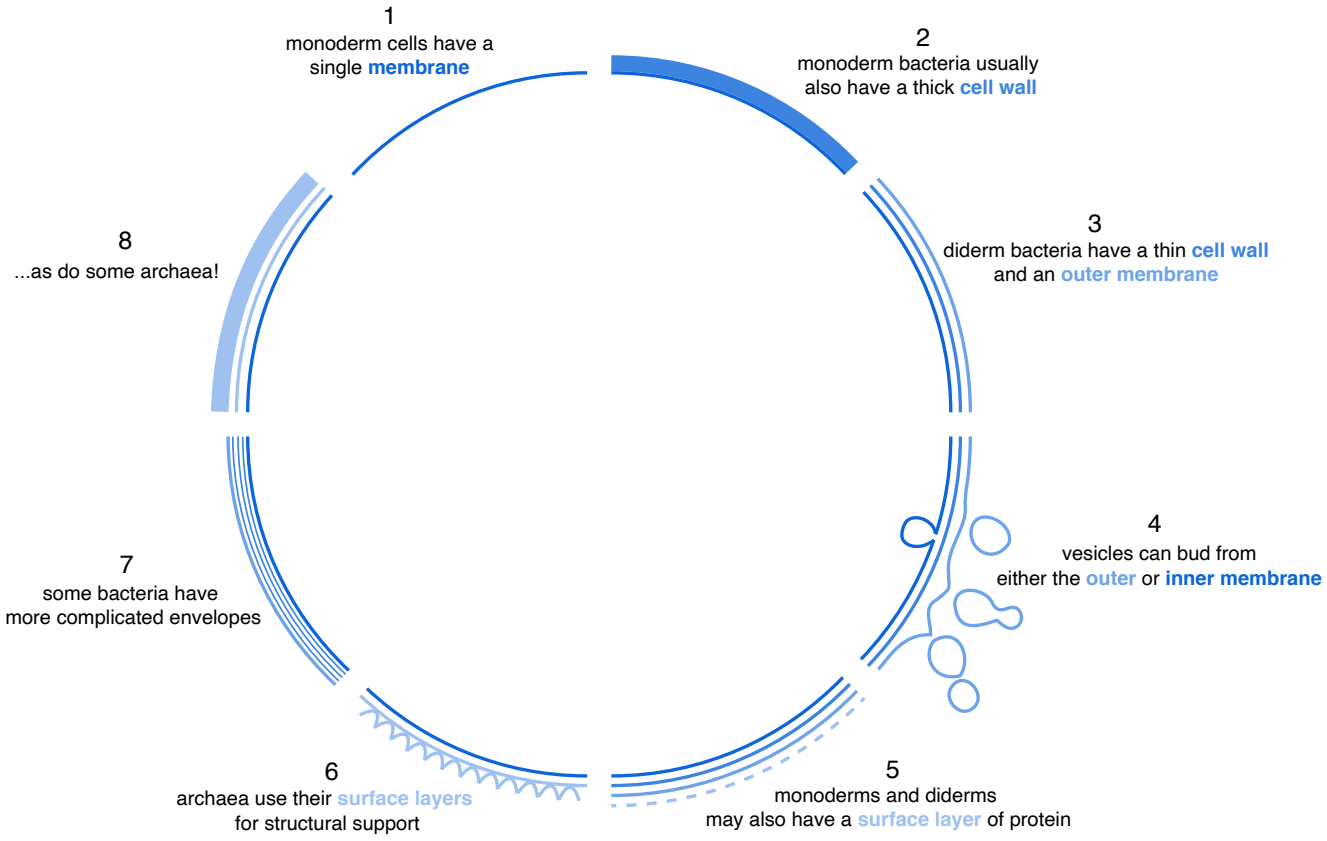

100 nm

10. Nucleoid

Cells contain enormous amounts of DNA. The single, circular chromosome of this *Bdellovibrio bacteriovorus* cell contains 3,782,950 individual nucleotide pairs, which means that if the circle were cut and laid out as a long piece, it would be about *one thousand times* longer than the cell itself. To fit and function inside the cell, the chromosome has to be extraordinarily organized and packed, a feat we still do not understand. Some of this packing is evident in nearly every cell: the center of the cell tends to have very few large macromolecular complexes like ribosomes, because they are excluded by the densely-packed chromosome(s). Look for these ribosome-excluding zones in the rest of the book; they indicate the location of the bulk of the cell's DNA. Since this region is not enclosed by an internal membrane, it is not called a nucleus (the "karyon" that defines eukaryotes). Instead, we use the term **nucleoid** to describe the cytoplasmic region where most of the DNA is concentrated.

At times, the nucleoid becomes easier to see. Imagine that your cell wanted to decrease its gene expression (we will discuss why in Chapters 8 and 9). One approach is simply to pack the chromosome so tightly that the transcriptional machinery cannot access the genes. This cell has done just that, condensing its nucleoid into a dense twisted braid we can easily visualize.

Bdellovibrio bacteriovorus Collected by: Yi-Wei Chang DOI: 10.22002/D1.1359


1
monoderm cells have a
single membrane
2
monoderm bacteria usually
also have a thick cell wall
3
diderm bacteria have a thin cell wall
and an outer membrane
4
vesicles can bud from
either the outer or inner membrane
5
monoderms and diderms
may also have a surface layer of protein
6
archaea use their surface layers
for structural support
7
some bacteria have
more complicated envelopes
8
...as do some archaea!

Summary

Concept Check Questions

- What kind of macromolecule makes up the membrane? The cell wall? The surface layer?
- What is the difference between a monoderm and a diderm?
- What is the main function of the cell wall?
- What is a nucleoid?

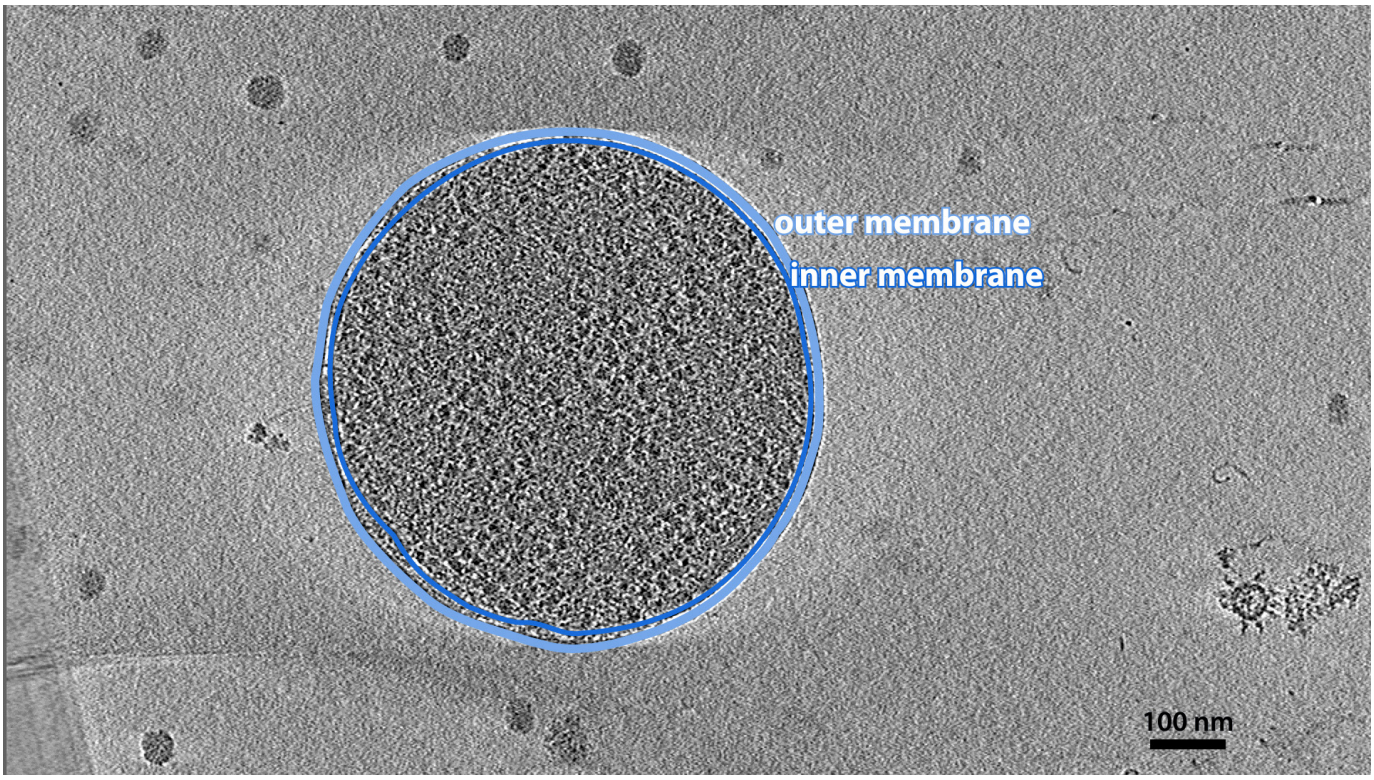
Further Reading

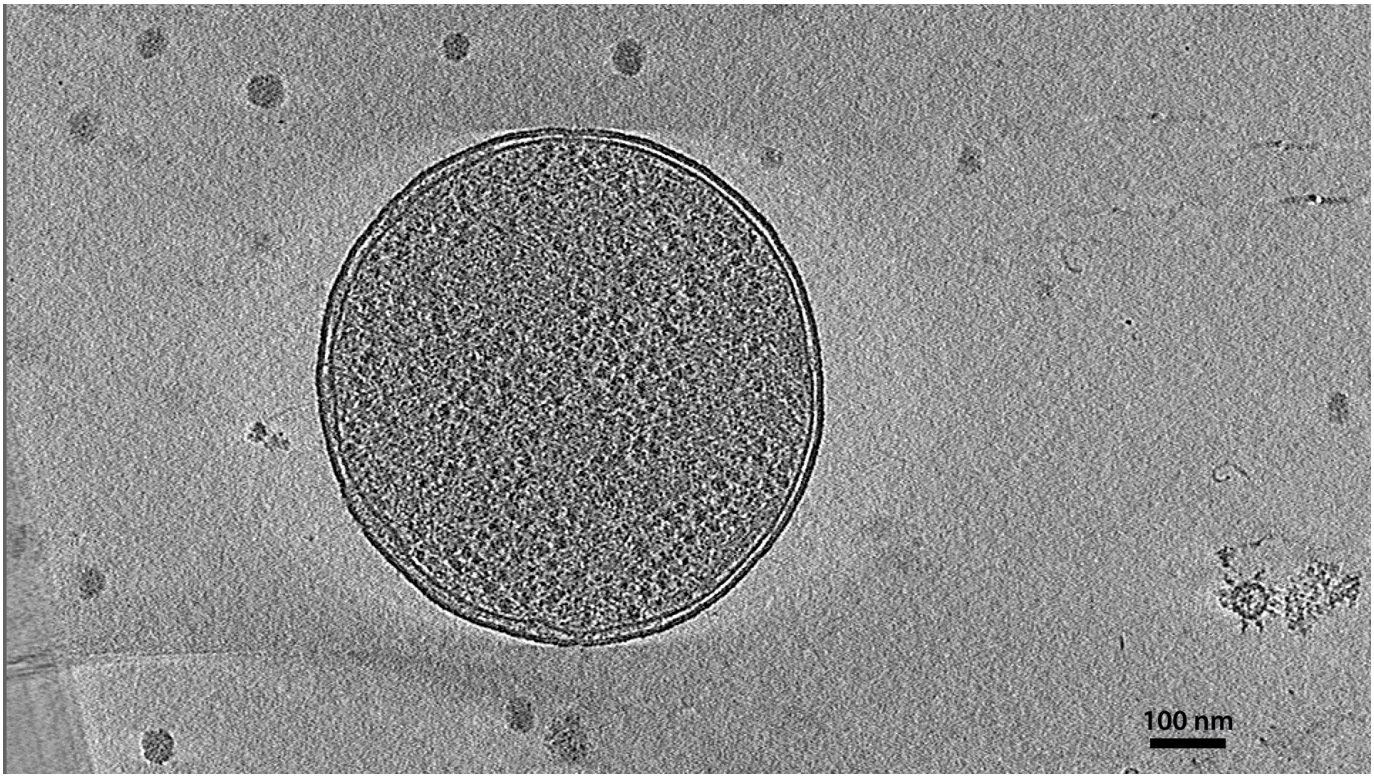
- Errington (2013). *L-form bacteria, cell walls and the origins of life* [26].
- Ptacin and Shapiro (2013). *Chromosome architecture is a key element of bacterial cellular organization* [27].
- Sleytr and Beveridge (1999). *Bacterial S-layers* [28].
- Strahl and Errington (2017). *Bacterial membranes: Structure, domains, and function* [29].

3

Shape

"To be brutally honest, few people care that bacteria have different shapes. Which is a shame, because the bacteria seem to care very much."
- Kevin Young [30]


outer membrane
inner membrane
100 nm


100 nm

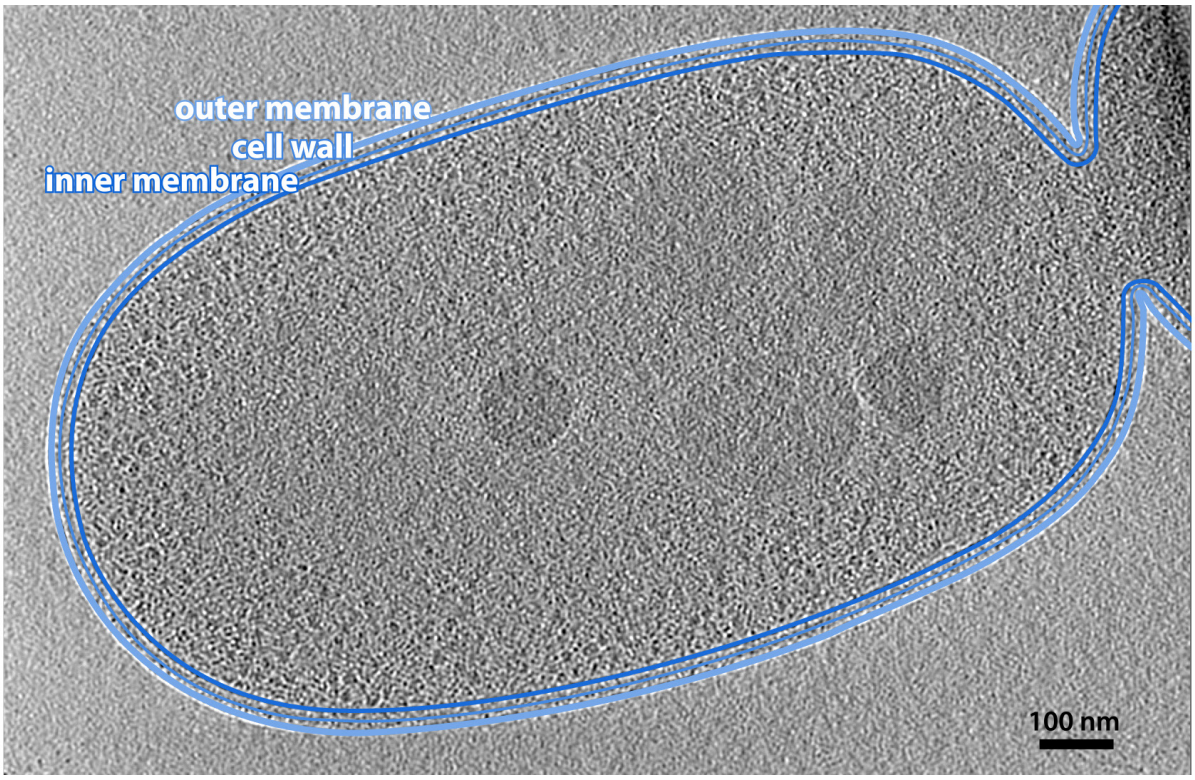
1. Coccoid

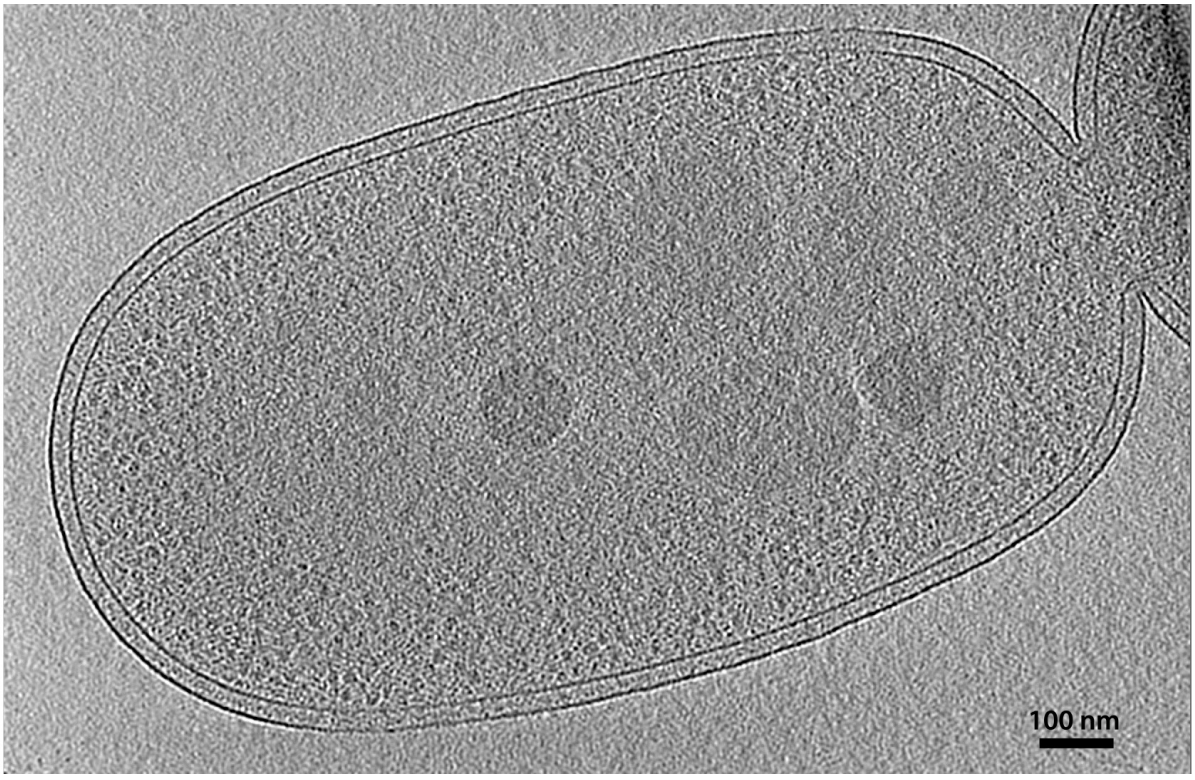
What kind of life do you envision for your cell? Just as the design of buildings reflects their purpose, different cell shapes suit different lifestyles. Does your cell need to soak up sunlight for photosynthesis? Burrow through the tissue of a host? Chase down prey? Each lifestyle is best served by a particular form.

How can you give your cell a particular form? The final shape of a building is determined by a shell erected around a system of internal beams and joists–its skeleton. Cells determine their shape using a similar system–the external constraint of the rigid cell wall and/or surface layer in concert with an internal cyto("cell")-skeleton. The **cytoskeleton** of bacteria and archaea comprises a set of proteins that form filaments or other superstructures that move or scaffold other material in the cell. In many cases, this cytoskeletal scaffolding is dynamic and ever-changing, appropriate for a living building.

Consider a cell like this *Simkania negevensis*. It takes the form of a sphere–the default shape for a membrane in water, uniformly resistant to pressure, and the shape that maximizes volume relative to surface area. We refer to spherical cells as **coccoid** ("berry-like"). To grow, a coccoid cell can simply add lipids to its membrane(s) and randomly insert new glycan strands into its cell wall, expanding to a larger radius.

Simkania negevensis Collected by: Martin Pilhofer DOI: 10.22002/D1.1477


outer membrane
cell wall
inner membrane
100 nm

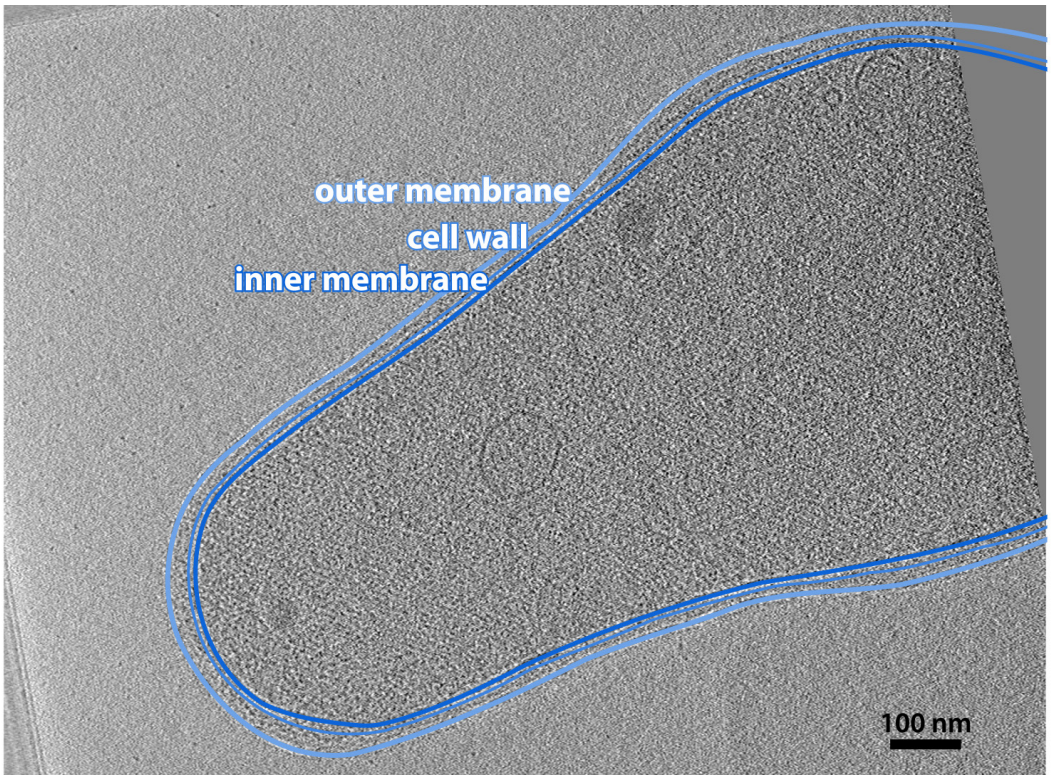

100 nm

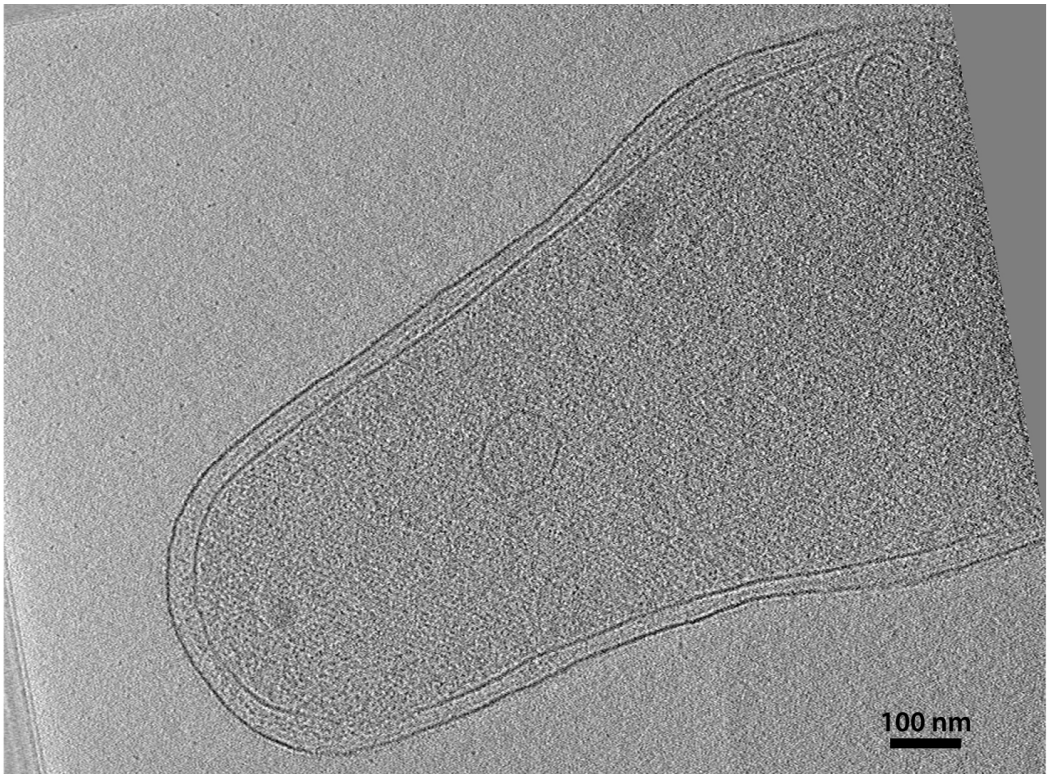
2. Rod

Instead of a sphere, maybe you would like to make your cell cylindrical, like this *Cupriavidus necator*. **Rod-shaped** cells (cylinders with hemispherical caps) are a very common form for bacteria and archaea, likely because they make efficient swimmers and swarmers (more on that in Chapter 6). Starting from a sphere, imagine that you had a construction contractor who could direct where workers lay in new cell wall. Instead of random insertion, you could, say, direct them to work around a single plane. As the workers laid in more and more hoops of peptidoglycan in this region, a cylinder would form with the same diameter as the initial sphere (which would now serve as the structure's end caps).

The contractor for most rod-shaped bacterial cells is a cytoskeletal protein named **MreB** (the name comes from a screen for mutants resistant to the cell-wall-targeting antibiotic mecillinam). MreB is a homolog of the eukaryotic cytoskeletal protein actin. It remains unclear exactly how it works, but small patches of MreB seem to shuttle rapidly around the circumference of the cell, directing where new peptidoglycan is added to the sacculus. MreB's circuit is restricted to the cylindrical portion of the cell, expanding the rod without affecting the ends. Not all rod-shaped bacteria use MreB, and we are still figuring out how the shape forms in many species (*Learn More* on the next page). For rod-shaped archaea (*Learn More* on following pages), the surface layer plays an important role in determining the shape.

Cupriavidus necator Collected by: Morgan Beeby DOI: 10.22002/D1.1478

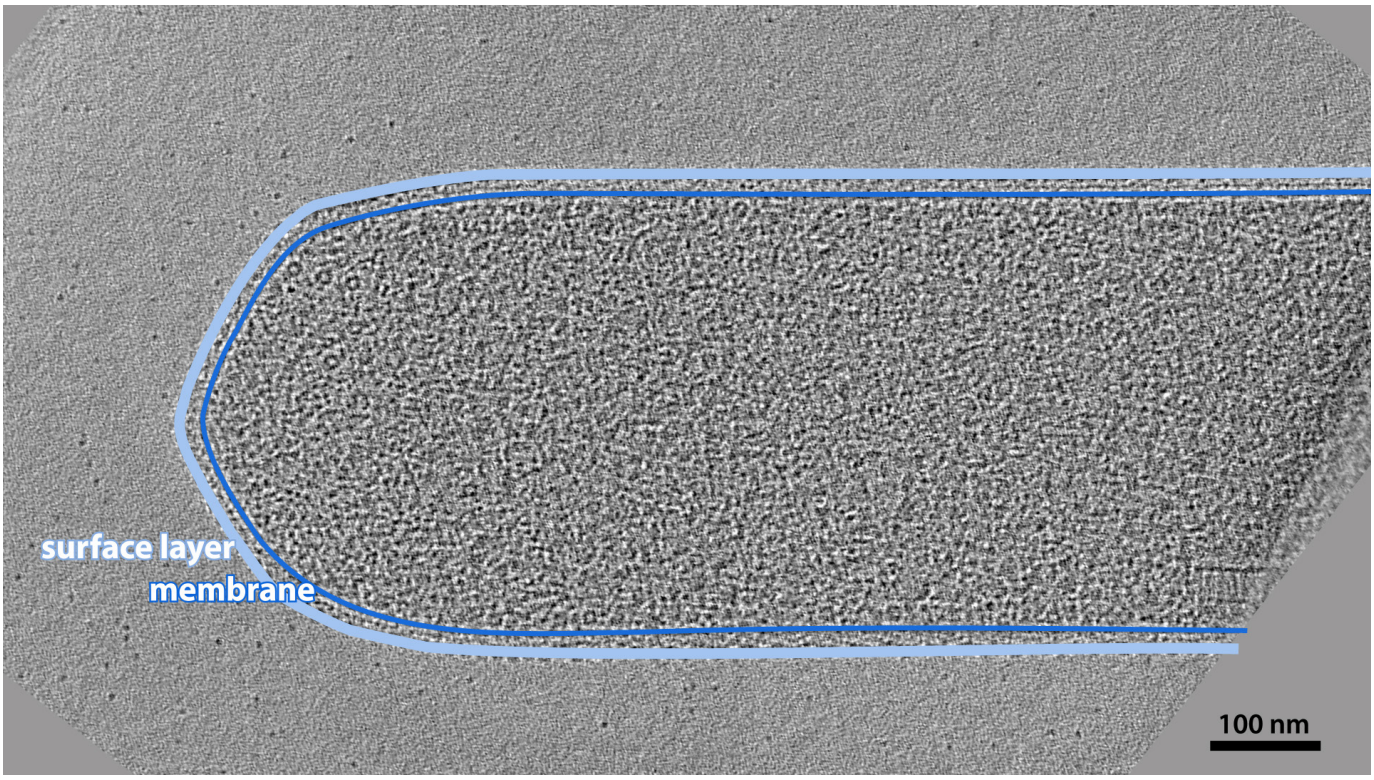

outer membrane
cell wall
inner membrane
100 nm

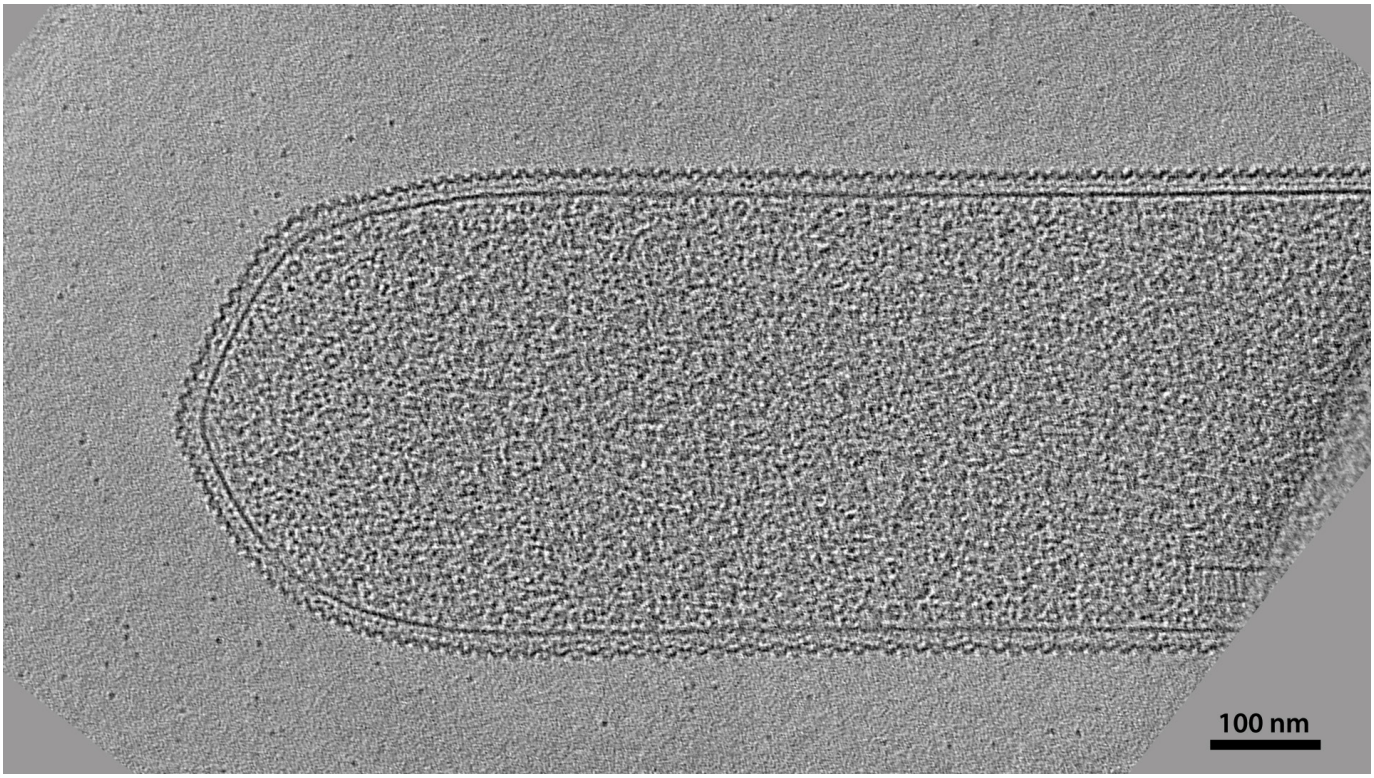

100 nm

Learn More: Variety

Not all rod-shaped cells are perfectly cylindrical. For instance, Brucella abortus *like this one are pear-shaped. This is one of the rod-shaped bacterial species that do not use MreB.*

Brucella abortus *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1484*


surface layer
membrane
100 nm

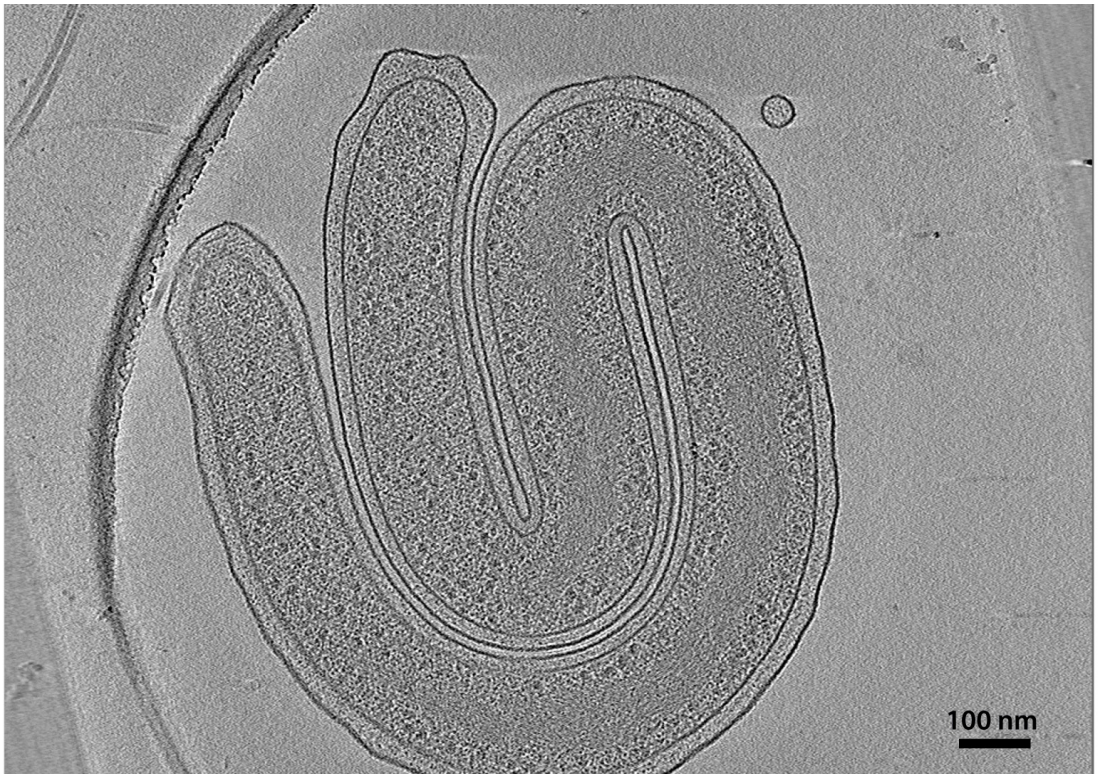

100 nm

Learn More: Archaeal Rods

Some archaeal species, like this Methanoregula formicica*, are rod-shaped. The rigid surface layer helps define the shape, but the details are still unknown.*

Methanoregula formicica *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1485*

inner membrane
cell wall
outer membrane
100 nm

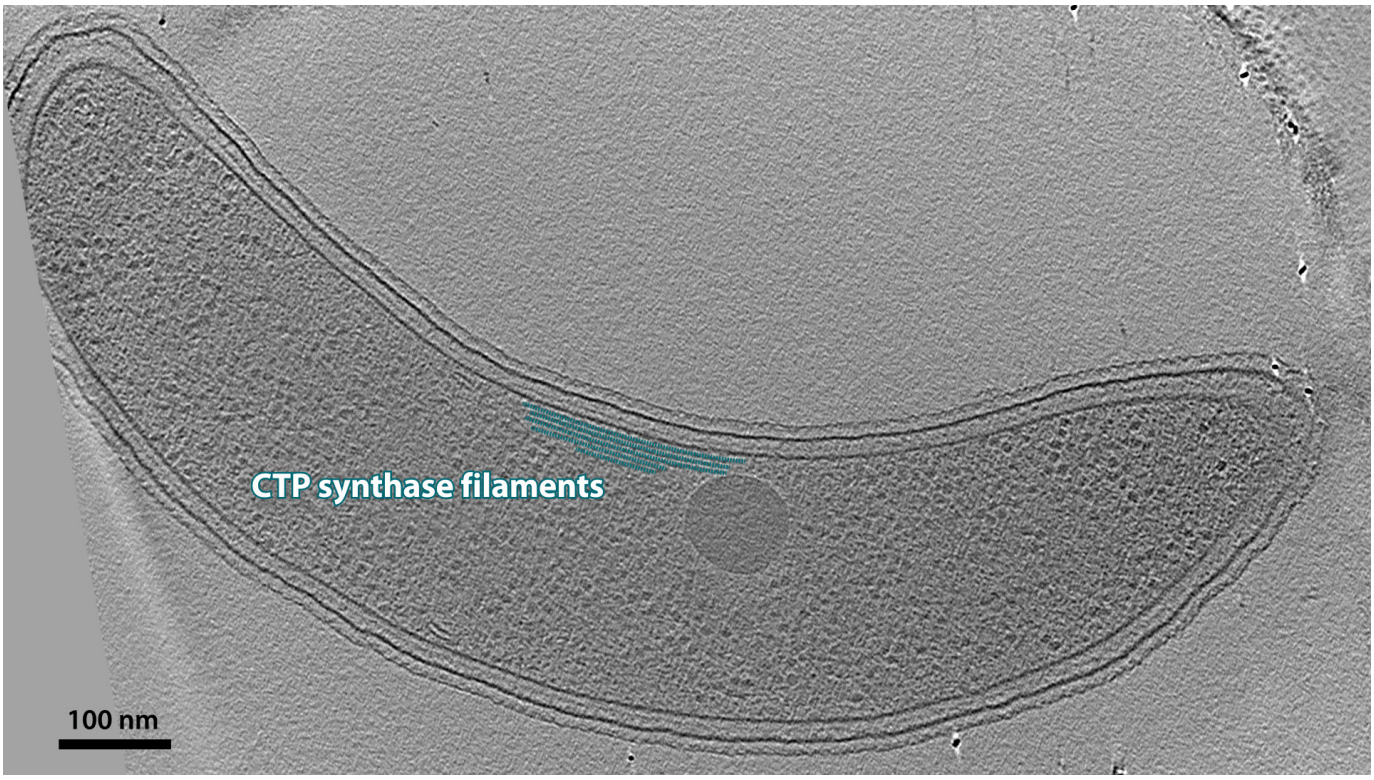

100 nm

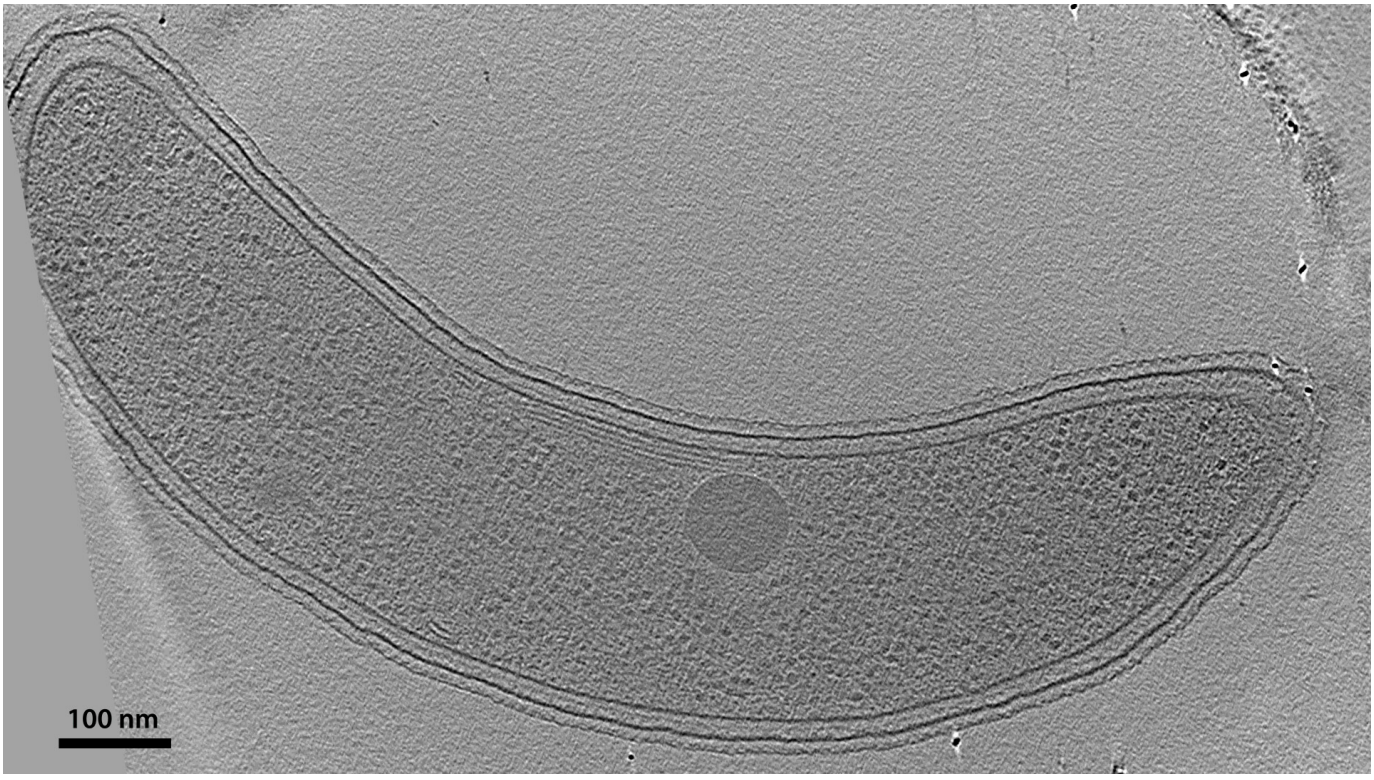
3. Length

Rod-shaped cells have a useful property: they can grow by extending their length without significantly changing the ratio of their surface area to volume, which would in turn change how efficiently they can take up nutrients from the environment. This property enables an impressive range of lengths for rod-shaped cells, from the short *Cupriavidus necator* you just saw, to this much longer *Hylemonella gracilis*.

The length of a cell (or, more generally, its size) varies depending on the environment or its stage of the lifecycle, but it does not vary much. Size tends to be strongly conserved within a species, ranging not much more than the factor of two dictated by replication. Sizes *between* species vary much more widely, as you will see throughout this book. Keep in mind, too, that the species we are able to image directly by cryo-ET are relatively small. Other species can be much larger, in some exceptional cases up to 100 μm across, visible to the naked eye.

Hylemonella gracilis Collected by: Yi-Wei Chang DOI: 10.22002/D1.1479


CTP synthase filaments
100 nm

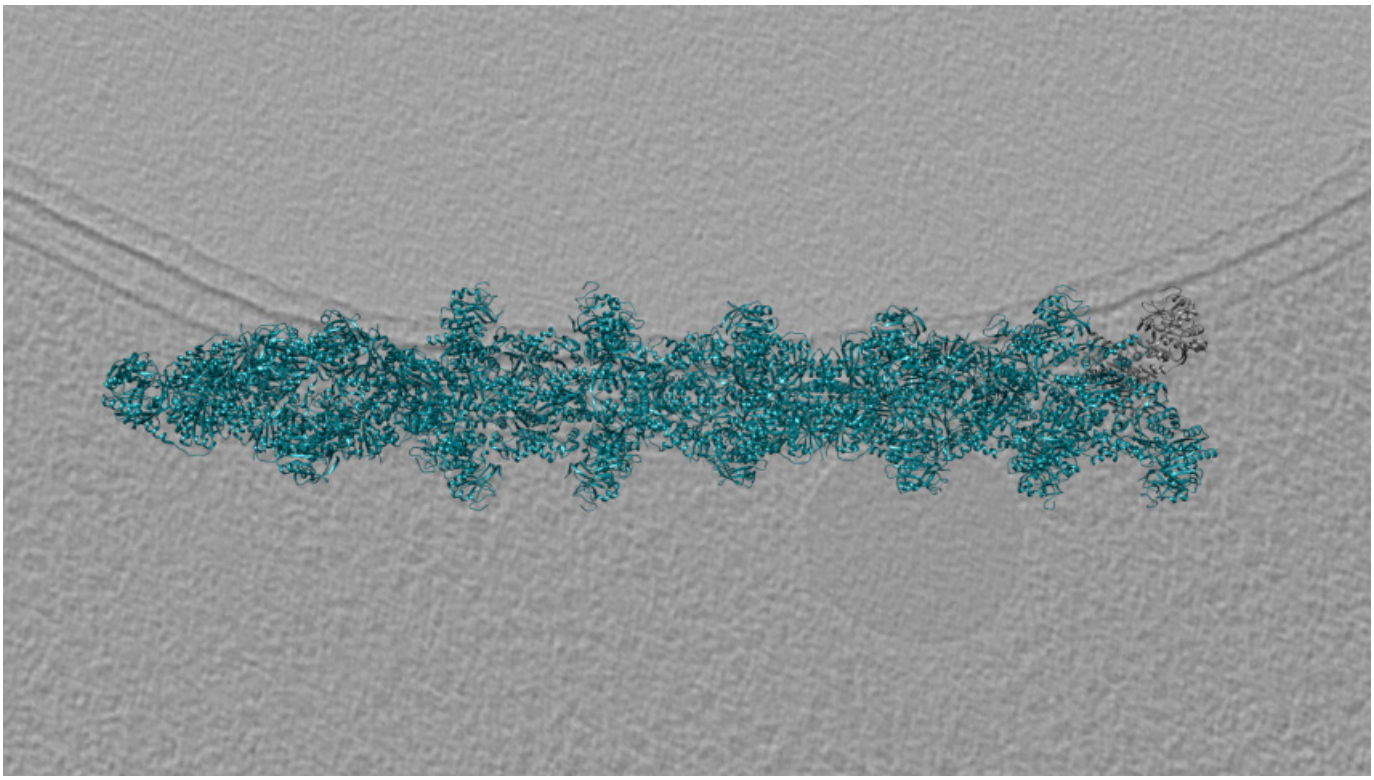

100 nm

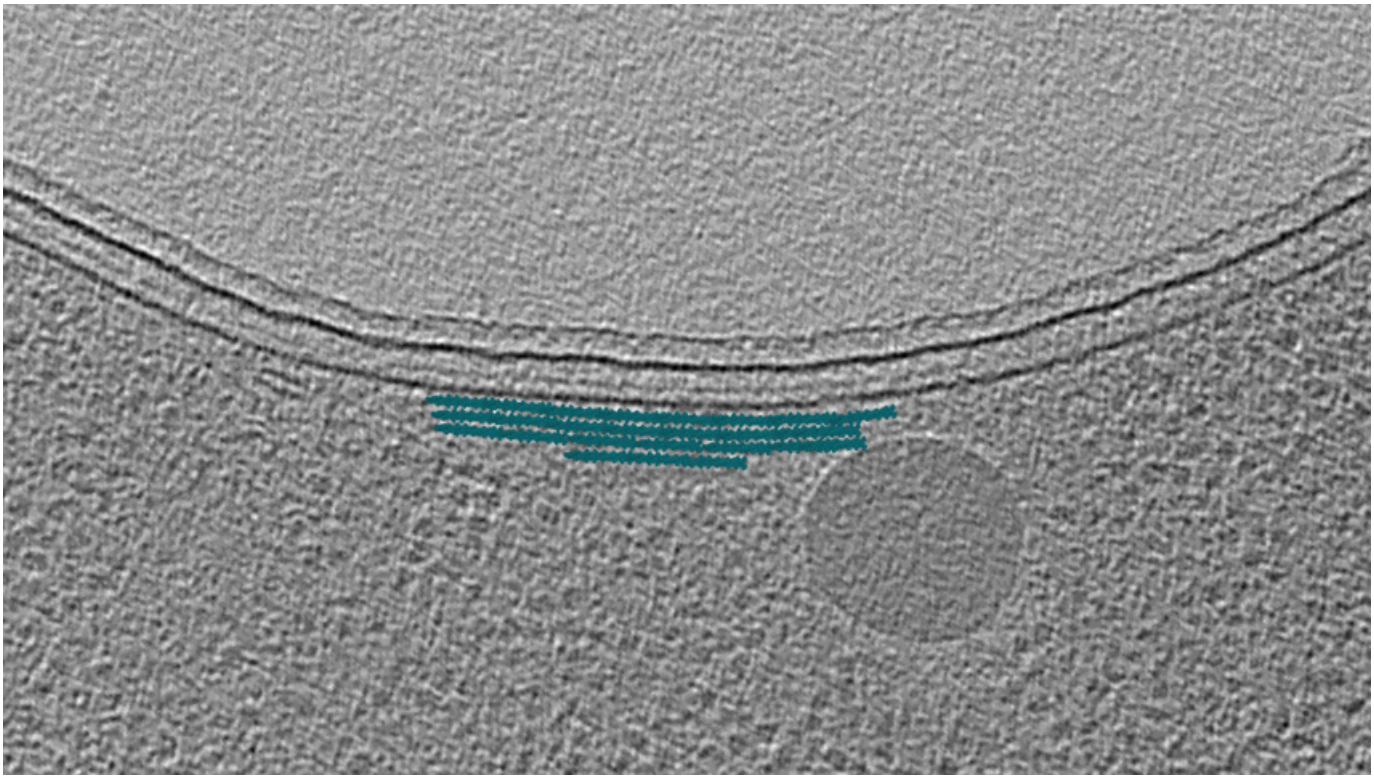
4. Vibrioid

What if you want to curve your rod-shaped cell into a comma? **Vibrioid** shape (named for the genus *Vibrio*, where it is common) may help cells swim faster. To make a vibrioid cell wall, you can imagine the contractor simply telling the workers to incorporate more material on one side of the rod relative to the other. In *Caulobacter crescentus* like this one, this is the function of two cytoskeletal proteins. The first, called (for an obvious reason) **Crescentin**, inhibits cell wall synthesis. It is kept in check by the second, called **CTP synthase**. (As its name implies, CTP synthase has another, metabolic, function in the cell (*Learn More* on the next page).) The balance between the two makes sure the cell curves, but not too much. Such checks and balances are a common theme in nature. Both cytoskeletal proteins localize to one side of the cell, resulting in more cell wall growth on the opposite side, and a curved cell. Here you can see a bundle of CTP synthase filaments on the inner curvature of the cell. The form of Crescentin is more elusive; we only see obvious filaments when it is artificially overexpressed, so its exact structure in the cell remains unclear.

This system is only one way of making a curved cell. There must be others since many vibrioid species (including *Vibrio*!) do not use Crescentin. As you will see throughout this book, there is no shortage of biological questions still to be figured out.

Caulobacter crescentus Collected by: Prabha Dias DOI: 10.22002/D1.1480

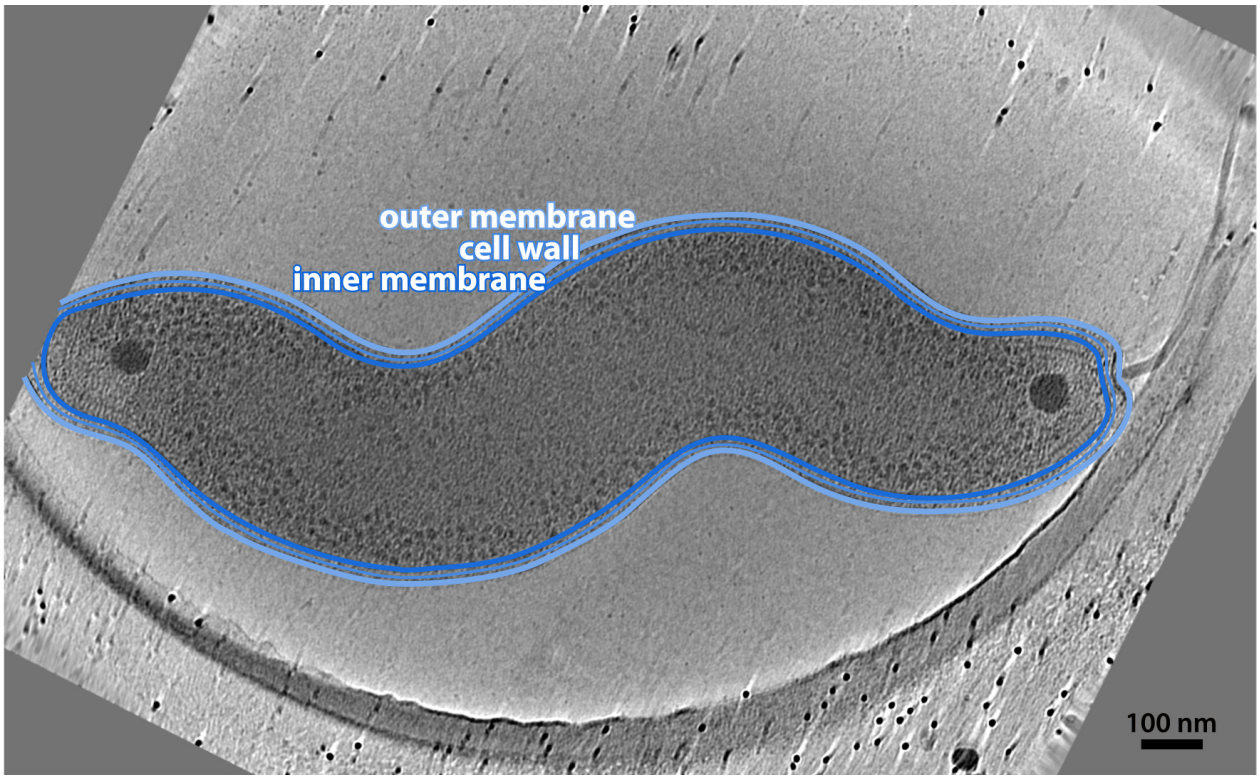


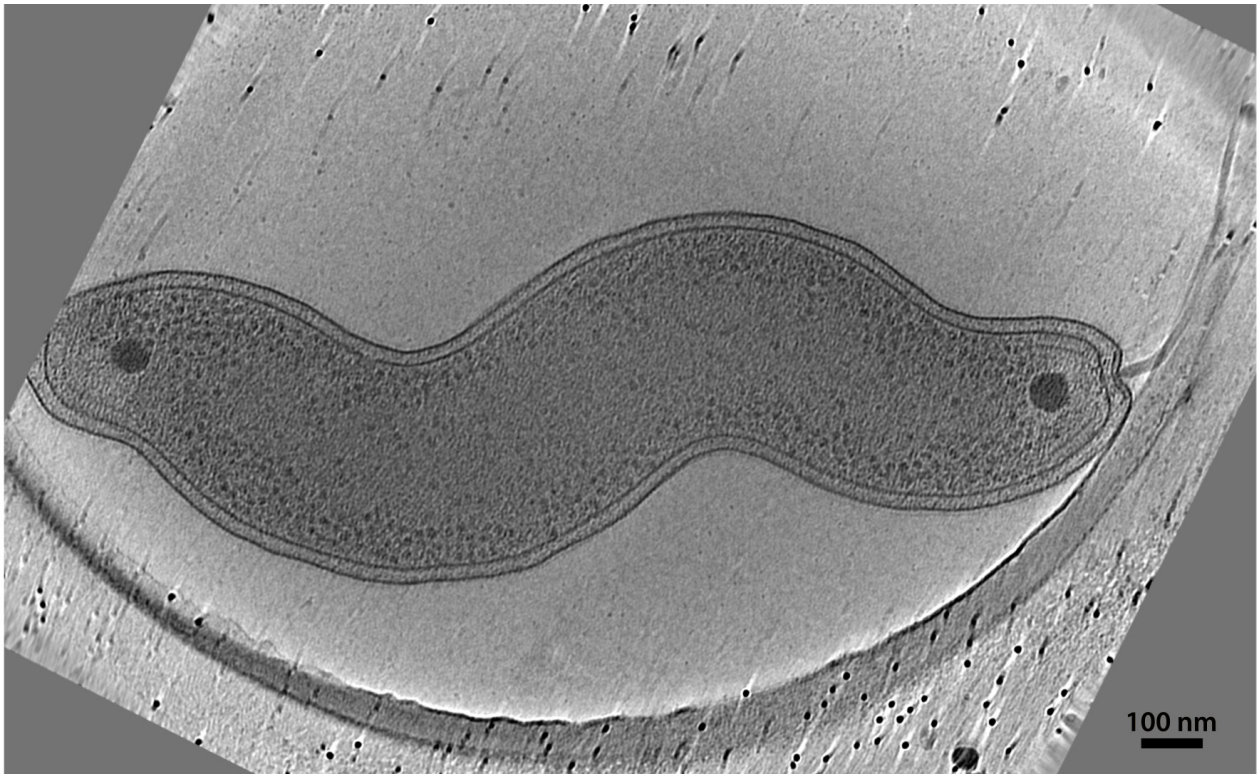


Learn More: CTP Synthase

CTP synthase is a ubiquitous metabolic protein found throughout all domains of life that helps make the building blocks of RNA and DNA. It also polymerizes into filaments. In eukaryotes, the filament structure activates the enzyme. In bacteria, the filament structure (shown here for Escherichia coli *[31]) inhibits the enzyme's metabolic function. Polymerization of enzymes is fairly common, providing an elegant way to quickly regulate the activity of a protein that may not always be needed, but would be costly or slow to degrade and synthesize again. You will see another example in Chapter 4. In the case of CTP synthase, the cytoskeletal role likely arose secondarily; once you have a long filament lying around, why not use it as a scaffold?*

Source: Lynch et al. (2017) *Structure: PDB 5U3C*


outer membrane
cell wall
inner membrane
100 nm

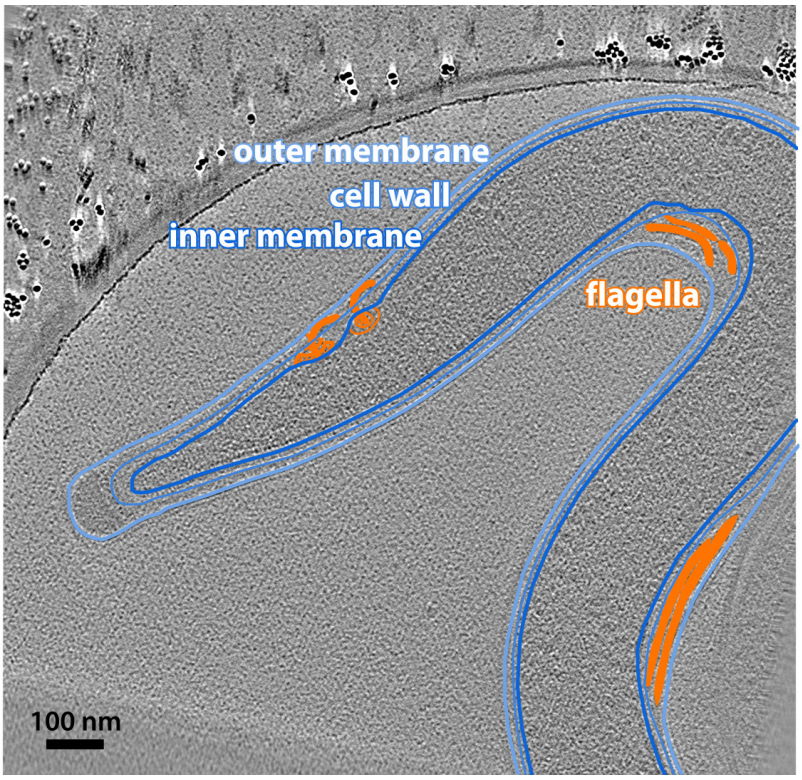

100 nm

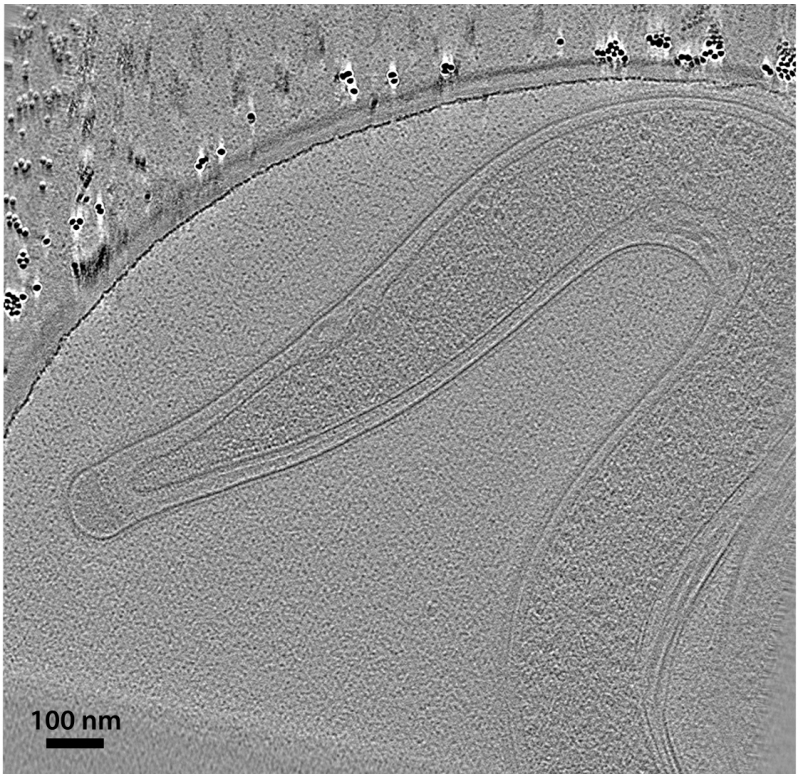
5. Helical

Why stop at a quarter turn when you can twist your cell into a full wave or even a corkscrew? Just as a corkscrew penetrates its target, helical pathogenic bacteria like this *Campylobacter jejuni* can burrow efficiently into the tissue of their target.

It can be tempting to group species based on a common characteristic, but appearances are often deceiving about relatedness. Undulating shape, for instance, was not a one-shot invention; it evolved independently multiple times. This is true of other bacterial and archaeal cell shapes as well. For wavy shape, these independent origins are reflected in different mechanisms of creating it. Some species, including *C. jejuni*, use dedicated proteins to regulate the pattern of peptidoglycan insertion–a continuation of the theme we have been discussing. Other species take different approaches (*Learn More* on the next page).

Campylobacter jejuni Collected by: Morgan Beeby DOI: 10.22002/D1.1481

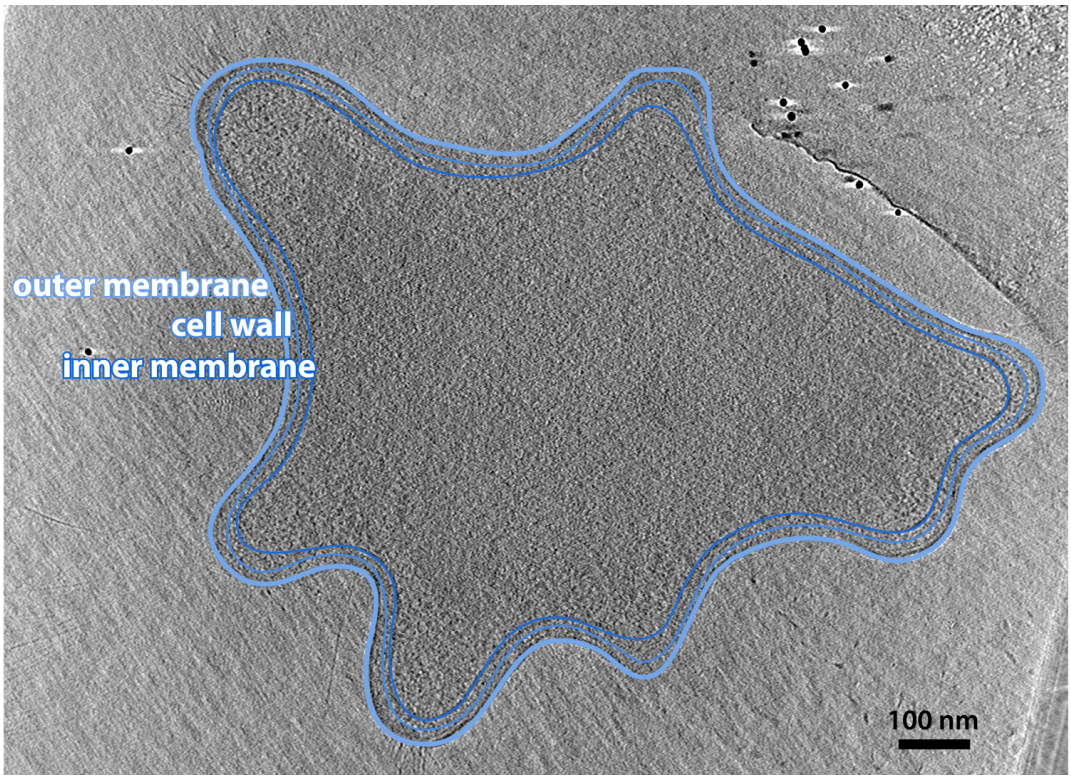

outer membrane
cell wall
inner membrane
flagella
100 nm

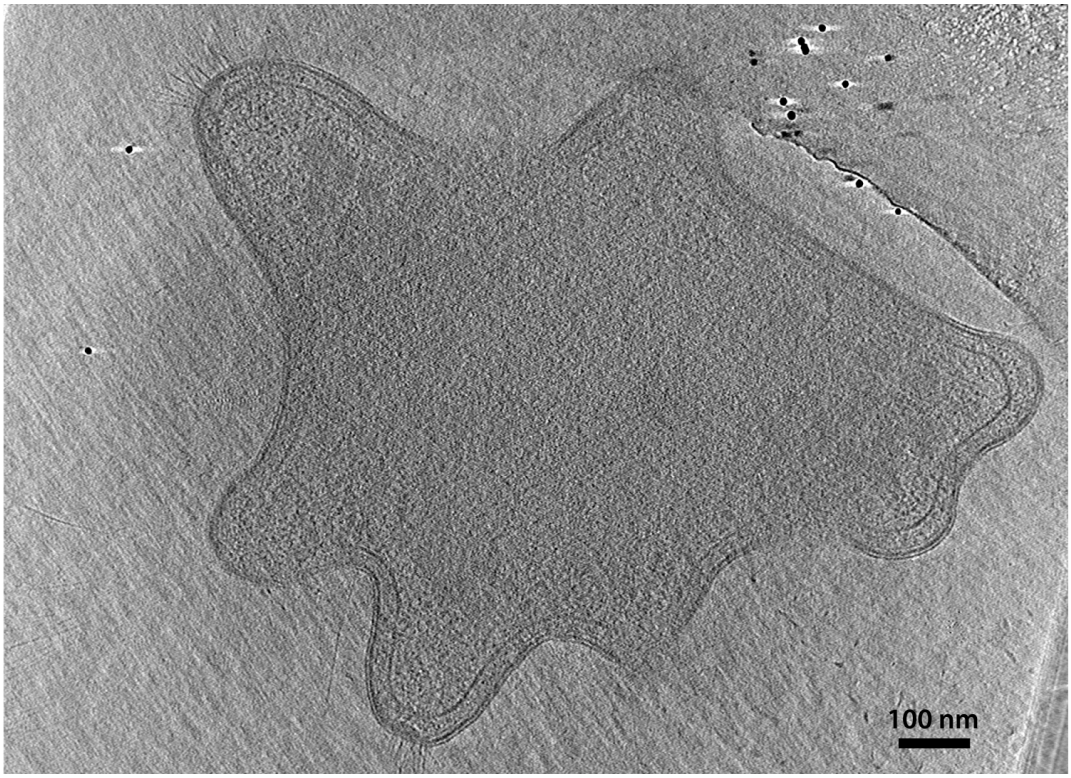

100 nm

Learn More: Borrelia Shape

Spirochete bacteria like this Borrelia burgdorferi *use the long filaments of their motility machinery (flagella, discussed in Chapter 6) as a kind of cytoskeleton. A bundle of flagella wraps around the cell in the periplasm, between the cell wall and the outer membrane. Spun by motors at their base (more on that in Chapter 6), the filaments impart a wave pattern to the growing cell wall. Without the motors' rotation, the cells develop a rod shape. The Spirochete phylum gets its name from this feature of spiral "hair" (the hair being the flagella). Note that* B. burgdorferi *are not helical like some other spirochetes, but rather adopt a two-dimensional waveform like that of a snake.*

Borrelia burgdorferi *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1486*


outer membrane
cell wall
inner membrane
100 nm

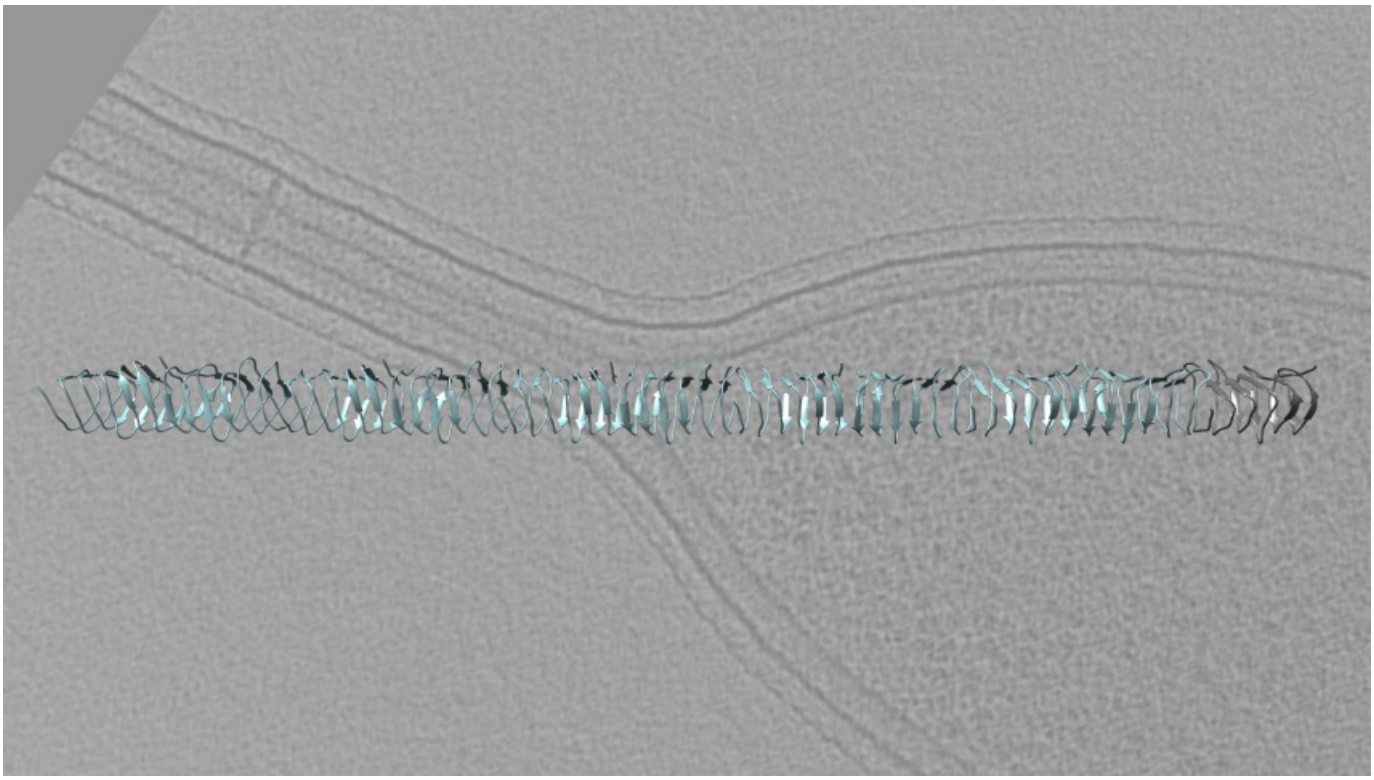

100 nm

6. Prosthecate

Motility is not everything. Another major force that shapes cells is metabolism. Nutrients are often scarce, and increasing your cell's ability to absorb them can give it a boost in the competitive game of life. So how can you do that? Remember that a sphere maximizes volume relative to surface area. To maximize surface area (for nutrient uptake) relative to volume, you would instead want something spikier. Some bacteria extend **prosthecae** ("add-ons" or appendages) for this purpose. Some, like *Caulobacter crescentus*, use a single prostheca, which is also called a **stalk**. Stalks are commonly located at the pole of the cell, where, as you'll see in Chapter 8.4, they can help cells attach to a surface and hang on even in turbulent flow. Other species have a stalk on either end. Still others, like this *Verrucomicrobium spinosum*, form astral shapes with prosthecae jutting out in all directions.

Prosthecae offer an architectural challenge: thin extensions are delicate. Prosthecate cells use cytoskeletal proteins to form and stabilize their stalks, although exactly how this works remains unclear. One of these cytoskeletal proteins is Bactofilin (*Learn More* on the next page), which is similar to the proteins that make intermediate filaments in eukaryotes. *C. crescentus* use Bactofilin polymers to help make their stalks. *Prosthecobacter* contain a different cytoskeletal element–microtubules–in their stalks, the function of which remains unclear (*Learn More* on following pages).

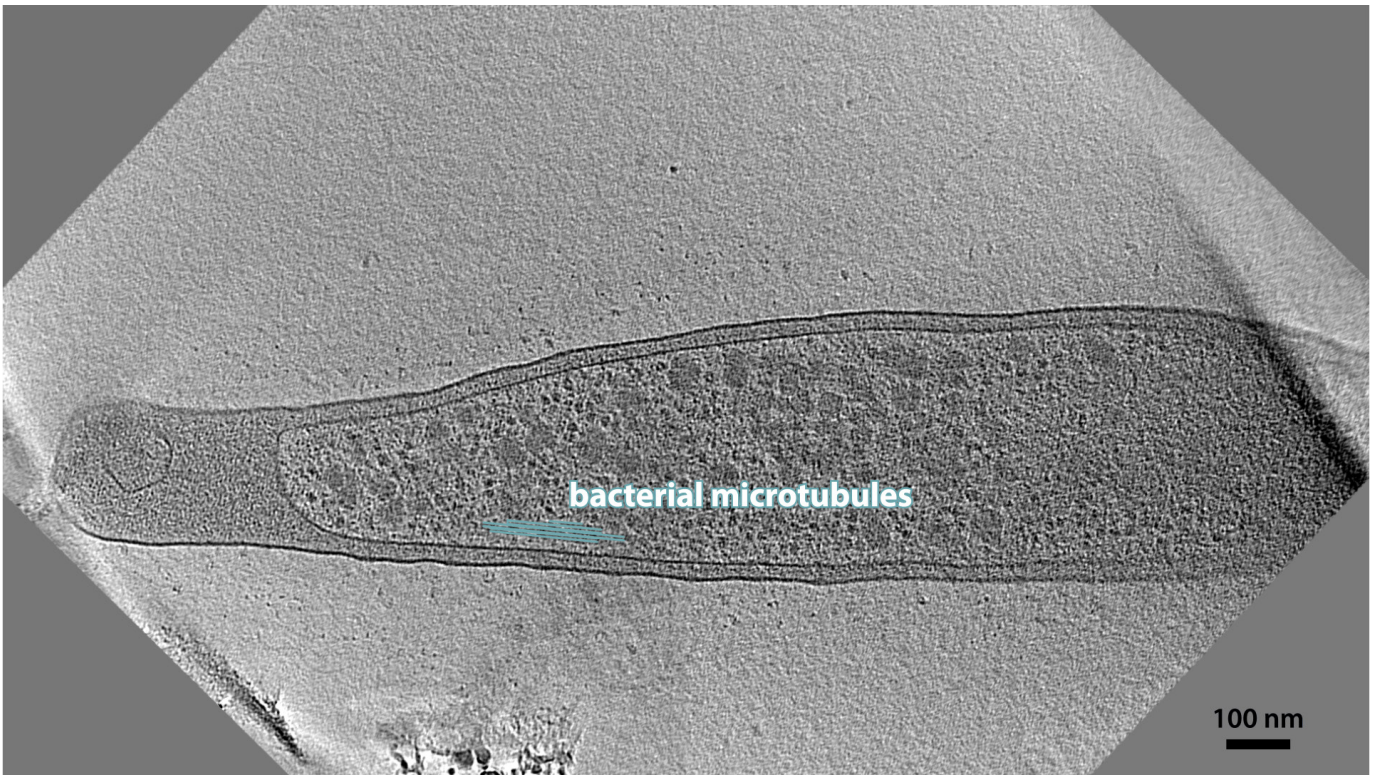
Verrucomicrobium spinosum Collected by: Martin Pilhofer DOI: 10.22002/D1.1482

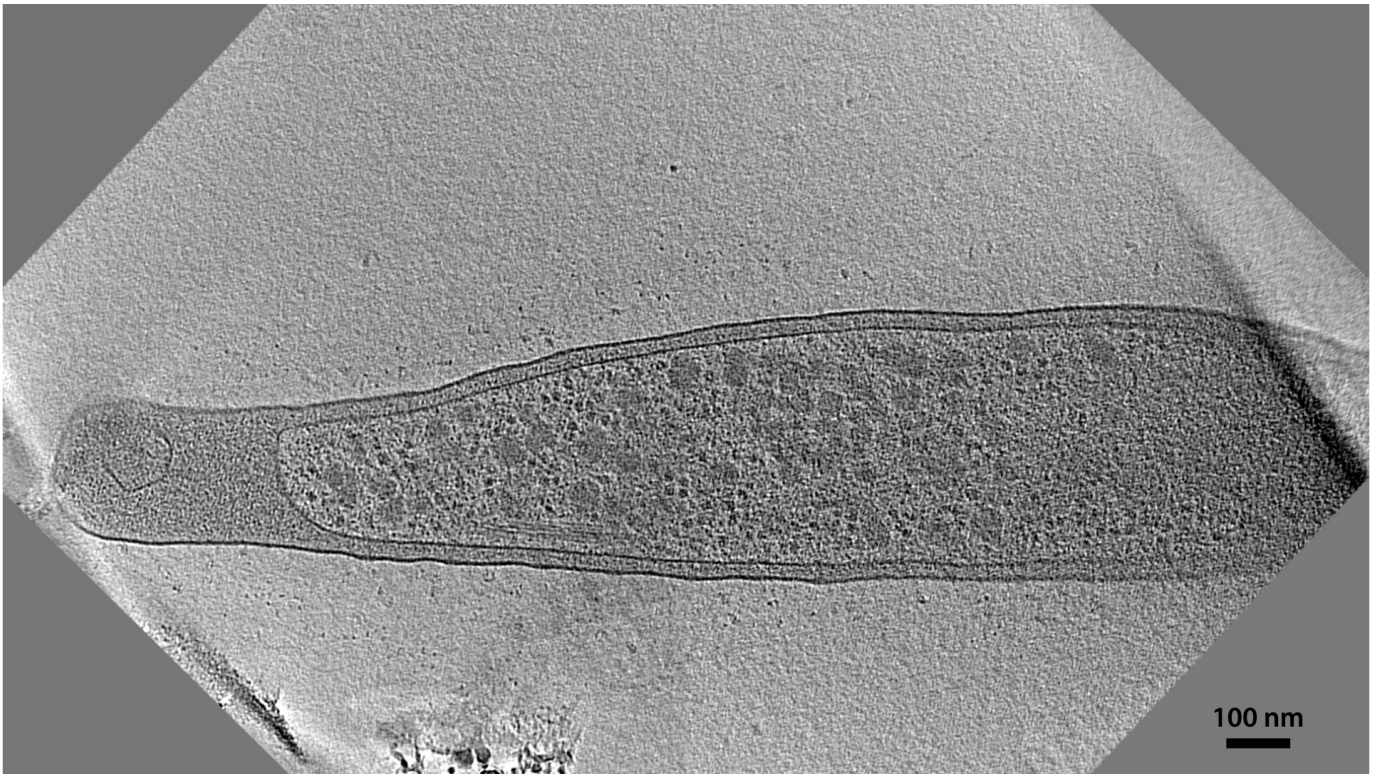


Learn More: Bactofilin

Bactofilins are found in many species of bacteria and archaea, suggesting that they perform diverse (and currently unknown) functions. They polymerize into very stable filaments with a triangular beta-helical structure, like this one from Thermus thermophilus *[32]. Bactofilin filaments lack two hallmarks of actin- and tubulin-based cytoskeletal elements: polarity and dynamic assembly/disassembly. In this way, they are similar to intermediate filaments in eukaryotic cytoskeletons.*

Source: Deng et al. (2019) *Structure: PDB 6RIB*


bacterial microtubules
100 nm

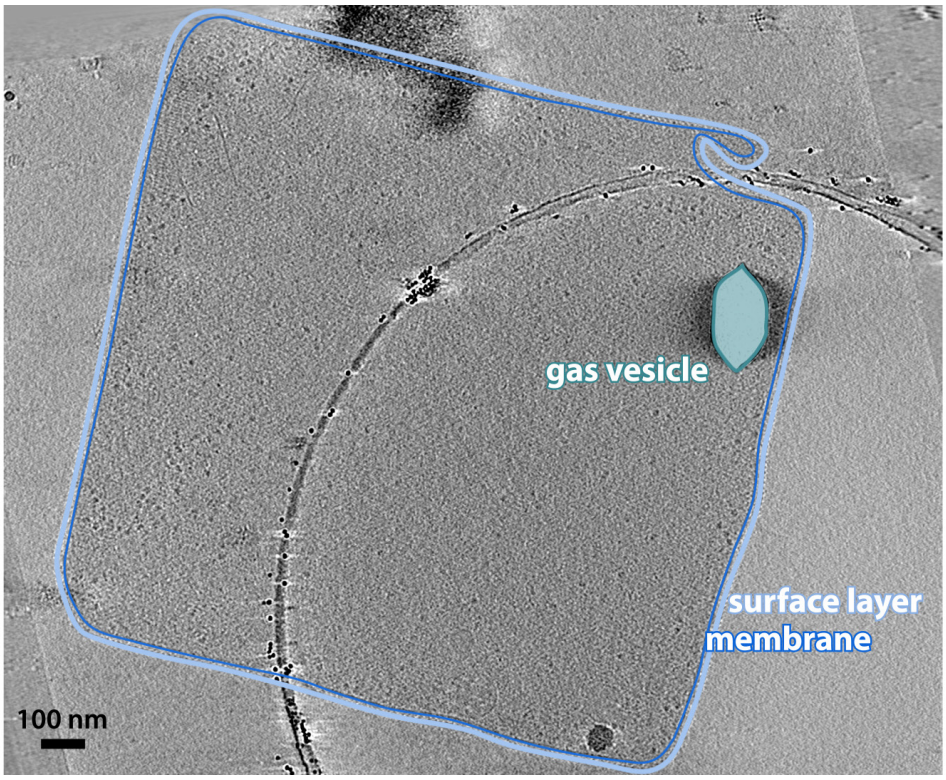

100 nm

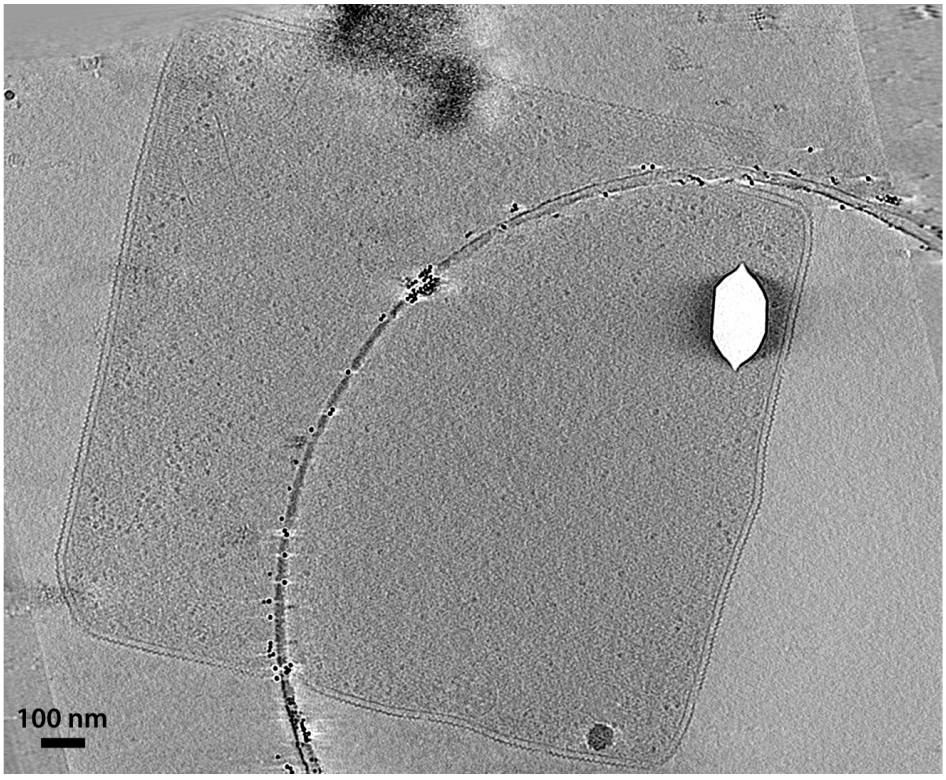
Learn More: Microtubules

Some bacterial species with prosthecae express structures similar to eukaryotic microtubules, made from two proteins called BtubA and BtubB to reflect their homology to eukaryotic tubulins. Eukaryotic microtubules are hollow tubes formed by 13 protofilaments; bacterial microtubules are smaller, with only ~5 protofilaments. Cells commonly contain a bundle of microtubules in their prosthecae, like this Prosthecobacter vanneervenii *cell, which has a bundle of four.*

Prosthecobacter *belong to an evolutionarily unique group of species that share characteristics unusual in the rest of the bacterial phylogenetic tree. We refer to the collective group as the PVC superphylum (because it contains* Planctomycetes, Verrucomicrobia, *and* Chlamydiae*). Having homologs of eukaryotic microtubule proteins is one of these unique characteristics; so far, Btubs have only been identified in species of* Prosthecobacter. *They seem to have come from a horizontal gene transfer from a eukaryotic cell (meaning that microtubules evolved first in eukaryotes and were later borrowed by the bacteria).*

Prosthecobacter vanneervenii *Collected by: Martin Pilhofer* *DOI: 10.22002/D1.1487*


gas vesicle
surface layer
membrane
100 nm

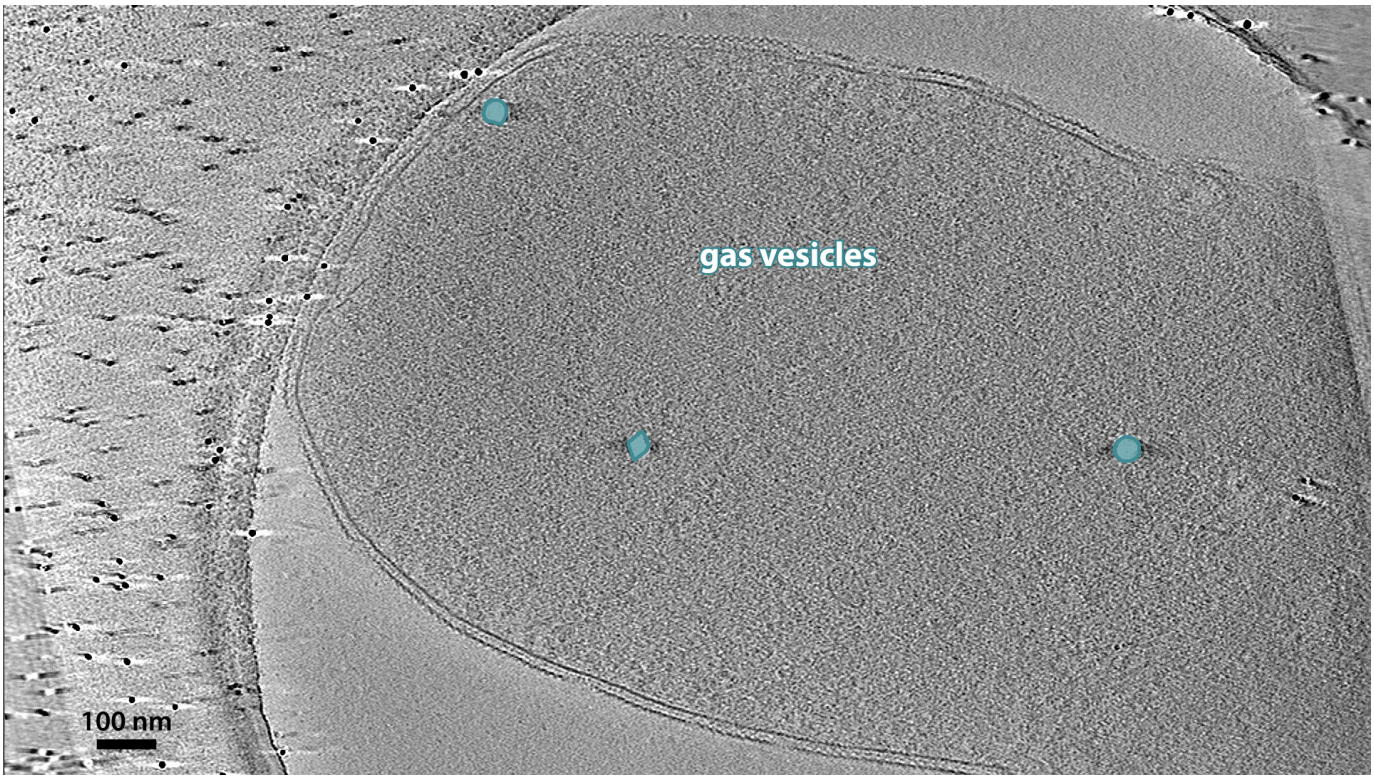

100 nm

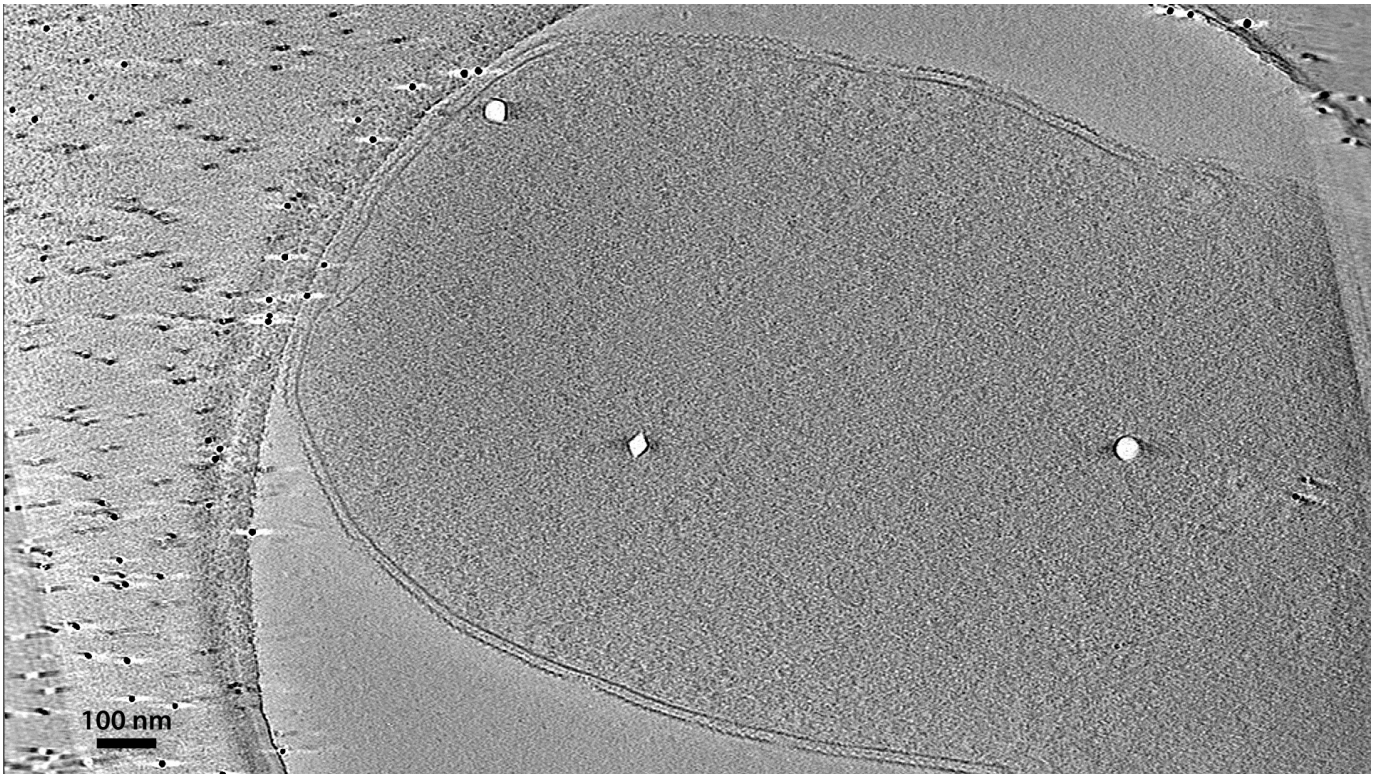
7. Square

So far we have focused on bacteria, but archaea hold their own in the specialized shape competition. In fact, one of the most extreme examples of maximizing surface area relative to volume comes from this archaeon, *Haloquadratum walsbyi*, which grows as thin, square tiles. *Very* thin, square tiles. This property helps keep them oriented with their broad sides to the sun, whose light they rely on for photosynthesis. To float at the surface of the super-salty lakes where they live, they use gas vesicles (*Learn More* on the next page).

We still do not know exactly how this shape is determined, but at least part of the mechanism seems to involve glycoproteins on the cell's surface layer.

Haloquadratum walsbyii Collected by: Zhuo Li DOI: 10.22002/D1.1483


gas vesicles
100 nm

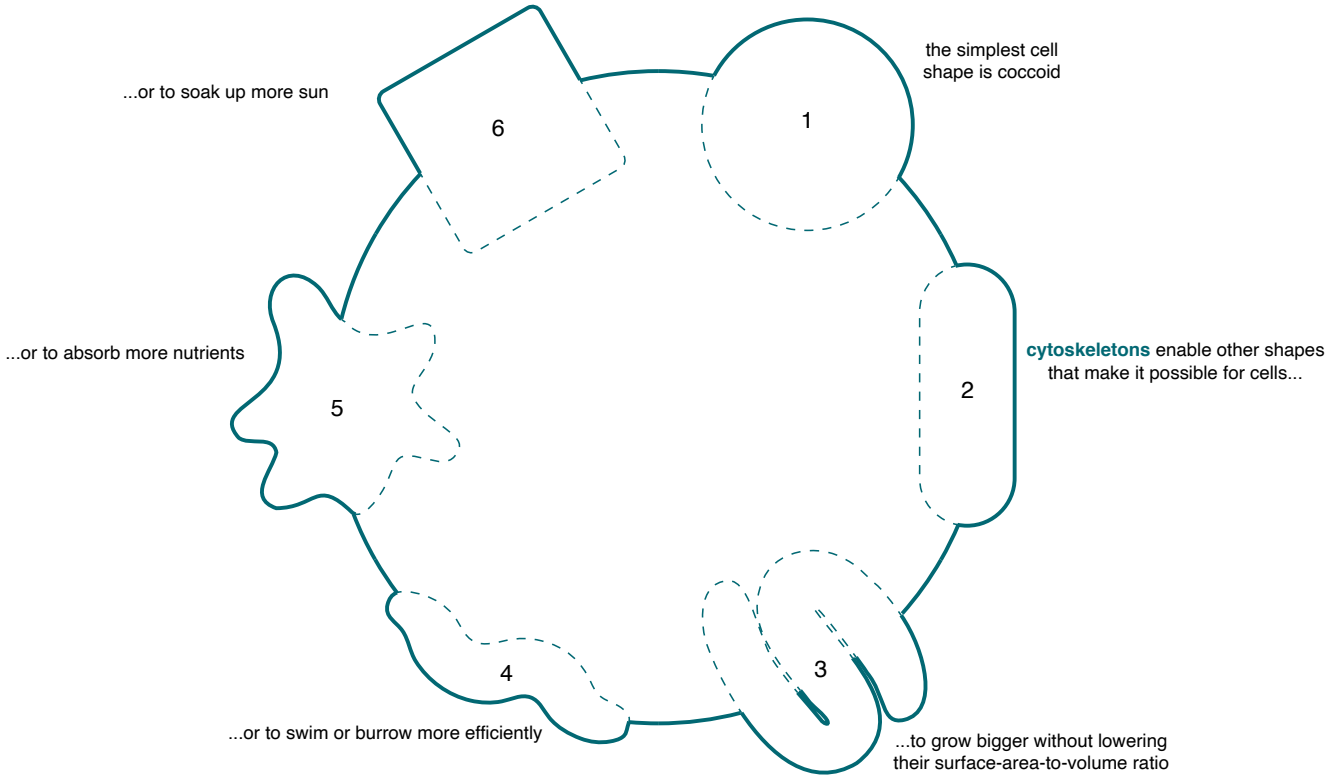

100 nm

Learn More: Gas Vesicles

Some species of archaea and bacteria use ***gas vesicles*** *to control their buoyancy. This can allow them to rise or fall in a water column, which can be a great advantage.* Halobacterium salinarum *like this one produce gas vesicles in response to cues from the environment, lifting themselves out of the sediment and into more favorable conditions of oxygen or sunlight for photosynthesis. This cell has just started producing gas vesicles, so they are small and isolated. Later, vesicles elongate into larger spindles or cylinders with conical ends, as you saw in* Haloquadratum walsbyi. *Each cell might contain dozens of vesicles, and they often cluster together.*

Gas vesicles are microcompartments enclosed by a hydrophobic shell made of a single layer of protein. (Sometimes some additional proteins reinforce the shell.) You will see more examples of microcompartments in Chapter 4. Gas vesicles do not actively store gas; they simply allow gas dissolved in the cytoplasm to diffuse in, while forming a tight barrier against anything else, like water. They are fragile and prone to collapse with even a slight increase in the surrounding pressure.

Halobacterium salinarum *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1488*


the simplest cell shape is coccoid
1
cytoskeletons enable other shapes that make it possible for cells...
2
...to grow bigger without lowering their surface-area-to-volume ratio
3
...or to swim or burrow more efficiently
4
...or to absorb more nutrients
5
...or to soak up more sun
6

Summary

Concept Check Questions

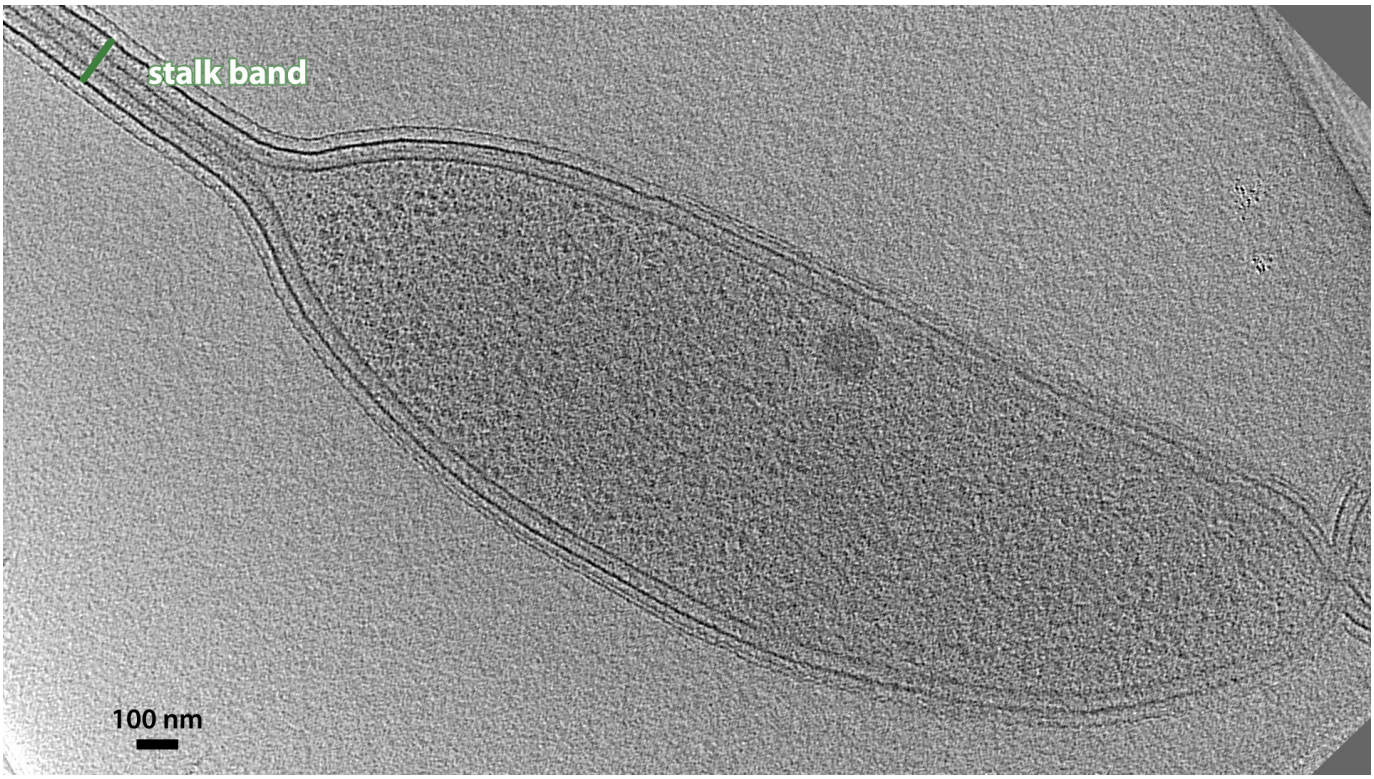
- What shape maximizes volume relative to surface area?
- What is a cytoskeletal element?
- How could a cytoskeletal element grow a rod shape from a coccoid?
- How could a cytoskeletal element grow a vibrioid shape from a rod?

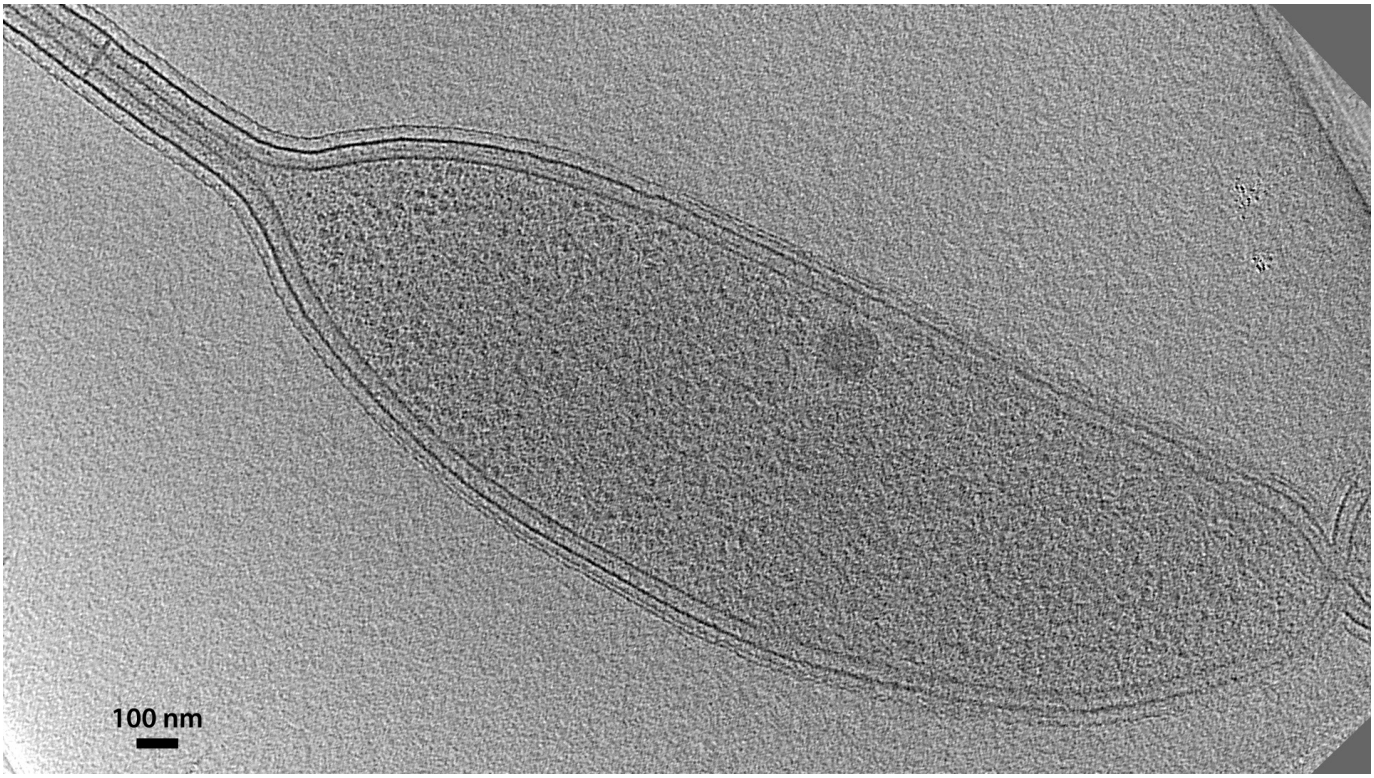
Further Reading

- Barry and Gitai (2011). *Self-assembling enzymes and the origins of the cytoskeleton* [33].
- Pfeifer (2012). *Distribution, formation and regulation of gas vesicles* [34].
- Pilhofer and Jensen (2013). *The bacterial cytoskeleton: More than twisted filaments* [35].
- Young (2006). *The selective value of bacterial shape* [30].

4
Growth

"Indeed, the entire cell can be viewed as a factory that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines."
- Bruce Alberts [36]


stalk band
100 nm

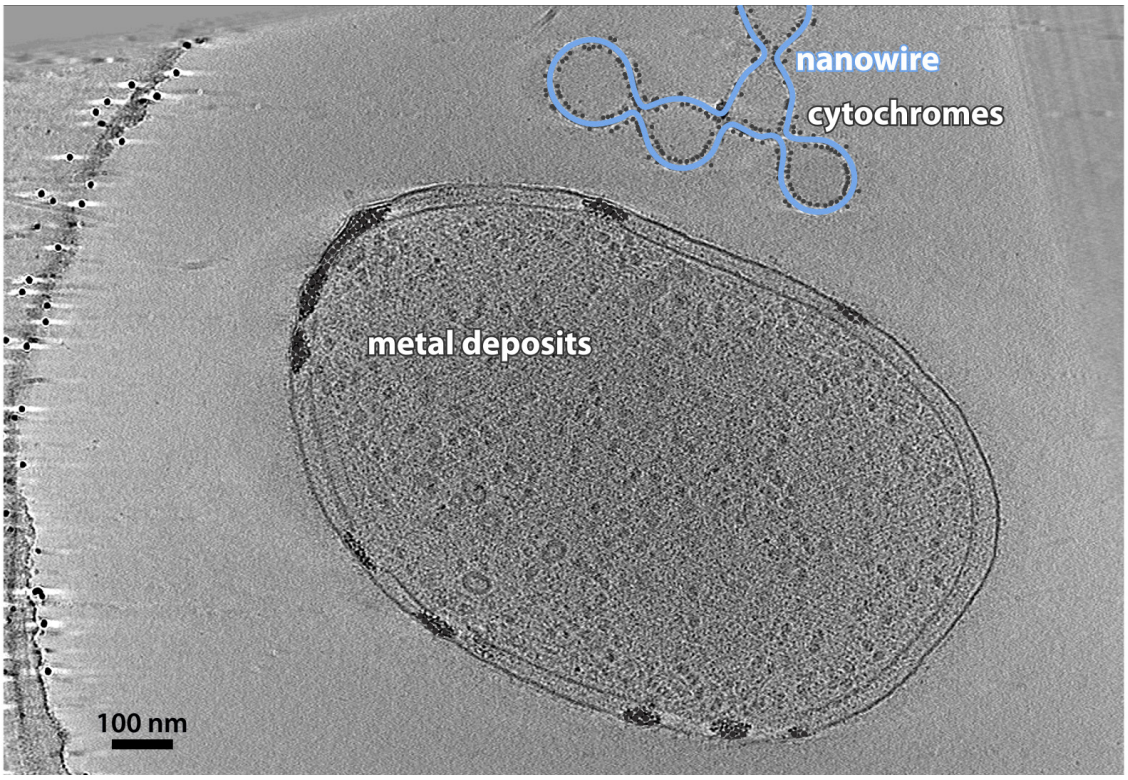

100 nm

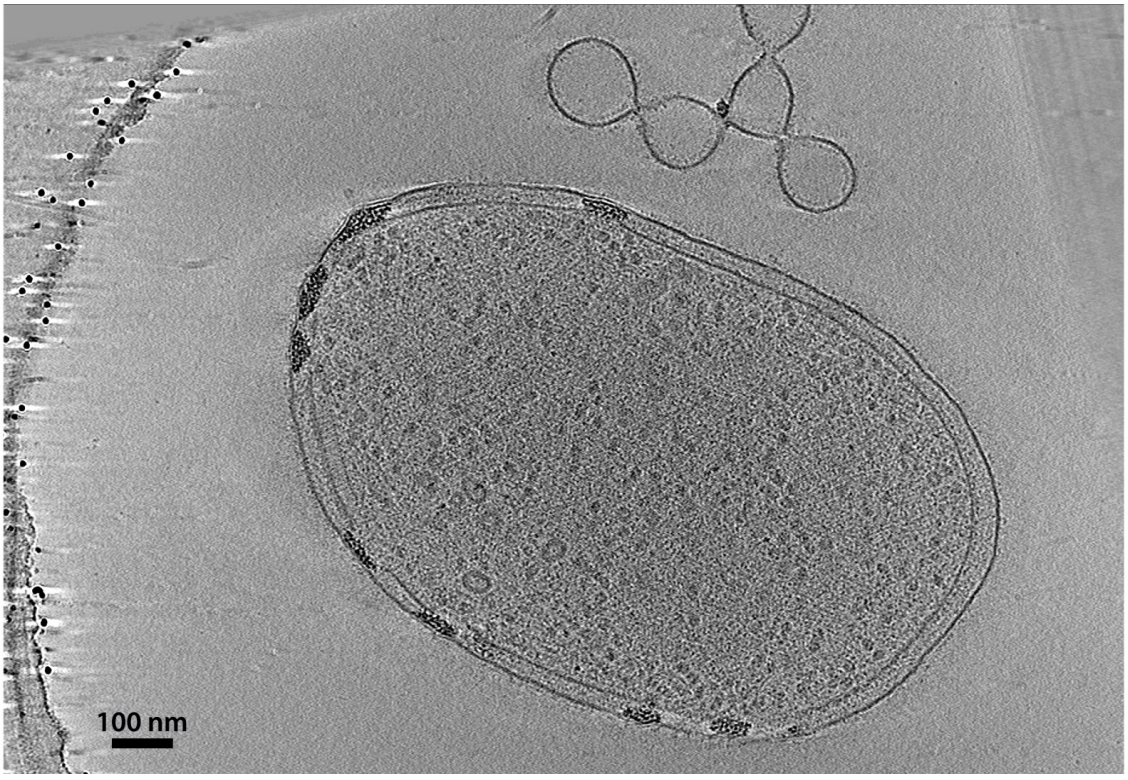
1. Stalk Bands

The life of a cell is simple in theory, and complex in practice. The evolutionary purpose of your cell is to grow, ultimately amassing enough resources to produce all the macromolecules needed to make another cell. How best to grow, though, depends on the environment and what fuel is available. Rather than focusing on the chemical reactions of metabolism, which are well covered in other texts, in this chapter we will examine a few architectural features that enhance growth in various conditions. In a world of scarce resources and fierce competition, any adaptation that lets your cell grow more efficiently can have a big effect on its success. And often, these adaptations are visible in the structure of the cell.

You have already seen how the shape of your cell can allow it to gather nutrients from the environment more efficiently. For example, prosthecate bacteria like this *Caulobacter crescentus* use long, thin extensions to increase surface area relative to volume, allowing them to absorb more nutrients. The extra surface area of a stalk increases a cell's ability to absorb nutrients, but it also adds more membrane, diluting membrane proteins and increasing the time it takes them to diffuse around the cell. *C. crescentus* stalks get longer throughout their lifetime, so the situation only gets more extreme with age. To solve this problem, you might want to separate the envelope of the stalk from the envelope of the rest of the cell. *C. crescentus* has evolved a structure to do just this, called a **stalk band**. These protein structures form diffusion barriers for the membranes and periplasm, but not the cytoplasm, so nutrients can still diffuse into the cell body. Each cell division produces another band in the elongating stalk, so you can tell a cell's divisional age by counting its bands.

Caulobacter crescentus Collected by: Zhuo Li DOI: 10.22002/D1.1489


nanowire
cytochromes
metal deposits
100 nm


100 nm

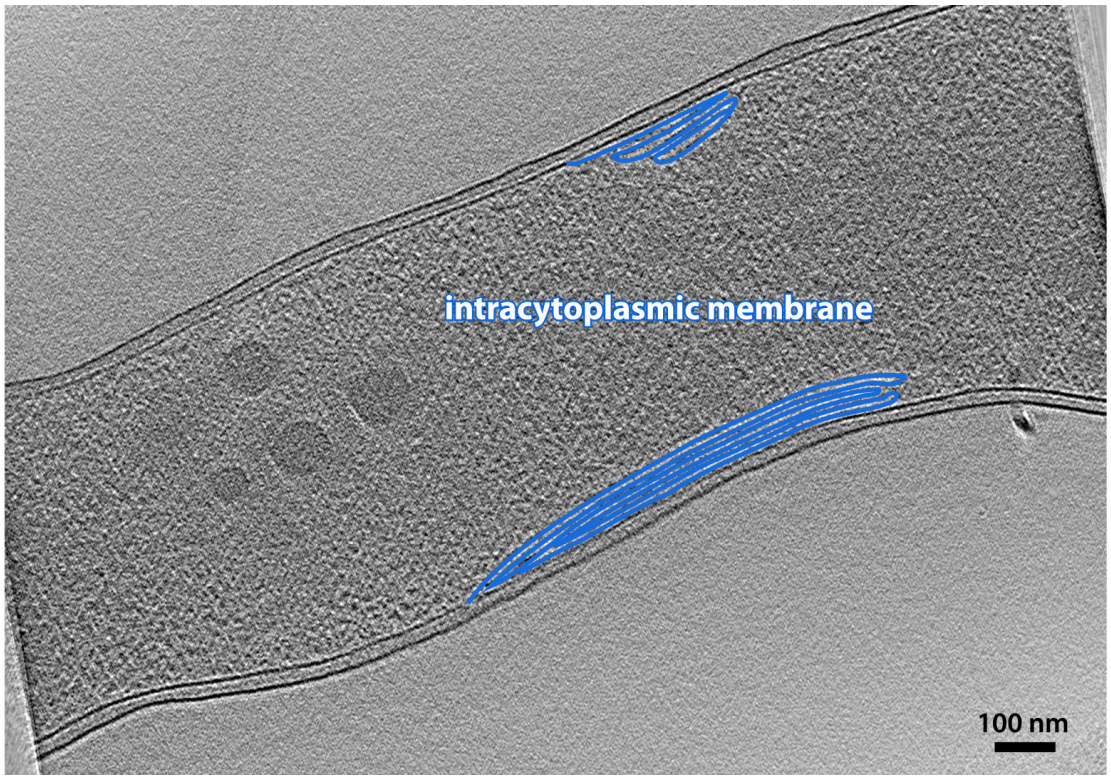
2. Nanowires

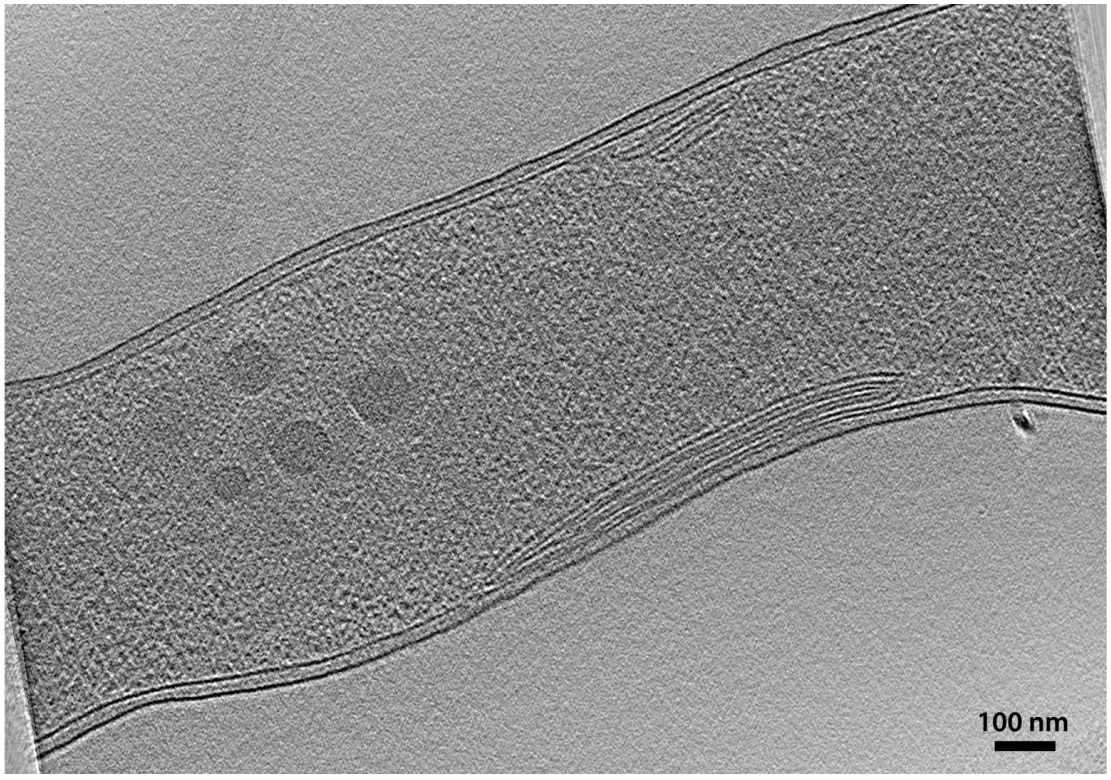
Other cell extensions also aid metabolism. Non-photosynthesizing cells break down nutrients into chemical energy (ATP) through respiration reactions. The chemistry is beyond our scope here, but it involves the ultimate transfer of electrons to an acceptor molecule, typically oxygen (the "aer" in aerobic respiration). When no oxygen is present, some cells can use an alternative electron acceptor, such as iron or sulfur, in a process called anaerobic respiration. This works well when the acceptor is soluble and can diffuse into the cell. You can see deposits of soluble metal on the membrane of this *Shewanella oneidensis* cell which it is likely using for anaerobic respiration.

What if the only available acceptor is trapped in a mineral, though? *S. oneidensis* have evolved a mechanism to take their metabolism to the mineral by extending chains of outer membrane vesicles. Electron-carrying proteins called cytochromes in the membrane shuttle electrons to metal oxide minerals in the environment, a conductive property that lends the appendages the name "**nanowires**." The morphology of *S. oneidensis* nanowires is similar to that of other outer membrane vesicle chains, with tubular and pearled sections, like the detached nanowire you see here.

Other species, like *Geobacter sulfurreducens*, also use nanowires to transfer electrons to an insoluble acceptor in the environment, but the structure of those nanowires is very different: a long filament made from stacked cytochrome proteins, resembling a pilus (thin protein filaments you will see in Chapters 6 and 9).

Shewanella oneidensis Collected by: Poorna Subramanian DOI: 10.22002/D1.1490


intracytoplasmic membrane
100 nm

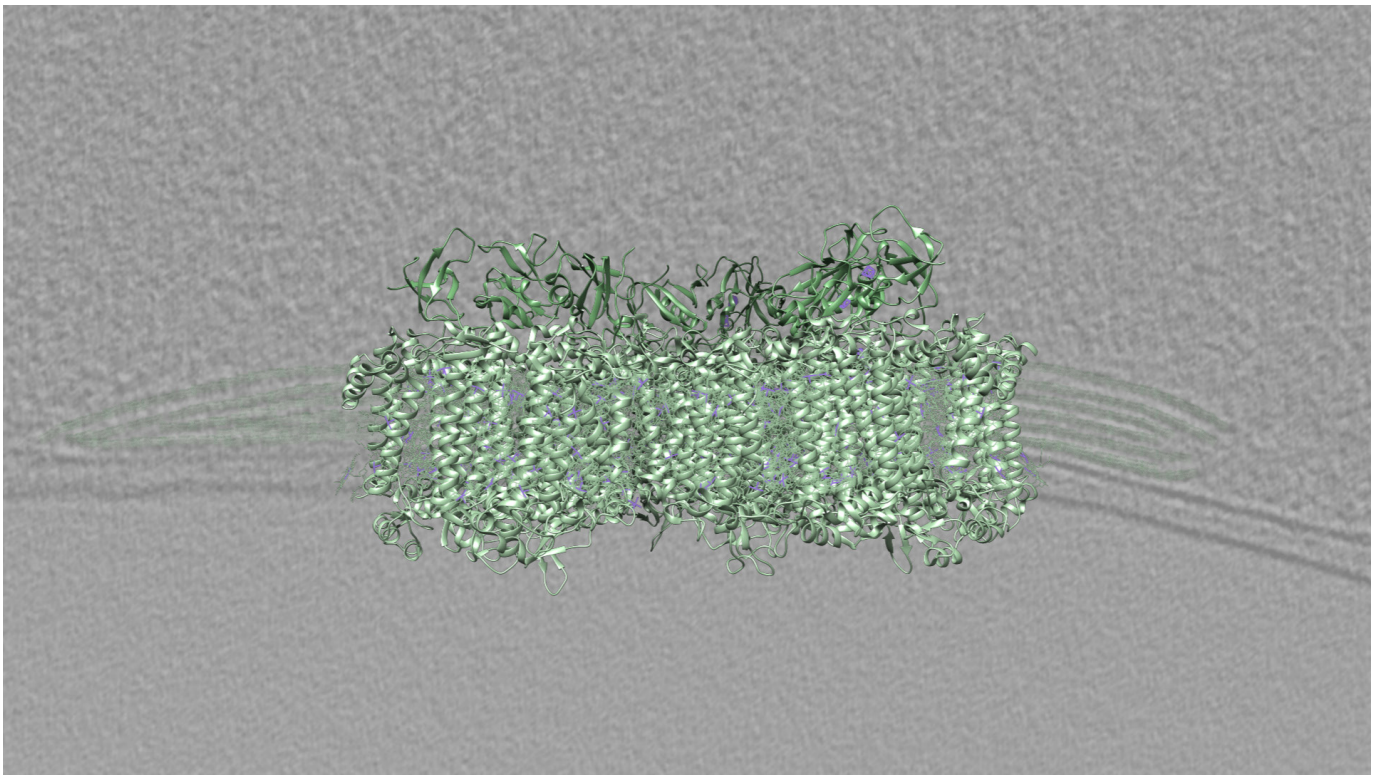

100 nm

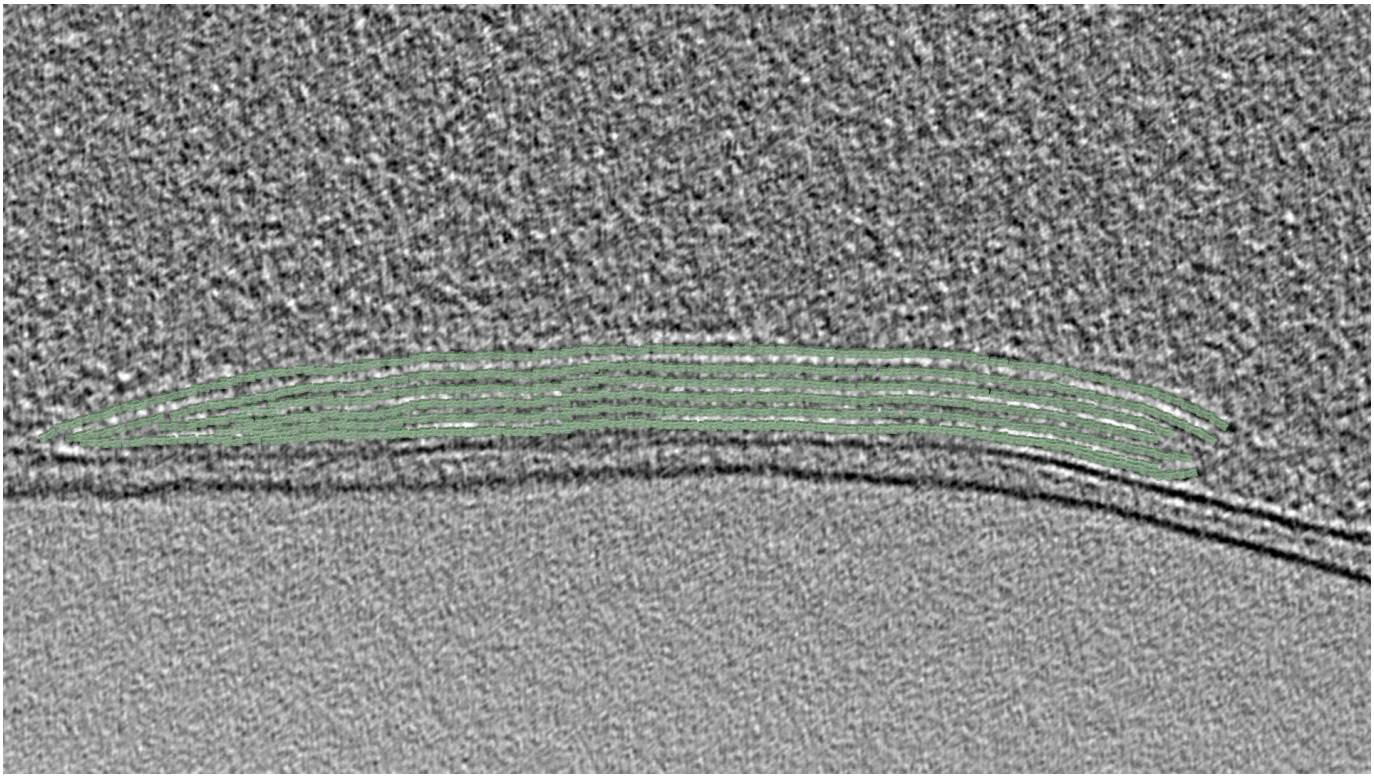
3. Intracytoplasmic Membrane

Photosynthetic bacteria harvest energy from sunlight using protein complexes (*Learn More* on the next page) embedded in their membrane. An easy way to increase the light-gathering capability of such a cell would be to expand the membrane, but then the cell's volume would also increase. To get around this problem, why not stack the extra membrane inside the cell? Many (but not all) photosynthetic bacteria contain intracytoplasmic membrane, or **ICM**. In cells like this *Rhodopseudomonas palustris*, the ICM is continuous with the inner membrane, forming stacks at the middle of the cell. These stacks grow and shrink as needed, depending on light conditions and the cell's metabolic state. Different species have different, and more extensive, arrangements (*Learn More* on following pages). ICM may remind you of the thylakoid membranes of eukaryotic chloroplasts, and for good reason; chloroplasts evolved from photosynthetic bacteria that likely lived symbiotically inside a eukaryotic precursor of modern plants.

Other photosynthetic bacteria have taken the specialization of their light-harvesting membranes a step further, expanding not the entire membrane, but simply the hydrophobic interior. The result is a sort of factory called a **chlorosome** with hundreds of thousands of photocomplexes packed tightly inside a lipid monolayer. Chlorosomes, and other compartments you will see soon, challenge the idea that organelles exist only in eukaryotes.

Rhodopseudomonas palustris Collected by: Alasdair McDowall DOI: 10.22002/D1.1491

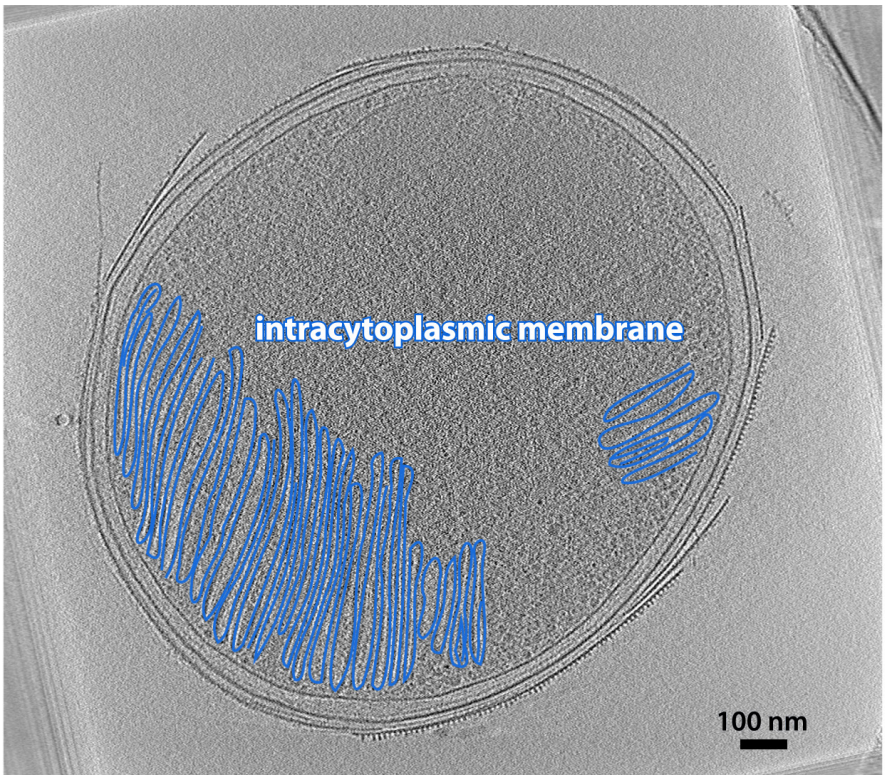


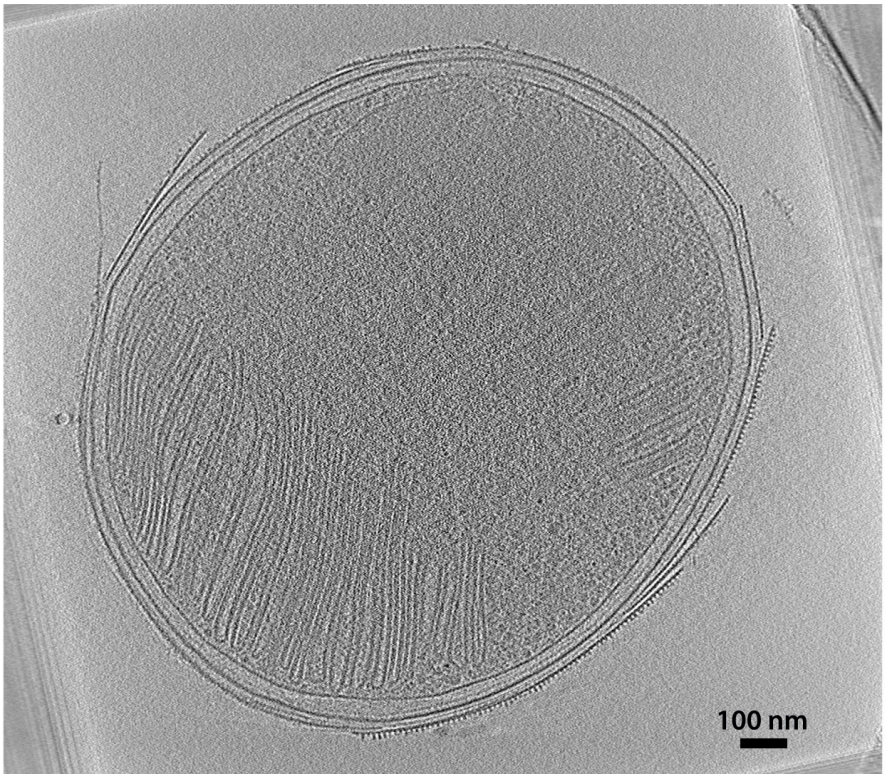


Learn More: Photocomplexes

Light-harvesting photocomplexes, like this one from a red alga (the machinery is closely related to that found in cyanobacteria) [37], can comprise hundreds of individual proteins and reach tens of nanometers in size. Physically tethering enzymes that function in the same pathway increases efficiency by allowing enzymes that catalyze subsequent steps to hand off substrates. You will see more examples of this theme in the following pages; metabolic enzymes in the cell often cluster together into relay teams.

Source: Ma et al. (2020) *Structure: PDB 6KGX*

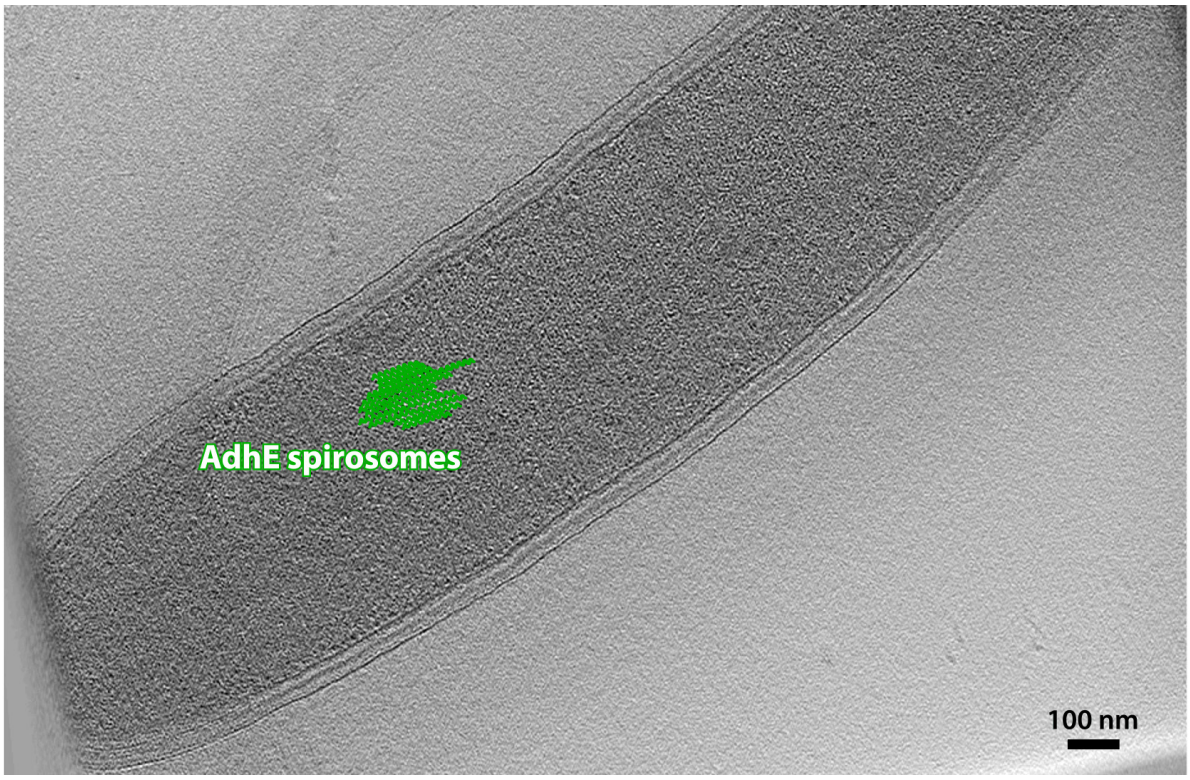

intracytoplasmic membrane
100 nm

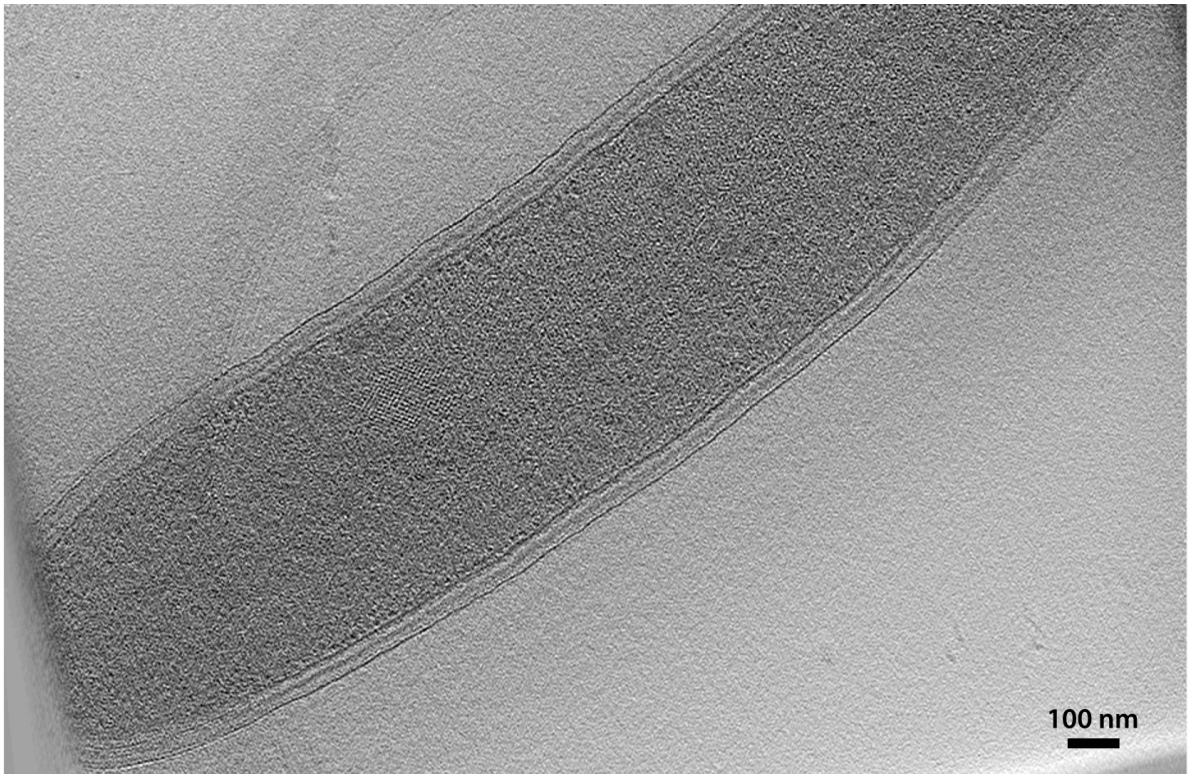

100 nm

Learn More: ICM Variety

In some species, like this Methyloprofundus sedimenti*, the intracytoplasmic membrane is very extensive and appears to be fully separated from the cell membrane. As you can see, it occupies much of the cytoplasmic space.*

Methyloprofundus sedimenti *Collected by: Elitza Tocheva* *DOI: 10.22002/D1.1499*


AdhE spirosomes
100 nm

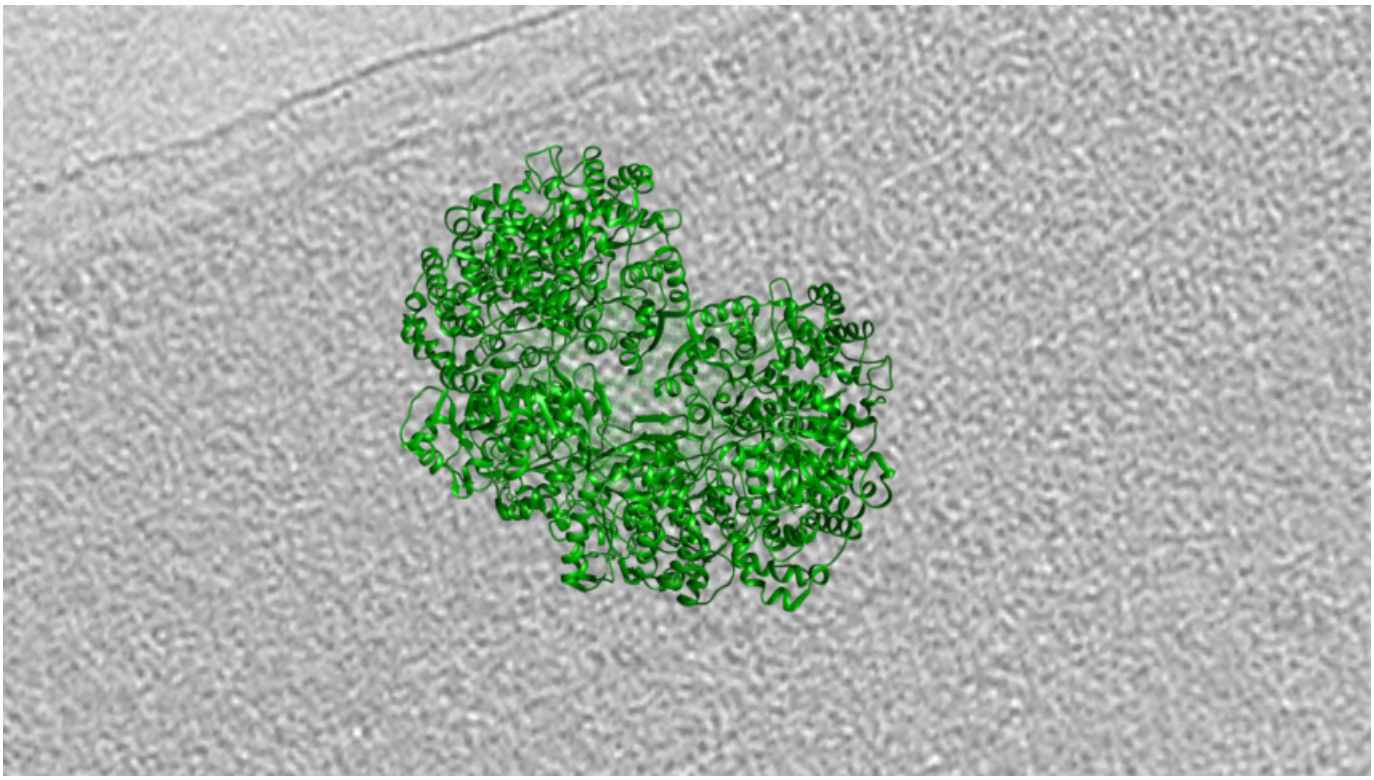

100 nm

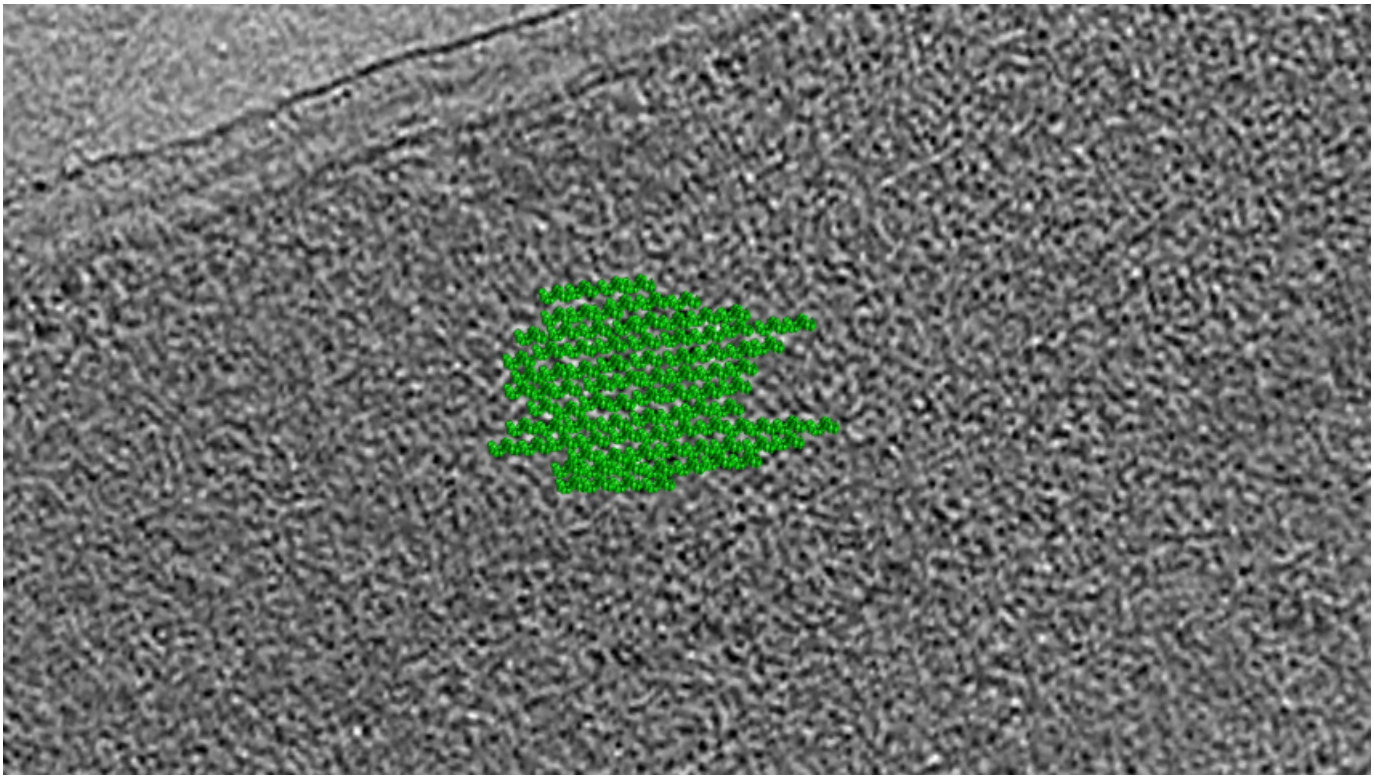
4. Enzyme Filaments

Cells are subject to the vagaries of an environment in which conditions often change. To respond, they must be metabolically agile. The production of enzymes is dialed up and down to meet demand, but they can also be regulated in other ways. Degrading and resynthesizing proteins wastes resources and time. So why not just grant workers a sabbatical when demand lags and call them back in when things pick up again? Cells across all domains of life have evolved an elegant sabbatical mechanism: enzyme polymerization. You already saw an example of this in Chapter 3: the metabolic enzyme CTP synthase. Another example is the alcohol-acetaldehyde dehydrogenase (AdhE) enzyme that allows *Clostridium thermocellum* like this one to digest cellulose into ethanol. As you can see, AdhE polymerizes into helical filaments called **spirosomes**. Polymerization can either inactivate an enzyme (e.g. by occluding its active site), or activate it (e.g. by changing its conformation to open an active site). Filaments can also regulate activity by changing their conformation; for AdhE, it is thought that spirosomes torque between a compact, inactive form to a more extended, active form (what you see here) (*Learn More* on the next page). Whatever the details, polymerization is a rapid way to mobilize (or demobilize) a large number of enzymes for a metabolic task.

Unoccupied workers can also be recruited to other projects. Remember that CTP synthase filaments play a secondary, cytoskeletal role. (And perhaps many, if not all, cytoskeletal elements evolved this way.) AdhE spirosomes seem to have a secondary function in cell adhesion, although the details are not yet clear.

Clostridium thermocellum Collected by: Elitza Tocheva DOI: 10.22002/D1.1493



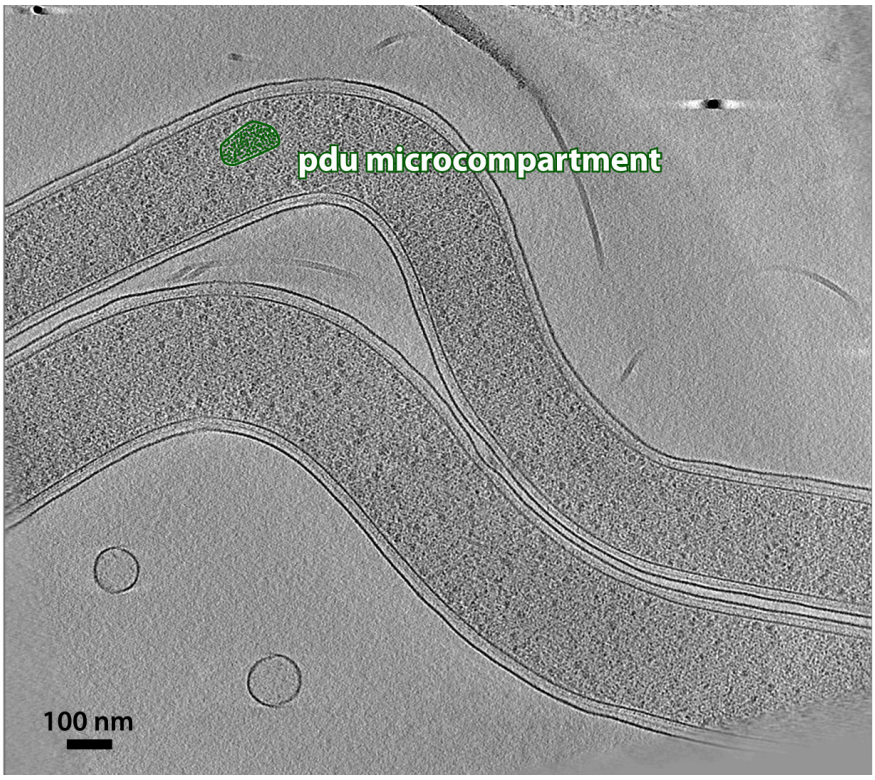


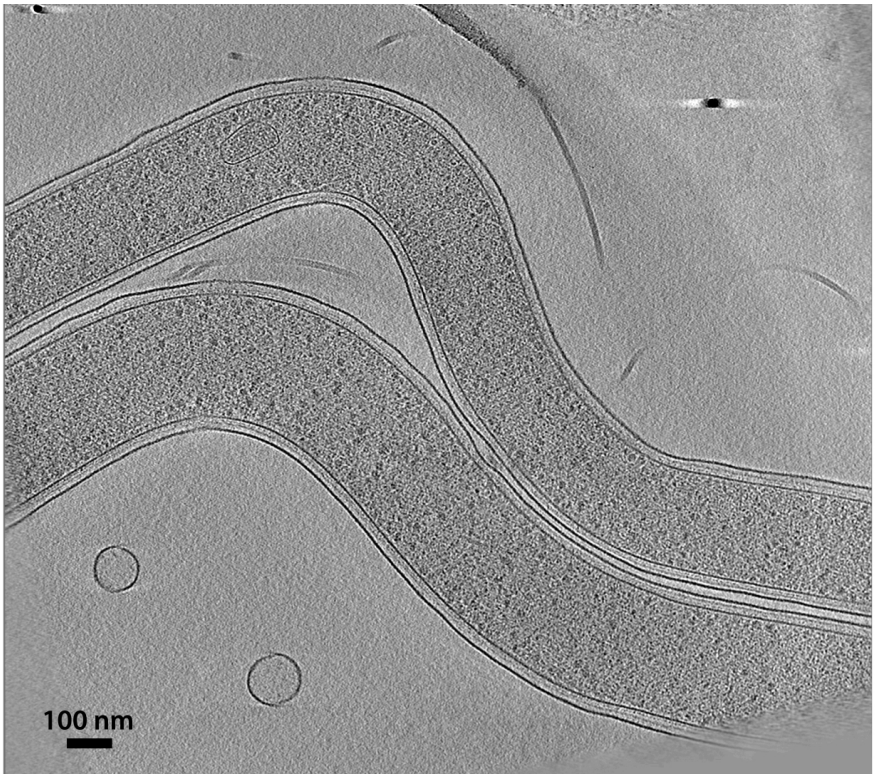
Learn More: Spirosome

Here you see a short segment of an AdhE spirosome from Escherichia coli *in the extended, active filament conformation [38].*

Source: Pony et al. (2020)

Structure: EMD-10552, PDB 6TQH

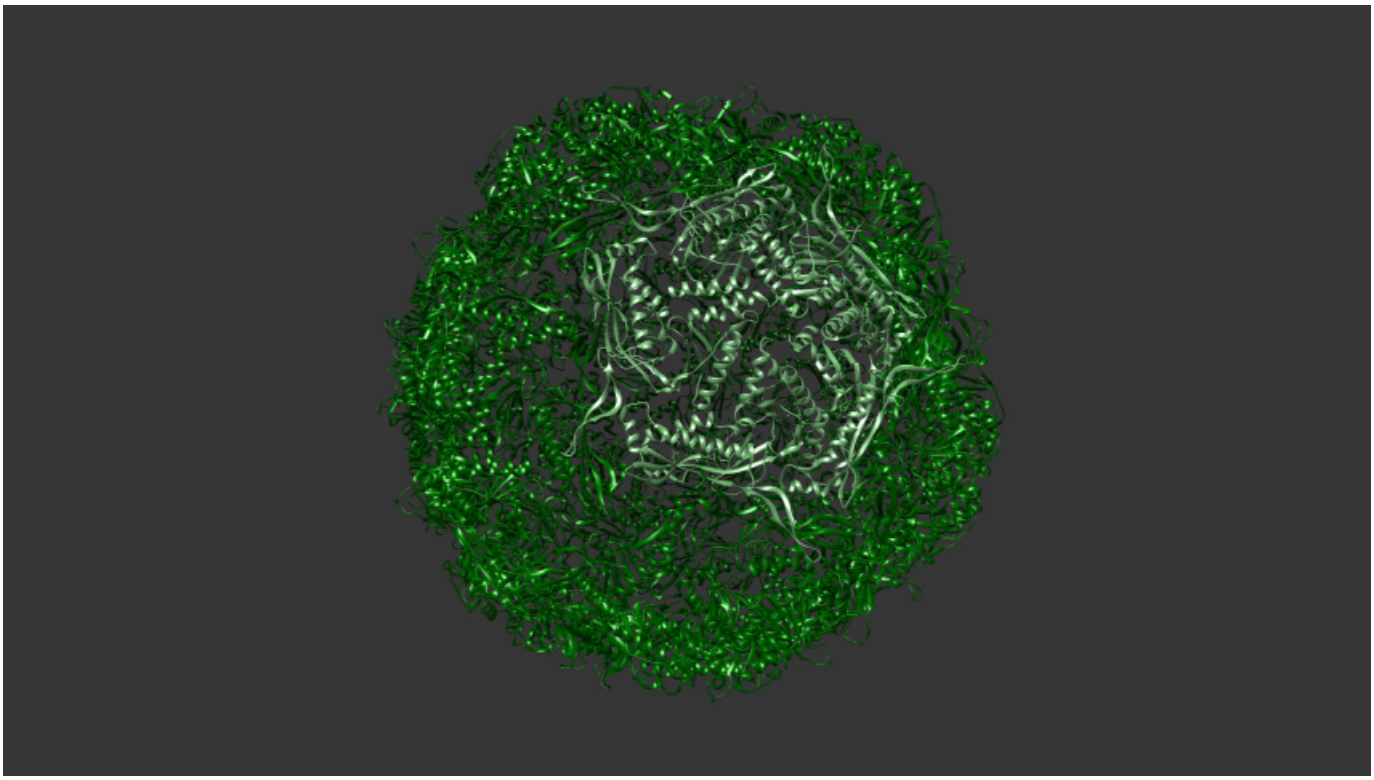

pdu microcompartment
100 nm

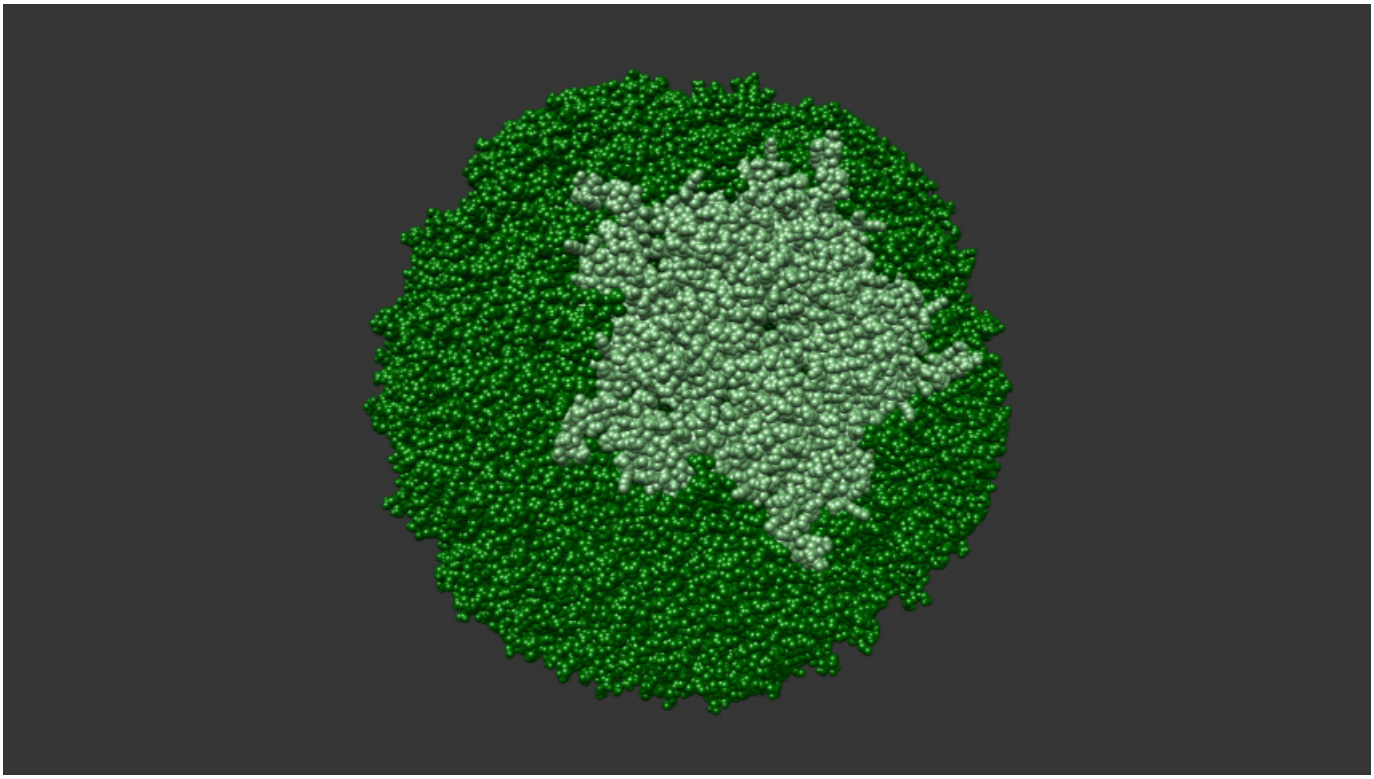

100 nm

5. Microcompartments

In addition to increasing the number of active workers to boost metabolic output, you could also make them more efficient. One way to do this is by bringing enzymes that work in the same pathway together into an assembly (or disassembly) line. Such factories are common inside cells. You could even go a step further and enclose the factory in a dedicated building. Such structures, found in bacteria but not (as far as we know) archaea, are known as **microcompartments**–areas of the cytoplasm walled off by a protein shell (*Learn More* on the next page). For instance, *Acetonema longum* like this use a factory called the propanediol utilization, or pdu, microcompartment to increase the efficiency of an enzymatic pathway that breaks down 1,2-propanediol. Illustrating the interlocking lives of microbial communities, 1,2-propanediol is itself the byproduct of the metabolism of other microbes, neighbors in *A. longum*'s environment in the gut of an animal.

Acetonema longum Collected by: Elitza Tocheva DOI: 10.22002/D1.1494



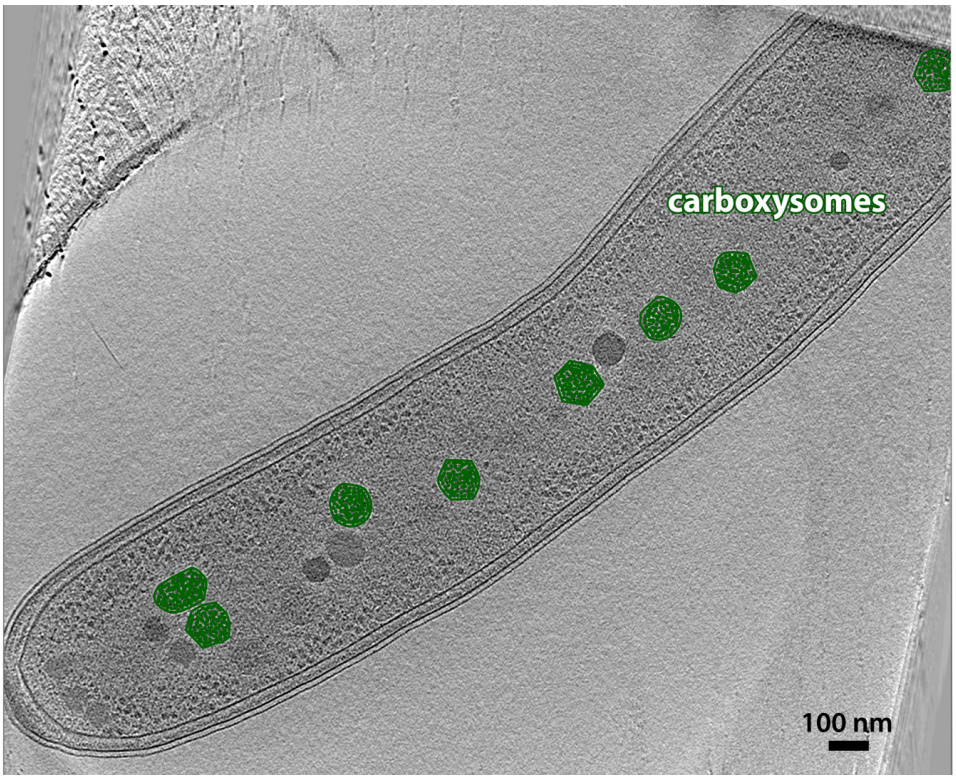


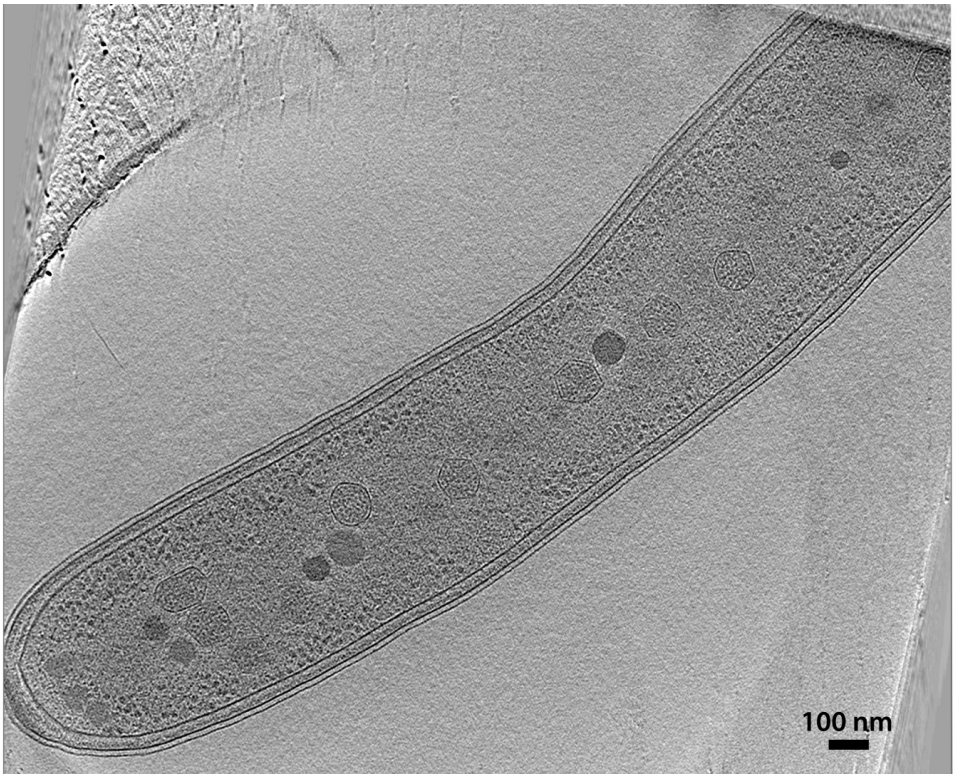
Learn More: Shells

Bacterial microcompartments are typically enclosed by a self-assembling shell formed by many copies of just a few proteins. The shell is icosahedral, consisting of hexameric units packed into flat planes, which are joined together by pentamers at the vertices. You can see this arrangement in this shell structure of a microcompartment called an encapsulin that helps Thermotoga maritima *use to sequester soluble iron so that it does not react with reactive oxygen species and damage the cell [39].*

Source: Sutter et al. (2008)

Structure: PDB 3DKT


carboxysomes
100 nm

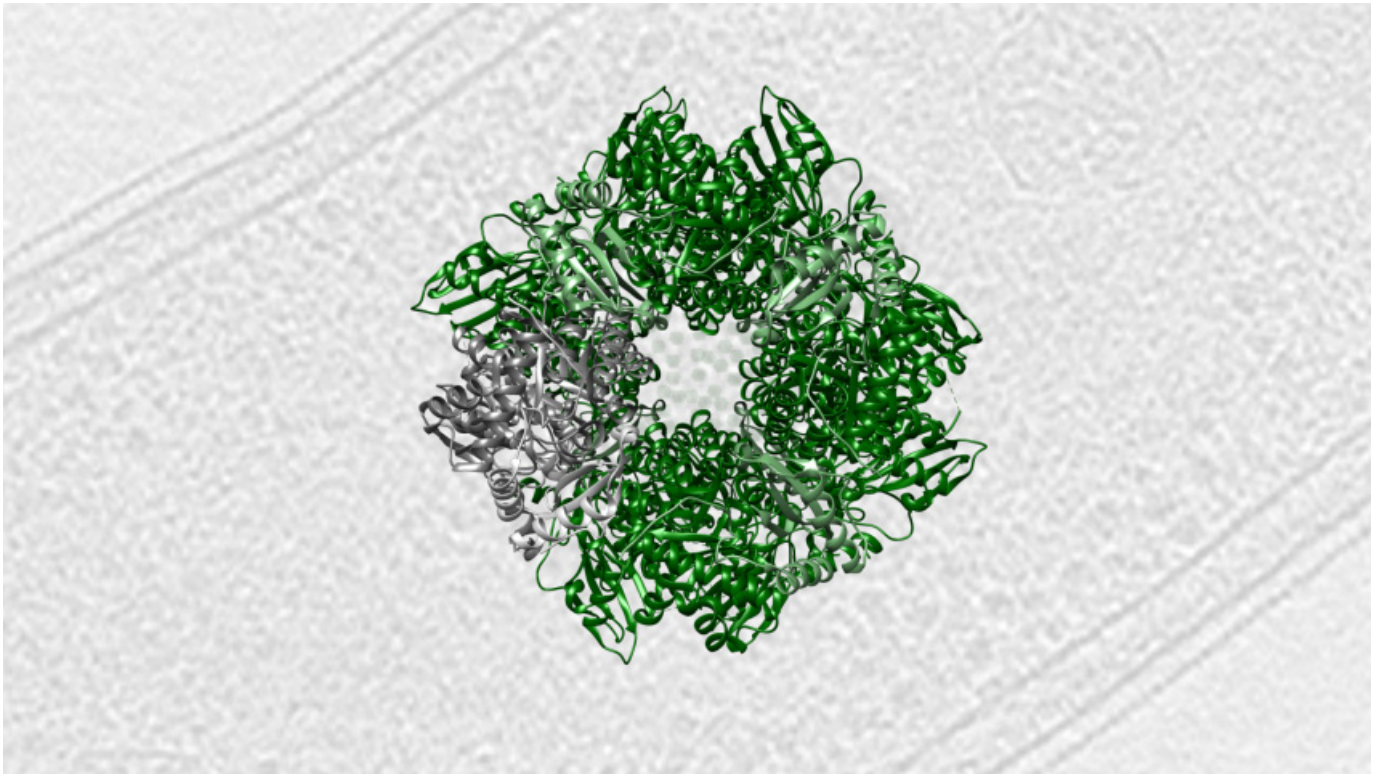

100 nm

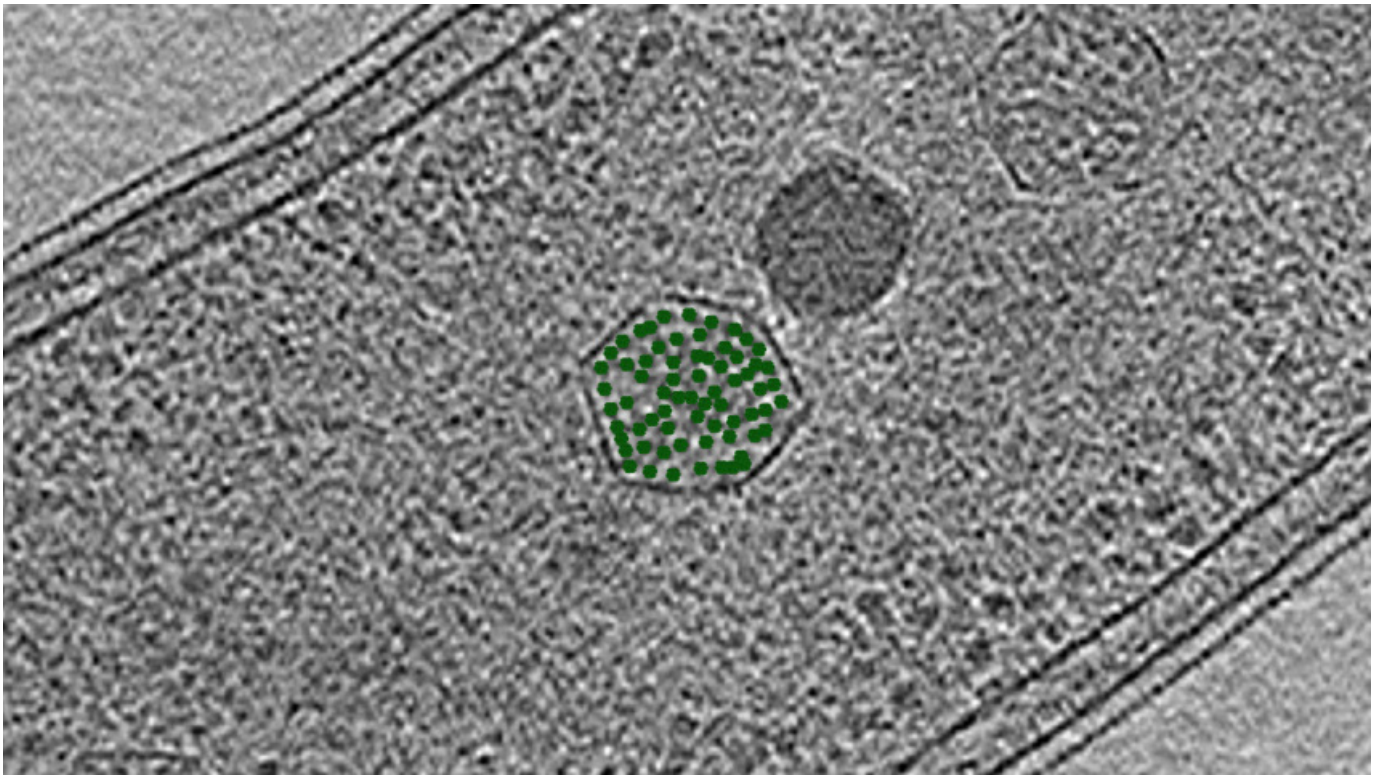
6. Carboxysomes

One of the most impressive bacterial microcompartments is the **carboxysome**, which some species use for carbon fixation (the conversion of carbon dioxide into usable fuel molecules). Carboxysomes contain tightly packed copies of an enzyme called ribulose bisphosphate carboxylase/oxygenase, or more succinctly, RuBisCO (*Learn More* on the next page), which is the most abundant enzyme on Earth. In this case, the shell does more than simply concentrate the enzymes. Oxygen competes with carbon dioxide for binding RuBisCO, so the bacteria include another enzyme in the compartment which converts bicarbonate (HCO_3) into CO_2, increasing the local concentration and making it readily available to the RuBisCO. The shell is permeable to HCO_3 but slows down the diffusion of CO_2 out, and O_2 in. Carboxysomes were probably unnecessary in early cells because the oxygen level of the environment was much lower. Cells, like this *Thiomonas intermedia*, can contain many carboxysomes. While most are icosahedral, there is significant variation in their forms (*Learn More* on following pages).

In this case, the microcompartment protects its contents from the rest of the cell, but they can also do the reverse. Some metabolic pathways generate toxic intermediates, and bacteria have evolved microcompartments that sequester them so that they do not interfere with other processes in the cell.

Thiomonas intermedia Collected by: Cristina Iancu DOI: 10.22002/D1.1495



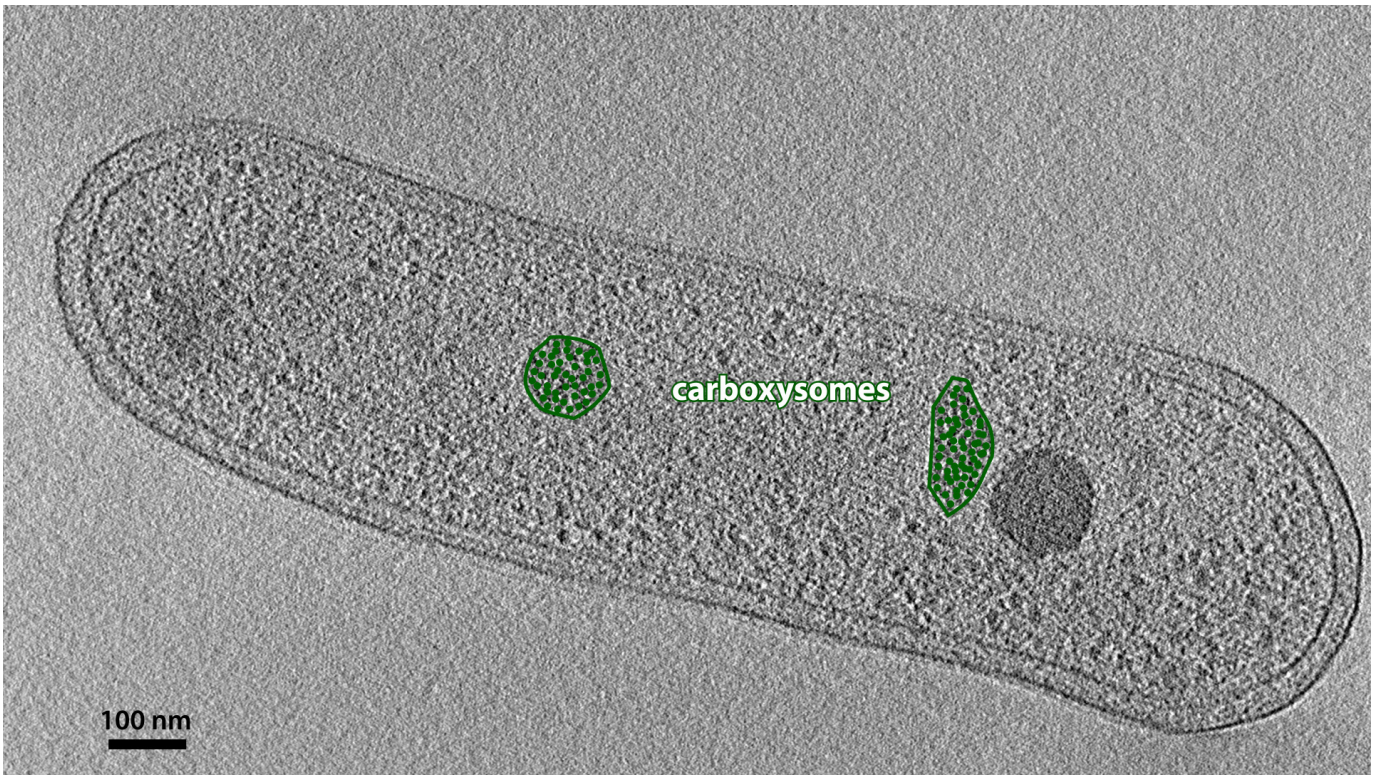


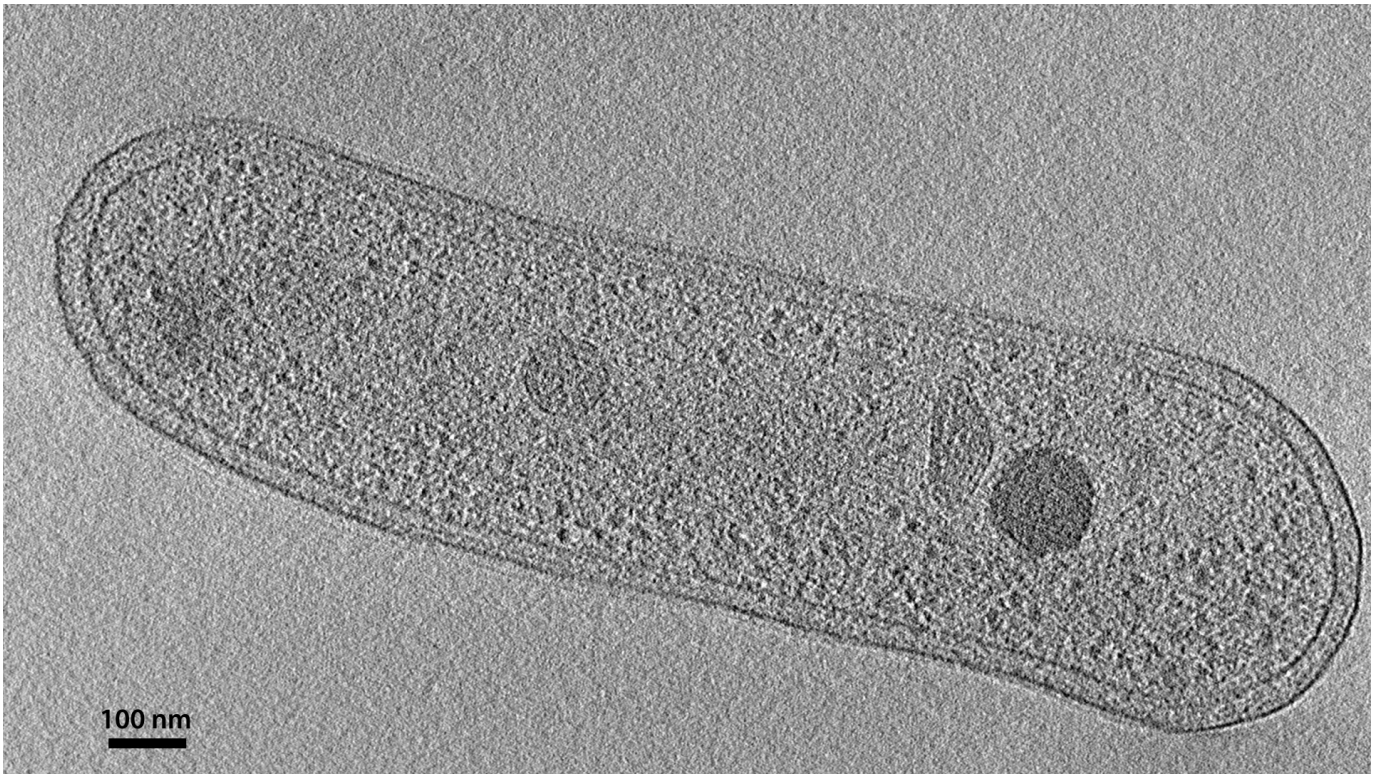
Learn More: RuBisCO

*RuBisCO is a compact protein complex, as you can see in this structure from T*hiomonas intermedia *[40]. It is made of eight copies each of a large and a small component (shown in dark and light green, respectively), forming parallel assembly lines to make useful organic carbon from CO_2. One set is highlighted in grey.*

Source: Oltrogge et al. (2020)

Structure: PDB 6UEW

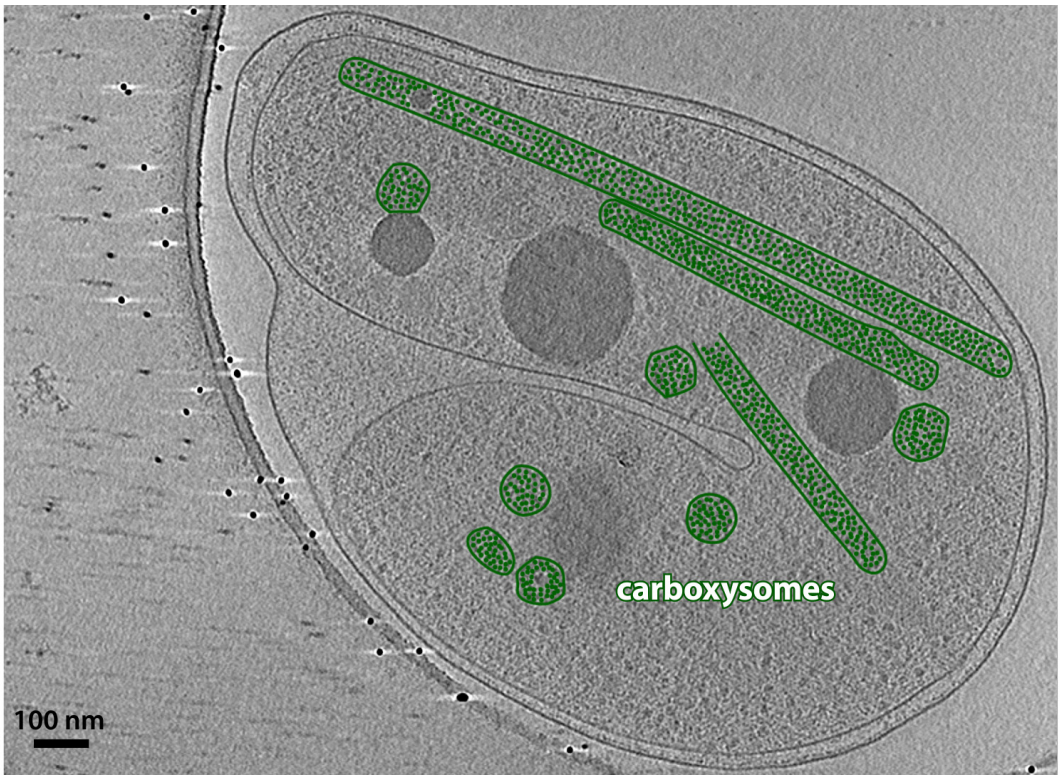

carboxysomes
100 nm

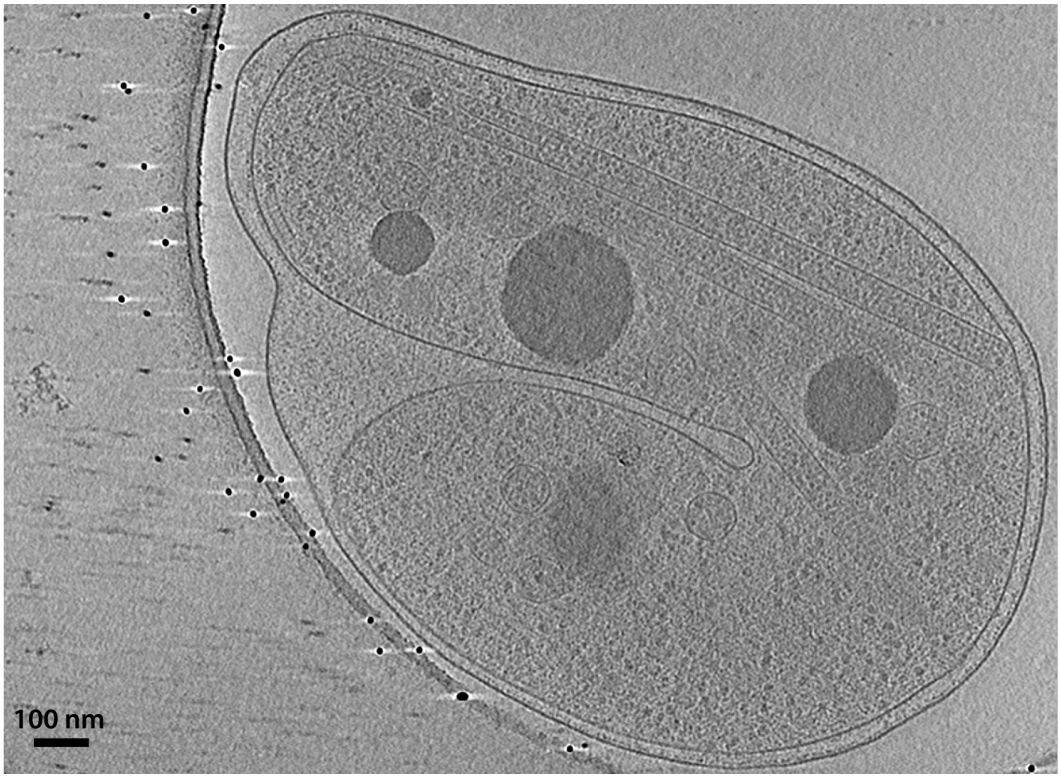

100 nm

Learn More: Variety

Most carboxysomes look like the ones you saw on the last page, but not all. Some are less regular polyhedra, as in this Halothiobacillus neapolitanus *cell. And some are stranger still (*Learn More *on the next page).*

Halothiobacillus neapolitanus *Collected by: Cristina Iancu* *DOI: 10.22002/D1.1500*


carboxysomes
100 nm

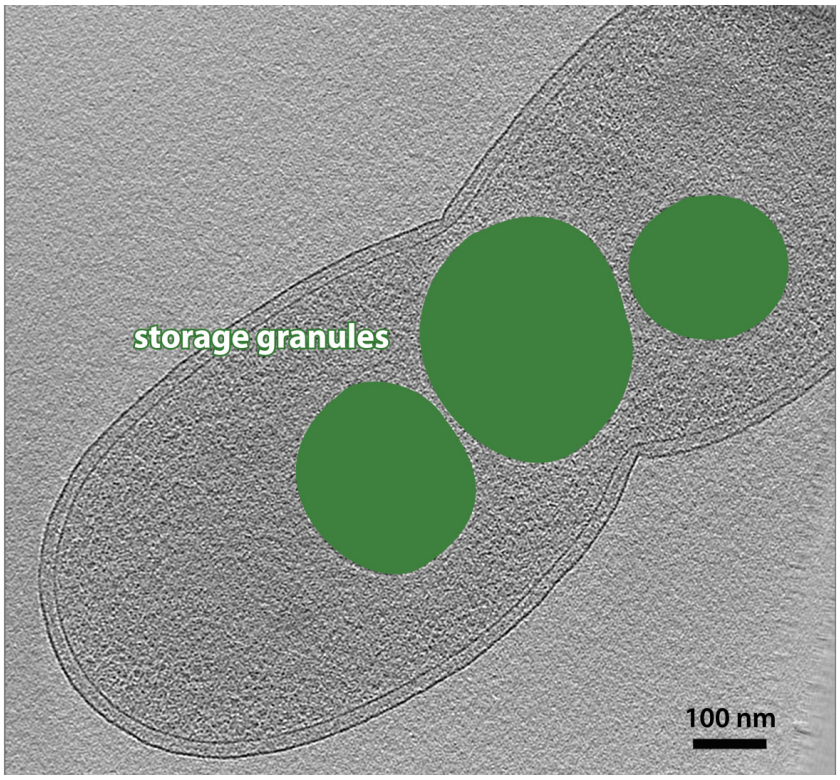

100 nm

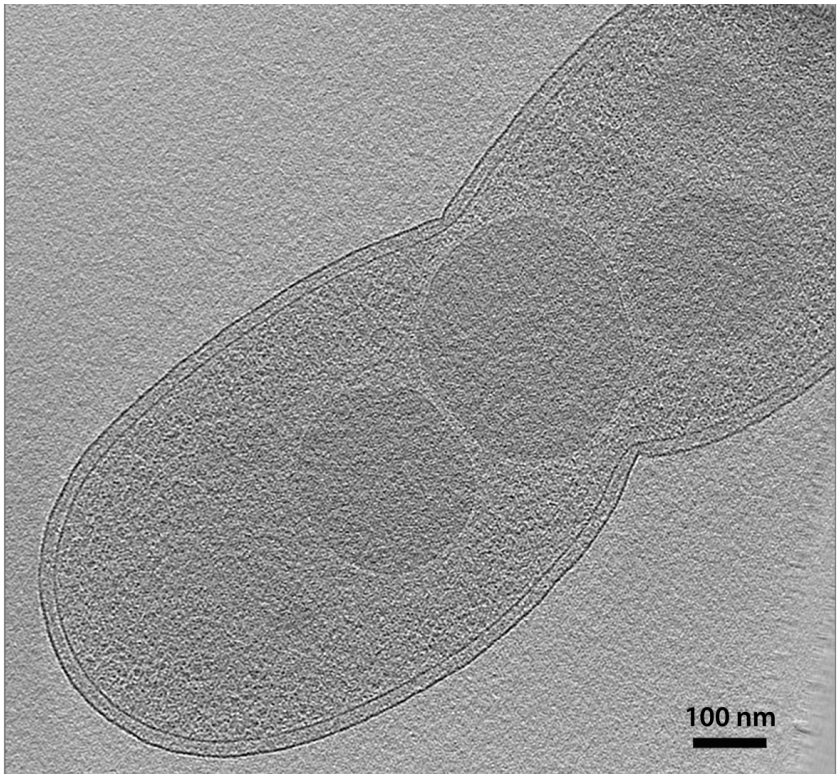
Learn More: Length

The carboxysomes in this Hydrogenovibrio crunogenus *have grown abnormally long, extending across the full width of the cell. The length of these is unusual, but minor elongations are more common.*

This cell also highlights the lability in the outer membrane common in some bacterial species, particularly pathogens. Note how the cell has curled inside its loose outer membrane. You can also see part of it stretching across to another cell in a neighboring hole; they were probably just finishing division when they were pulled apart by sample preparation.

Hydrogenovibrio crunogenus *Collected by: Cristina Iancu* *DOI: 10.22002/D1.1501*


storage granules
100 nm

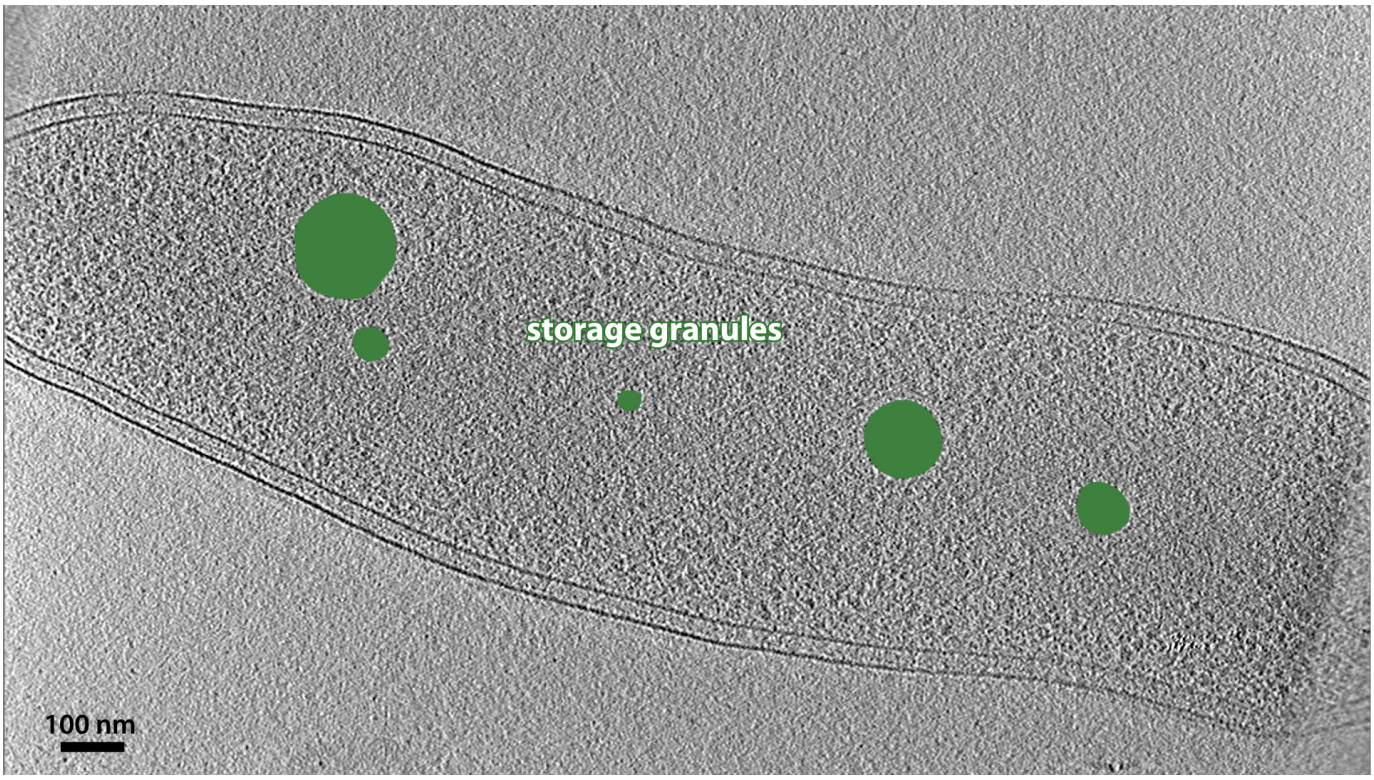

100 nm

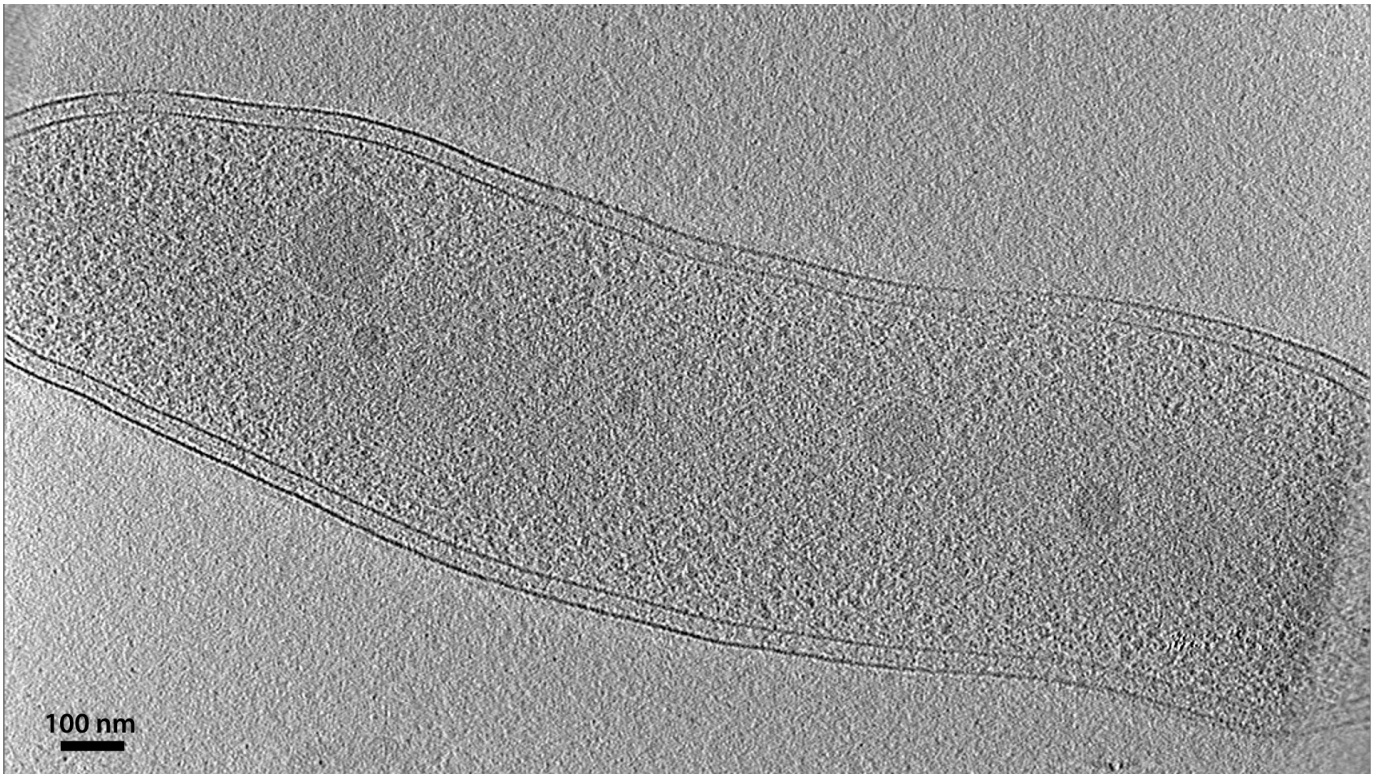
7. Bacterial Storage Granules

No matter how efficient your factories are, they need raw materials, and in an ever-changing environment, a cell cannot always depend upon a steady supply chain. How can you help your cell cope with occasional shortages? If you said stockpile, you are in good agreement with nature. Both bacteria and archaea use **storage granules** to stockpile essential nutrients. The substrate is usually polymerized for easier packing, like poly-phosphate or the poly-hydroxybutyrate (a carbon store) in these *Cupriavidus necator* granules. No matter what they pack, storage granules are generally spherical, and exhibit a range of sizes (*Learn More* on the next page). When the environment is rich in one nutrient but poor in another, cells may stop growing, but keep adding to their cellular stores. You can see that effect in this *C. necator* cell, which has been cultured in a medium with carbon but not enough nitrogen; as a result, it has accumulated very large poly-hydroxybutyrate granules which take up much of the cytoplasmic space.

Storage granules are ubiquitous in bacteria, and you will see them in many of the cells in this book. The most common type is poly-phosphate, which has a characteristic dark appearance in electron microscopy images.

Cupriavidus necator Collected by: Morgan Beeby DOI: 10.22002/D1.1496

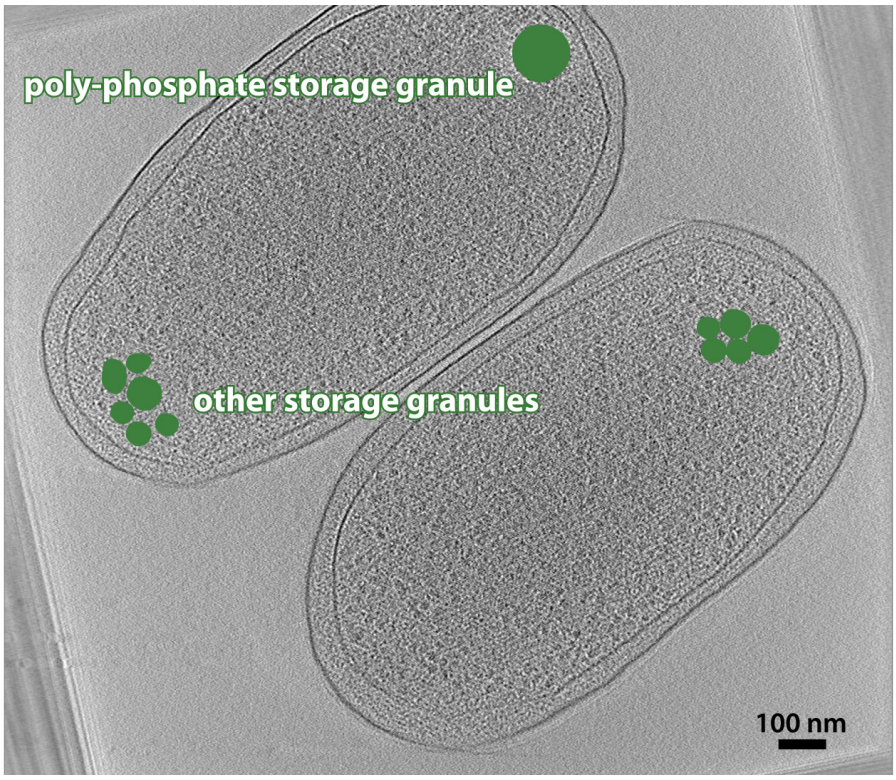

storage granules
100 nm

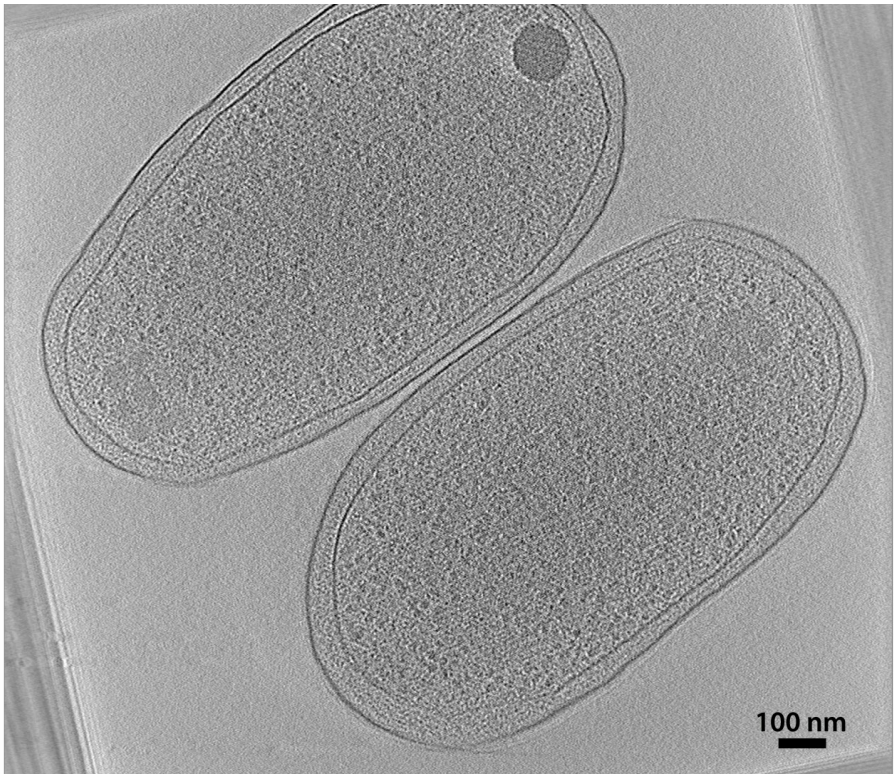

100 nm

Learn More: Growth

Storage granules expand and contract with the amount of material they contain. This Lysobacter antibioticus *cell exhibits a typical range of sizes of poly-phosphate granules.*

Lysobacter antibioticus *Collected by: Morgan Beeby* *DOI: 10.22002/D1.1502*


poly-phosphate storage granule
other storage granules
100 nm


100 nm

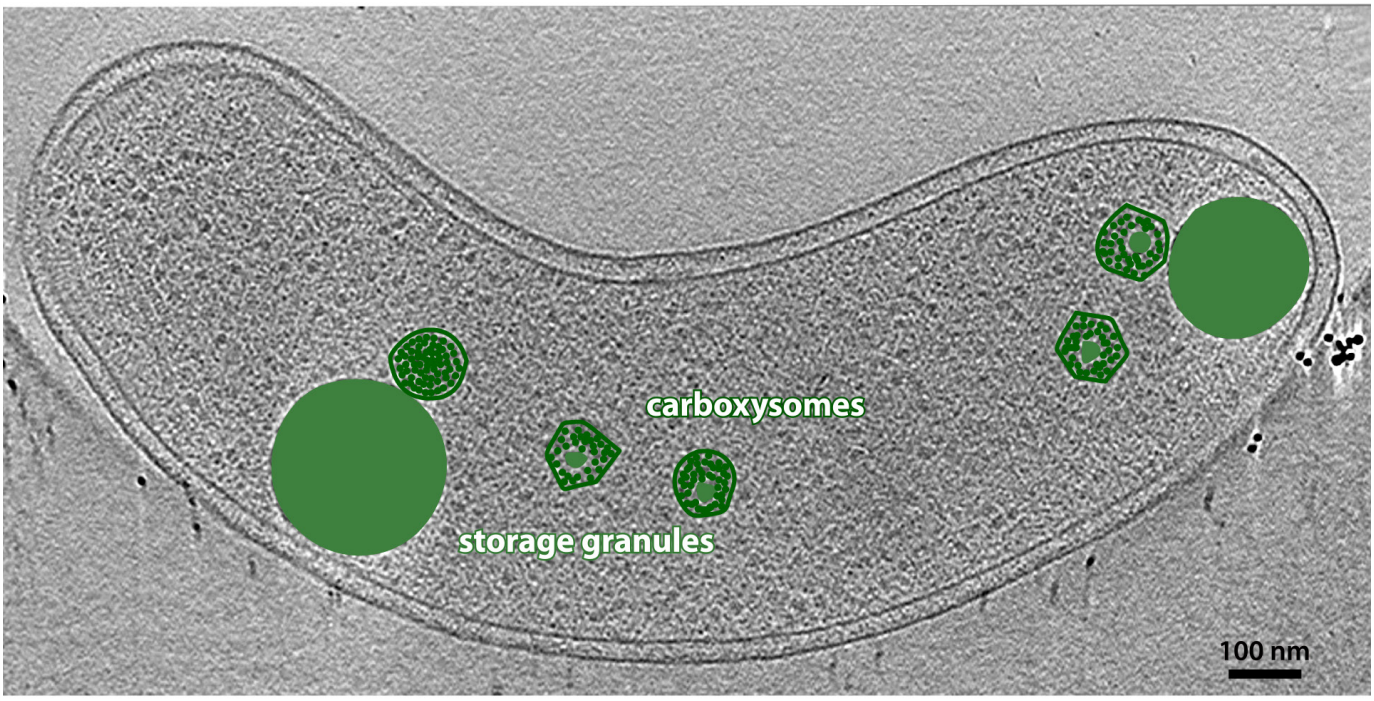
8. Storage Granule Variety

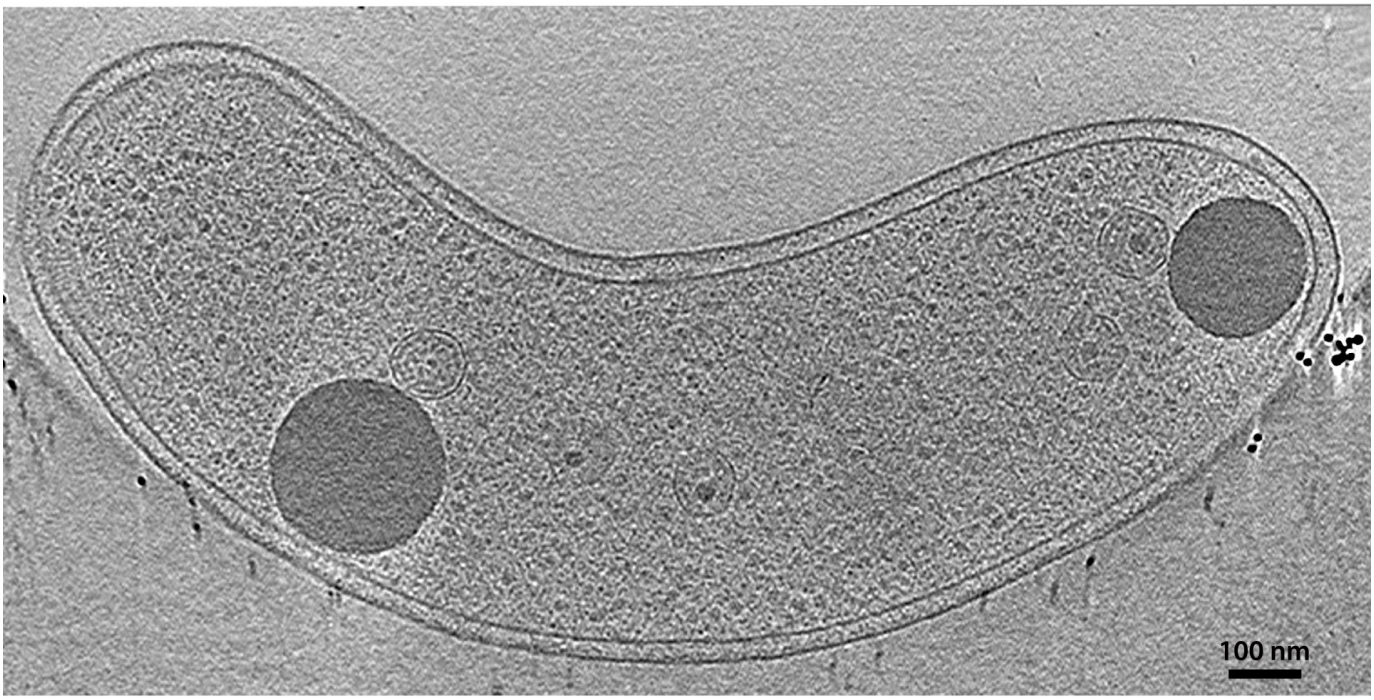
Some types of storage granules, like poly-phosphate and poly-hydroxybutyrate, are very densely packed and clearly delineated from the rest of the cytoplasm. Others are less tightly packed and more amorphous, like the ones in this *Agrobacterium tumefaciens* cell. We do not yet know what these contain.

In some species, like the *Cupriavidus necator* on the last page, storage granules seem to be positioned randomly in the cell. Other species have more regulated arrangements. *A. tumefaciens* cells always have one poly-phosphate storage granule, located near a cell pole. Other species have one at each end of the cell. As we will discuss in Chapter 5, this arrangement can help mother cells deliver a storage granule to each of their daughters during division.

You might expect a close relationship between cellular factories and storage depots. In fact, there does seem to be a relationship between carboxysomes and storage granules, although the details remain unclear (*Learn More* on the next pages).

Agrobacterium tumefaciens Collected by: Elitza Tocheva DOI: 10.22002/D1.1497

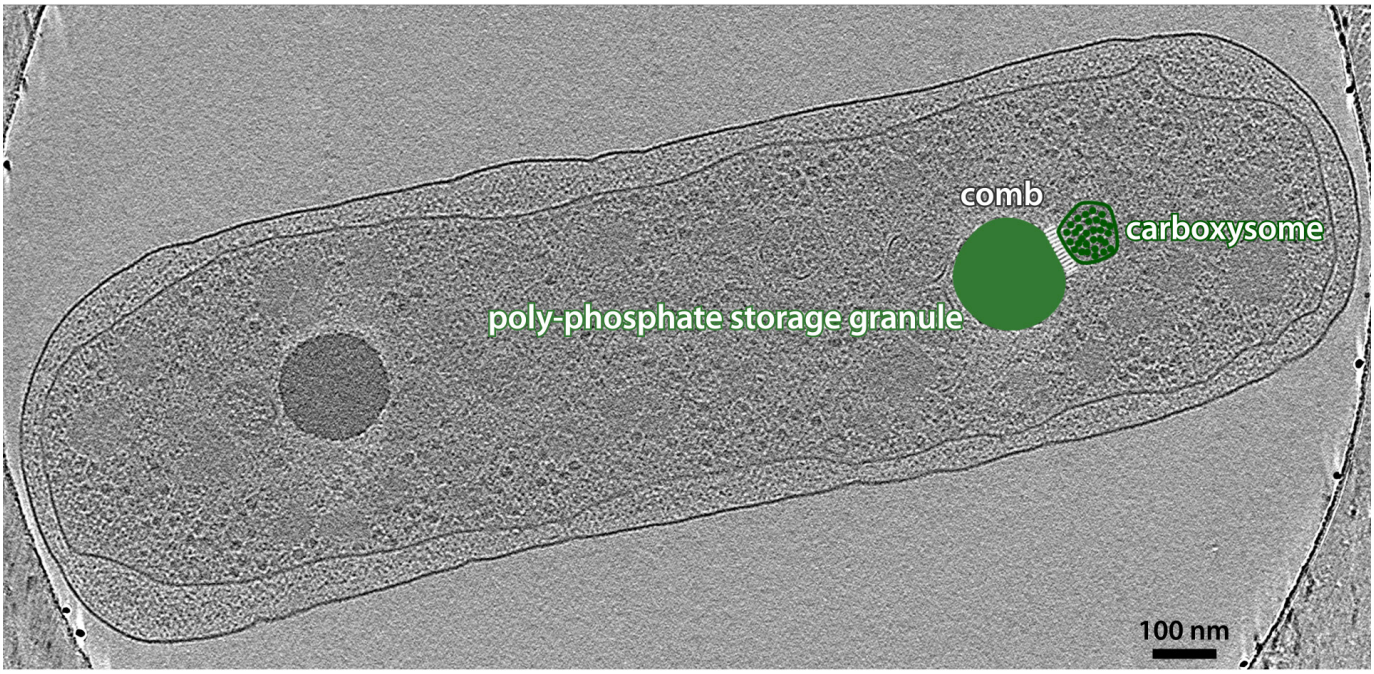

carboxysomes
storage granules
100 nm

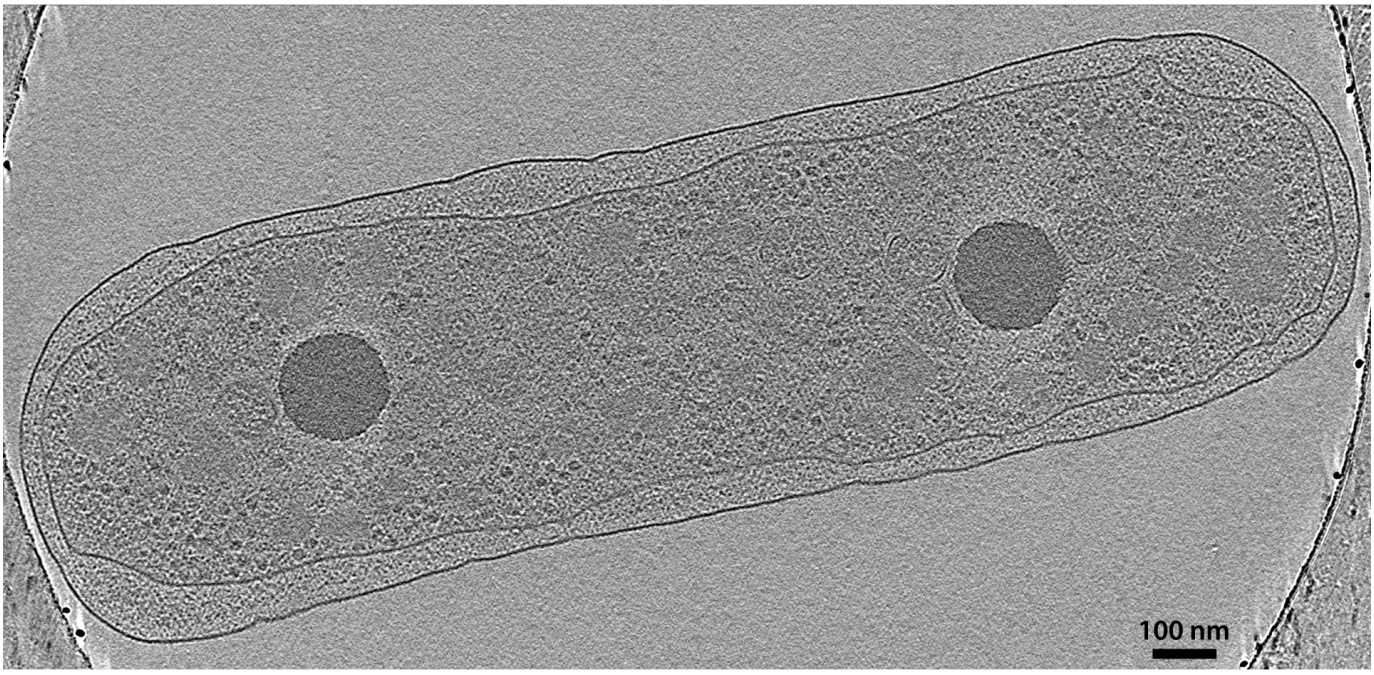

100 nm

Learn More: Carboxysomes

Small storage granules are sometimes seen inside *carboxysomes, as in this* Hydrogenovibrio crunogenus *cell. Their function there is unknown.*

Hydrogenovibrio crunogenus *Collected by: Cristina Iancu* *DOI: 10.22002/D1.1503*

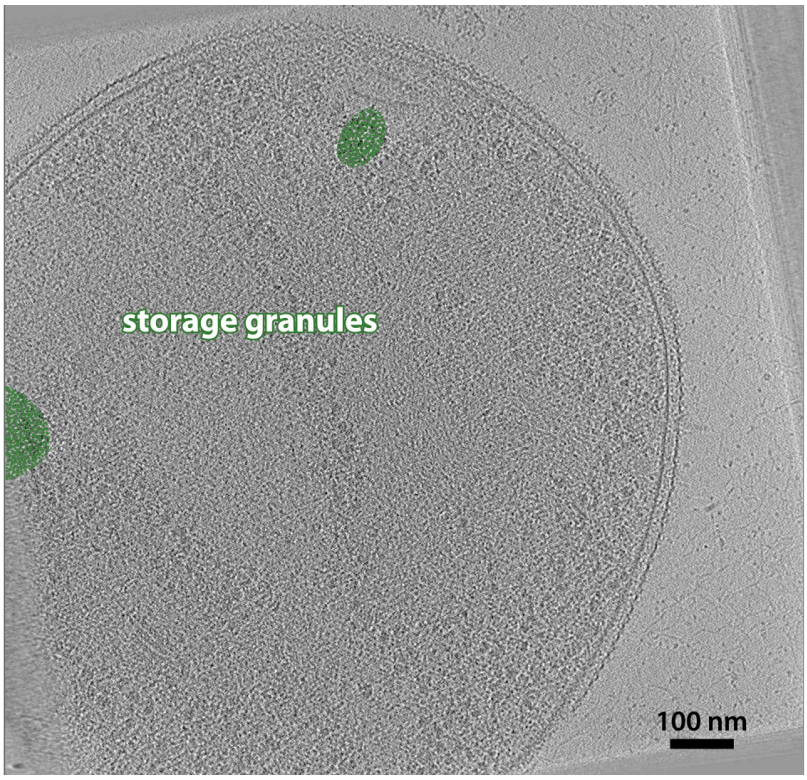

comb
carboxysome
poly-phosphate storage granule
100 nm

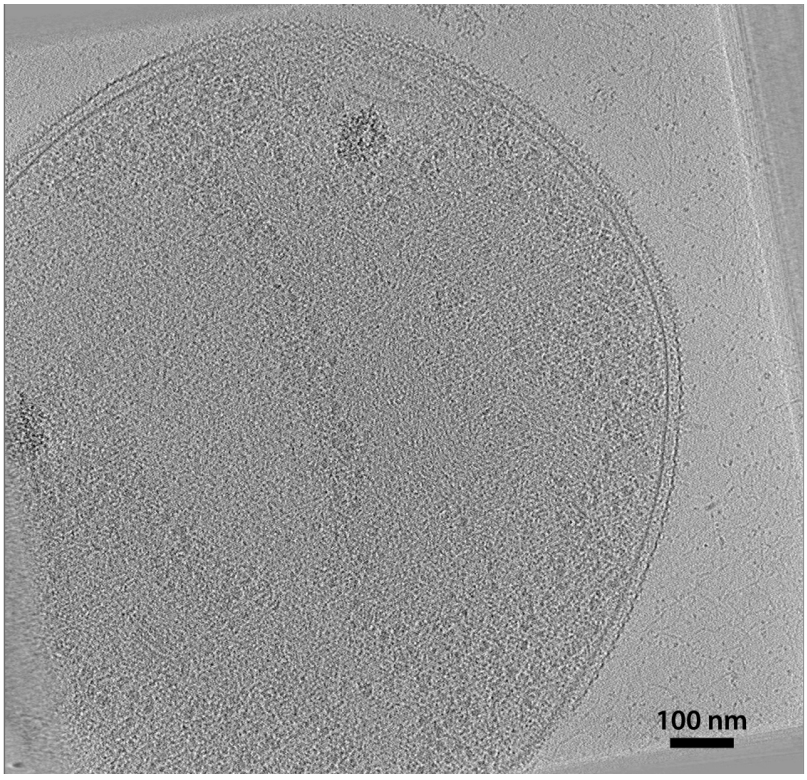

100 nm

Learn More: Connections

We sometimes see comb-like structures connecting poly-phosphate storage granules with carboxysomes, as in this Halothiobacillus neapolitanus *cell. The nature of the relationship between the structures, and the identity and function of the combs, remains a mystery.*

Halothiobacillus neapolitanus *Collected by: Cristina Iancu* *DOI: 10.22002/D1.1504*

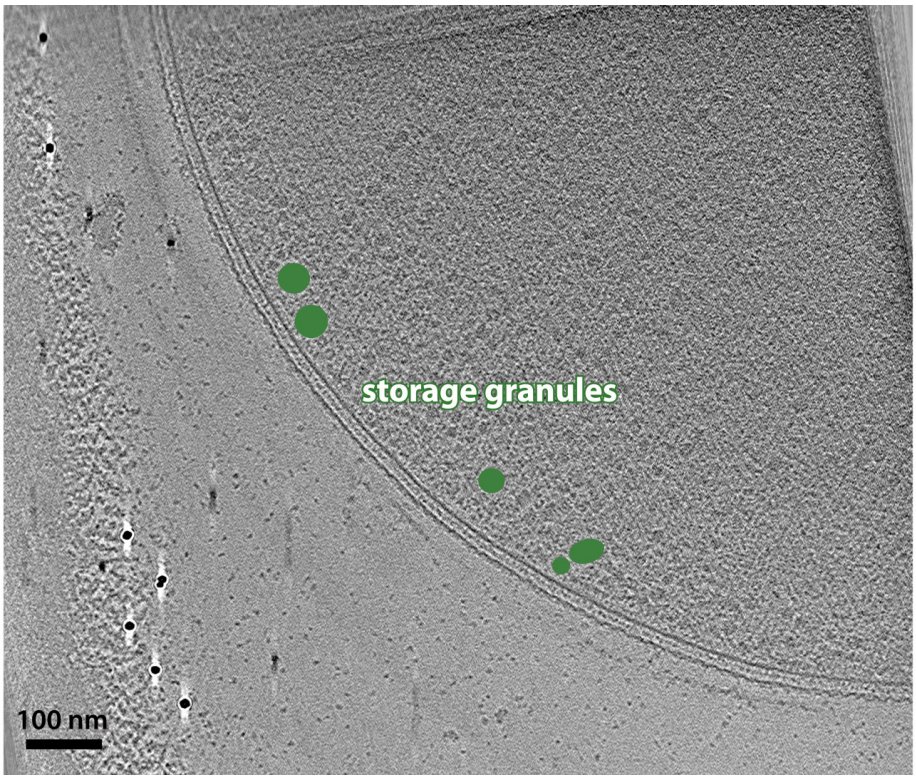

storage granules
100 nm

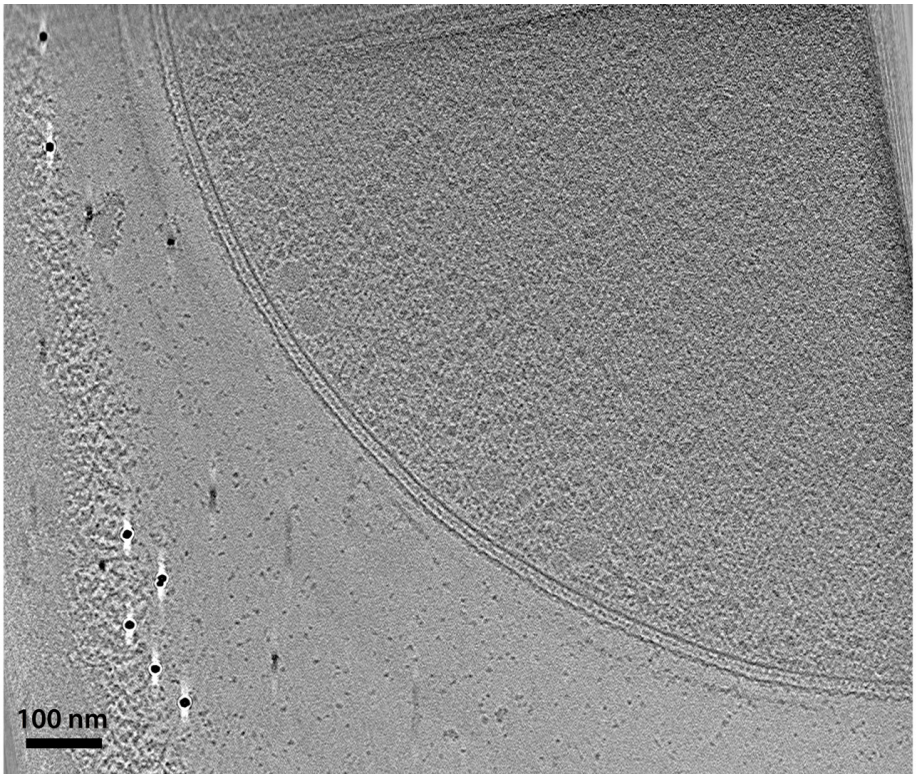

100 nm

9. Archaeal Storage Granules

Archaea also use storage granules, and you will see many examples throughout the book. Compared to the relatively smooth surface of bacterial granules, the edges of some archaeal granules are spikier, giving them a rougher appearance, as in this *Halorubrum litoreum* cell. Others are smooth and, just like in bacteria, they exhibit a range of morphologies depending on what they store. For instance, compare the granules in the *Learn More* sections on the next pages.

Halorubrum litoreum Collected by: Ariane Briegel DOI: 10.22002/D1.1498

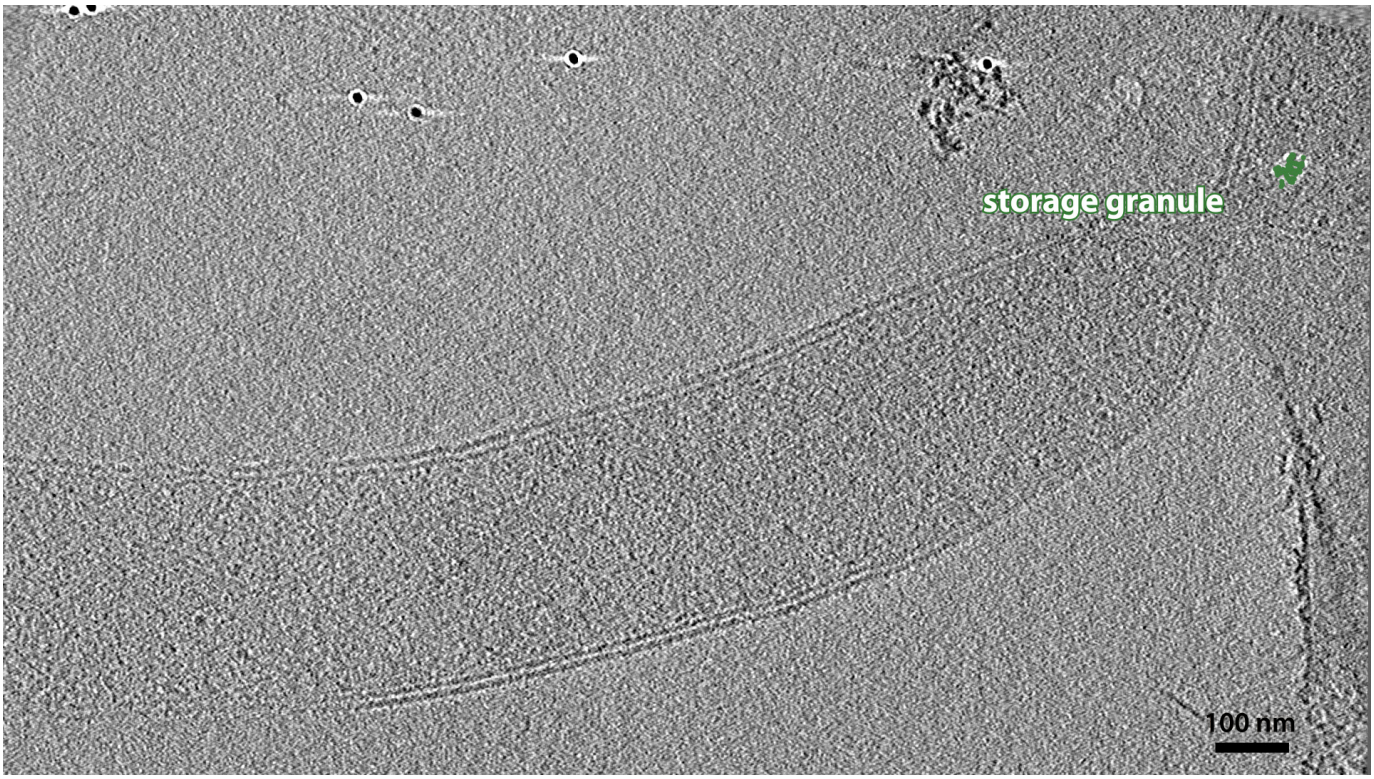

storage granules
100 nm

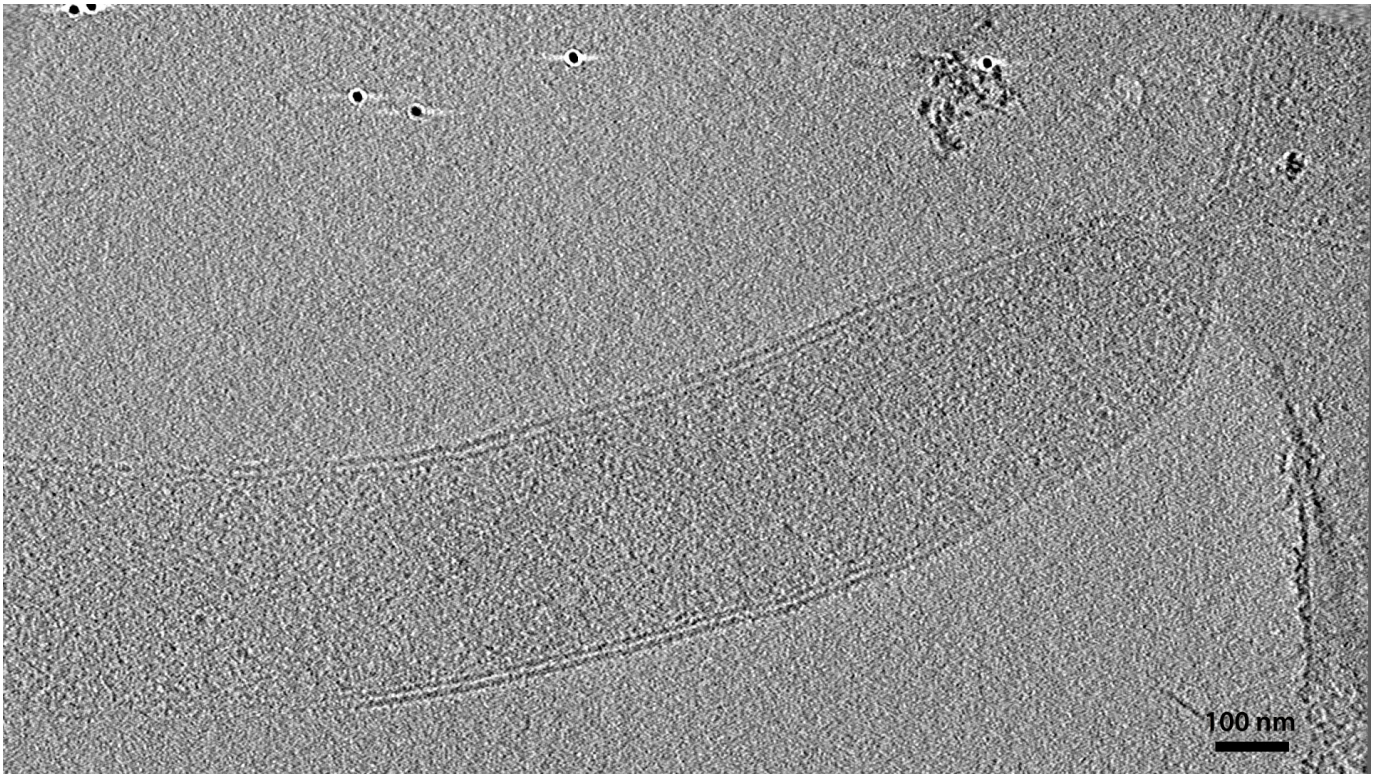

100 nm

Learn More: Light

Some archaeal cells, like this Haloferax gibbonsii*, contain many small granules. We do not yet know what they store, but based on their electron density (how dark they appear in EM images), it may be a carbon source.*

Haloferax gibbonsii *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1505*

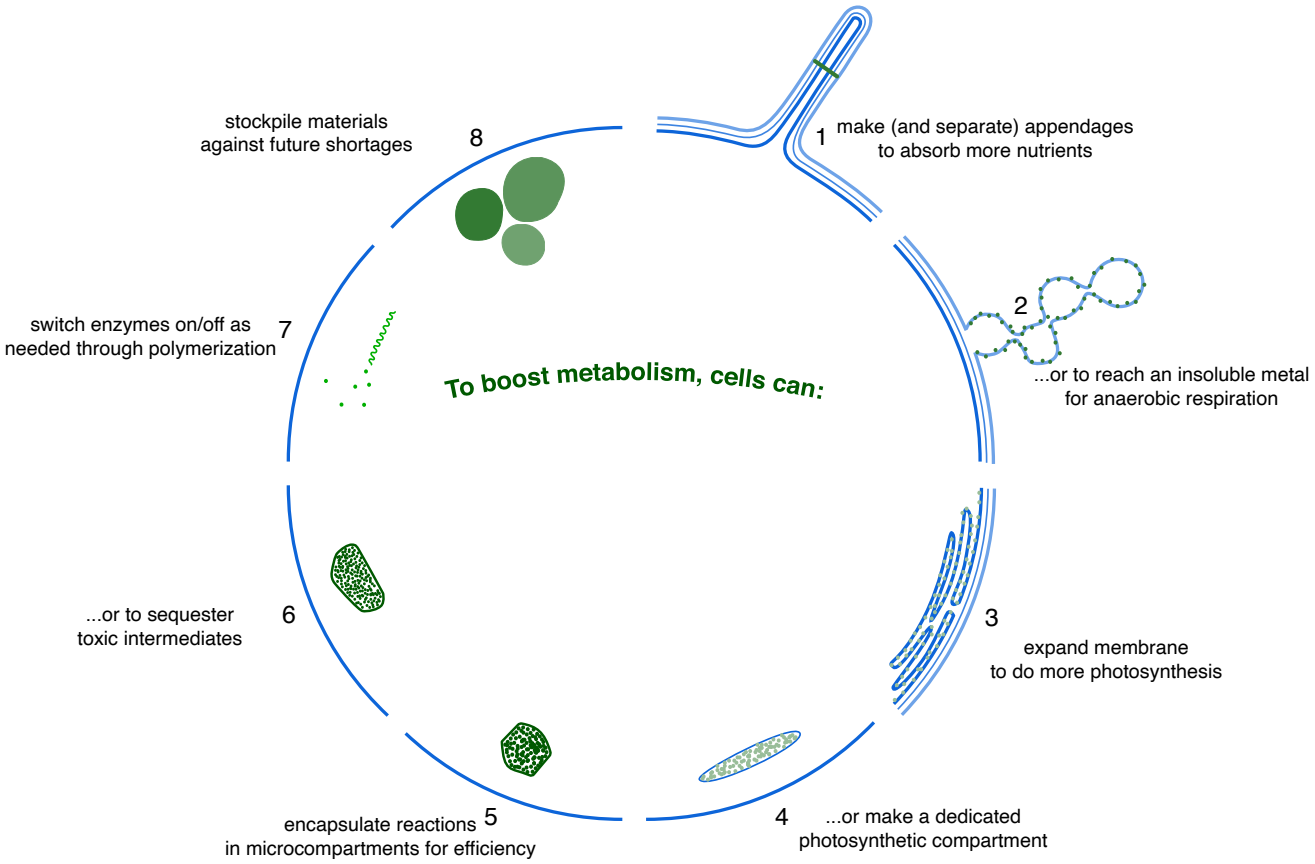

storage granule
100 nm


100 nm

Learn More: Dark

This Halohasta litchfieldiae *cell has more unusual granules. The high electron density (darkness) of these punctate structures suggests that they are iron storage granules, known as ferrosomes.*

Halohasta litchfieldiae *Collected by: Zhuo Li* *DOI: 10.22002/D1.1506*


To boost metabolism, cells can:
1 make (and separate) appendages to absorb more nutrients
2 ...or to reach an insoluble metal for anaerobic respiration
3 expand membrane to do more photosynthesis
4 ...or make a dedicated photosynthetic compartment
5 encapsulate reactions in microcompartments for efficiency
6 ...or to sequester toxic intermediates
7 switch enzymes on/off as needed through polymerization
8 stockpile materials against future shortages

Summary

Concept Check Questions

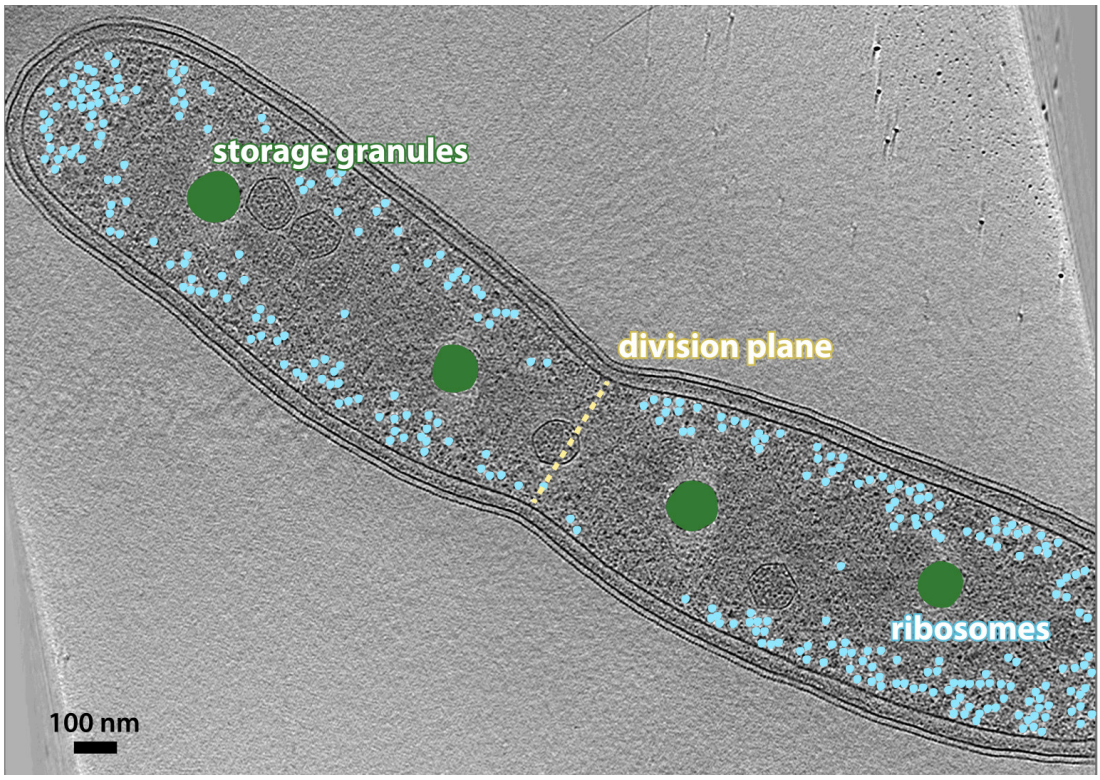
- How can cells perform anaerobic respiration without a soluble electron acceptor?
- How does a microcompartment make a metabolic reaction more efficient?
- What kind of macromolecule makes up a bacterial microcompartment shell?
- What is a secondary use for enzyme filaments?

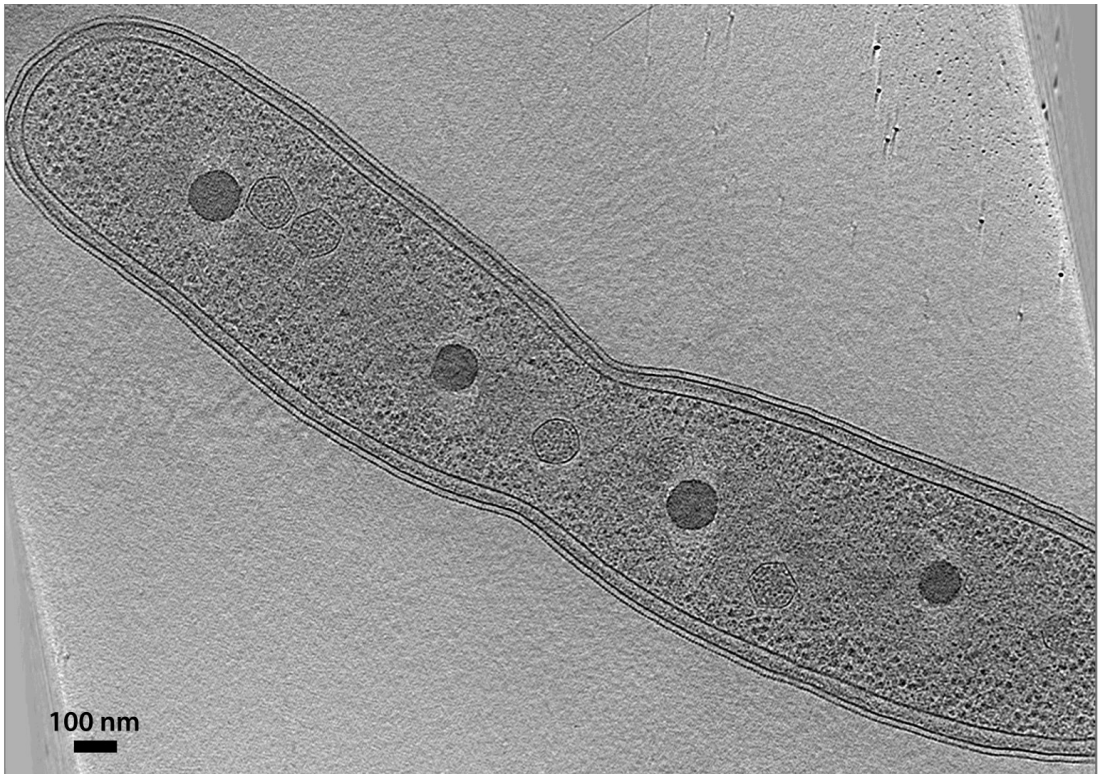
Further Reading

- Barry and Gitai (2011). *Self-assembling enzymes and the origins of the cytoskeleton* [33].
- Hoppert and Mayer (1999). *Principles of macromolecular organization and cell function in bacteria and archaea* [41].
- Kerfeld et al. (2018). *Bacterial microcompartments* [42].
- Oostergetel et al. (2010). *The chlorosome: A prototype for efficient light harvesting in photosynthesis* [43].

5
Division

"…la rêve de la bactérie : produire deux bactéries."
(the dream of the bacterium: to generate two bacteria)
- François Jacob [44]


storage granules
division plane
ribosomes
100 nm


100 nm

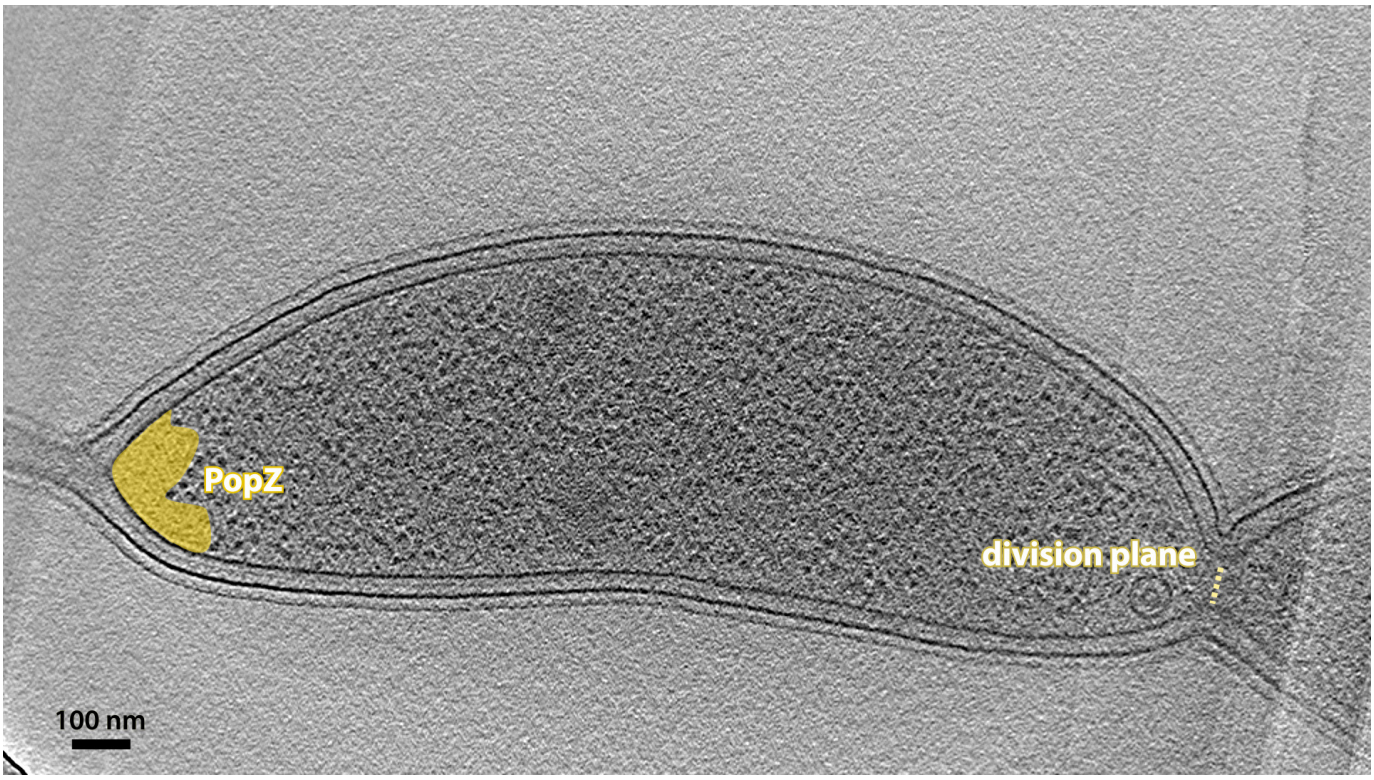
1. Copy Number

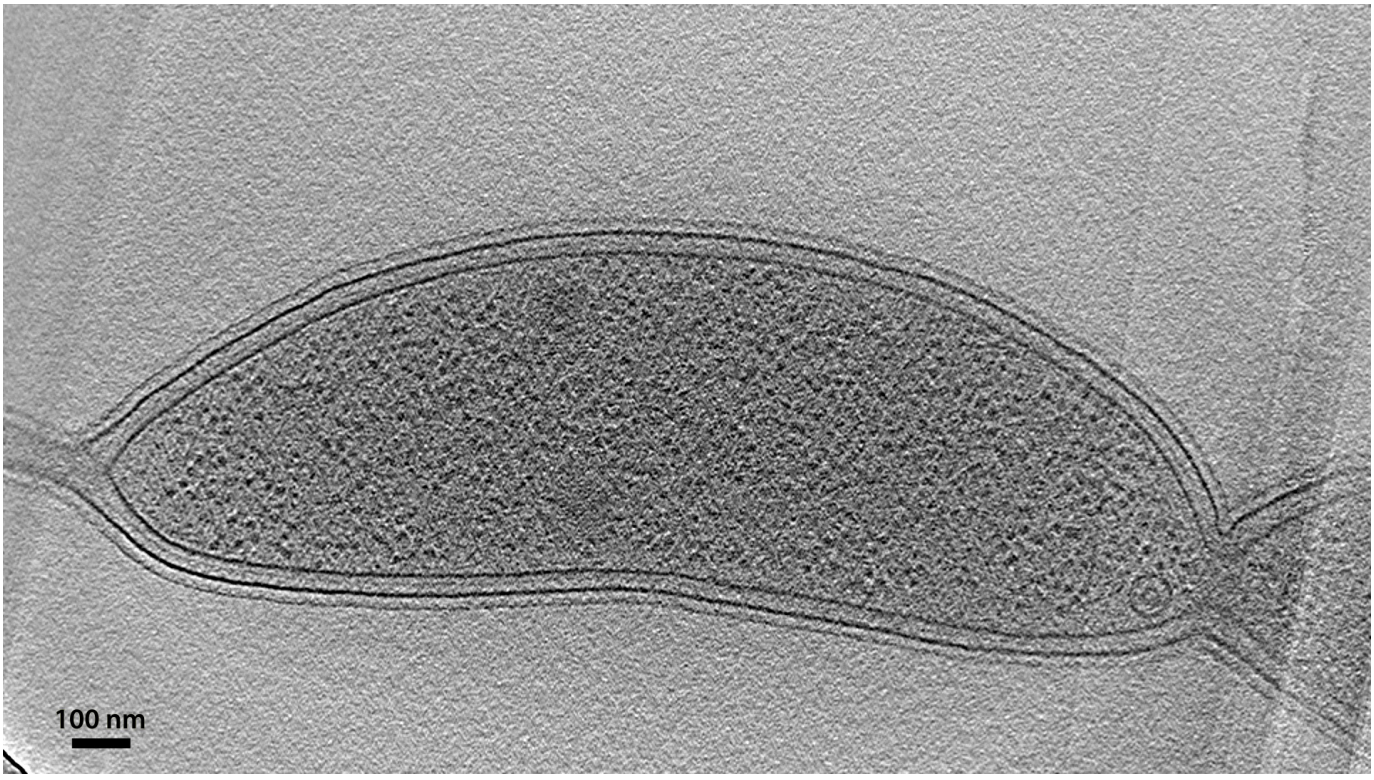
Growth only gets you so far. For a coccoid cell, growth increases volume more rapidly than surface area, which is a problem if the cell relies on nutrients imported from the environment. Even if a cell is rod-shaped (so its surface area to volume ratio remains relatively constant with growth), increased volume increases diffusion times, making metabolism less efficient. So what can you do to keep your cell thriving?

It may be time to divide. In essence, division simply splits a cell, the "mother," into two "daughters." Each daughter will be roughly half the size of the mother. A fair split, though, particularly of critical components like the genome, requires careful coordination. Think about the things in your cell. Some of them are present in many copies, like the lipids in the membrane(s) and most of the proteins. Others are present in very few copies, like the chromosome(s). Conceptually, we can sort components into two broad categories: high copy-number and low copy-number items. How would you split the high copy-number items? Easy, right? Just split the cell in the middle and each half will have plenty. This is true, for instance, for the ribosomes you see in this *Thiomonas intermedia* cell.

What about low copy-number components? Look at the poly-phosphate storage granules in the same cell. To ensure each daughter cell gets the same number, they are evenly spaced along the length of the cell, ready for division in the middle.

Thiomonas intermedia Collected by: Cristina Iancu DOI: 10.22002/D1.1507


PopZ
division plane
100 nm


100 nm

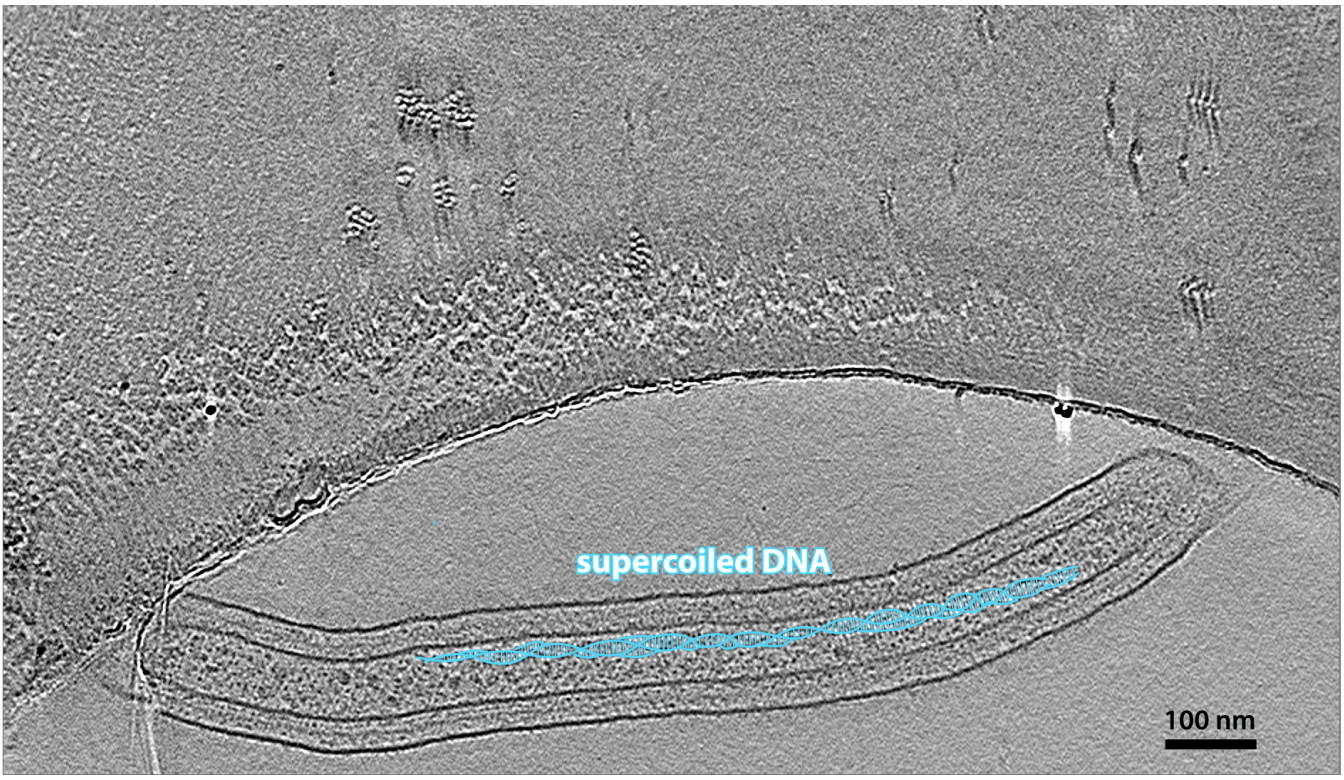
2. Fission

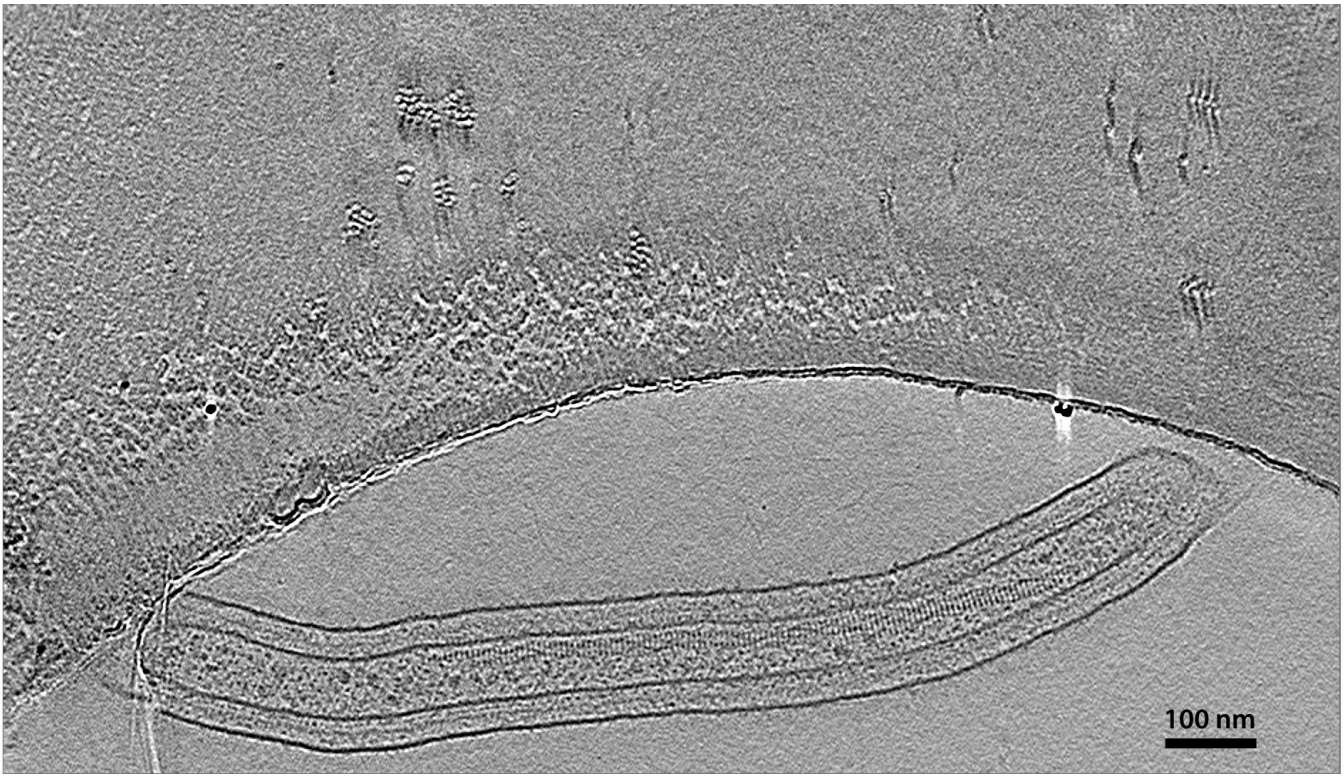
The most important low-copy number component in your cell is its genome; without the instructions, nothing gets built. Reflecting its importance, cells have evolved complex mechanisms to coordinate DNA replication and segregation in time as well as space. The details are beyond our scope here, but we can touch on some general structural principles.

If your cell divides by splitting in the middle, what is the easiest way to get one complete genome copy to each daughter? (Assume your cell has a single chromosome like most bacteria and archaea.) Why not just tether each copy of the chromosome to an opposite pole of the cell? Nearly all bacteria, and many archaea, have a protein called **ParB** (for Partitioning) that recognizes a specific sequence (**ParS**) on the chromosome, creating a molecular handle. In *Caulobacter crescentus* like this, ParB also binds a scaffolding protein at the pole called **PopZ**, hooking the handle to the pole. The PopZ scaffold is not highly ordered, so we see it as a diffuse blob of DNA and protein, noticeable because it excludes other large protein complexes like ribosomes. Several species of bacteria use PopZ or other hub-organizing proteins to tether a genome copy, as well as other low-copy-number things like chemosensory machinery, to the cell pole. Other species use a different mechanism involving many copies of a dynamic protein called ParA that bind and release ParB, ratcheting the ParS handle of the chromosome across the cell. Other low-copy-number components, like the carboxysomes and storage granules you just saw, also use ParA for segregation.

Remember that your cell's chromosome is colossal, so getting the ParS handle to one side is only part of the battle. An army of other proteins work to condense the chromosome to a more manageable volume and wrangle with the division machinery to make sure no straggling loops get caught off-sides.

Caulobacter crescentus Collected by: Ariane Briegel DOI: 10.22002/D1.1508


supercoiled DNA
100 nm

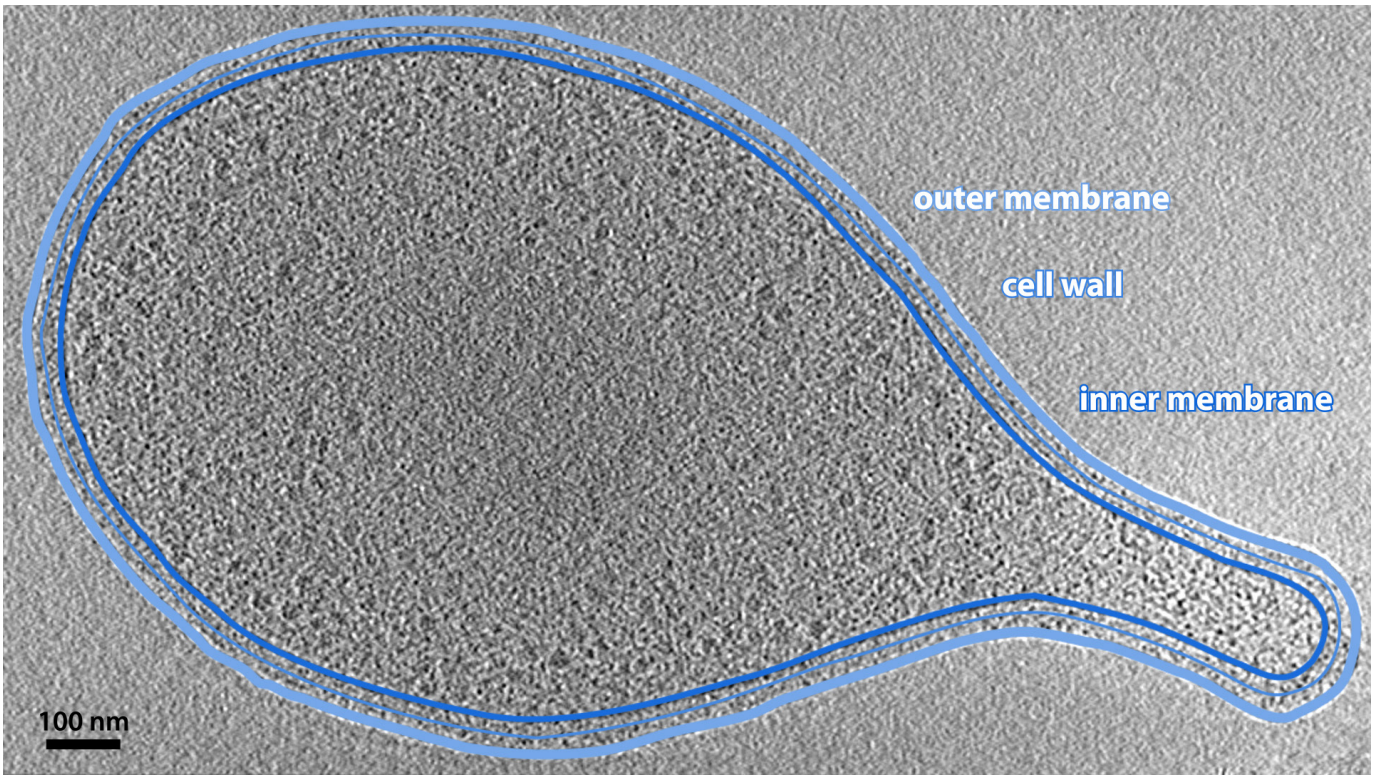

100 nm

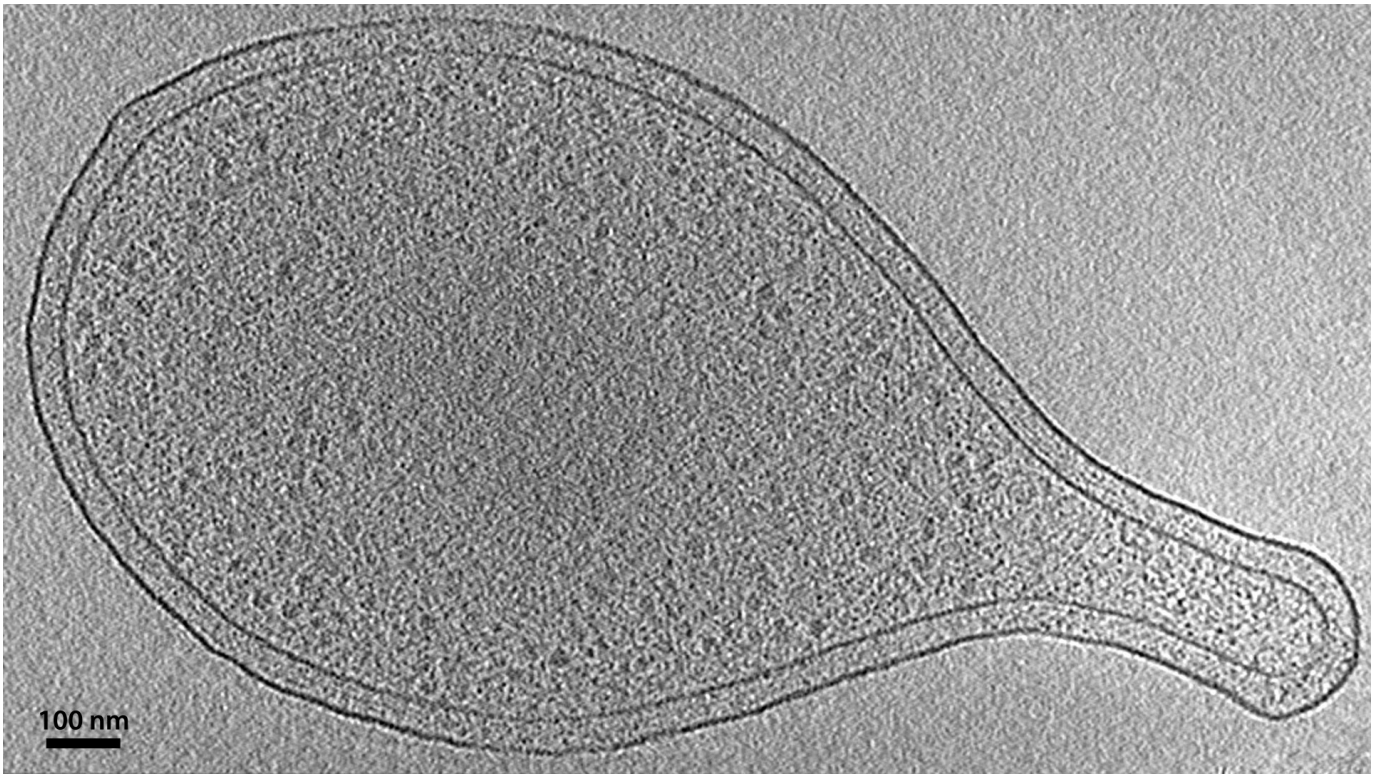
3. Budding

What if your cell divides a different way? Some bacteria produce daughters not by fission, but by budding, like this *Hyphomonas neptunium* cell. These cells concentrate their growth at the end of a stalk (*Learn More* on the next page), producing a daughter cell like blowing a bubble. When the bud becomes big enough, they divide at the end of the stalk to release it. First, though, they have to make sure all the necessary components make it into the bud. The process is most dramatic for the genome; here you can see a copy being transferred through the stalk. The chromosome here resembles a double-stranded DNA helix, but it is actually a higher-order structure of supercoiled DNA. (We think the cross-bands are proteins that help pack the DNA, not hydrogen-bonded bases.)

Several other bacterial species divide by budding, although not all have stalks. Some simply bud from the main cell body; you will see an example later in this chapter.

Hyphomonas neptunium Collected by: Jian Shi DOI: 10.22002/D1.1509


outer membrane
cell wall
inner membrane
100 nm

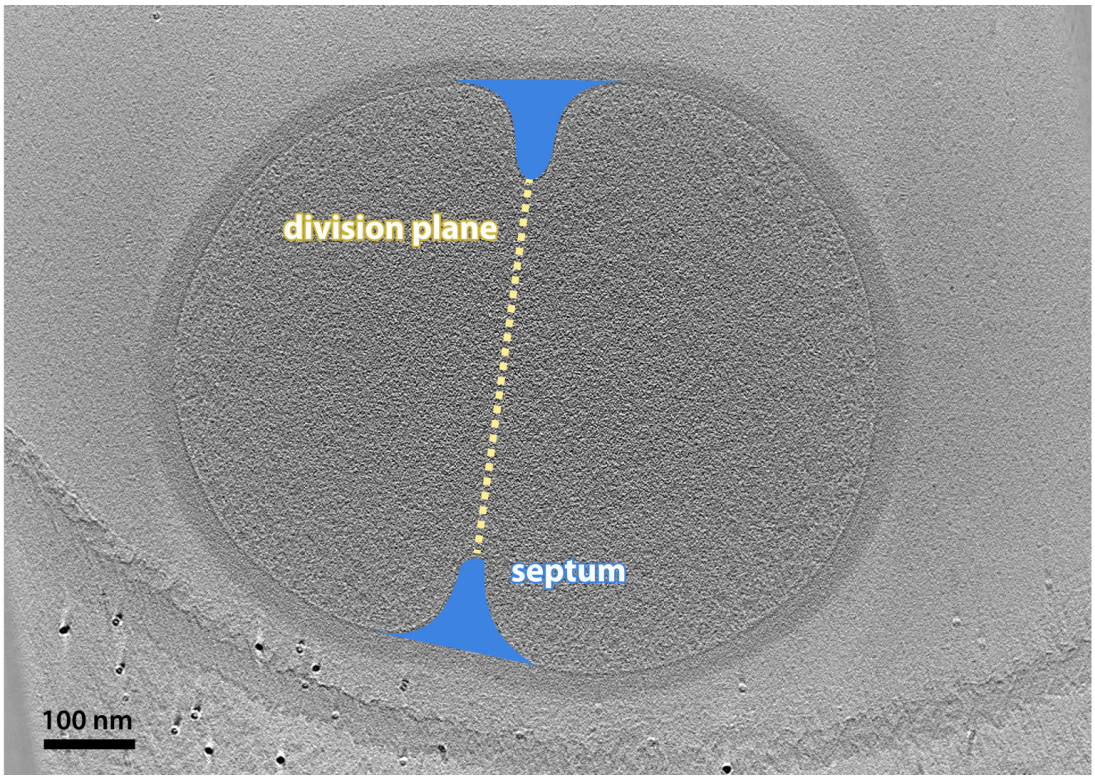

100 nm

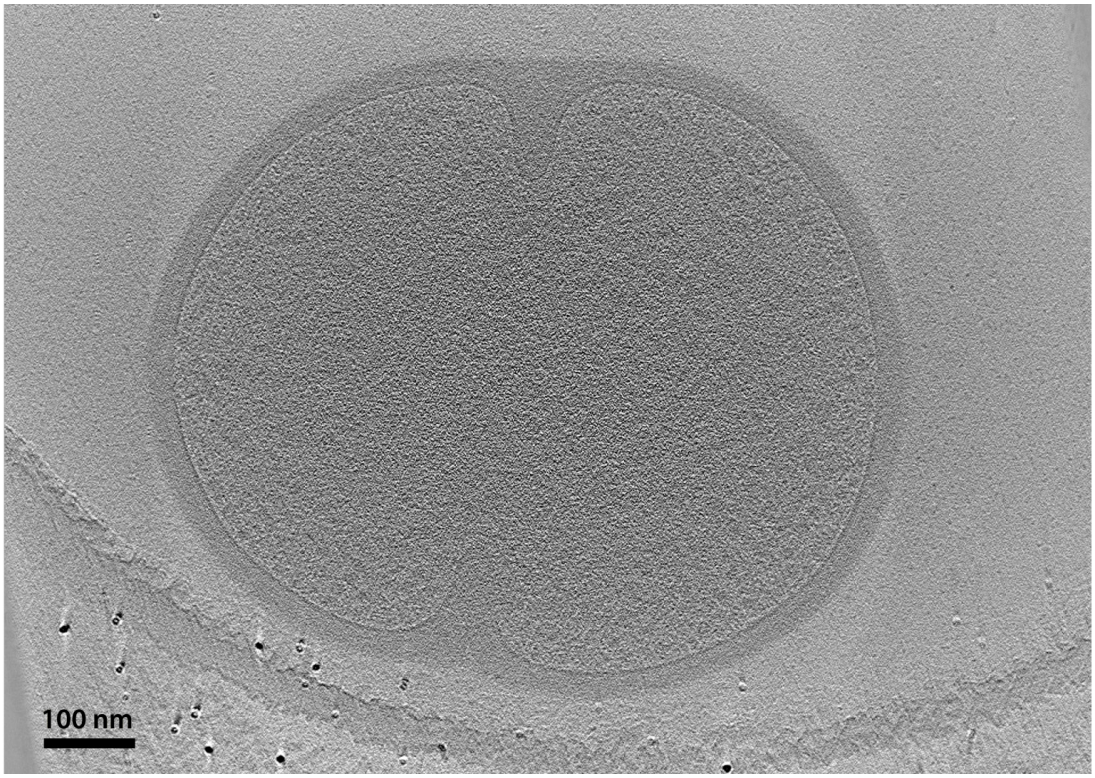
Learn More: Lifecycle

Hyphomonas neptunium *grow a single stalk from one end of their cell body, similar to the* Caulobacter crescentus *you saw in Chapter 4.1. The function of the stalk, though, is different in this budding bacterium than in* C. crescentus*, which divides by more conventional fission.*

H. neptunium *have evolved a program of stages they pass through in the course of their life. When a newborn cell is released, it is in the "swarmer" stage, using a flagellum (discussed in the next chapter) to swim away in search of a favorable location to settle down, then jettisoning its flagellum and growing a stalk like this with which to make its own bud. Once a cell settles down into the "stalked" stage, it spends the rest of its life sending off buds as long as conditions are good. We will discuss this lifecycle, and its advantages, more in Chapter 8.4.*

Hyphomonas neptunium *Collected by: Jian Shi* *DOI: 10.22002/D1.1519*


division plane
septum
100 nm

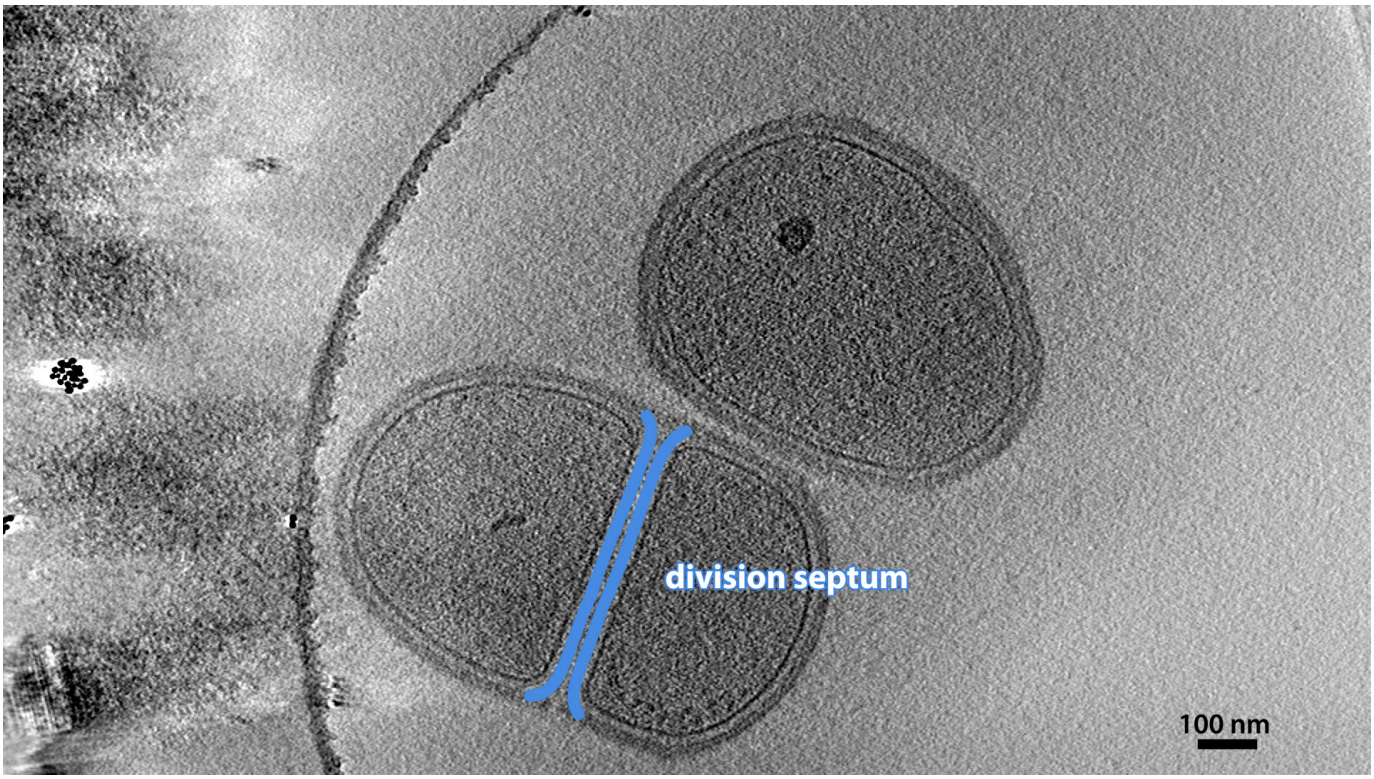

100 nm

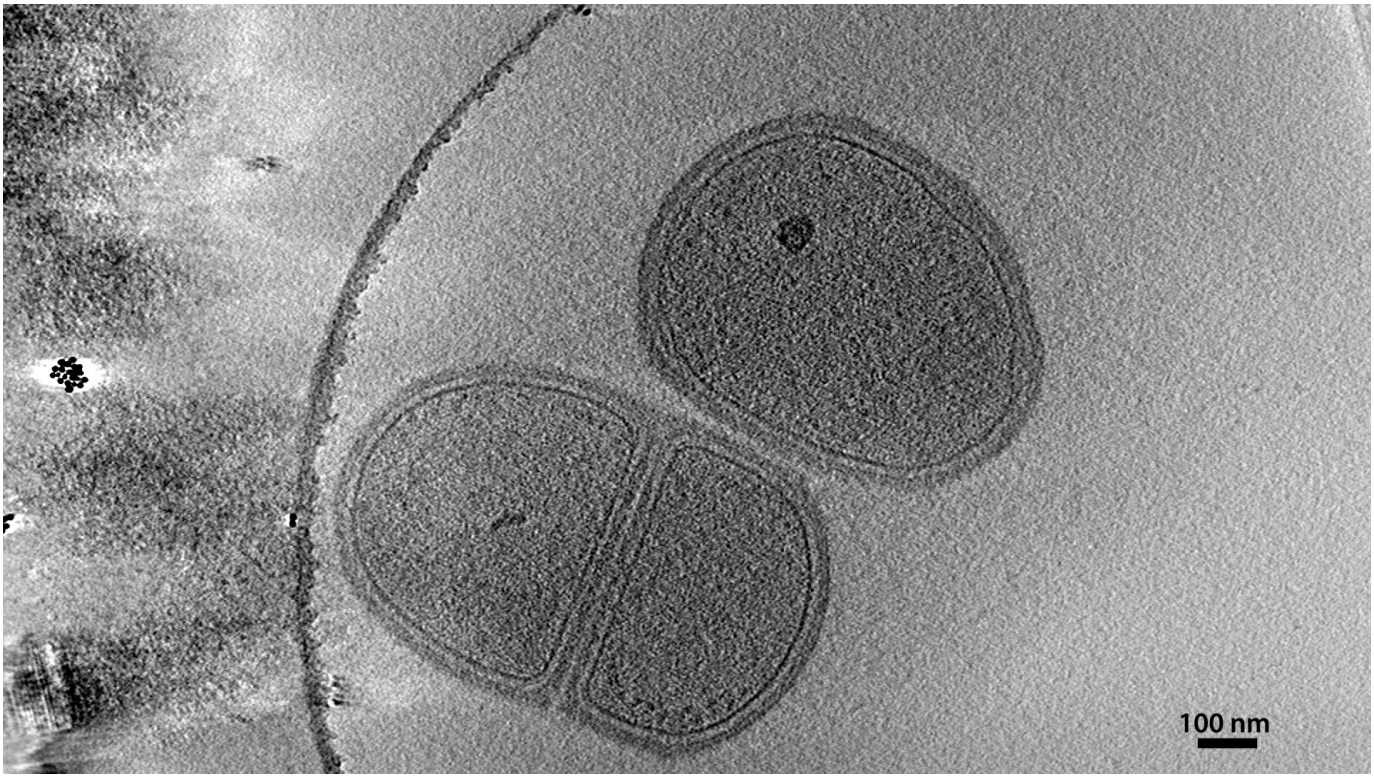
4. Monoderm Septum

Once your cell has gotten everything where it needs to go, how can it actually divide? Just as fences separate neighbors, why not use the cell wall to build a **septum** ("fence") between the two daughters? That's what this Staphylococcus aureus cell is doing, using its cell wall to begin **cytokinesis**–the physical division of the cytoplasm. In monoderm bacteria like this, with a thick cell wall, the septum is easy to see.

Staphylococcus aureus gets its name from the clusters of cells produced by repeated rounds of division, resembling grapes (or "staphyli" in Greek).

Staphylococcus aureus Collected by: Georges Chreifi DOI: 10.22002/D1.1510


division septum
100 nm

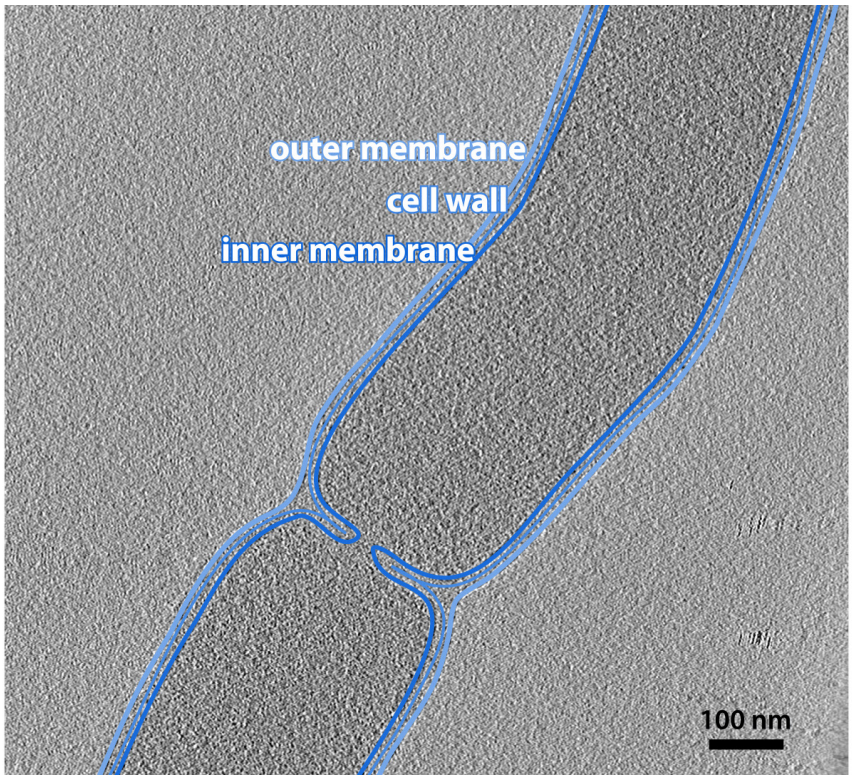

100 nm

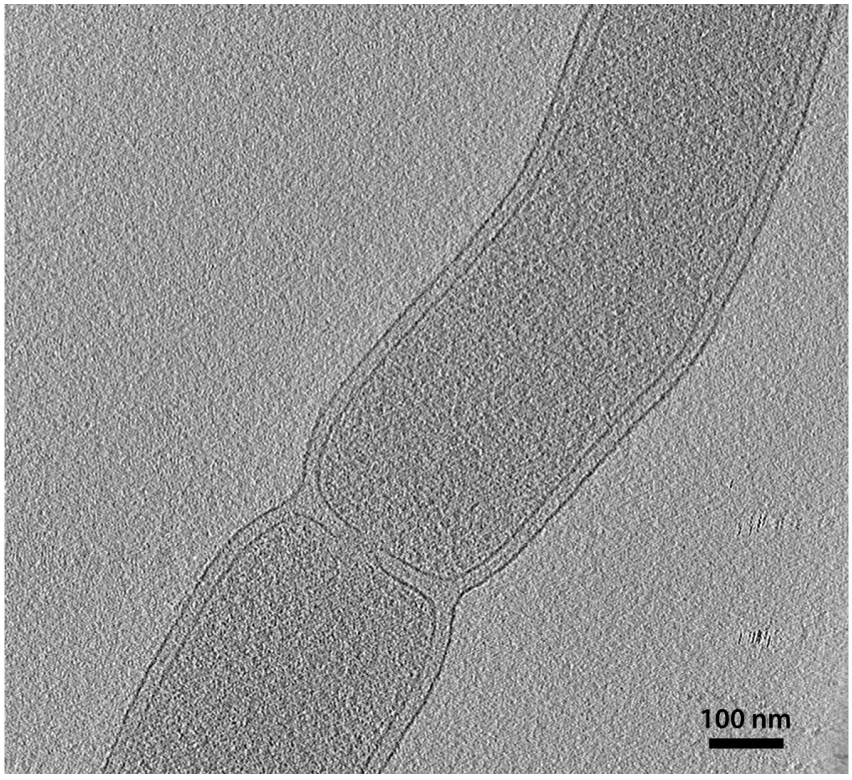
5. Monoderm Cytokinesis

The division septum grows in from the periphery of the dividing cell toward the middle, drawing the membrane with it. When it reaches the middle, the membrane seals off on each side and the septum separates to release the two cells. The bottom *Tetrasphaera remsis* cell here is at a fairly late stage of this process. This cell also illustrates another point about division: the division plane is not always in the exact middle of the cell. Depending on the species, division may produce daughter cells of unequal sizes.

There can also be more than two daughter cells. Some species undergo simultaneous divisions, with two or more septa forming more or less at the same time. The genus of *Tetrasphaera remsis* gets its name from this property–cells can either divide into two (as in this case) or four roughly spherical cells.

Tetrasphaera remsis Collected by: Ariane Briegel DOI: 10.22002/D1.1511

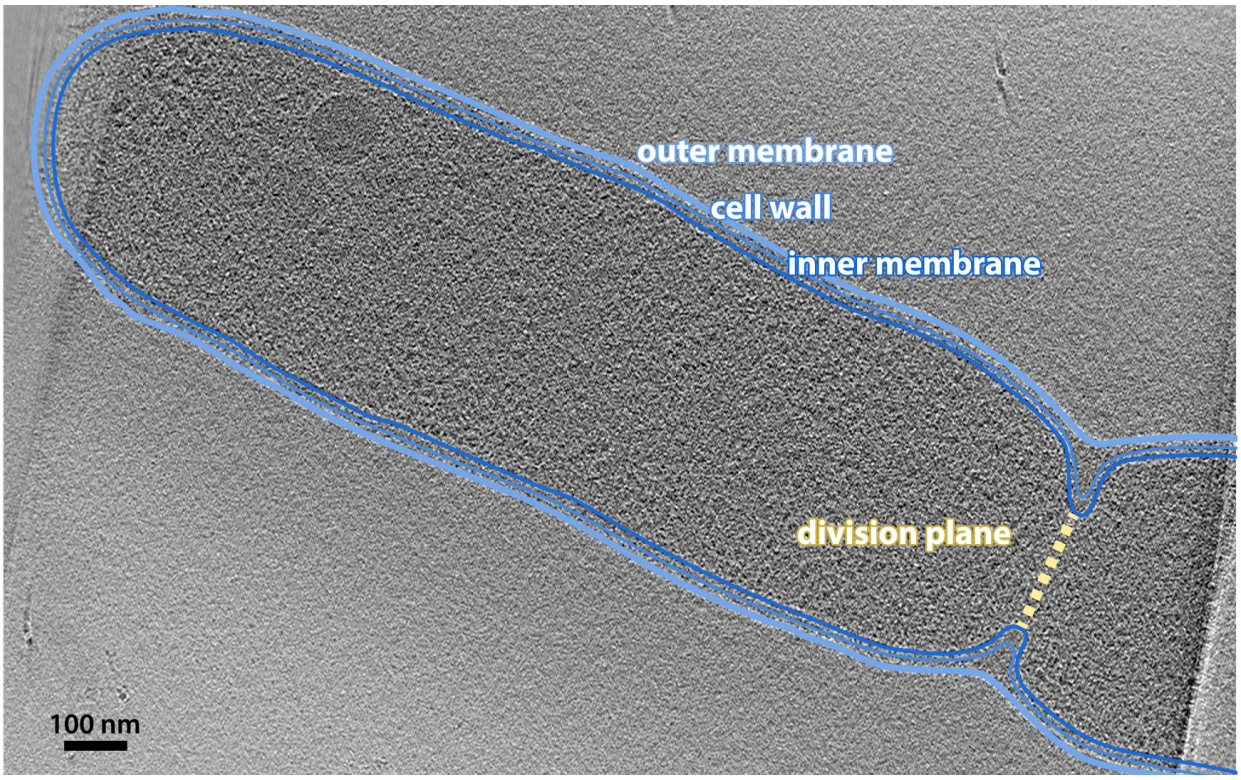

outer membrane
cell wall
inner membrane
100 nm

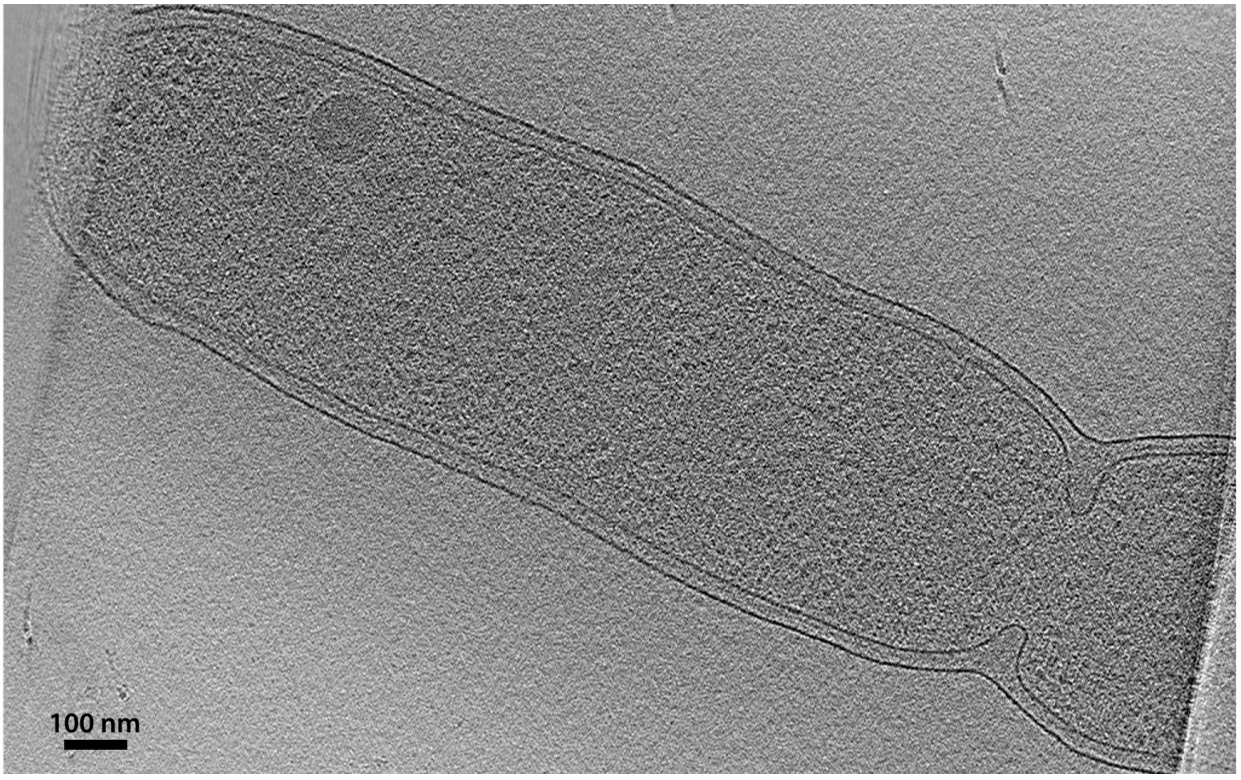

100 nm

6. Diderm Cytokinesis

The cytokinesis process is similar for diderm bacteria, as you can see in this *Idiomarina loihiensis* cell, with the cell wall and inner membrane growing inward. The process is almost complete here, with just a thin channel of cytoplasm in the very middle connecting the daughter cells. Notice the cell wall zippering apart at the edges of the septum. For another example slightly earlier in the process, *Learn More* on the next page.

Idiomarina loihiensis Collected by: Morgan Beeby DOI: 10.22002/D1.1512

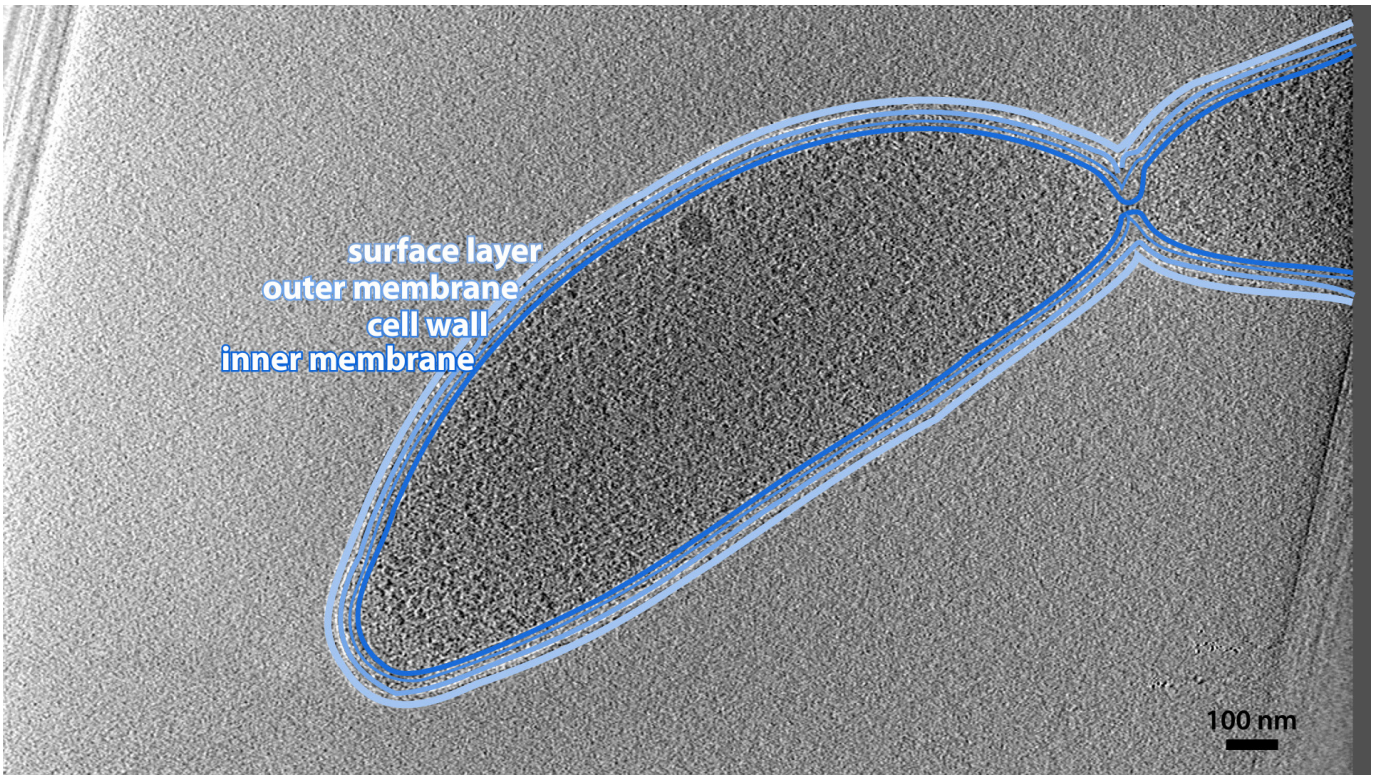

outer membrane
cell wall
inner membrane
division plane
100 nm

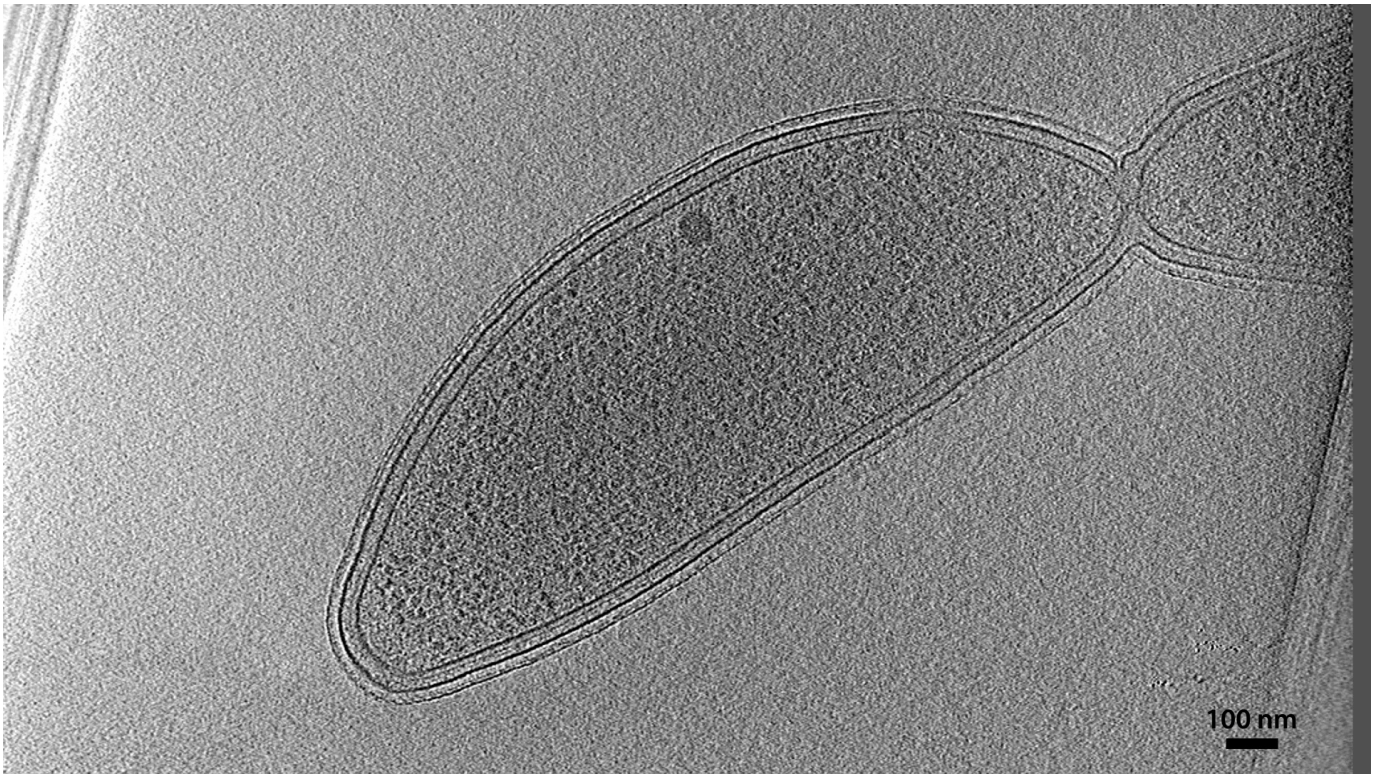

100 nm

Learn More: Mid-Constriction

This diderm cell is about halfway through cytokinesis. The two daughter cells are still connected by a wide bridge of cytoplasm, but the inner membrane and cell wall have drawn in all around.

Sphingopyxis alaskensis *Collected by: Morgan Beeby* *DOI: 10.22002/D1.1521*

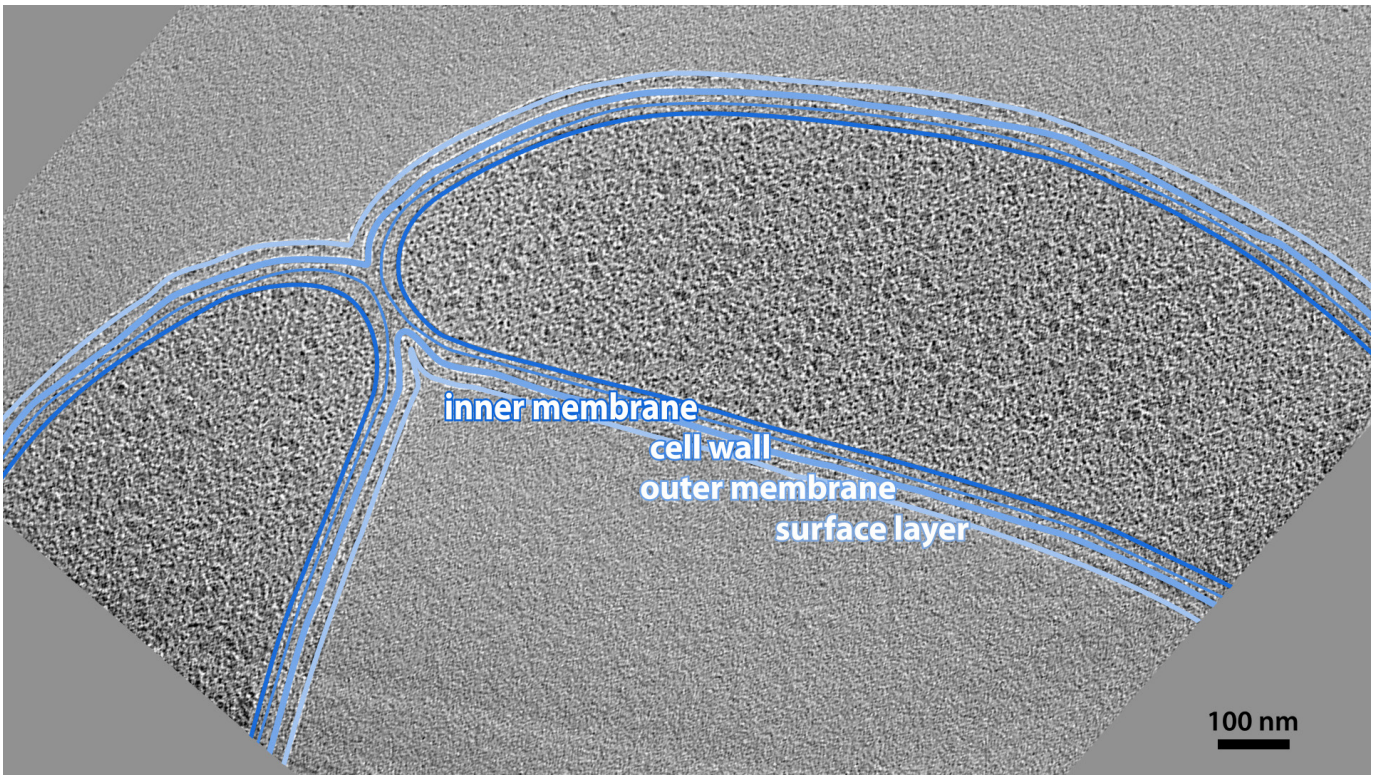

surface layer
outer membrane
cell wall
inner membrane
100 nm

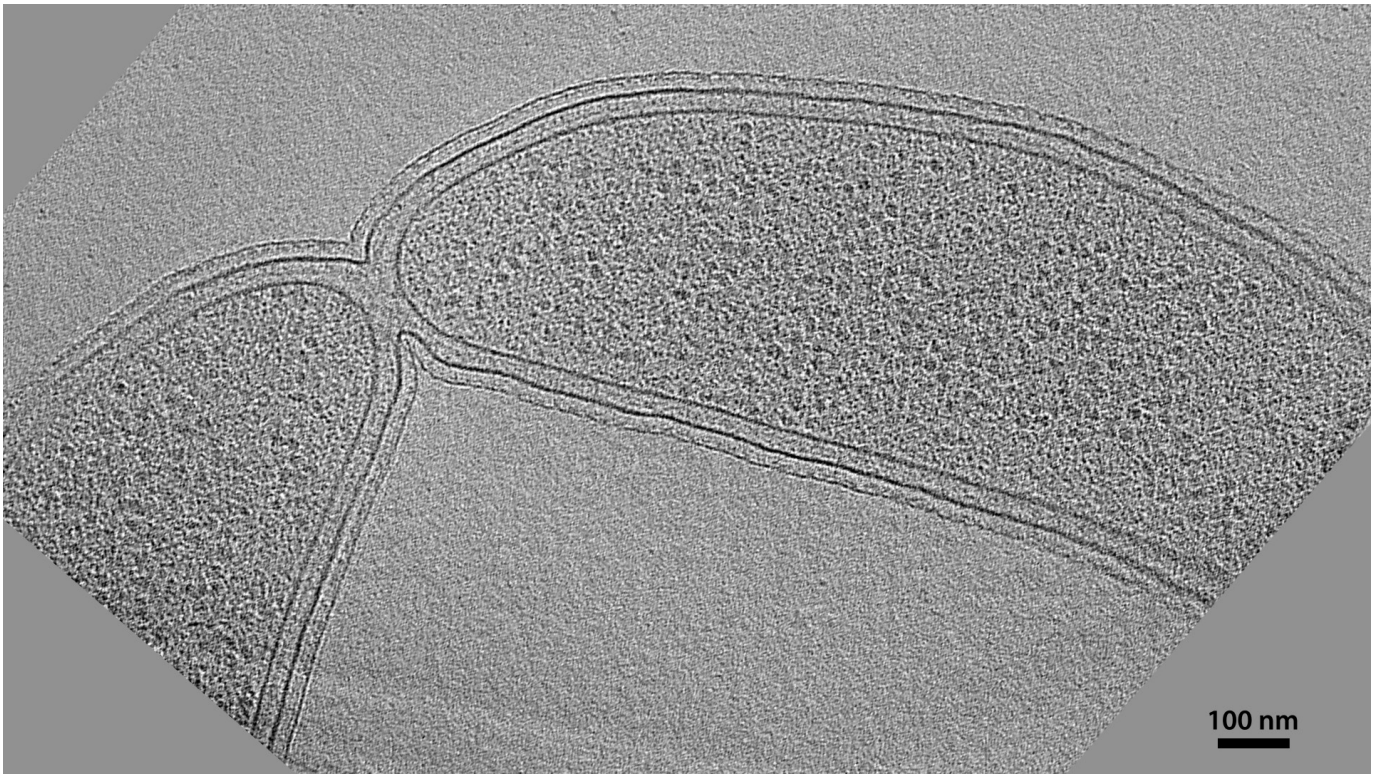

100 nm

7. Outer Membrane Constriction

In some species, the outer membrane constricts at the same time as the inner membrane, as you saw, for instance, in the *Thiomonas intermedia* cell at the beginning of this chapter. In others, including the cells you saw on the last page, the outer membrane is mainly remodeled at the end of the process, as the cells separate. Other species are intermediate. In *Caulobacter crescentus*, the outer membrane constricts along with the inner membrane and cell wall. At the end of division, though, the inner membrane seals off before the outer membrane, as you can see in this cell, which is at a very late stage of division. For an even later stage, see the example in the *Learn More* section on the next page.

Caulobacter crescentus Collected by: Zhuo Li DOI: 10.22002/D1.1513

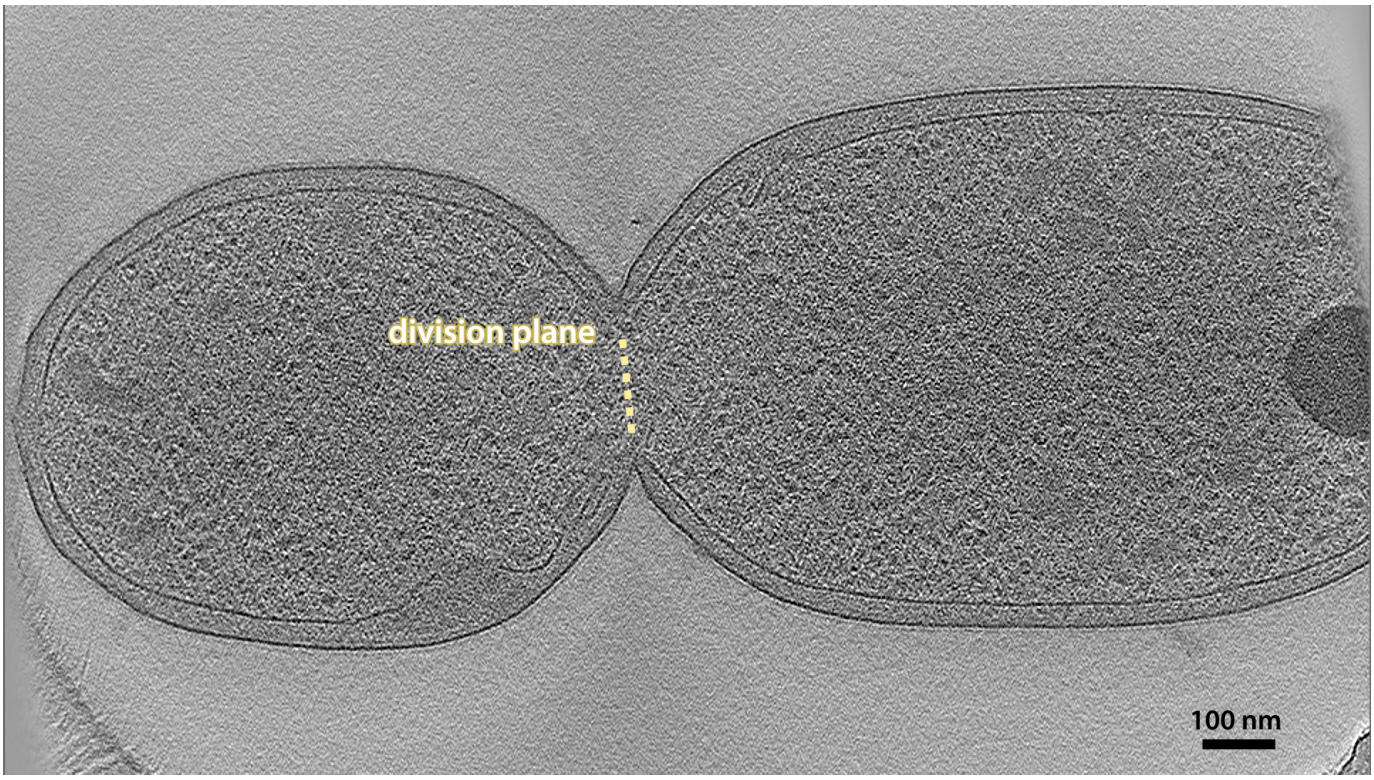

inner membrane
cell wall
outer membrane
surface layer
100 nm

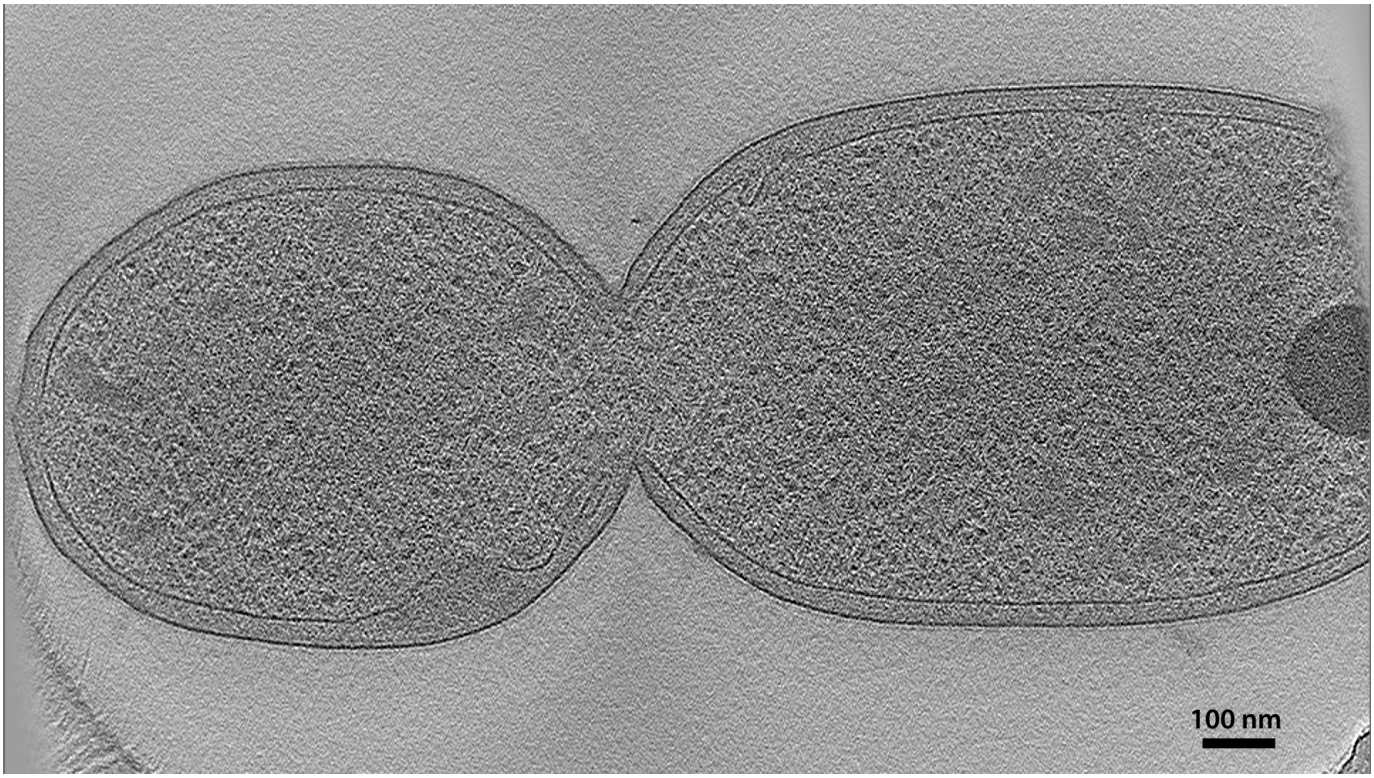

100 nm

Learn More: Final Constriction

This Caulobacter crescentus *cell has completely separated its inner membrane and cell wall, but not yet the outer membrane and surface layer.*

Caulobacter crescentus *Collected by: Qing Yao* *DOI: 10.22002/D1.1523*

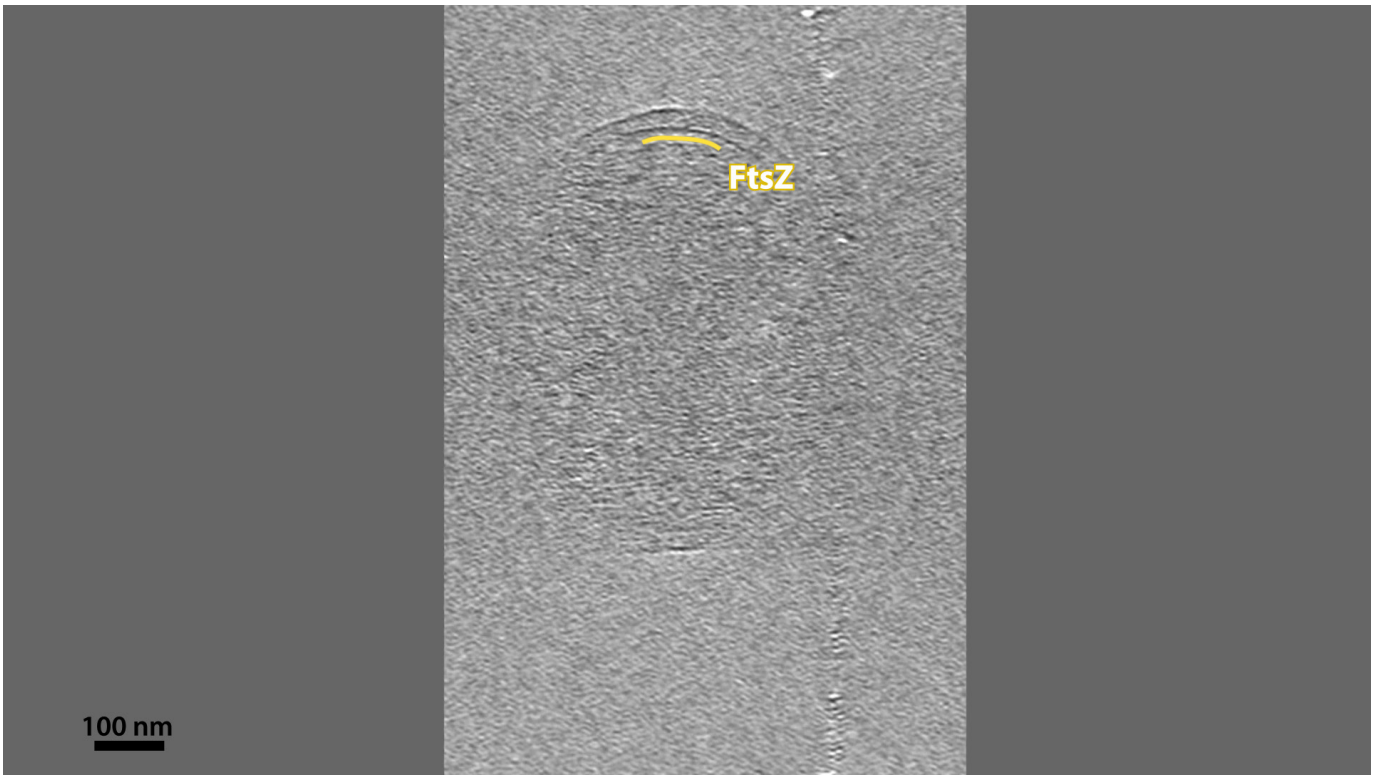

division plane
100 nm

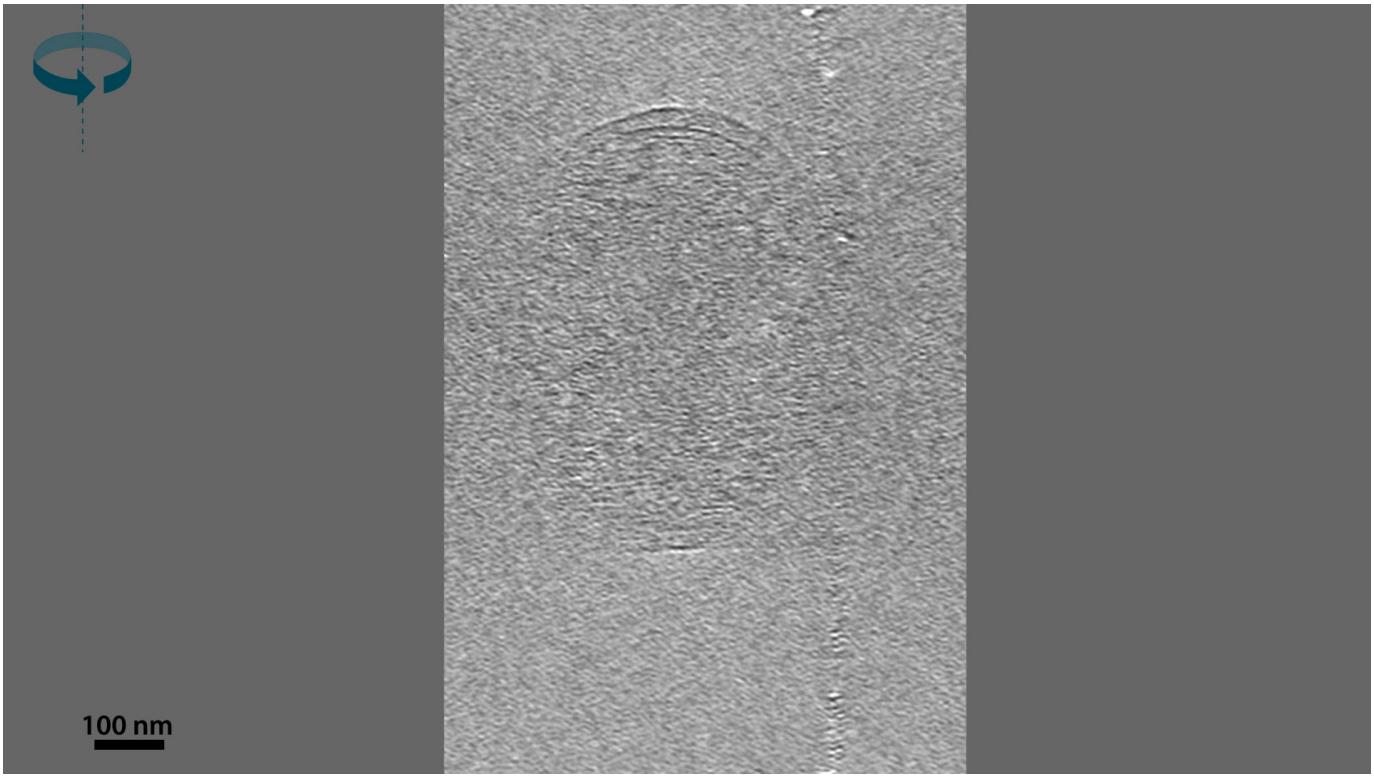

100 nm

8. Asymmetric Division

Like monoderms, most diderms divide into roughly equally-sized daughter cells, but not all do. Just like the *Tetrasphaera remsis* you saw earlier, some diderm species produce one larger, and one smaller, daughter, like this *Agrobacterium tumefaciens*. In this case, this is because the smaller daughter is technically a bud. *A. tumefaciens* concentrate their growth at one pole, pushing out a bud that, once it becomes large enough, is pinched off and released. The process is very similar to what you saw in *Hyphomonas neptunium*, just without the intervening stalk.

Agrobacterium tumefaciens Collected by: Elitza Tocheva DOI: 10.22002/D1.1514


FtsZ
100 nm


100 nm

9. Asynchronous Constriction

Division can also be asymmetric in a different way: how constricted one side of the cell is relative to the other. You can see that in this *Cupriavidus necator* cell. Asymmetric constriction is most common early in division, but it sometimes persists longer (*Learn More* on the next page). To understand why this happens, we need to take a closer look at how cells divide.

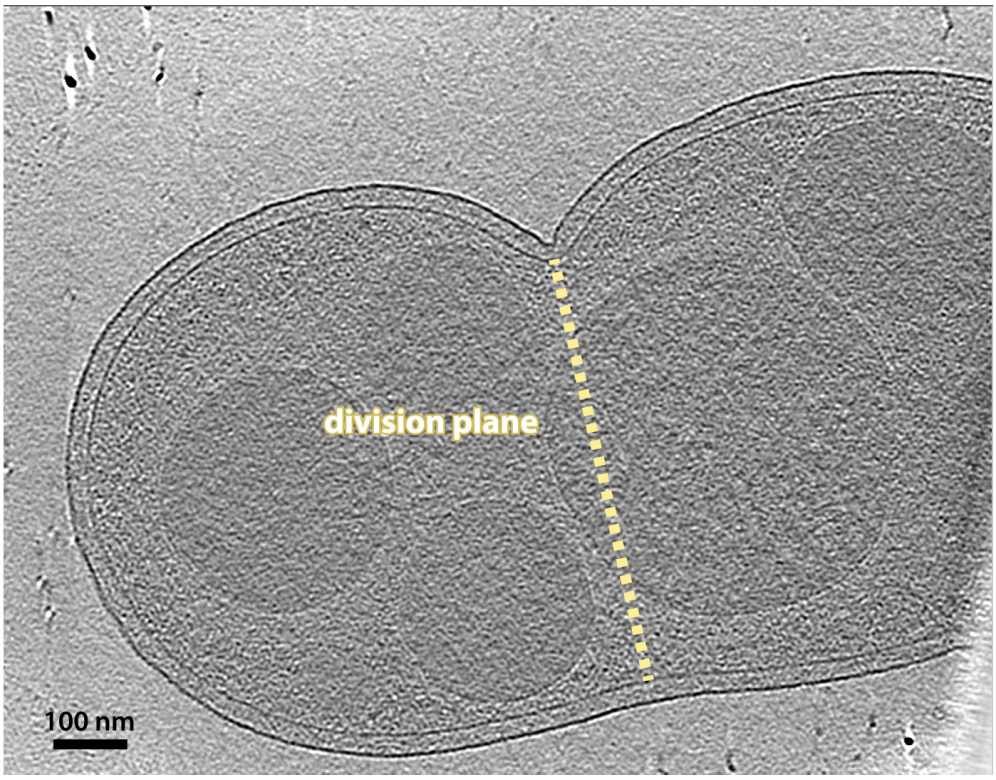
To divide, your cell's membrane and cell wall constrict. But remember that the cell wall is resisting the non-trivial force of turgor pressure pushing outward. So how can it overcome this pressure and grow inward? For almost all bacteria and many archaea, the answer is a protein contractor called **FtsZ** (Filamenting Temperature-Sensitive mutant Z, named for a genetic screen for cells that failed to divide, and therefore grew into long filaments). FtsZ is another piece of your cell's cytoskeleton, and is homologous to eukaryotic tubulin. FtsZ polymerizes into filaments that are linked to the cell membrane around the division plane (*Learn More* on following pages). The filaments are highly dynamic, forming, disassembling and reassembling within seconds.

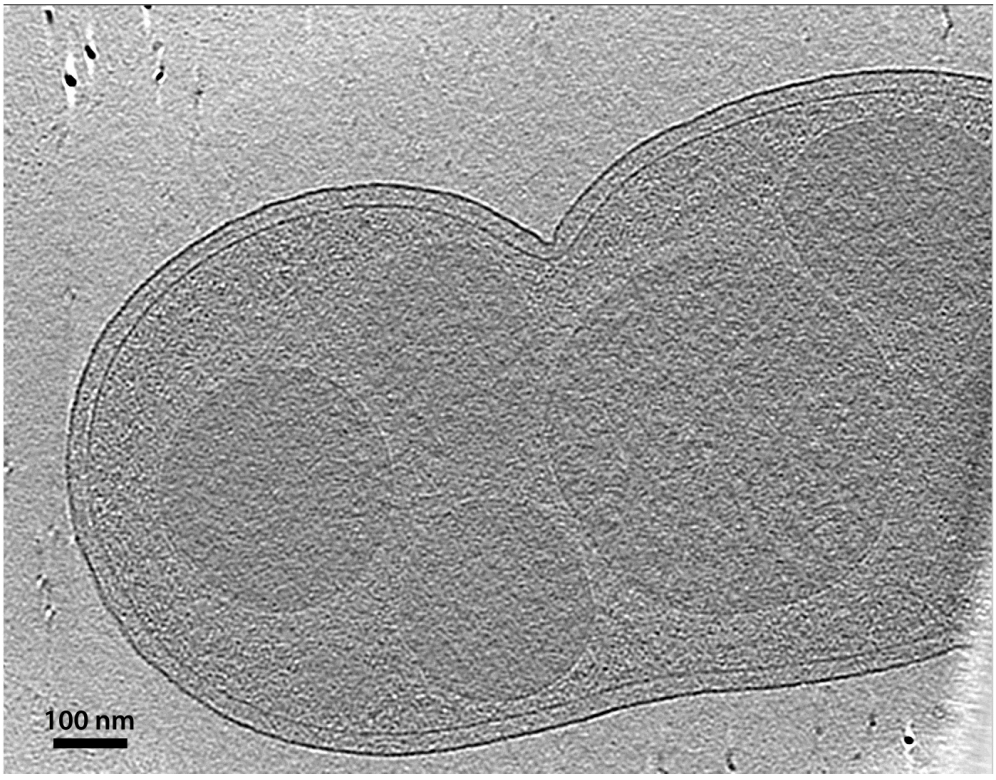
How does FtsZ find the right spot? There are multiple mechanisms, but an elegant one in cells like this involves an inhibitor that localizes to the cell poles, making a repressive gradient that is strongest at the ends of the cell and weakest in the middle. As the rod-shaped cell grows, the inhibitor concentration at the middle eventually drops low enough that FtsZ can polymerize. Most cells also inhibit FtsZ polymerization when the genome is still in the way, a mechanism called "**nucleoid occlusion**." Some species do this with an FtsZ-inhibiting protein called MipZ that concentrates around the ParB chromosomal handle, ensuring that FtsZ filaments do not form until the chromosome is clear.

Once everything is ready (the cell is long enough and the nucleoid out of the way), FtsZ filaments form at the division site. Initially, a single, short filament (or a few) appears. Already, this is enough to begin constricting the cell, which explains why many bacterial cells, including this one, start to constrict on one side only. FtsZ filaments run parallel to the cell membrane, so they appear as dots in cross-section; if we rotate the cell around to look down its long axis, we can see them.

Note: we cannot see the membrane (and any associated FtsZ filaments) all the way around the cell due to the "missing wedge" of information in cryoET imaging. See Chapter 1.6 for an explanation.

Cupriavidus necator Collected by: Morgan Beeby DOI: 10.22002/D1.1515

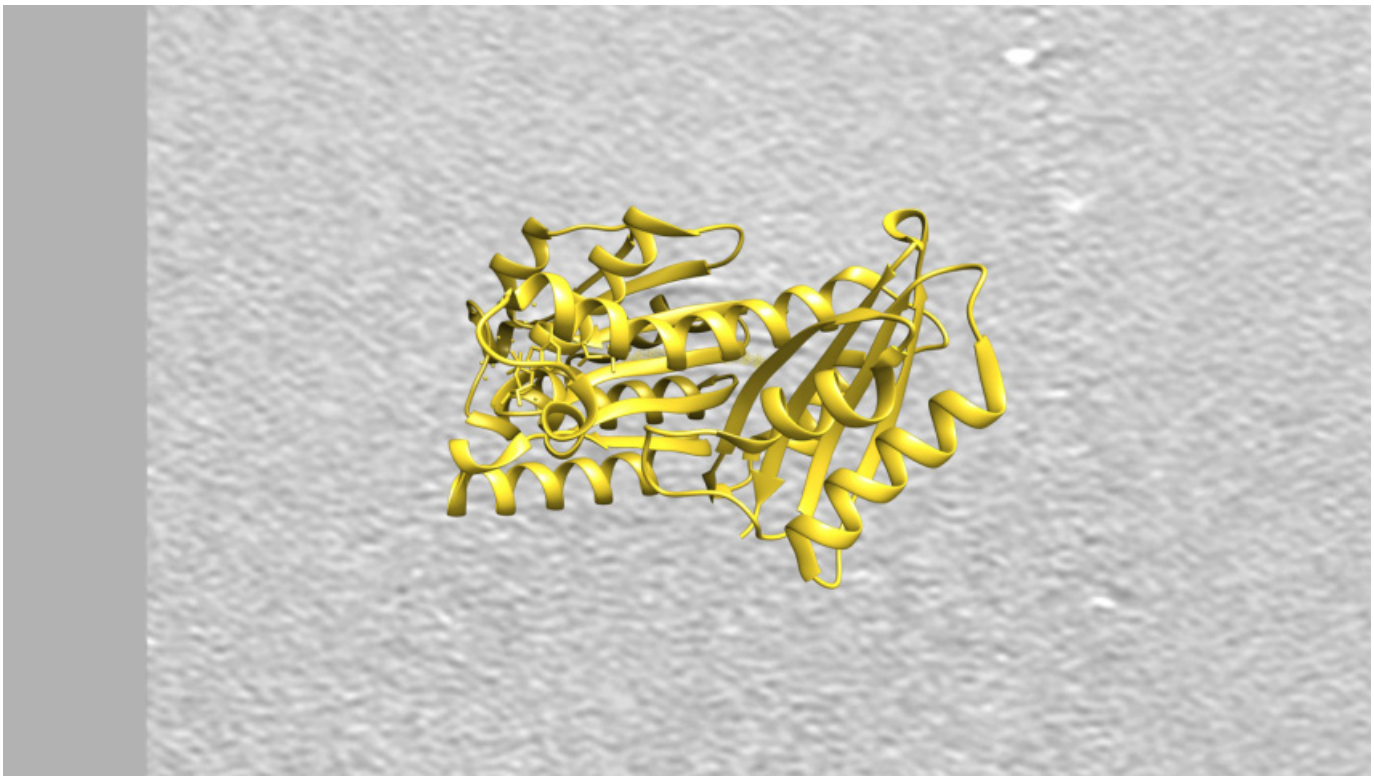

division plane
100 nm

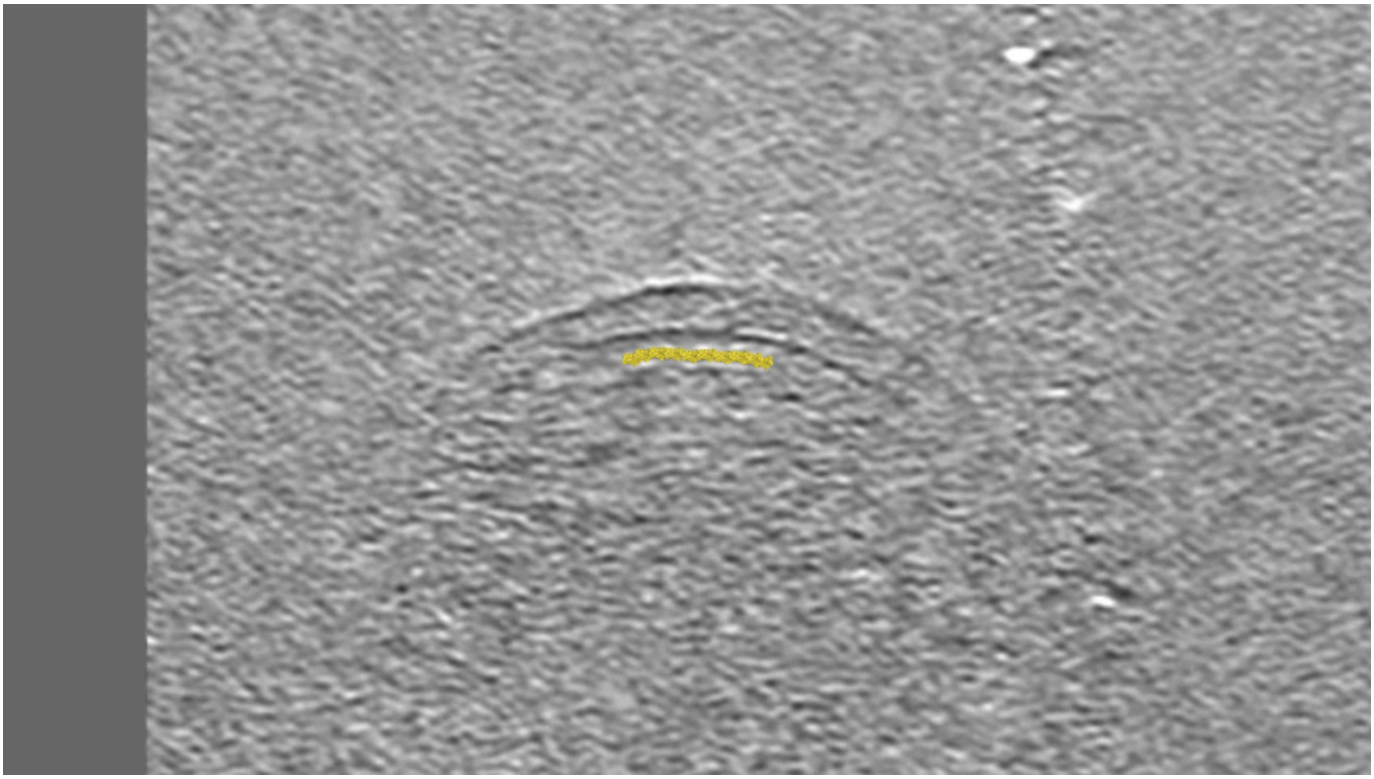

100 nm

Learn More: Asymmetric Constriction

This C. necator *cell is at a later stage of cytokinesis. In most cells, constriction would be more or less uniform around the circumference by now, but in some cells, including this one, the asymmetry persists.*

Cupriavidus necator *Collected by: Morgan Beeby* *DOI: 10.22002/D1.1524*



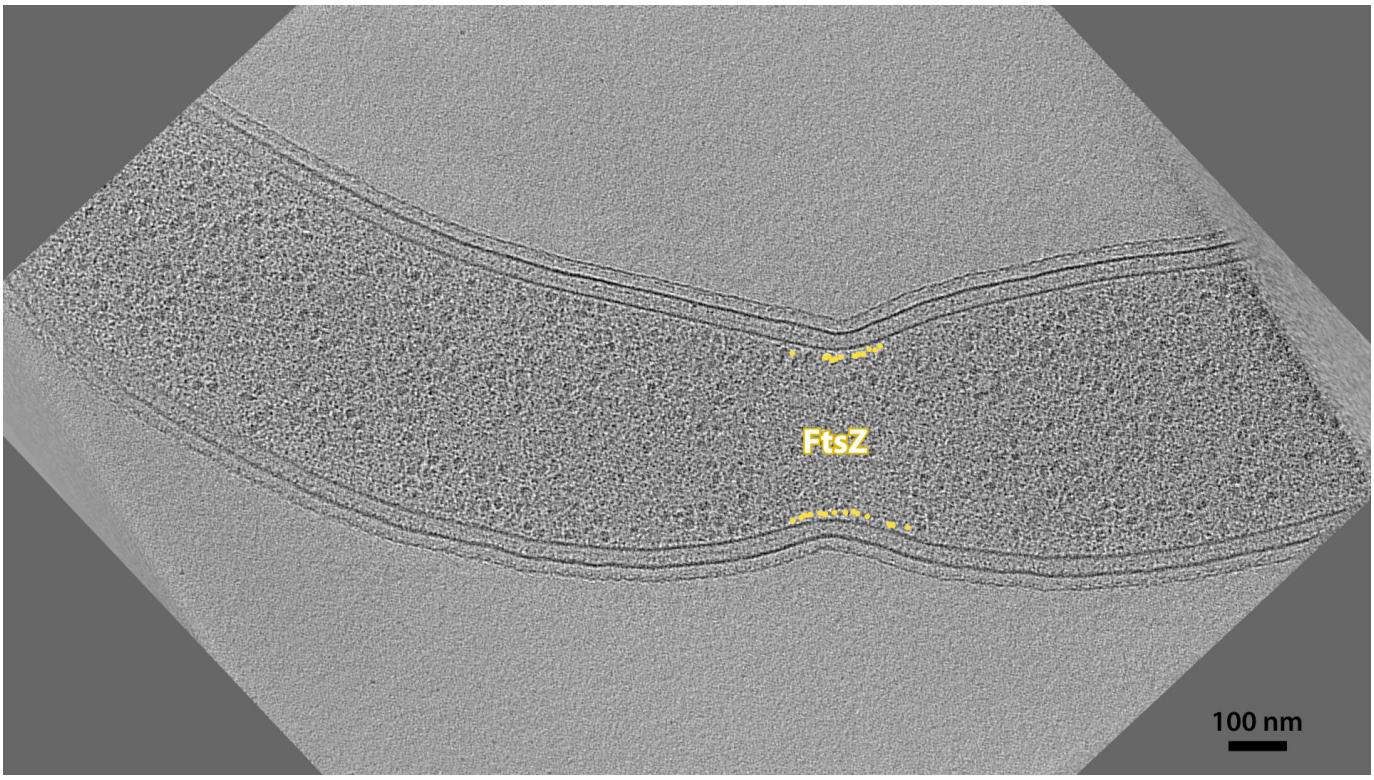


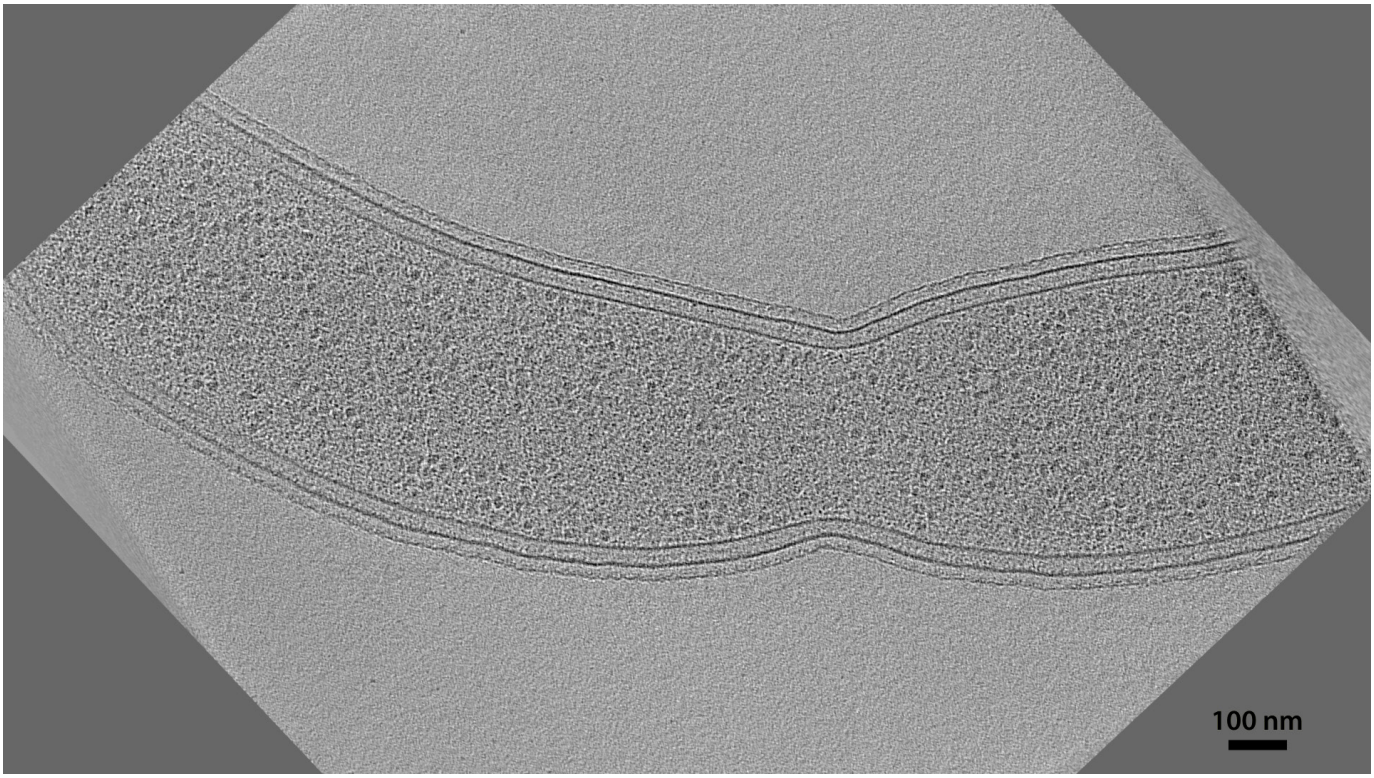
Learn More: Structure

While the structure of FtsZ is known, as seen here from Staphylococcus aureus *[45], its mechanism is not. It is an enzyme that hydrolyzes GTP, so one hypothesis is that the curvature of the filament changes as the chemical reaction drives subunits from one conformation to another. This different filament conformation may pull inward on the attached membrane, allowing the cell wall to build inward behind it.*

Source: Wagstaff et al. (2017)

Structure: PDB 5MN4

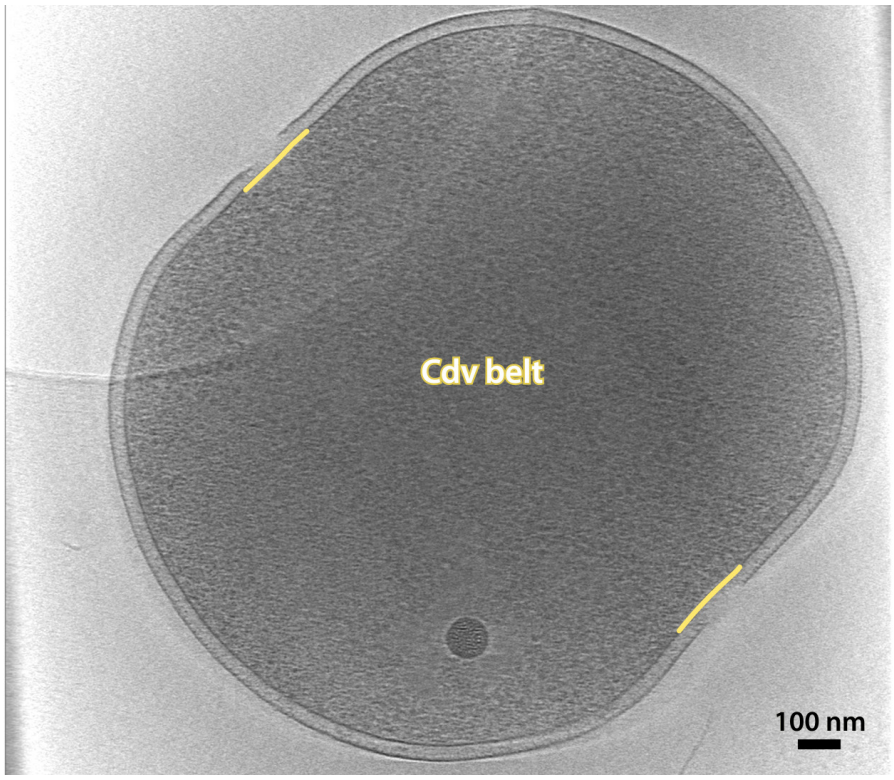

FtsZ
100 nm

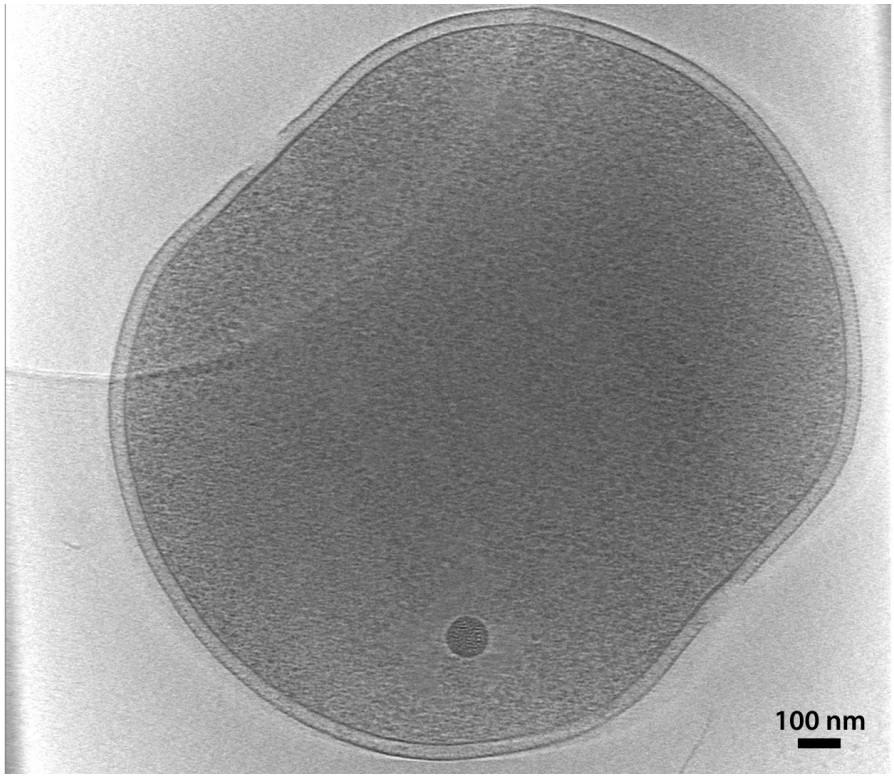

100 nm

10. FtsZ

As cytokinesis progresses, FtsZ filaments keep assembling and growing, soon forming a complete ring of long, overlapping filaments around the full circumference of the cell. This drives further constriction more or less uniformly. In this *Caulobacter crescentus* cell in a later stage of division, you can see many FtsZ filaments lined up in cross-section–the dots on both sides of the division plane. If we rotate the cell around, you can see filaments extending on both sides as far as the membrane is visible. (*See the note on the previous page about why we cannot trace the membrane and filaments all the way around*.) They will continue to pull the membrane inward, and direct cell wall to be built behind it, until cytokinesis is complete.

Caulobacter crescentus Collected by: Qing Yao DOI: 10.22002/D1.1516

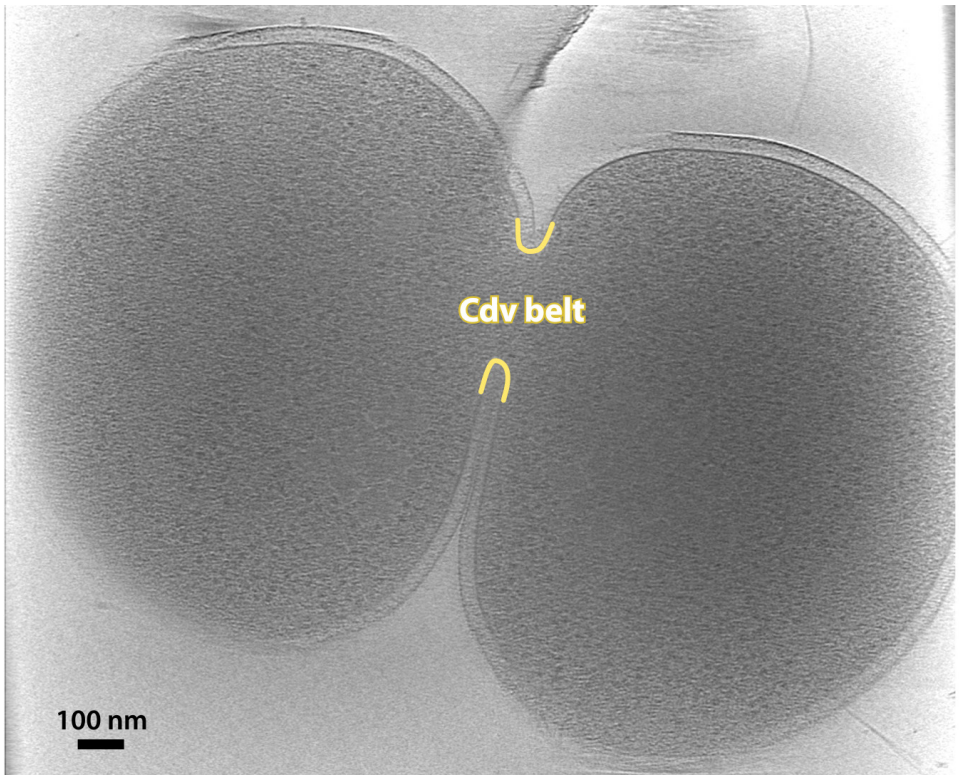

Cdv belt
100 nm

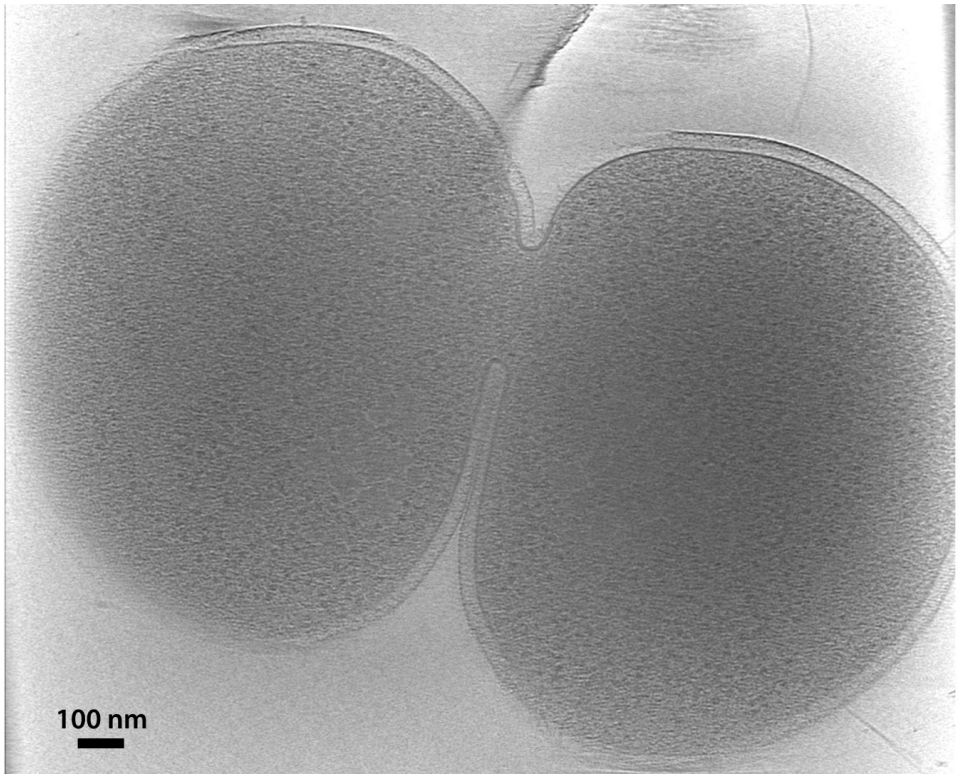

100 nm

11. Archaeal Cytokinesis

Nearly all bacteria, and many archaea, use FtsZ to divide. Other species of archaea, belonging to the Crenarchaeota phylum, use a different cytoskeletal system called **Cdv** (for Cell division). Cdv proteins are homologous to proteins of the Endosomal Sorting Complexes Required for Transport (ESCRT). ESCRT proteins were discovered in eukaryotes, where they are involved in many processes that involve cinching off membranes, from the final stage of cell division to virus budding to endocytosis (hence the name), by which cells engulf things from the environment. Again, despite its fundamental importance, we still do not know exactly how the Cdv machinery works to constrict membranes. In archaeal cells like this *Sulfolobus acidocaldarius*, Cdv proteins form a belt of parallel filaments around the middle of the cell, defining the division plane. The belt consists of a single closely-packed layer of filaments and lies directly next to the membrane. Notice that the rigid surface layer is dismantled outside the belt, presumably so that the membrane can be pulled inward.

Sulfolobus acidocaldarius Collected by: Shrawan Mageswaran DOI: 10.22002/D1.1517


Cdv belt
100 nm

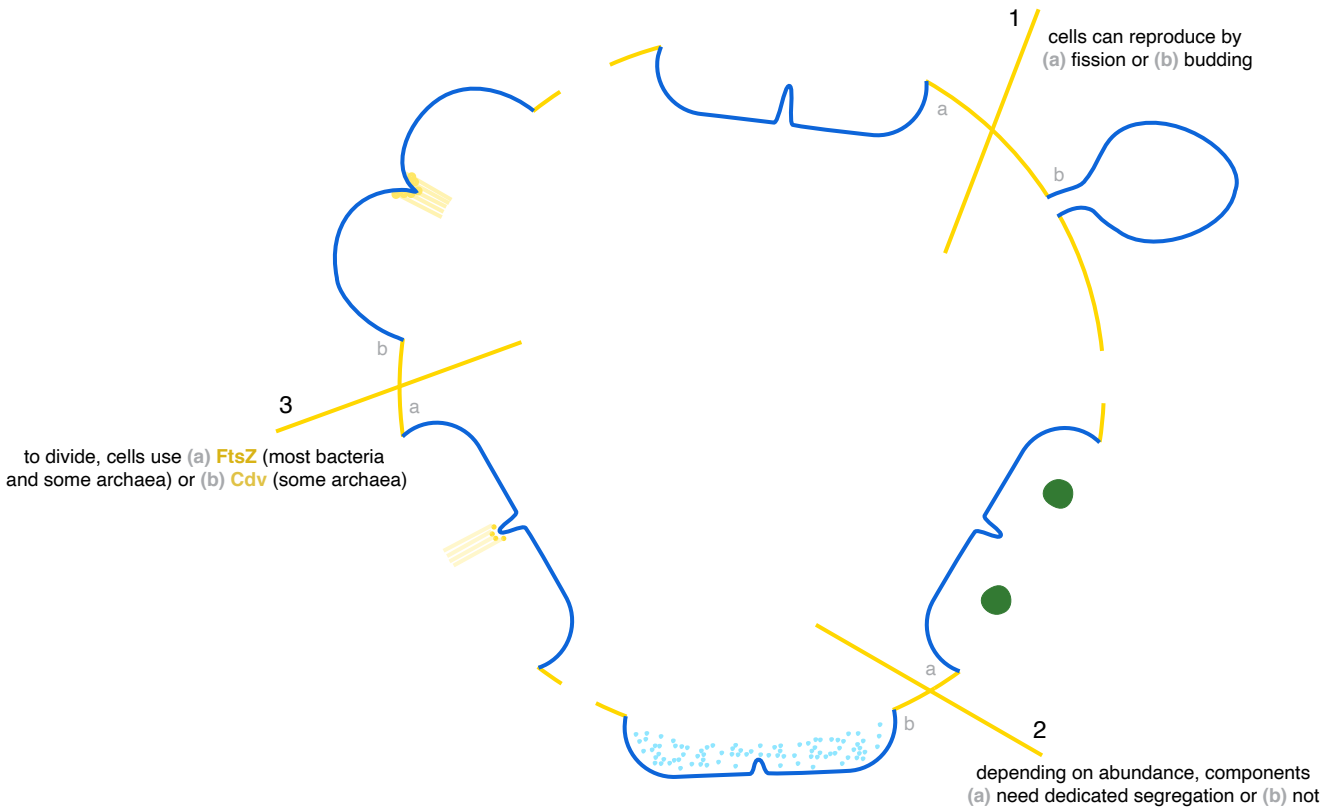

100 nm

12. Cdv

The Cdv filaments then pull the membrane inward, constricting the cell, as you can see in this *Sulfolobus acidocaldarius* at a later stage of division. Again, the exact mechanism is unknown.

The majority of bacterial and archaeal cells divide by one of these two mechanisms–FtsZ or Cdv–but not all do. Some species have neither FtsZ nor Cdv, and we are still figuring out how they divide.

Sulfolobus acidocaldarius Collected by: Shrawan Mageswaran DOI: 10.22002/D1.1518


1
cells can reproduce by
(a) fission or (b) budding
a
b
3
to divide, cells use (a) FtsZ (most bacteria
and some archaea) or (b) Cdv (some archaea)
b
a
2
depending on abundance, components
(a) need dedicated segregation or (b) not
a
b

Summary

Concept Check Questions

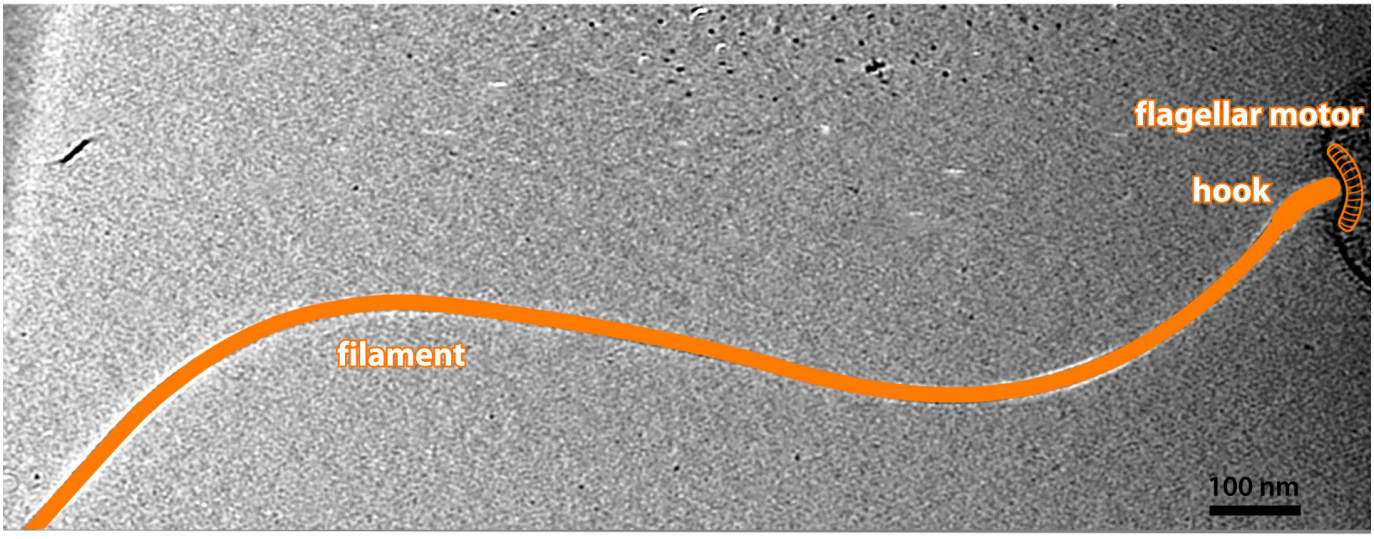
- What is the most important low-copy-number component of the cell?
- What are the two ways bacteria can form daughter cells?
- What cytoskeletal element do most bacteria use for cytokinesis?
- What eukaryotic constriction system is related to the archaeal Cdv system?

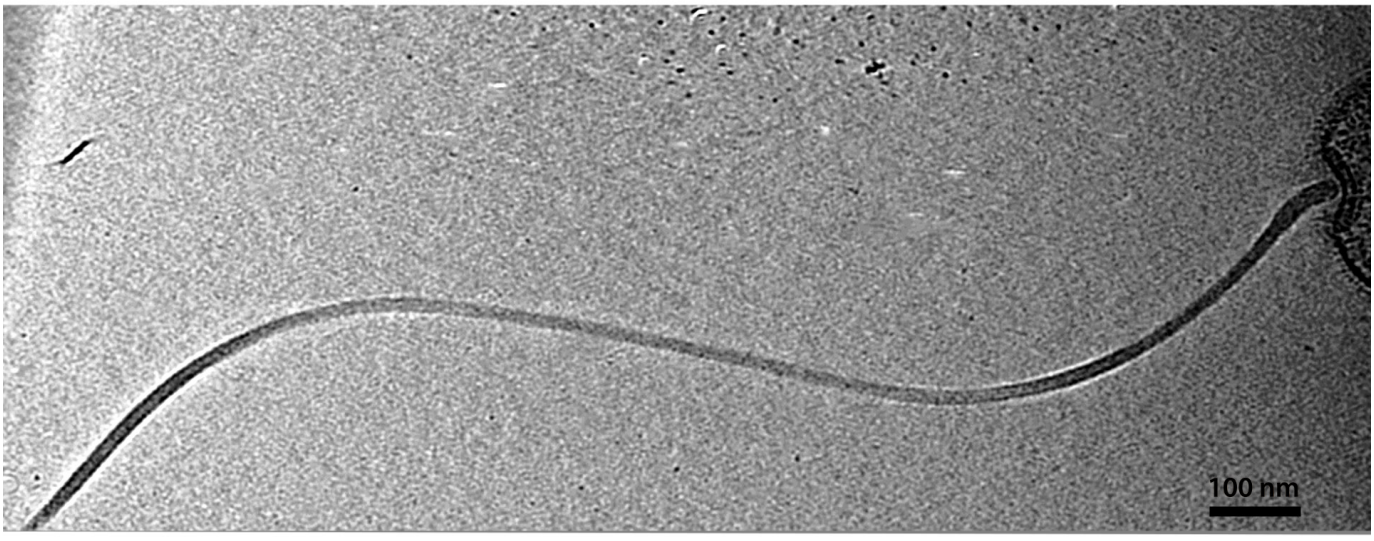
Further Reading

- Badrinarayanan et al. (2015). *Bacterial chromosome organization and segregation* [46].
- Hirsch (1974). *Budding bacteria* [47].
- Laloux and Jacobs-Wagner (2014). *How do bacteria localize proteins to the cell pole?* [48].
- Reyes-Lamothe et al. (2012). *Chromosome replication and segregation in bacteria* [49].

6
Motility

"…internal combustion engines do no better."
- Howard Berg [50]


flagellar motor
hook
filament
100 nm


100 nm

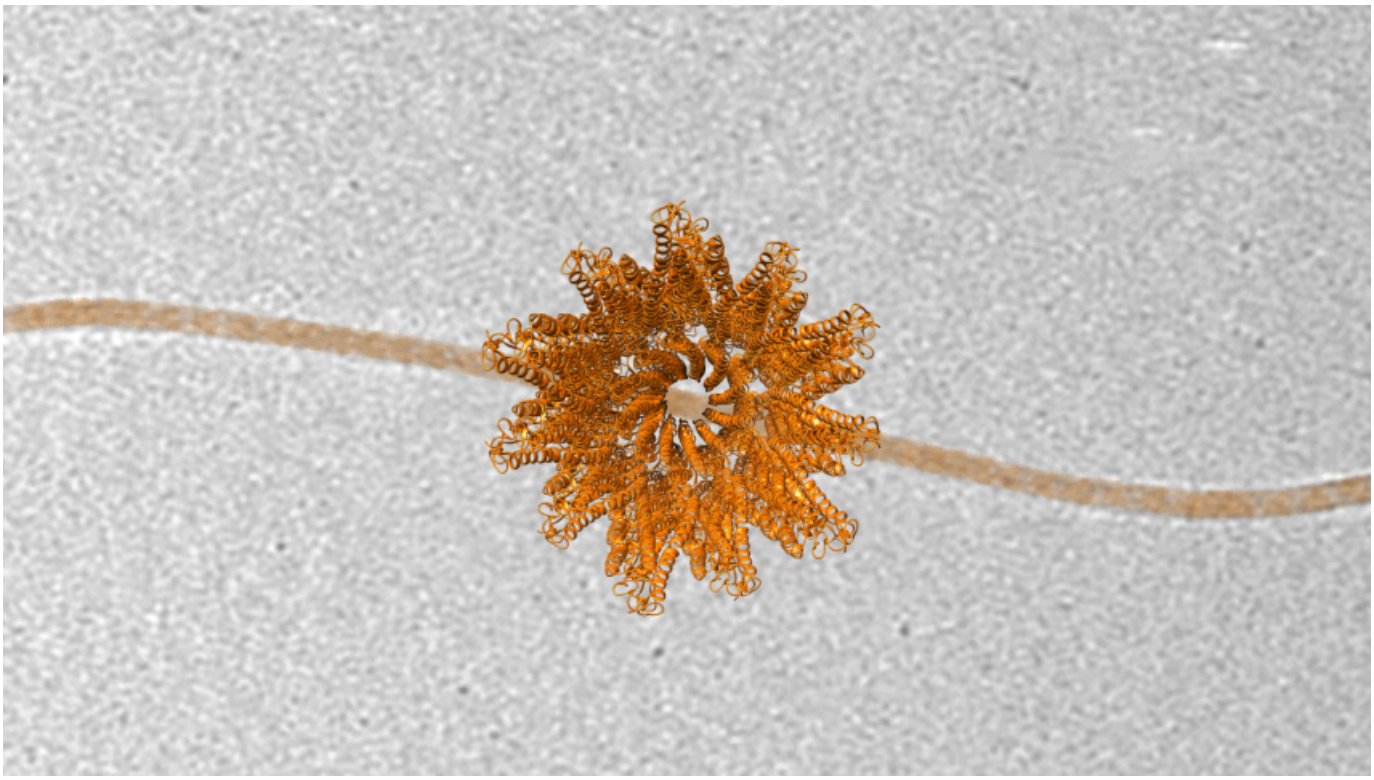
1. Flagellum

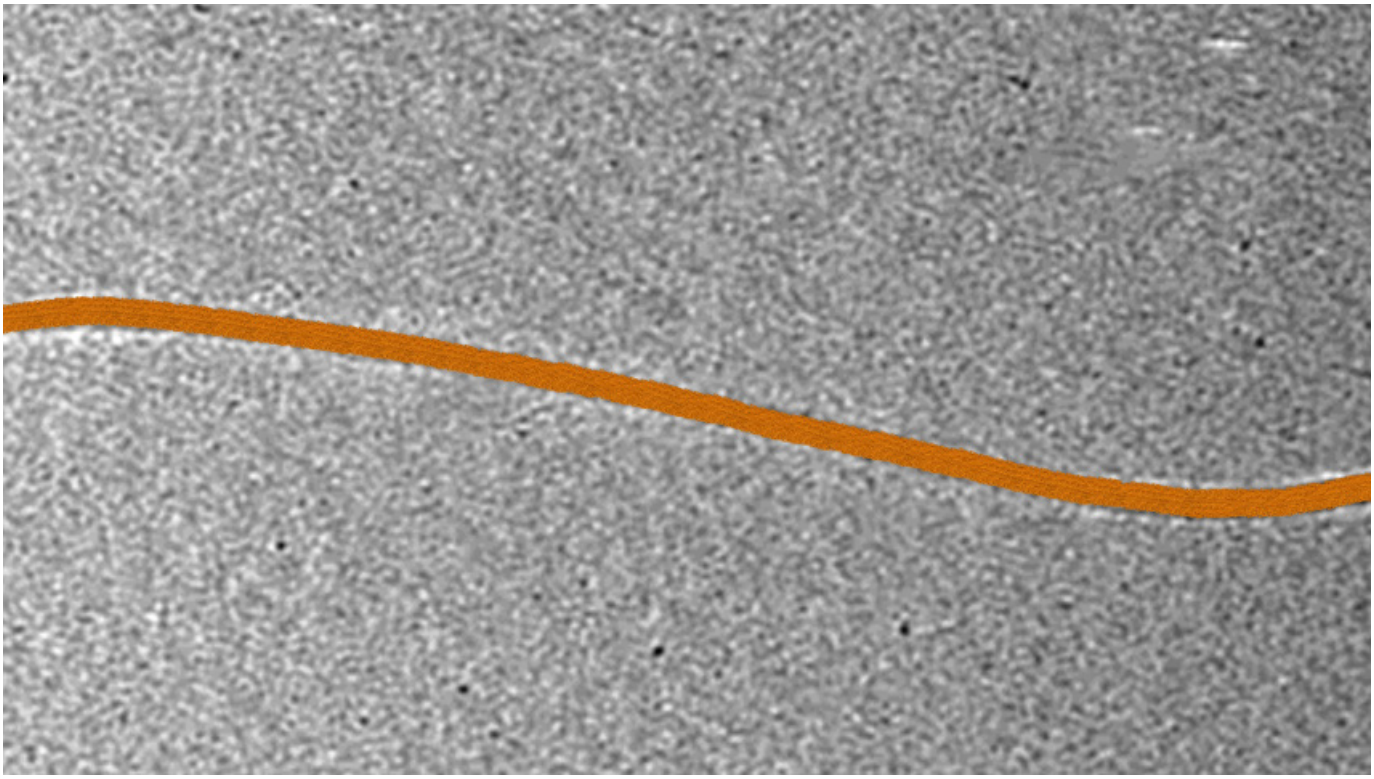
Location, location, location. So far, we have focused on how your cell can take the best advantage of its spot in the world. But why not find a better spot? Some cells live stationary lives, attached to a surface or embedded in a biofilm. Many, though, are explorers, using an impressive variety of techniques to move through their environments, finding advantages in places with more food or fewer competitors. In this chapter, we explore how your cell might make a mobile home.

Most bacteria and archaea live in liquid, so **motility** means swimming. When you are the size of a cell, though, the backstroke does not get you very far. A rough measure called the Reynolds number describes the relative influence of inertia and viscosity on liquid flow, and this parameter scales with an organism's size. In the low-Reynolds-number world of microbes, inertia is virtually nonexistent. When a rod-shaped bacterium stops swimming, it gets to coast only about the diameter of a hydrogen atom (~1 Å) before stopping. In this molasses-like environment, rotary propellers work much better than paddles.

Nearly all bacteria that swim use the same propeller: a rotary motor embedded in their envelope that spins a long helical fiber called a **flagellum** (*Learn More* on the next page). A universal joint called the hook connects the filament to the motor, translating the rotation. Flagella, like the one on this *Campylobacter jejuni*, are typically many times longer than the cell and take the form of a three-dimensional wave. The filament is highly dynamic, as you would expect, and throughout this book, you will see examples caught in various conformations: straight, curved, or in a typical loose helical waveform like this. (You can watch videos of *Escherichia coli* swimming with flagella on Howard Berg's website: http://www.rowland.harvard.edu/labs/bacteria/movies/ecoli.php.)

Campylobacter jejuni Collected by: Morgan Beeby DOI: 10.22002/D1.1525



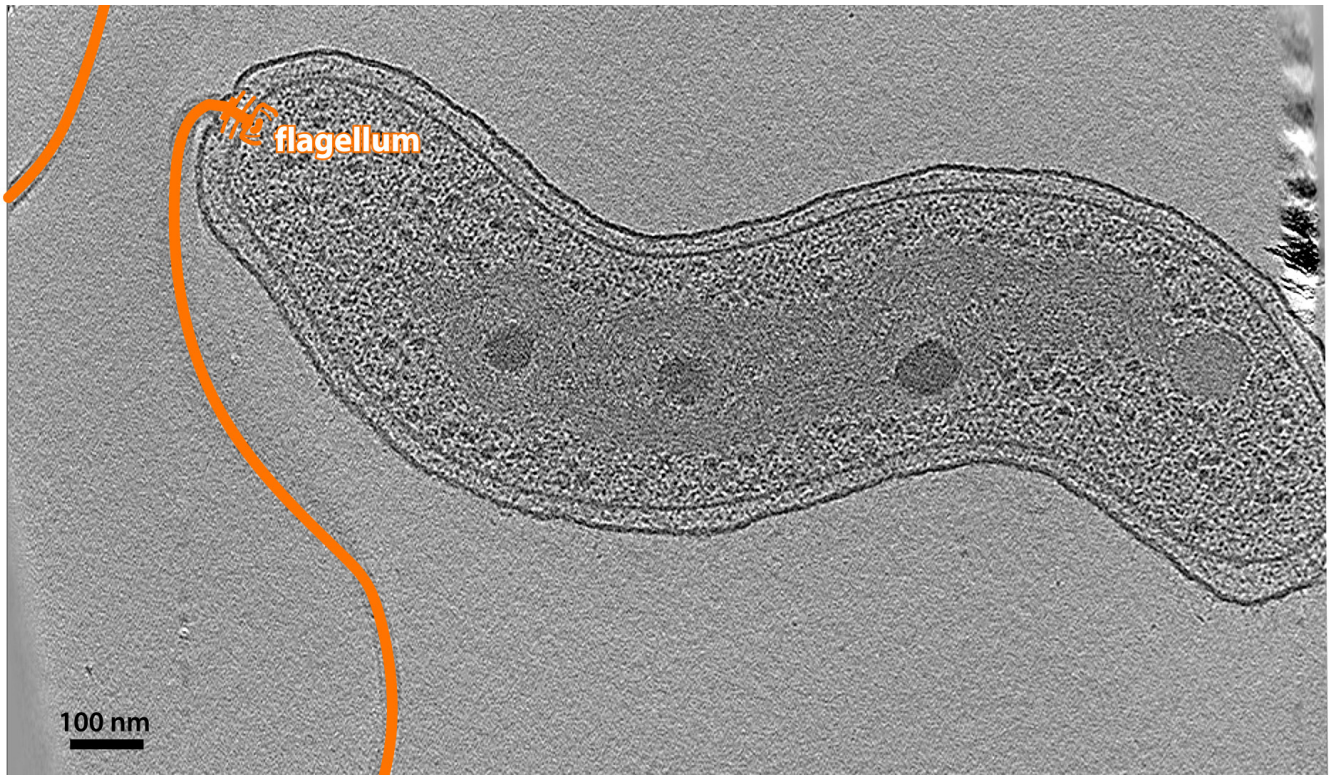


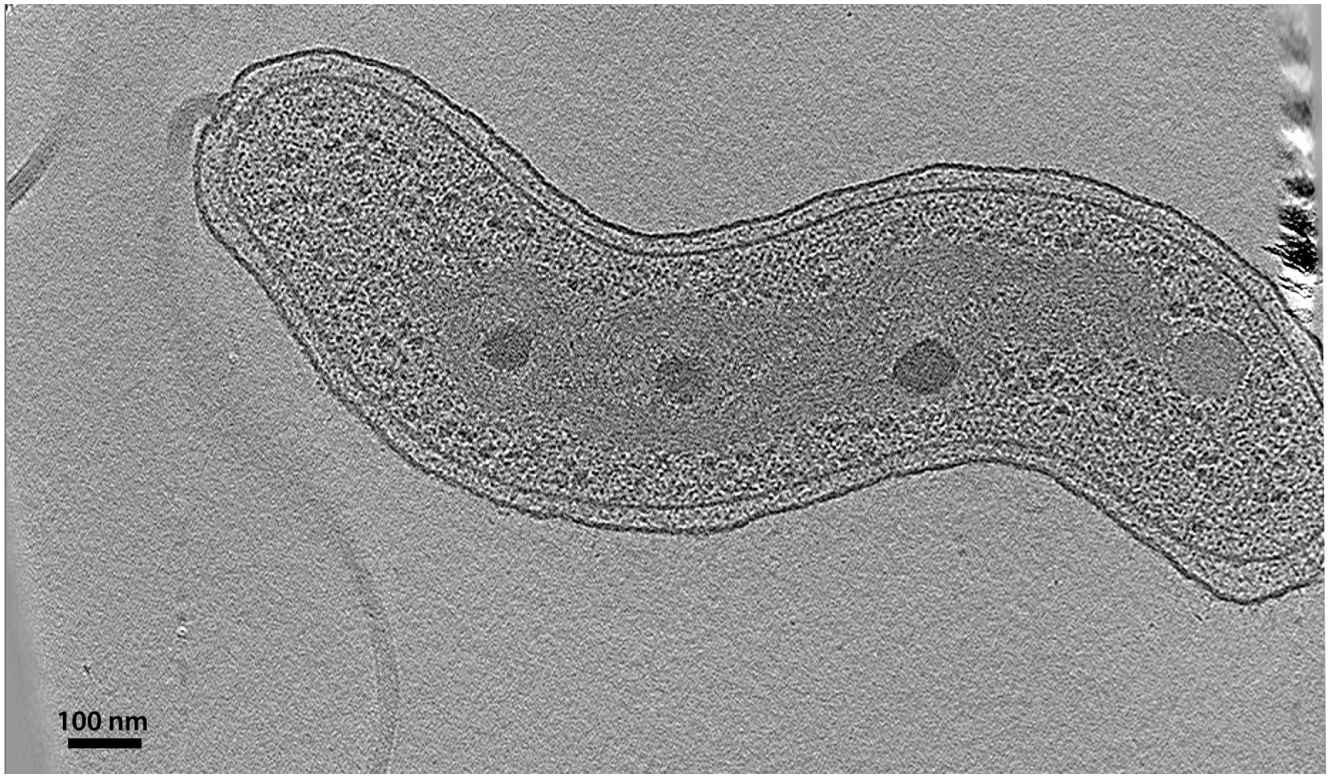
Learn More: Filament

The helical filament of the flagellum is made up of many copies of one or a few flagellin proteins. Each flagellin monomer has a soluble head domain and a hydrophobic alpha-helical tail that bundles together with the tails of other monomers to form a hollow tube. The tube comprises 11 twisting protofilaments, as you can see in this section of a flagellum from Bacillus subtilis *[51].*

Source: Wang et al. (2017)

Structure: PDB 5WJT


flagellum
100 nm

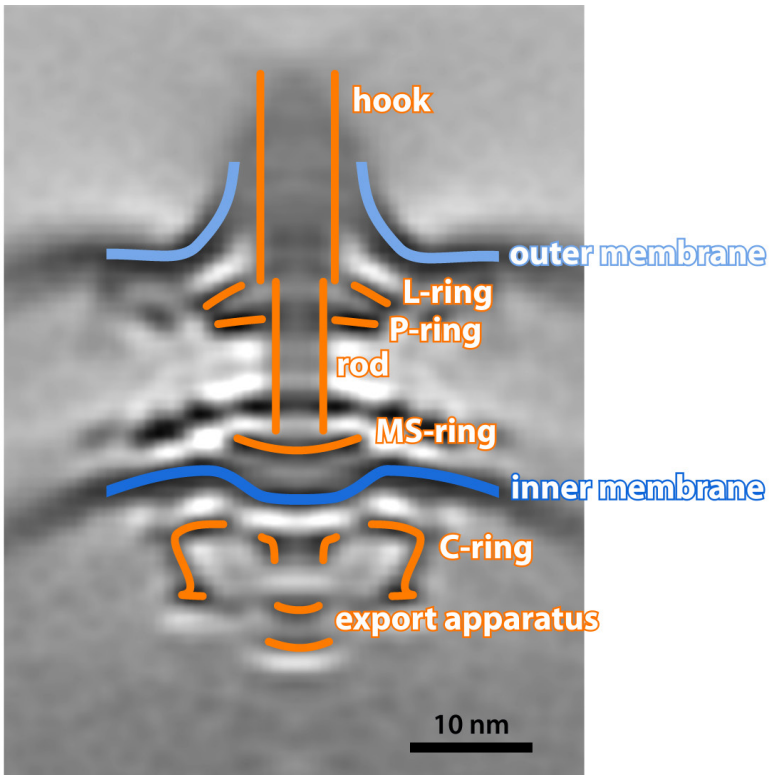

100 nm

2. Flagellar Motor

The motor that spins the flagellum is a complicated molecular machine made of many copies of dozens of different proteins, spanning the cell envelope with components in the cytoplasm, the periplasm (in diderms), and outside the cell, as you can see in this *Bdellovibrio bacteriovorus*. To get a closer look, we can average the individual motors from many cells (*Learn More* on the next page). Broadly, the motor consists of stationary "**stators**" that drive rotation of the "**rotor**" to spin the filament. The torque for spinning the flagellum comes from small movements in the stators that kick the rotor in a circle. The energy for these movements comes from the ion potential across the cell membrane that we discussed in Chapter 2; the stators provide a conduit for protons (in most species) or sodium ions (in some marine species) to diffuse down their chemical gradient into the cytoplasm, powering a conformational change in the stators in the process. The energy demands of the machine are high: a single rotation requires about 1,000 protons to flow through the stators, and the motor may spin at more than 100 rotations per second. The fact that cells pay this energetic cost indicates a strong evolutionary selection for motility, or in other words, the powerful advantage your cell can gain by learning to swim.

While the basic plan of the motor is the same in different species, there are structural differences that reflect the different environments those species encounter (*Learn More* on following pages).

Bdellovibrio bacteriovorus Collected by: Yi-Wei Chang DOI: 10.22002/D1.1526

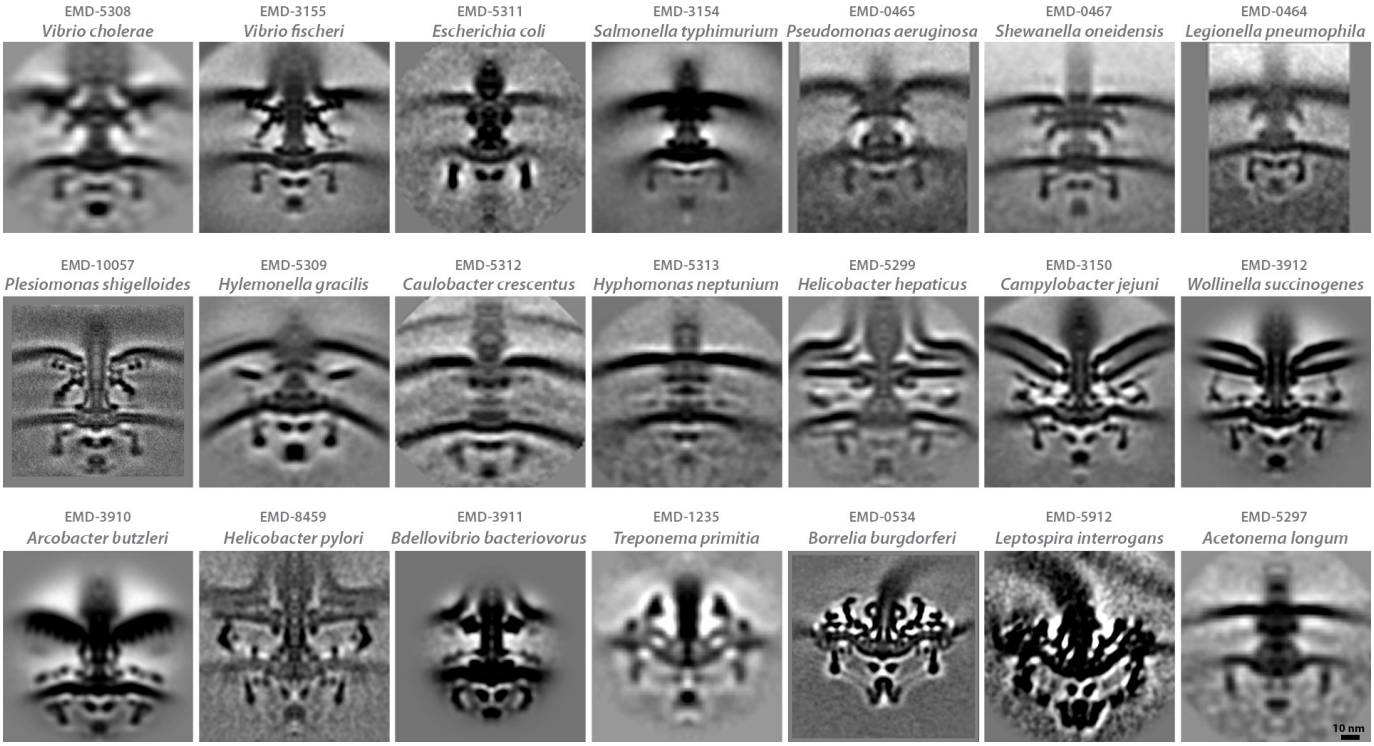

hook
outer membrane
L-ring
P-ring
rod
MS-ring
inner membrane
C-ring
export apparatus
10 nm

10 nm

Learn More: Motor Structure

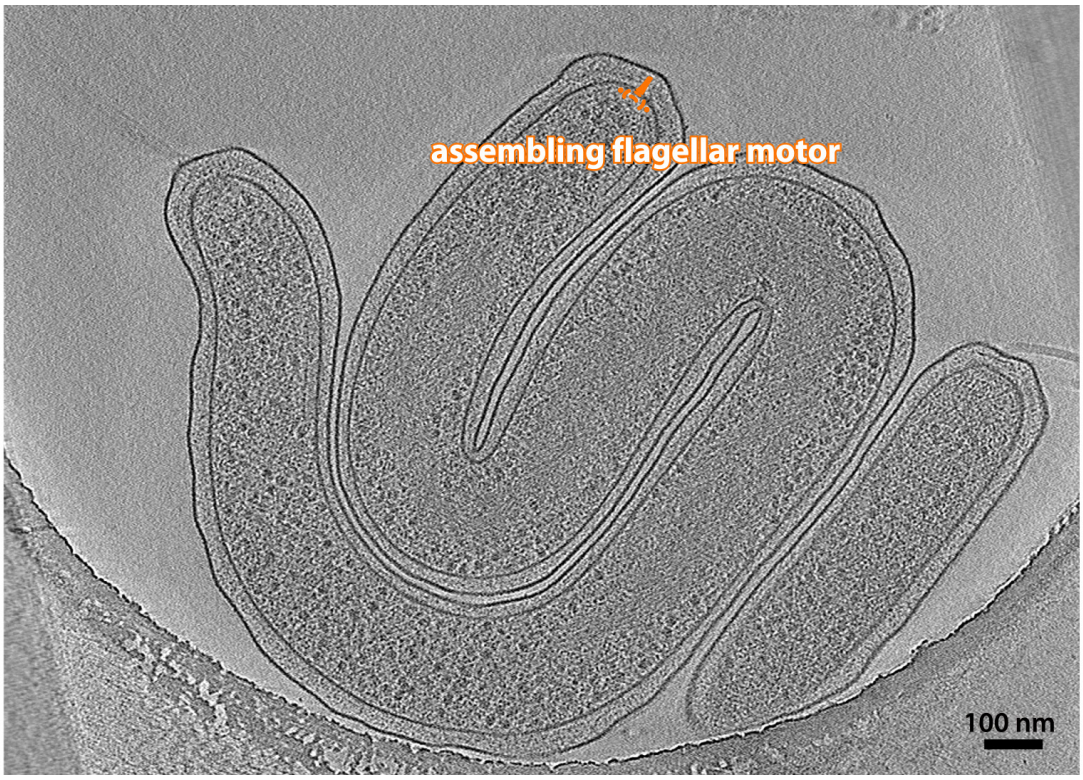
This is an average of the flagellar motors from more than one thousand Bdellovibrio bacteriovorus *cells. Working upward from the base, the major parts of the rotor are the C-ring (for Cytoplasmic), the MS-ring (for Membrane and Supramembrane), the rod, the hook, and finally the filament. The hook and filament are at different angles in different images so they wash out in the average. The rotor is surrounded by non-rotating parts: the stator ring (which is dynamic, with various conformations that wash out when averaged, so we cannot resolve the stators as they cross the inner membrane and connect to the C-ring) and a series of bushings that allow rotation within the cell wall (the Periplasmic or P-ring) and outer membrane (Lipopolysaccharide or L-ring). Additional cytoplasmic components form the export apparatus, which is involved in assembly (discussed on the next page). The unlabeled components you see are specific to this and closely-related species (*Learn More *on the next page).*

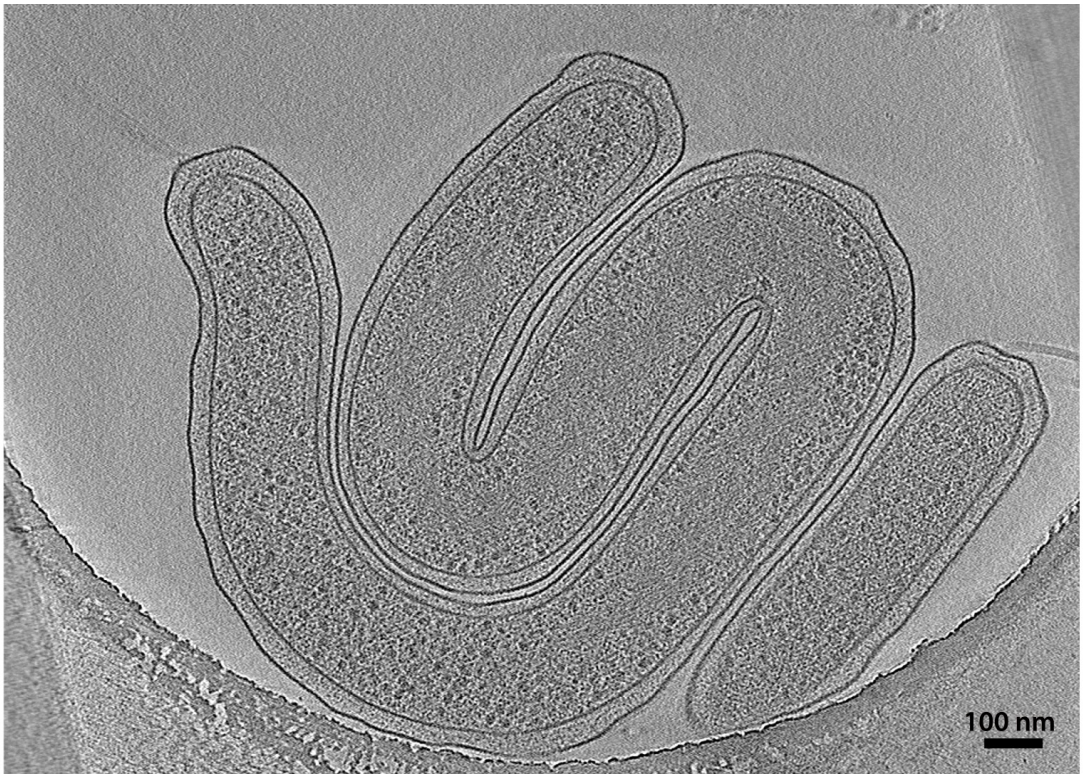
Bdellovibrio bacteriovorus *Collected by: Yi-Wei Chang* *DOI: 10.22002/D1.1537*


EMD-5308
Vibrio cholerae
EMD-3155
Vibrio fischeri
EMD-5311
Escherichia coli
EMD-3154
Salmonella typhimurium
EMD-0465
Pseudomonas aeruginosa
EMD-0467
Shewanella oneidensis
EMD-0464
Legionella pneumophila
EMD-10057
Plesiomonas shigelloides
EMD-5309
Hylemonella gracilis
EMD-5312
Caulobacter crescentus
EMD-5313
Hyphomonas neptunium
EMD-5299
Helicobacter hepaticus
EMD-3150
Campylobacter jejuni
EMD-3912
Wollinella succinogenes
EMD-3910
Arcobacter butzleri
EMD-8459
Helicobacter pylori
EMD-3911
Bdellovibrio bacteriovorus
EMD-1235
Treponema primitia
EMD-0534
Borrelia burgdorferi
EMD-5912
Leptospira interrogans
EMD-5297
Acetonema longum
10 nm

Learn More: Diversity

As you can see in these averages of flagellar motors from different species [52] [53] [54] [10] [55] [56] [57] [58] [59], bacteria have evolved various structural adaptations of their motors to better suit their environments. For instance, if your cell is a pathogen colonizing an animal's intestinal tract (like Campylobacter jejuni*, second from the right in the middle row), it will be swimming in more viscous conditions and may therefore have evolved a wider stator ring to generate more torque, along with reinforced anchors in the cell wall and outer membrane to withstand that added torque. The motors are arranged here according to the species' evolutionary relatedness.*


assembling flagellar motor
100 nm

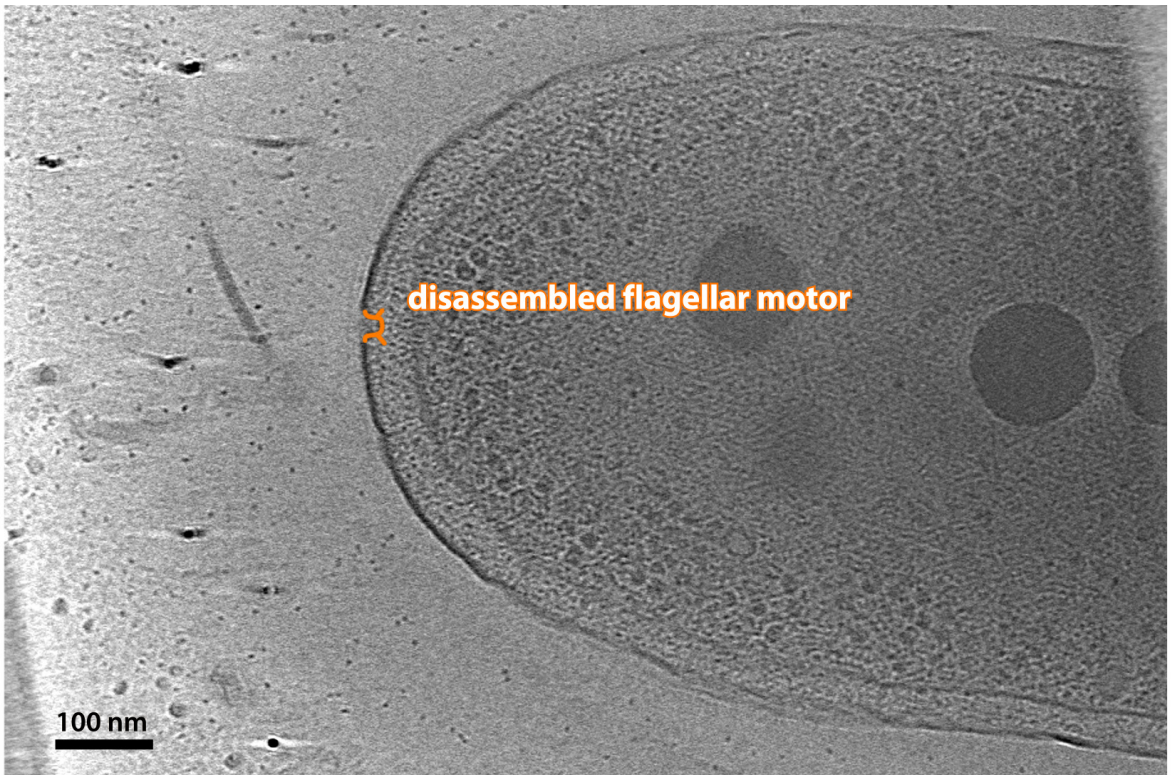

100 nm

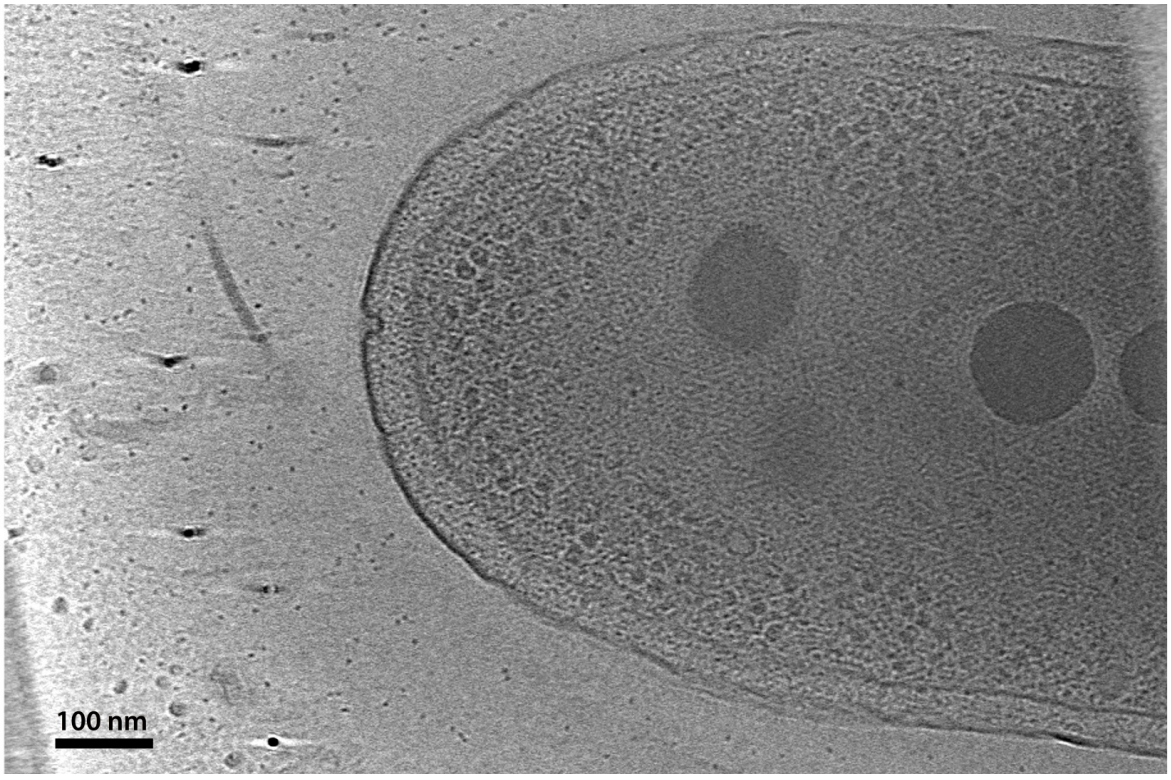
3. Flagellar Assembly

Operating the flagellar motor is impressive, but so is building it in the first place. Remember that the envelope of bacterial cells is a complicated multilayered barrier. The flagellar motor has about two dozen unique components, each present in many copies, embedded in every layer of the cell envelope. How can your cell get all hundred or more components (tens of thousands if you count the components of the filament) where they need to go? Making the feat even more impressive, the machine builds itself, assembling from the inside out. First the components associated with the inner membrane come together, forming an "export apparatus" which pumps subsequent components across the membrane to assemble in the periplasm and outer membrane (if the cell is a diderm). The energy for this process comes from an ATPase at the base of the machine. You can see a late stage in the assembly process in this *Hylemonella gracilis*. The final piece to be assembled (still missing here) is the flagellar filament, which continues the pattern of outward assembly; flagellin monomers travel through the hollow tube to take their places at the tip. Flagellar motors can also disassemble, for instance when the filament is broken (*Learn More* on the next page), and cells may make many new flagella throughout their lifetime.

So the flagellar motor is not just a machine for motility; it is also a machine to secrete molecules outside the cell. Bacteria and archaea contain many such "**secretion systems**," each specialized to transport specific macromolecules (e.g. DNA or a toxin protein) across cell envelopes–both their own and sometimes others. Secretion systems are classified by evolutionary relatedness; there are currently ~10 types recognized in bacteria, some of which are also present in archaea. The flagellar motor is an example of a **type III secretion system**. You will see another member of this family in Chapter 9, and examples of many other types in the rest of the book, starting in just a few pages.

Hylemonella gracilis Collected by: Yi-Wei Chang DOI: 10.22002/D1.1527

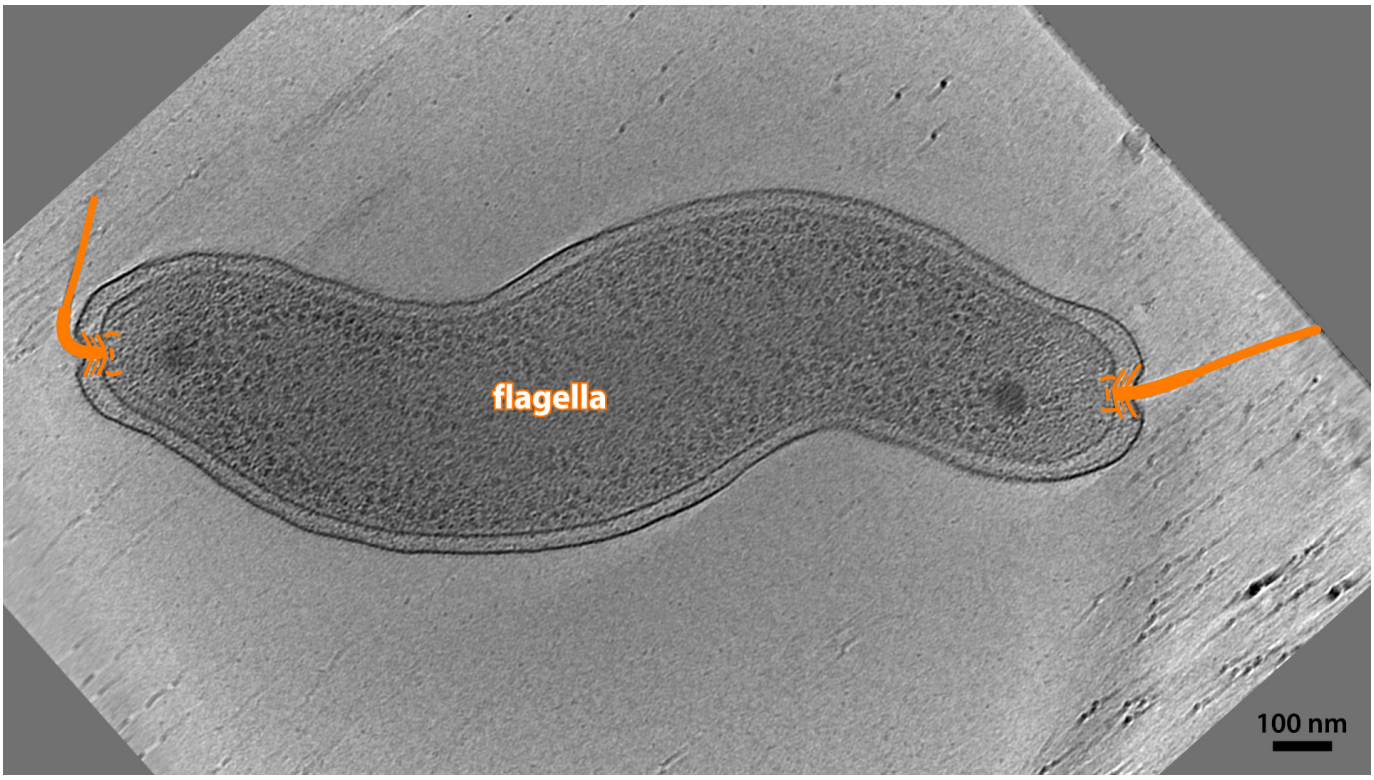

disassembled flagellar motor
100 nm

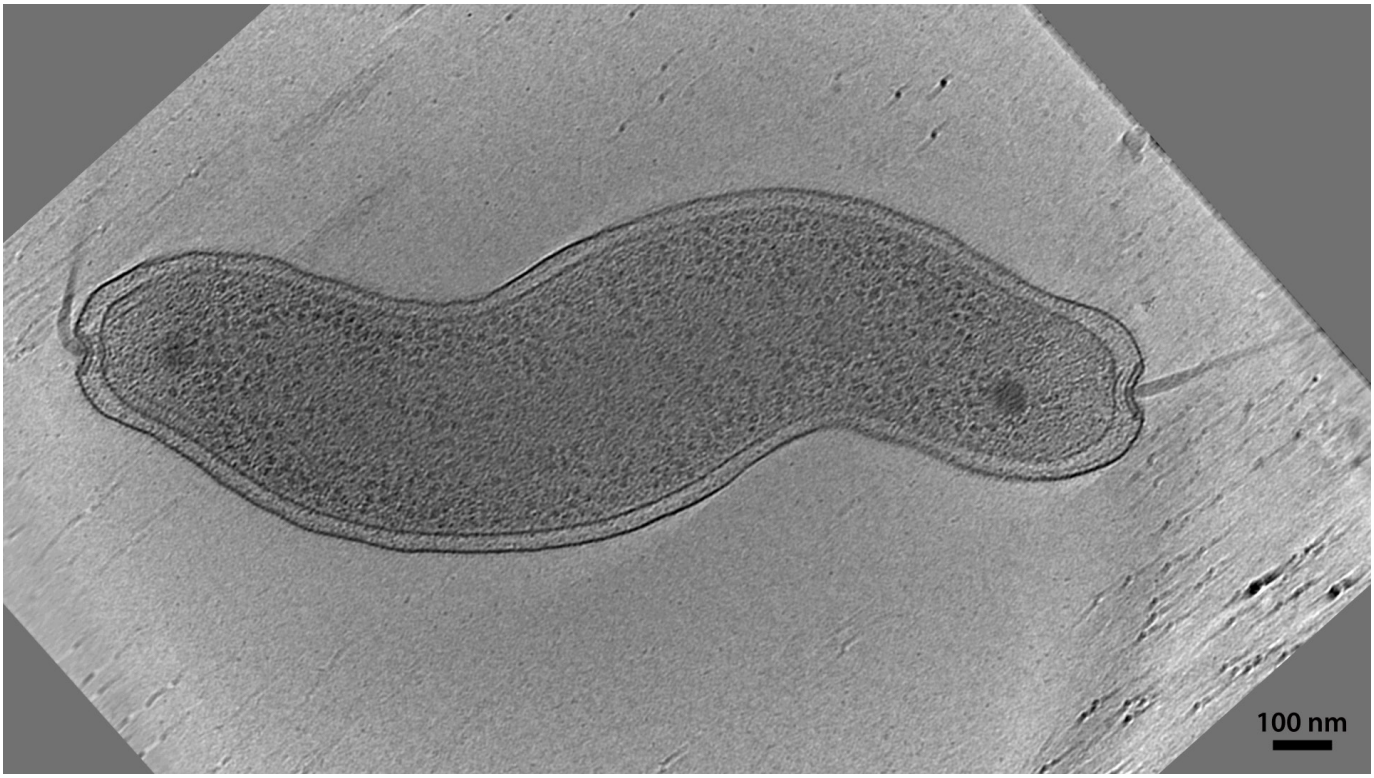

100 nm

Learn More: Disassembly

When flagella break (or in some species, like Caulobacter crescentus*, are jettisoned so the cell can attach to a surface), the motor is disassembled, usually beginning with the export apparatus. Not everything is dismantled, though; the P- and L-rings remain in place in the cell wall and outer membrane, respectively, as you can see in this* Pseudomonas aeruginosa*. We do not know if the cell has a reason to leave them there, but one possibility is that they may function as a plug so that loss of the hook does not create a hole in the outer membrane.*

Pseudomonas aeruginosa *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1538*

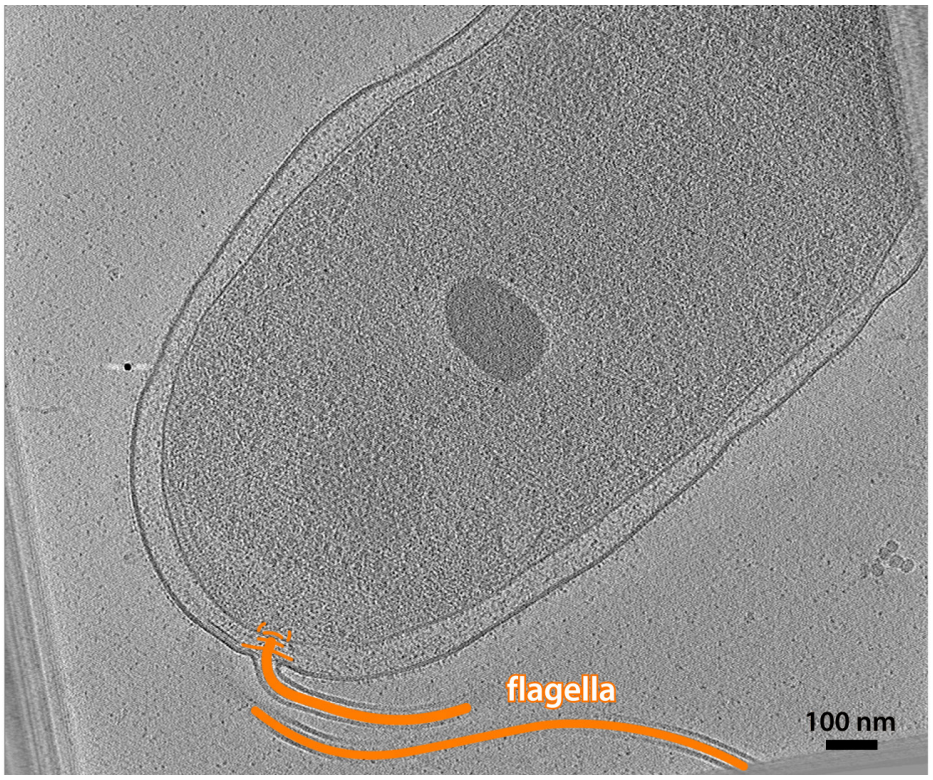

flagella
100 nm

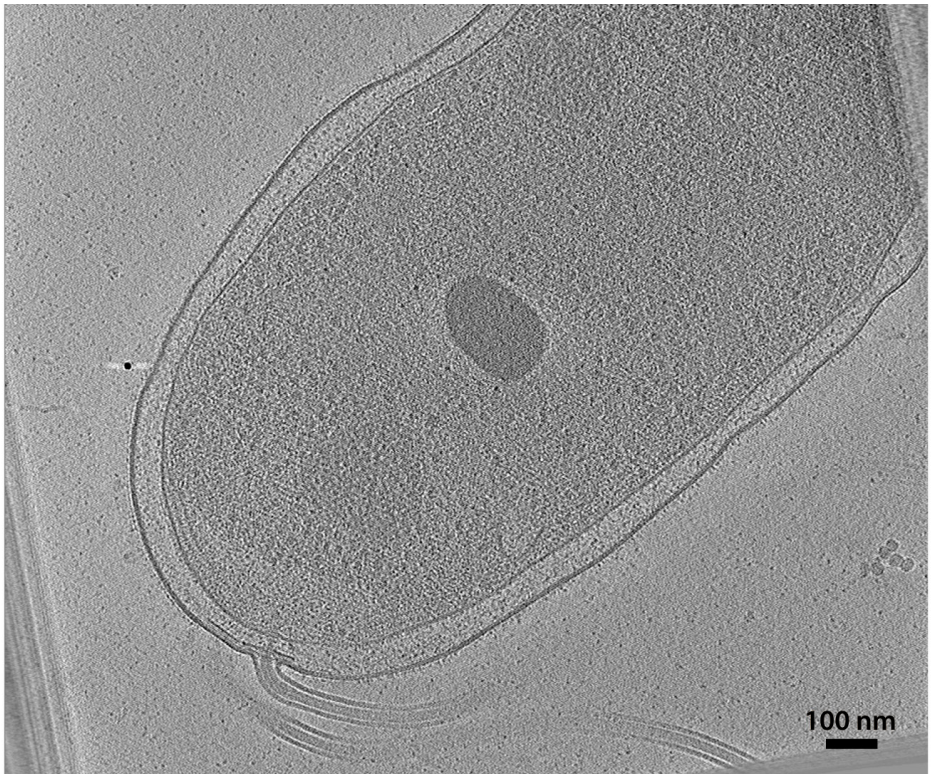

100 nm

4. Flagella Patterns

Once assembled, flagella can work in different ways. The motor is bidirectional, and can rotate either clockwise or counterclockwise. Depending on the number and location of flagella on the cell (and the cell's shape), this can push the cell, pull it, or give rise to even more complicated swimming behavior. Some bacterial species, like the *Bdellovibrio bacteriovorus* you just saw, are **monotrichous** ("single haired"), with one flagellum located at one pole to push/pull the cell. Other species, like the *Campylobacter jejuni* here, have bipolar flagella, one at each end. Still others are **lophotrichous** ("crest-haired"), with a clump of flagella (*Learn More* on the next page).

Campylobacter jejuni Collected by: Morgan Beeby DOI: 10.22002/D1.1528

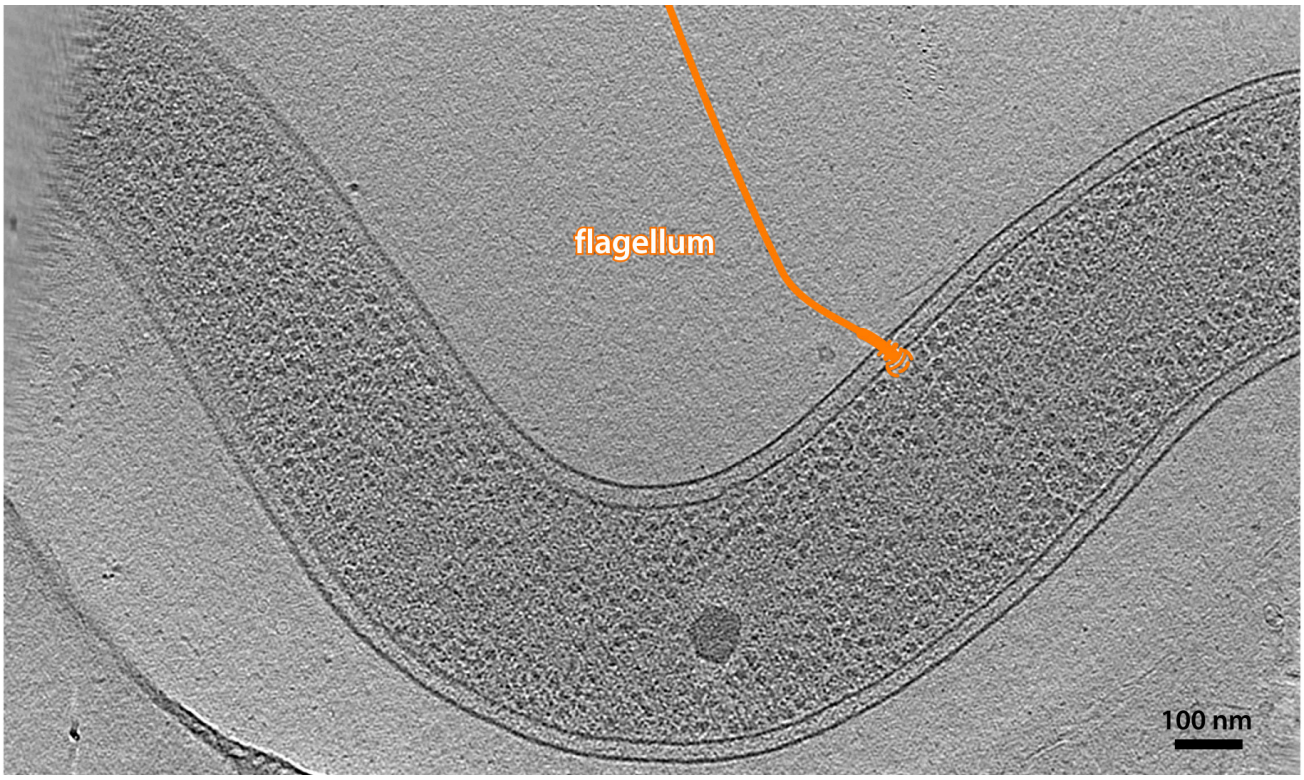

flagella
100 nm

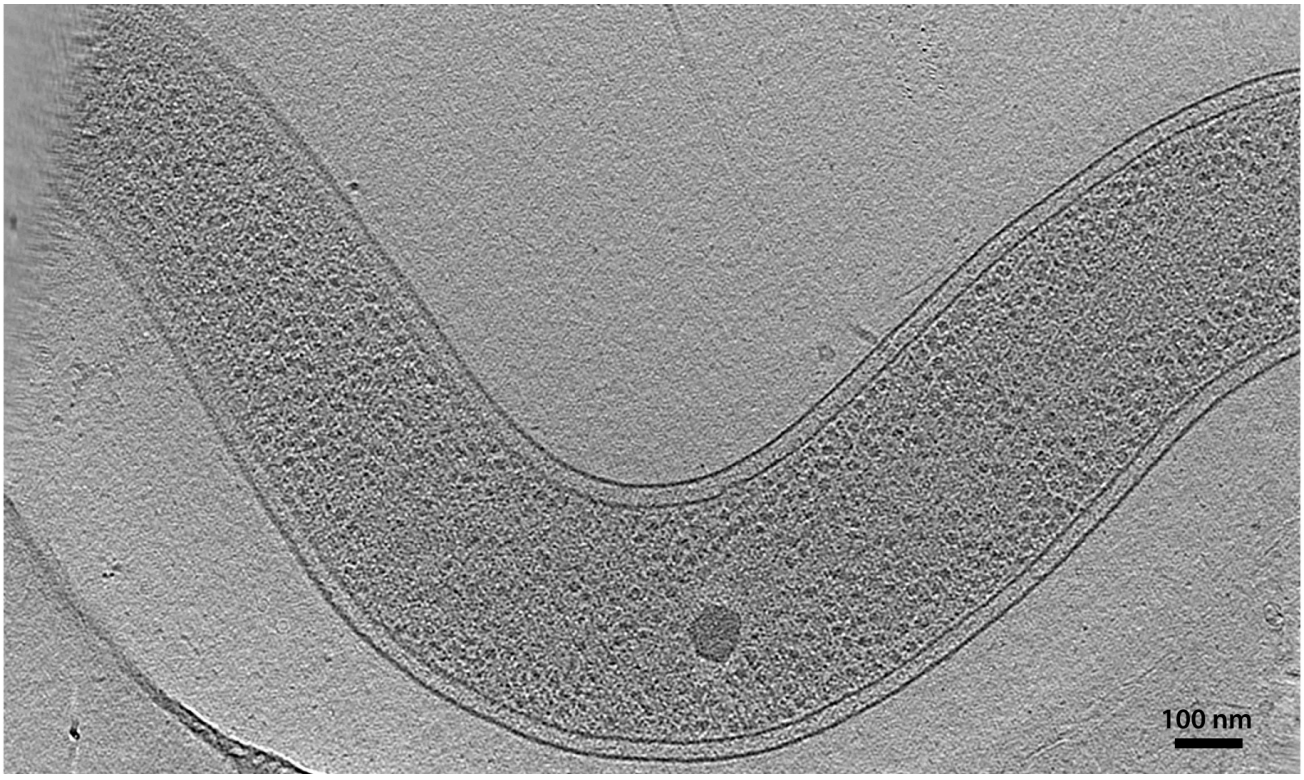

100 nm

Learn More: Lophotrichous

Many lophotrichous species, like the Hylemonella gracilis *you saw in Chapter 3 or this* Helicobacter pylori*, have a tuft of flagella at their cell pole. In some species, though, the tuft is located elsewhere; for example, a clump of flagella on the concave side of banana-shaped* Selenomonas artemidis *pushes the cells sideways in a seesawing swimming pattern.*

Helicobacter pylori *Collected by: Yi-Wei Chang* *DOI: 10.22002/D1.1539*

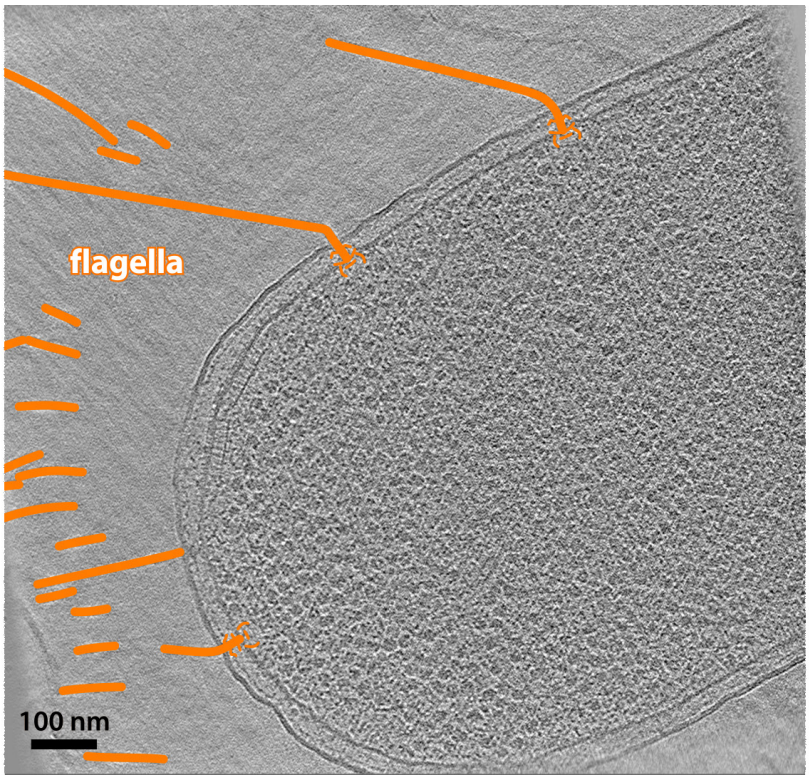

flagellum
100 nm

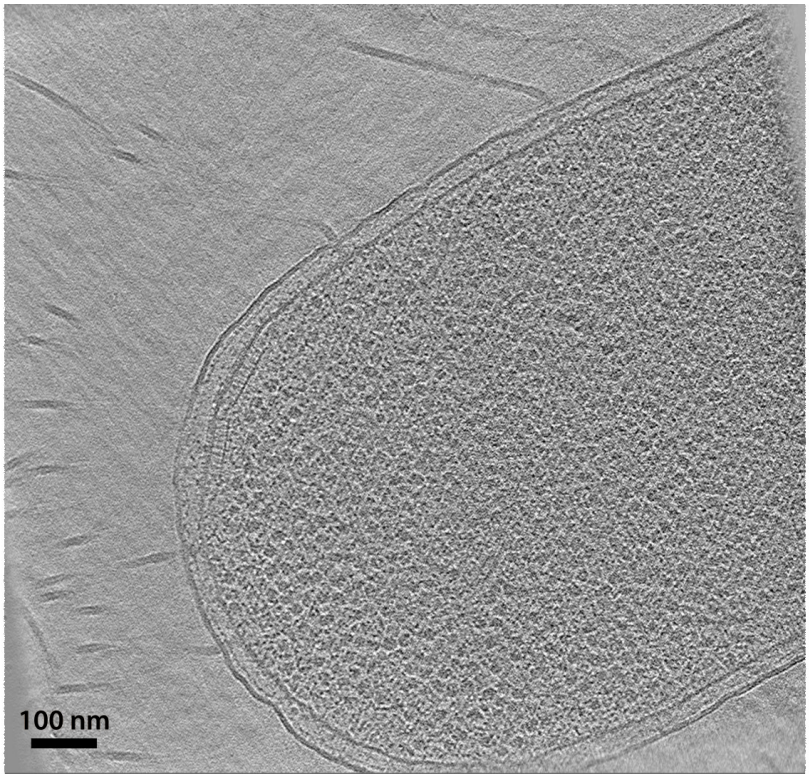

100 nm

5. Peritrichous Flagella

Still other species are **peritrichous** ("hair all around"), with multiple flagella distributed randomly around the cell, as you can see on this *Pseudomonas flexibilis*. The number of flagella varies between different species, from relatively few here to considerably more (*Learn More* on the next page). The well-known model system *Escherichia coli* is also peritrichously flagellated. In this arrangement, when the flagellar motors are all rotating one direction (counter-clockwise), the flagella form a whip-like bundle that propels the cell to "run" in a straight line. When one or more motors switch to clockwise rotation, the flagella dissociate from the bundle and "tumble" the cell to face a new direction. In the next chapter, you will see how cells use this behavior to seek out favorable spots.

Pseudomonas flexibilis Collected by: Morgan Beeby DOI: 10.22002/D1.1529

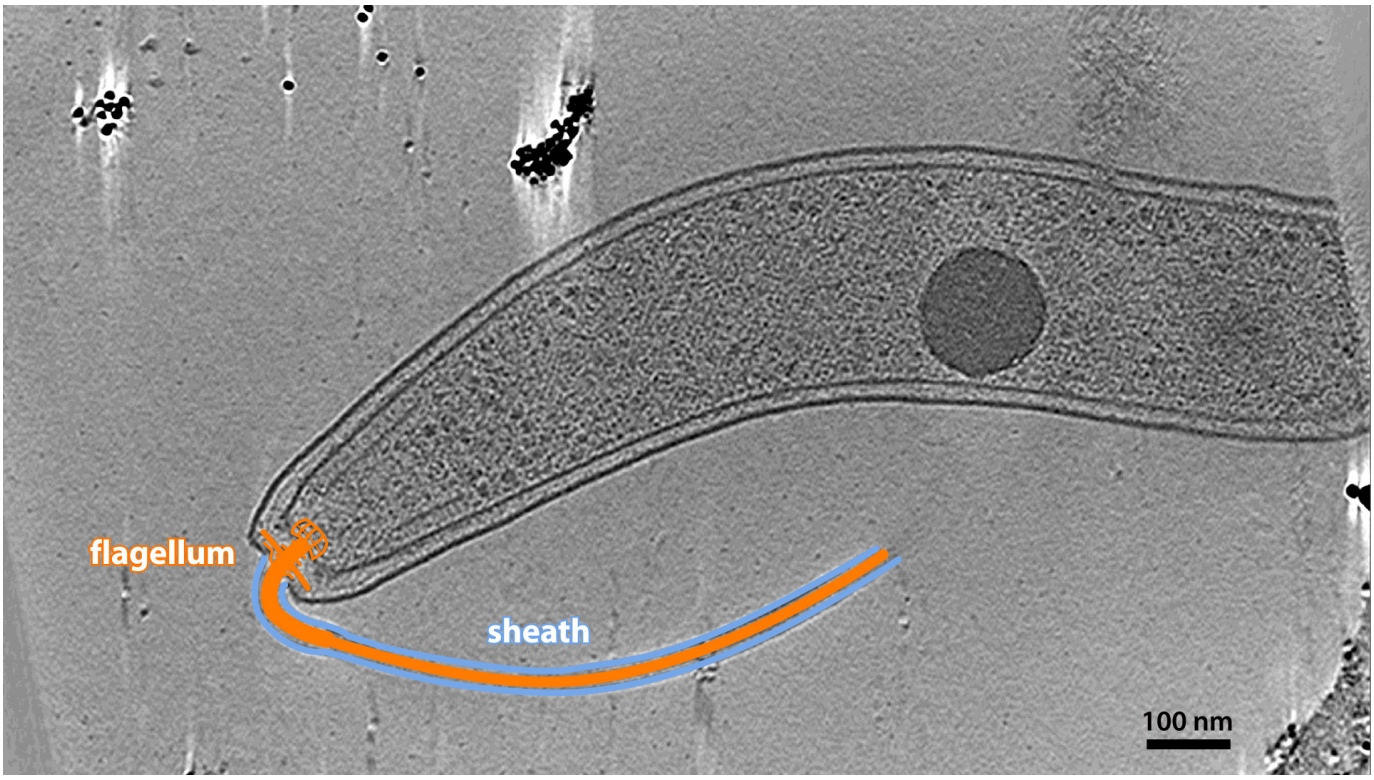

flagella
100 nm


100 nm

Learn More: Swarming

Proteus mirabilis *adapt their motility machinery to their environment. In liquid, the short rod-shaped cells swim with the help of a handful of flagella distributed peritrichously around their cell body. When they encounter a solid surface, the cells elongate and build many more flagella, as you can see on this cell. Instead of swimming, they now use their flagella to propel themselves in groups across the surface, a motility mode known as "swarming." This is an example of a differentiated lifecycle, which will come up again in Chapter 8.*

Proteus mirabilis *Collected by: Qing Yao* *DOI: 10.22002/D1.1540*

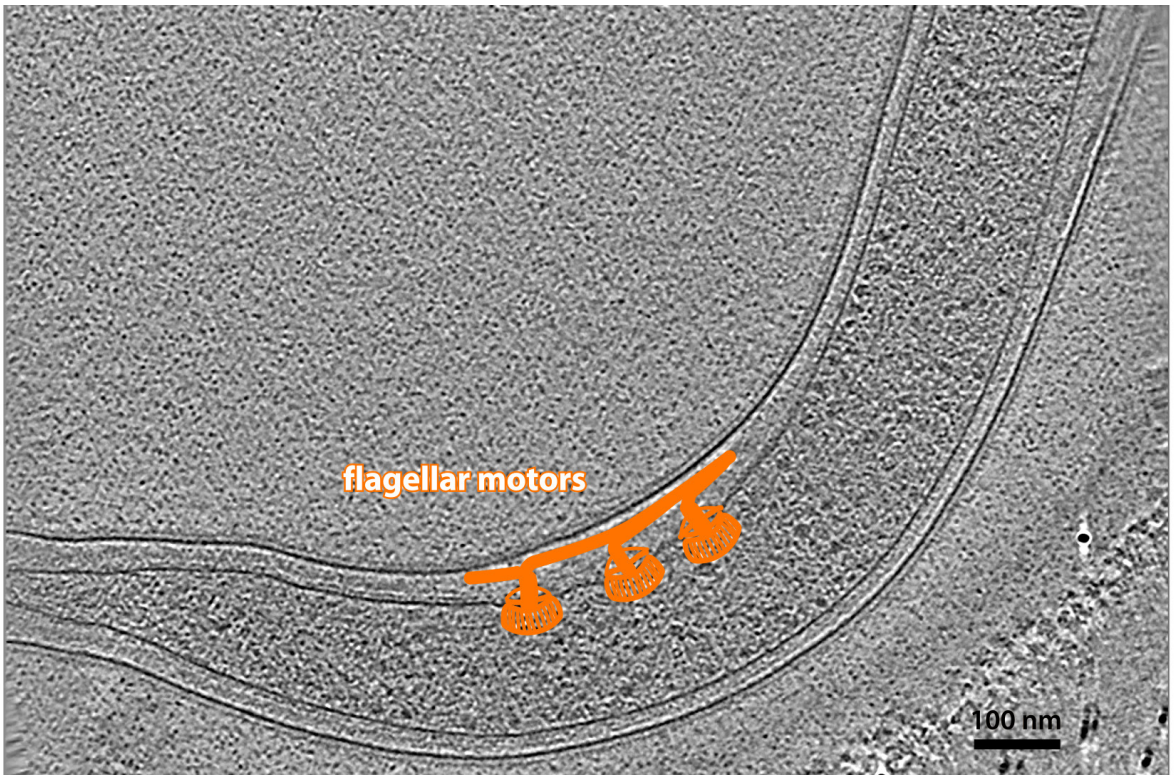

flagellum
sheath
100 nm

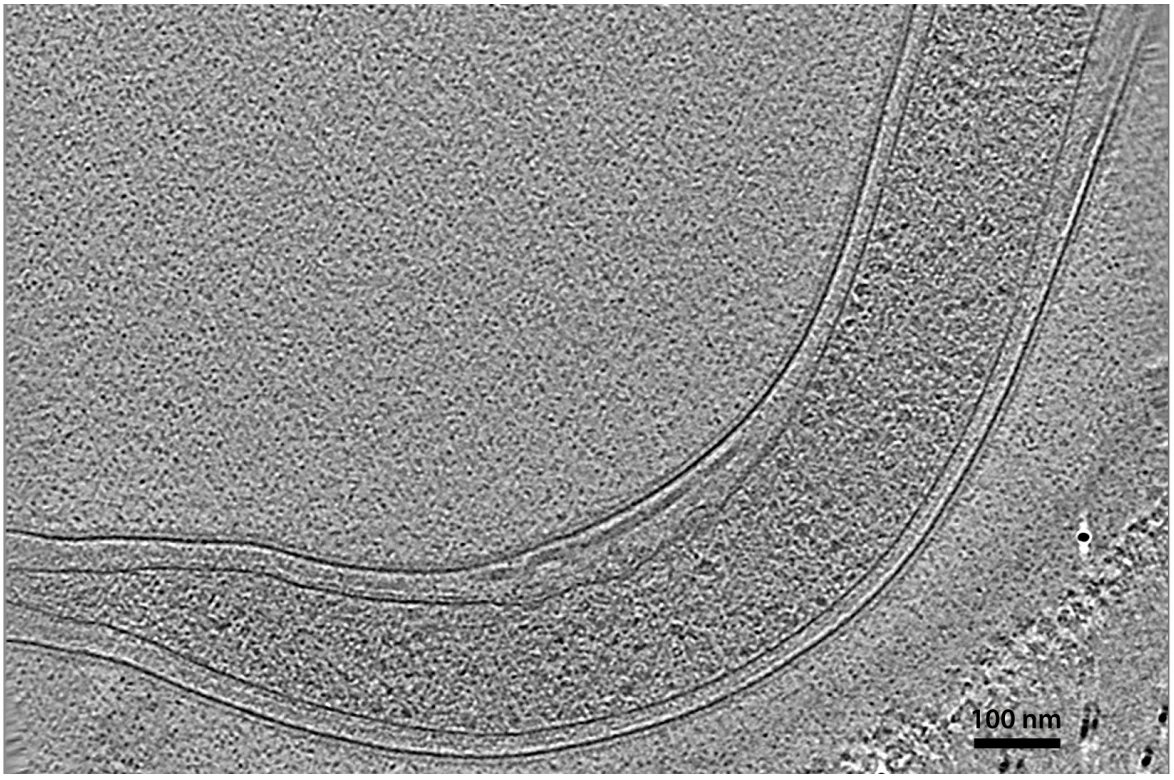
100 nm

6. Sheathed Flagella

You may have noticed that some of the flagella in this chapter were enclosed within the outer membrane of the cell. We call these "**sheathed**" **flagella**. It is a common adaptation in pathogenic species, like this *Helicobacter hepaticus*. Flagella offer pathogens a great advantage in colonizing their hosts; hosts in turn have learned to use them to identify potential invaders. As a result, the innate immune response of many eukaryotes, from plants to insects to humans, has evolved to recognize the telltale and abundant signal of flagellin proteins in the long filament. If your cell aims to take up residence in such a host, it could therefore benefit from cloaking this strongly antigenic feature.

Helicobacter hepaticus Collected by: Megan Dobro DOI: 10.22002/D1.1530

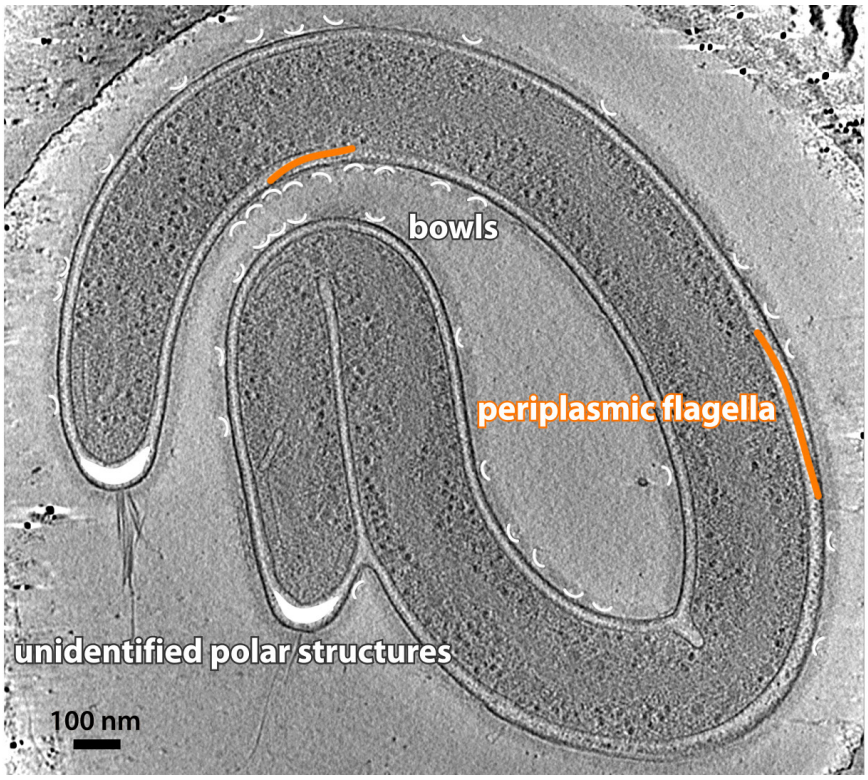

flagellar motors
100 nm

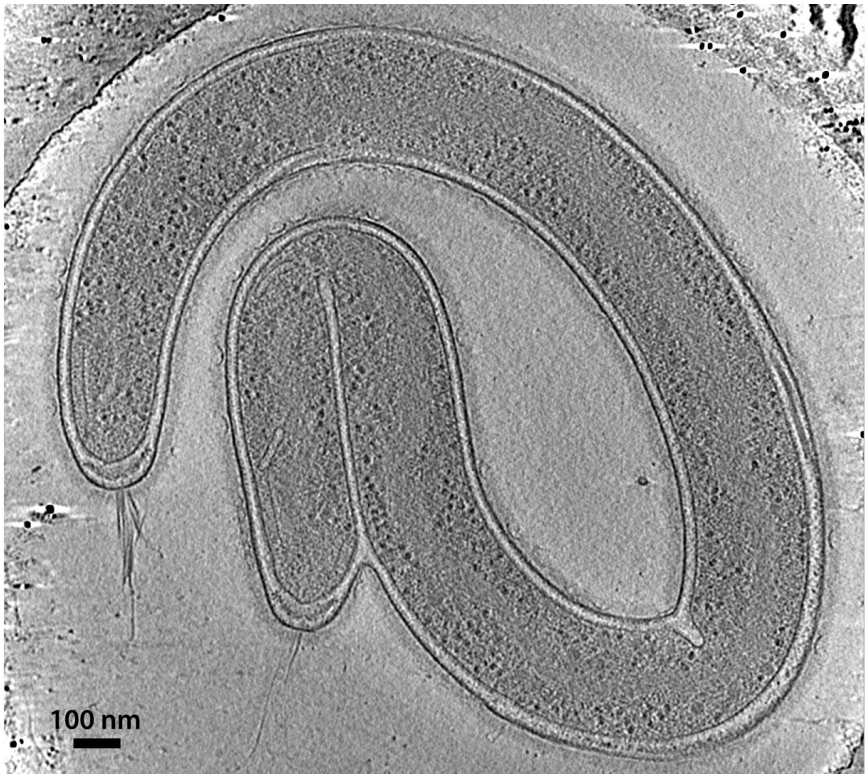

100 nm

7. Periplasmic Flagella

If your cell is a pathogen, swimming can be very useful, but so can burrowing, for instance between cells in host tissue. To do this, why not turn your cell into a corkscrew with the equipment at hand? Some cells do just this, wrapping their flagellum around their body to back out of a tight spot, or burrow into one. Other, diderm species like the *Borrelia burgdorferi* here have turned the temporary adaptation into a permanent one: they assemble their flagella *inside* the cell envelope, with the filaments wrapping around between the cell wall and outer membrane. These "**periplasmic**" **flagella** are usually multiple, arising from one or both ends of the cell, and pack together into a helical ribbon whose rotation drives wave-like motion of the cell (you can watch a video of this movement on YouTube: https://youtu.be/ODYu--TNPDE). The helical ribbon helps give these spirochetes ("spiral haired") their characteristic shape; mutants that cannot make flagella are simple rods. Some spirochetes also have additional features that may help them move around in animal hosts (*Learn More* on the next page).

Borrelia burgdorferi Collected by: Ariane Briegel DOI: 10.22002/D1.1531

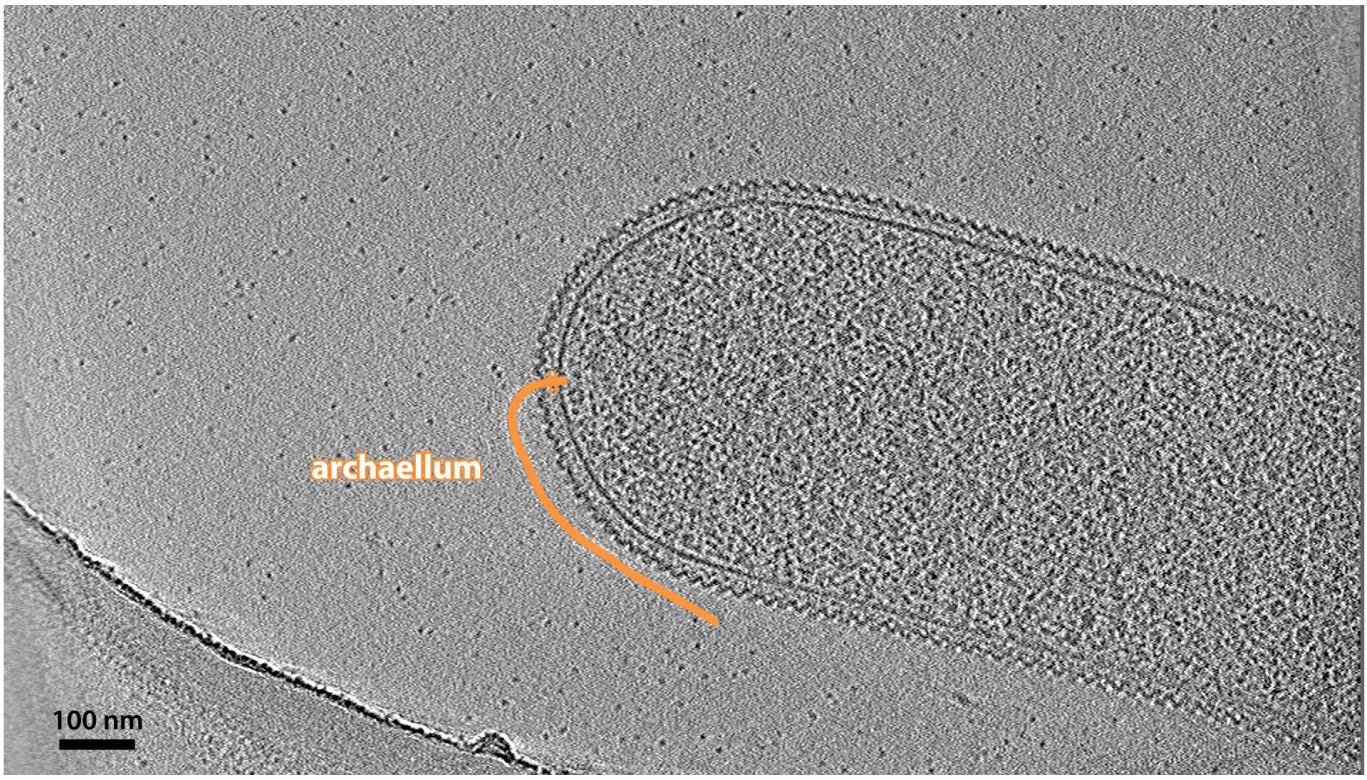

bowls
periplasmic flagella
unidentified polar structures
100 nm

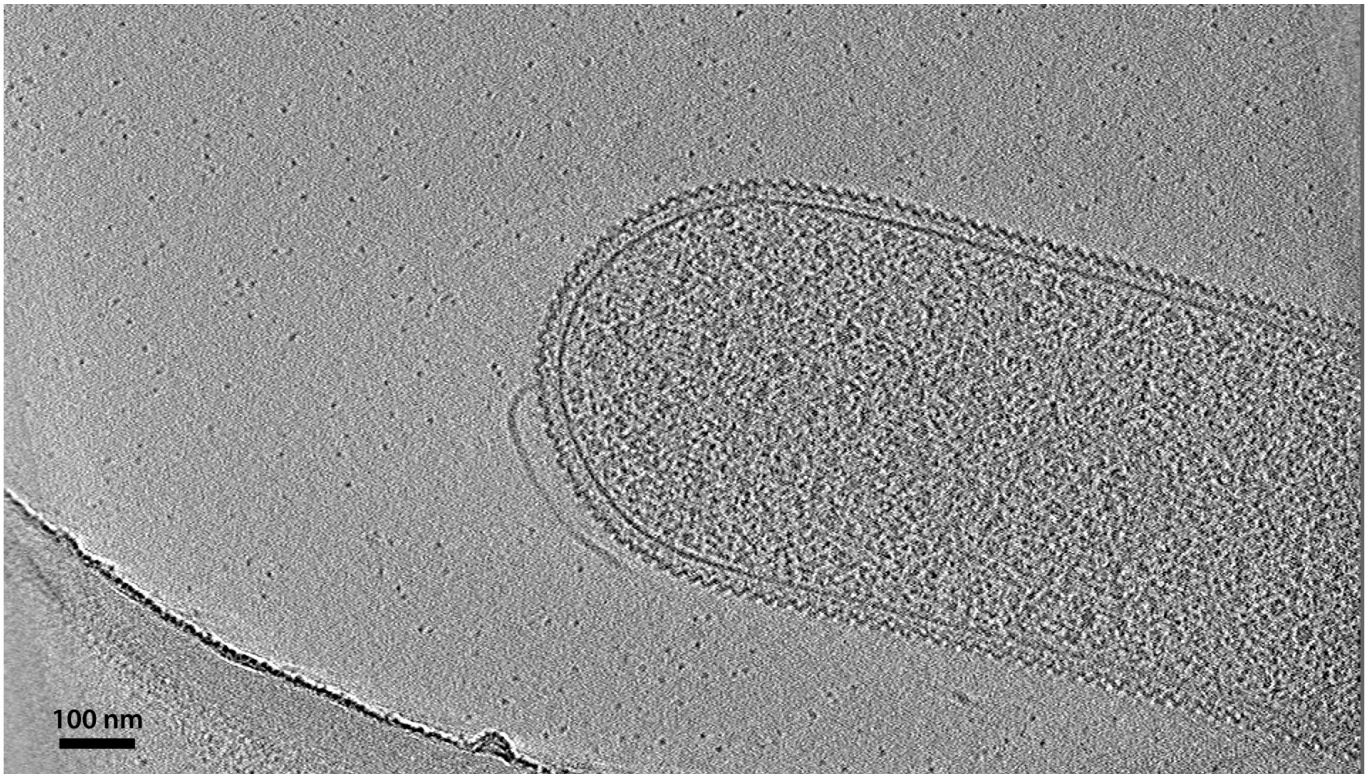

100 nm

Learn More: Surface Structures

Treponema primitia *like this are commensal residents of the termite gut, helping break down cellulose. In addition to two periplasmic flagella, the cells have arrays of bowl- and hook-like structures on their surface, the function of which, likely related to motility, remains mysterious.*

Treponema primitia *Collected by: Gavin Murphy* *DOI: 10.22002/D1.1541*

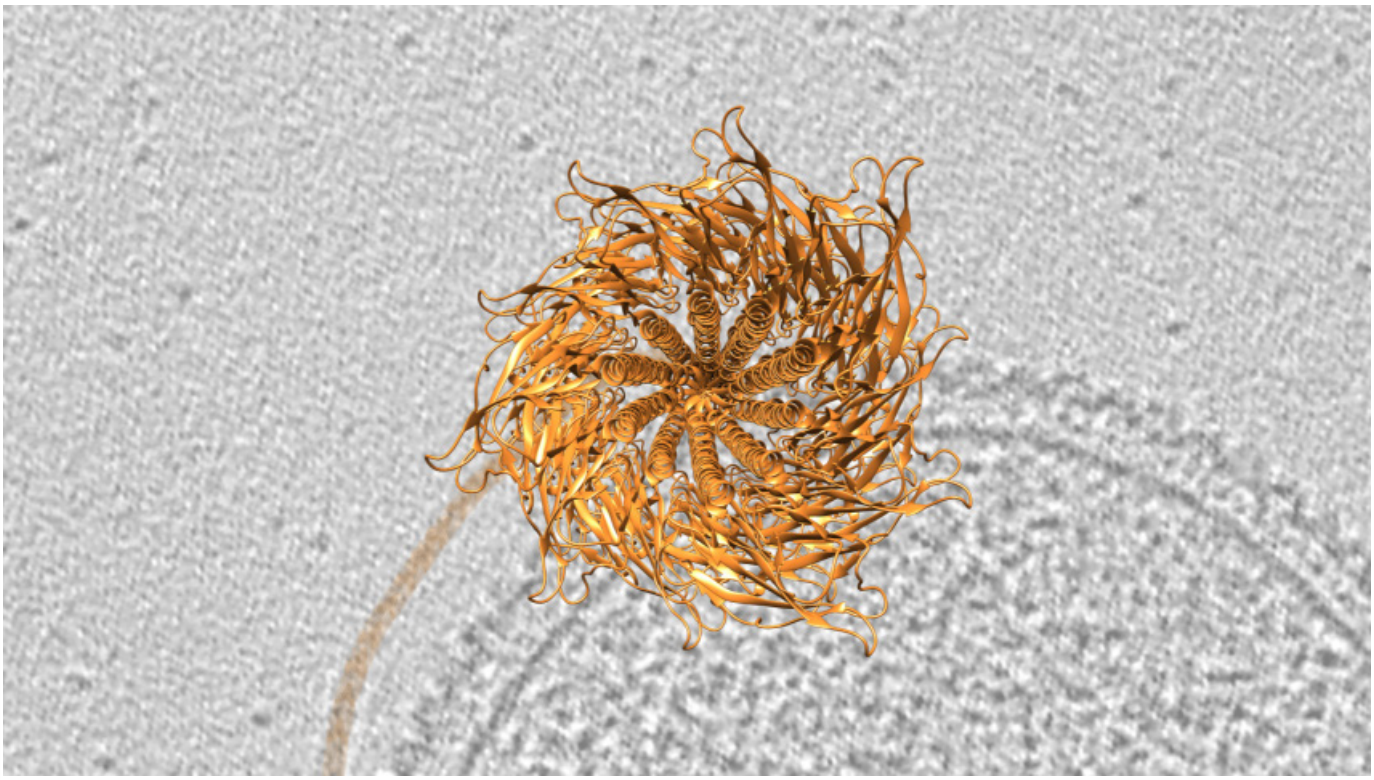

archaellum
100 nm

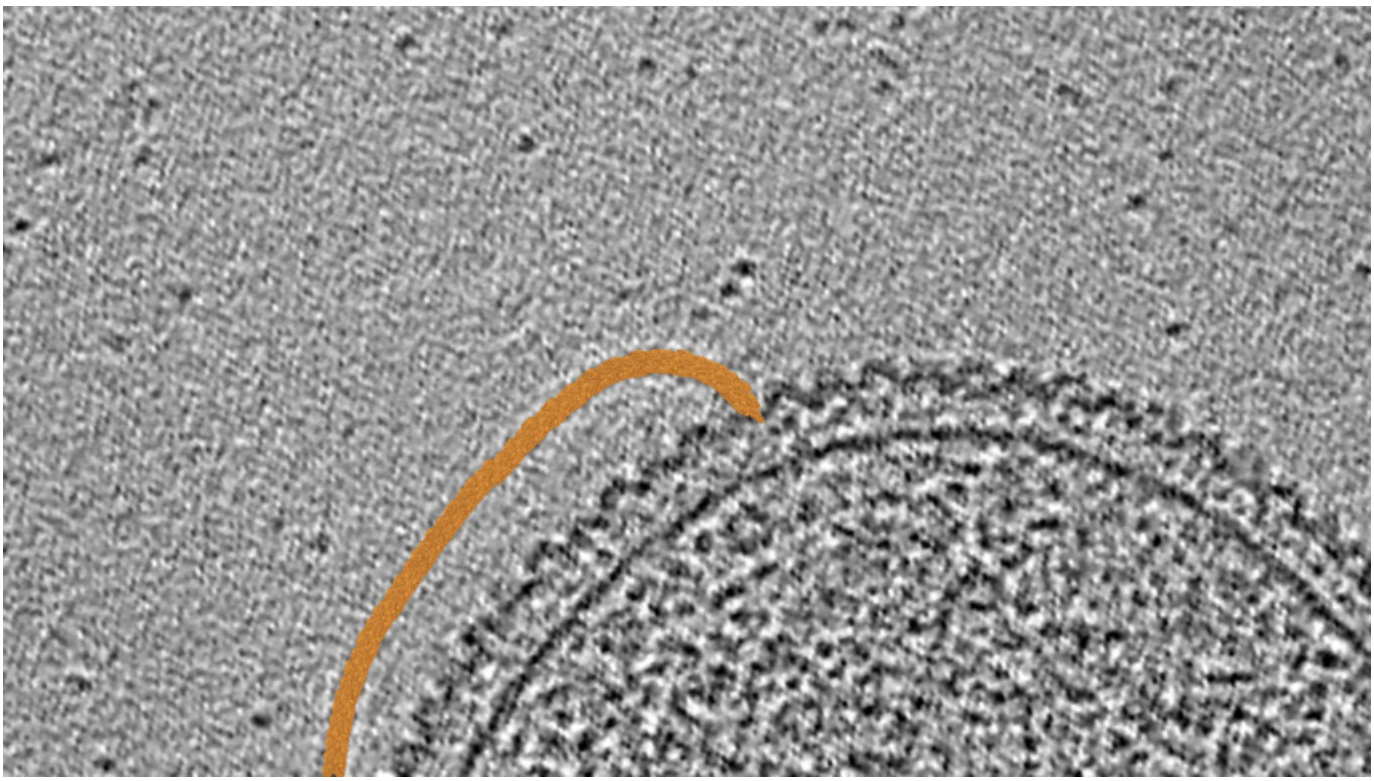

100 nm

8. Archaellum

Archaea swim, too. And as you might expect, they use similar machinery to do so: an envelope-embedded motor that spins a long extracellular filament. Despite the structural similarity, the machinery evolved independently, another indication of the strong advantage conferred by swimming. To reflect this similar-but-not-the-same character, we call the archaeal analogue of the bacterial flagellum the **archaellum**. Unlike the flagellum, which is a type III secretion system, the archaellum is a **type II secretion system**. As you can see on this *Methanoregula formicica*, archaella are narrower than flagella (*Learn More* on the next page). The motor is also different, and uses the ATPase at the base not just for assembly, but also to power rotation for swimming. Like flagella, archaellar motors can rotate in either direction, resulting in the filaments pushing or pulling the cell.

Methanoregula formicica Collected by: Ariane Briegel DOI: 10.22002/D1.1532



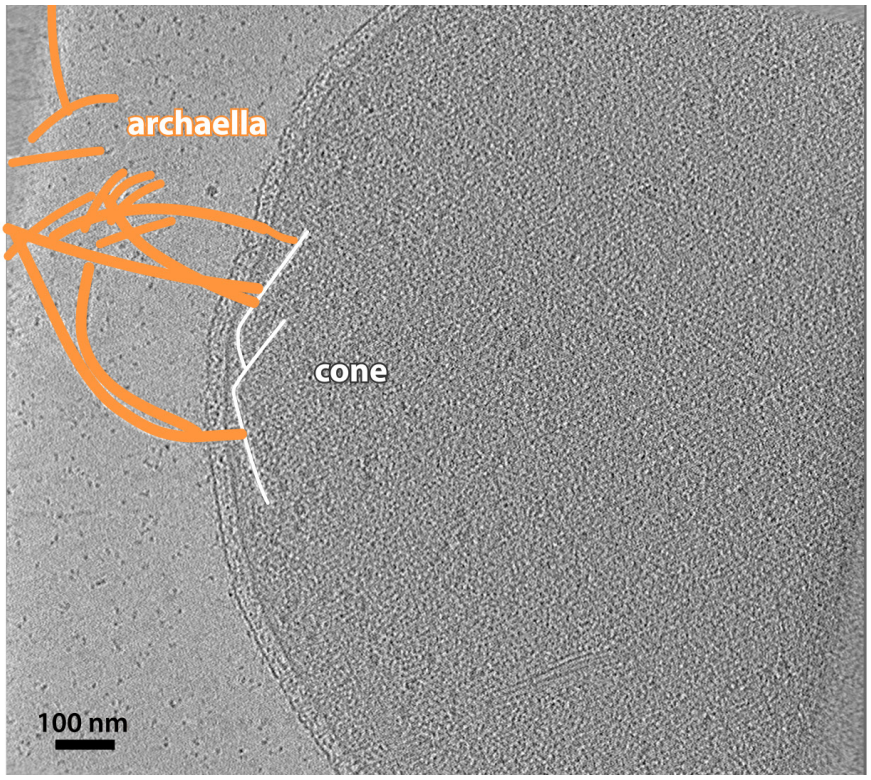


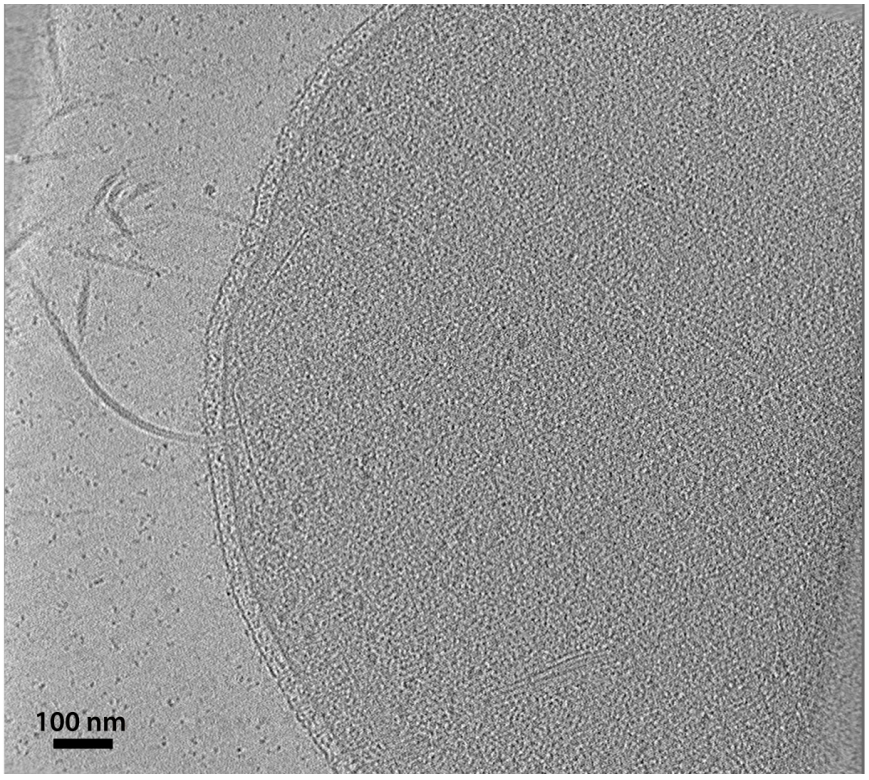
Learn More: Filament Structure

The overall architecture of the helical archaellum is similar to that of the bacterial flagellum, as you can see in this structure from Methanospirillum hungatei *[60]. Each protein subunit is smaller, however, resulting in a narrower filament diameter: ~10 nm, compared to ~24 nm for the flagellum. They are also more tightly packed, so there is no central channel. And, unlike flagella, the filament assembles from the base.*

Source: Poweleit et al. (2016)

Structure: PDB 5TFY


archaella
cone
100 nm

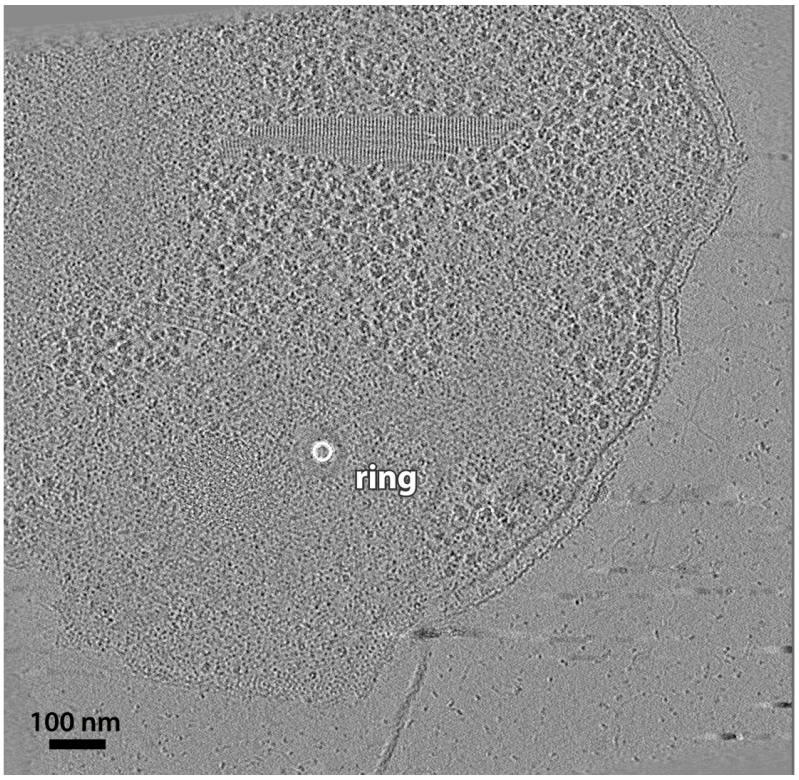

100 nm

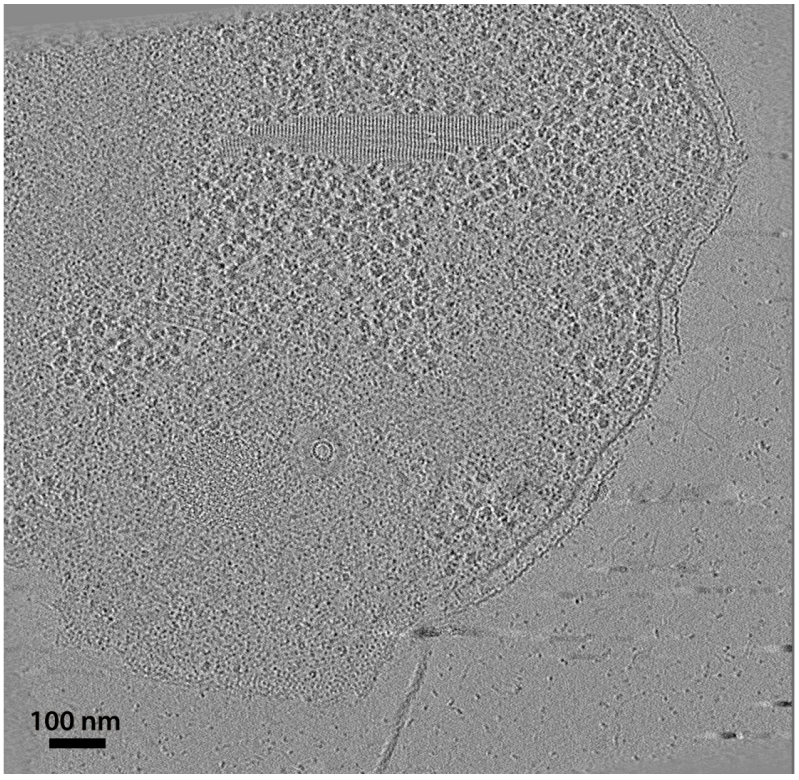
9. Archaella Patterns

Similar to flagella in bacteria, different archaeal species employ different numbers and patterns of archaella. Some species have one, others have many, either distributed peritrichously (all around) as in the *Methanoregula formicica* you just saw, or lophotrichously (clumped) as in this *Thermococcus kodakaraensis*. In *T. kodakaraensis* and related species, an additional structure–a large conical plate–is seen in the cytoplasm, perhaps providing leverage for the multiple motors. The plate has a unique structure (*Learn More* on the next page) and may act as an organizing center akin to the polar PopZ structure we discussed in the last chapter (*Learn More* on following pages). Note the two peaks on this cone; it may be in the process of replicating in preparation for division.

A leveraging plate must not be essential, however, because not all lophotrichous archaea use one (*Learn More* on following pages).

Thermococcus kodakaraensis Collected by: Ariane Briegel DOI: 10.22002/D1.1533

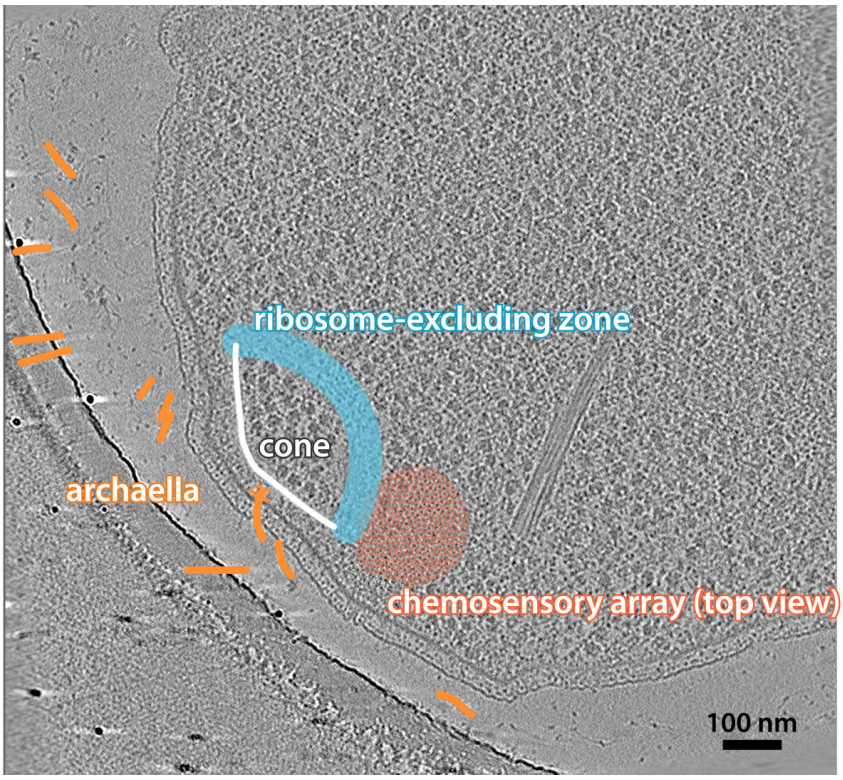

ring
100 nm

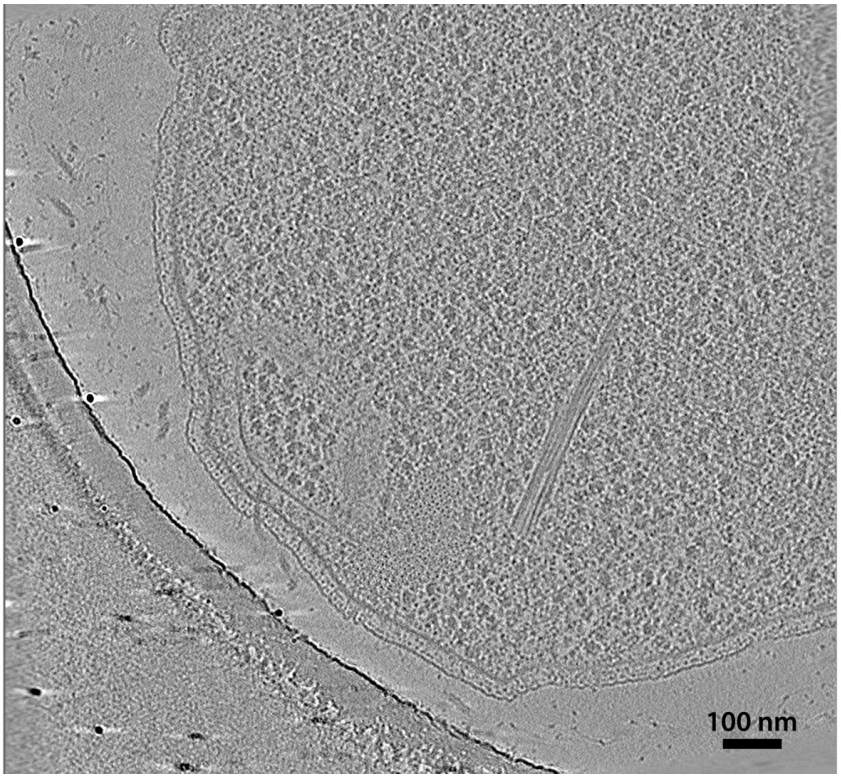

100 nm

Learn More: Cone

The archaellar plate does not come to a point at the tip, but rather is a conical frustum (open at the top), resembling a lampshade. In the center of the tip is a small ring, as you can see in a top view in this lysed, and therefore flattened, Thermococcus kodakaraensis *cell. The function of the ring remains unknown; perhaps it nucleates the rest of the structure?*

Thermococcus kodakaraensis *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1542*

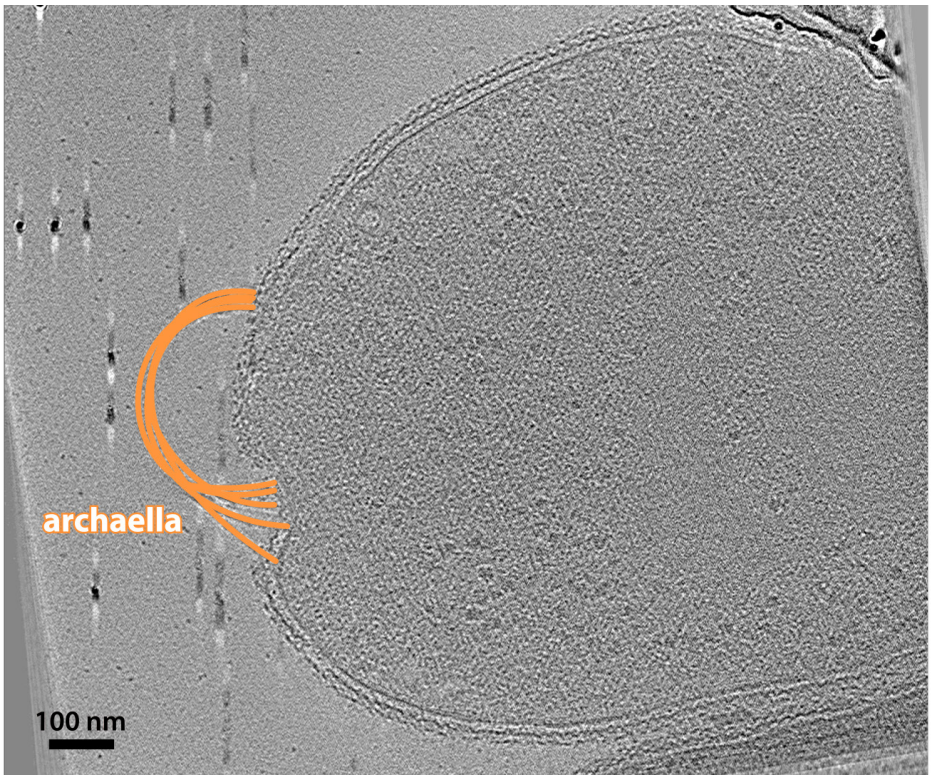

ribosome-excluding zone
cone
archaella
chemosensory array (top view)
100 nm

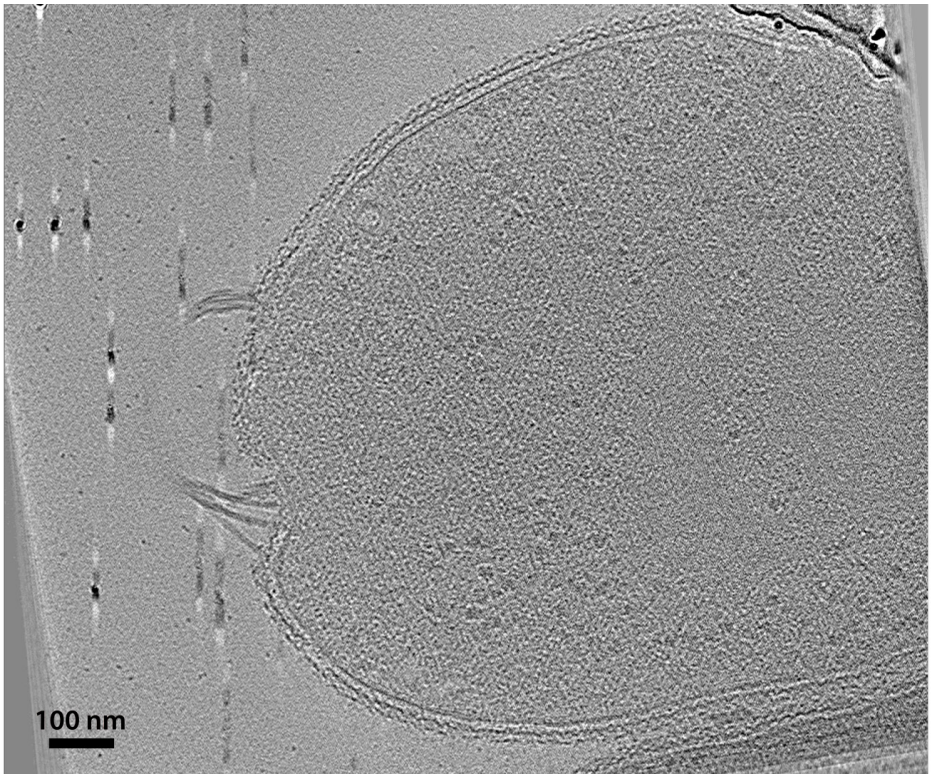

100 nm

Learn More: Organizer

As you can see more clearly in this lysed, flattened Thermococcus kodakaraensis *cell, the conical plate in the cytoplasm is attached to more than just the archaella. It is also associated with chemosensory arrays (discussed in the next chapter) and DNA, as you can see from the ribosome-excluding zone. This structure may therefore perform an analogous function to bacterial organizing proteins such as PopZ, tethering cellular components into a* de facto *pole for the (in this case round) cell.*

Thermococcus kodakaraensis *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1543*

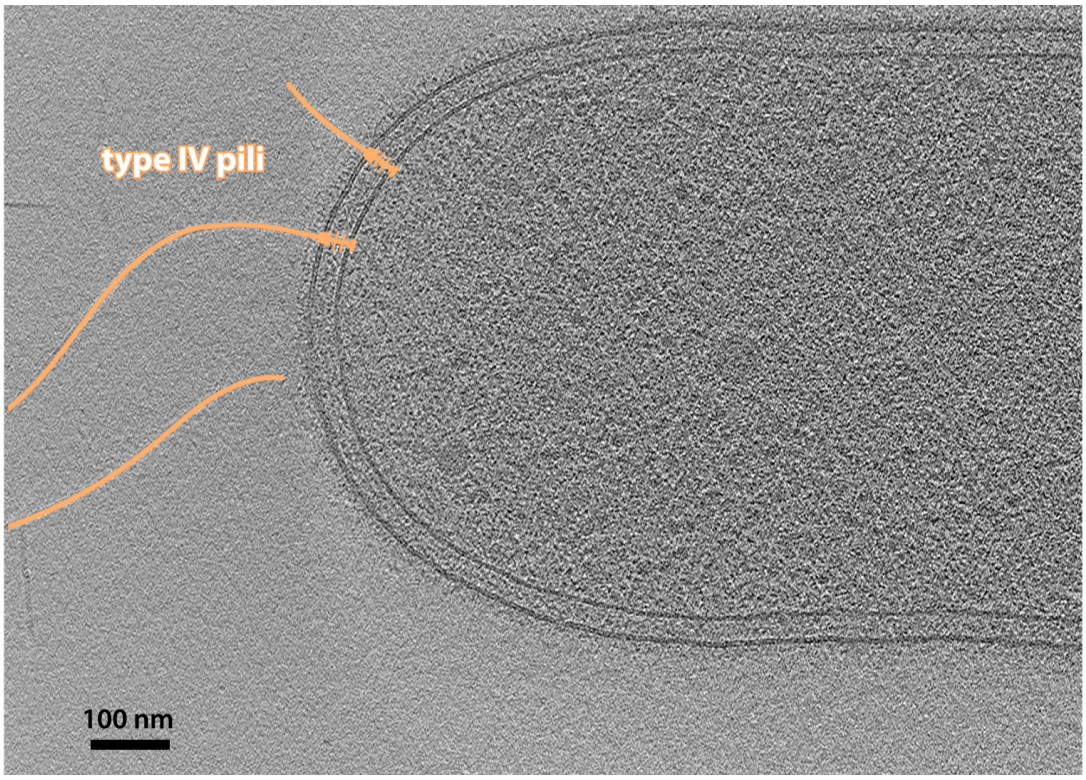

archaella
100 nm

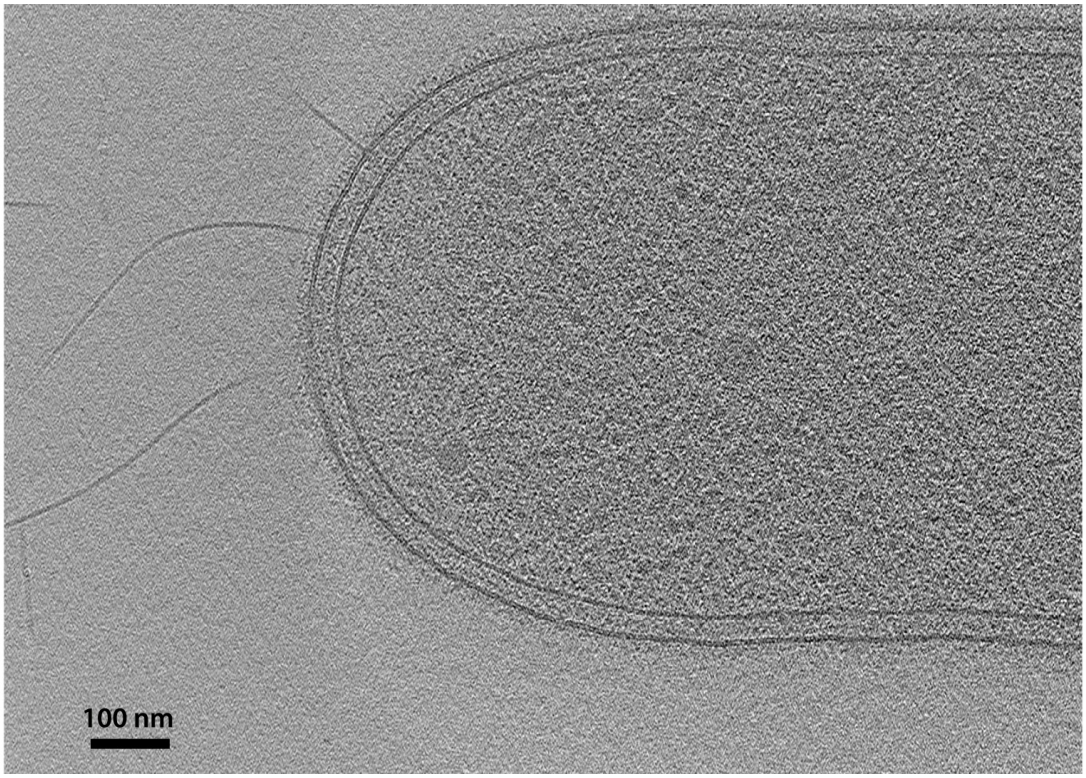

100 nm

Learn More: Diversity

While a cytoplasmic plate may help distribute the force of multiple, closely-packed archaella, it is clearly not necessary since other lophotrichous species, like this Halobacterium salinarum*, do not use one.*

Halobacterium salinarum *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1544*


type IV pili
100 nm

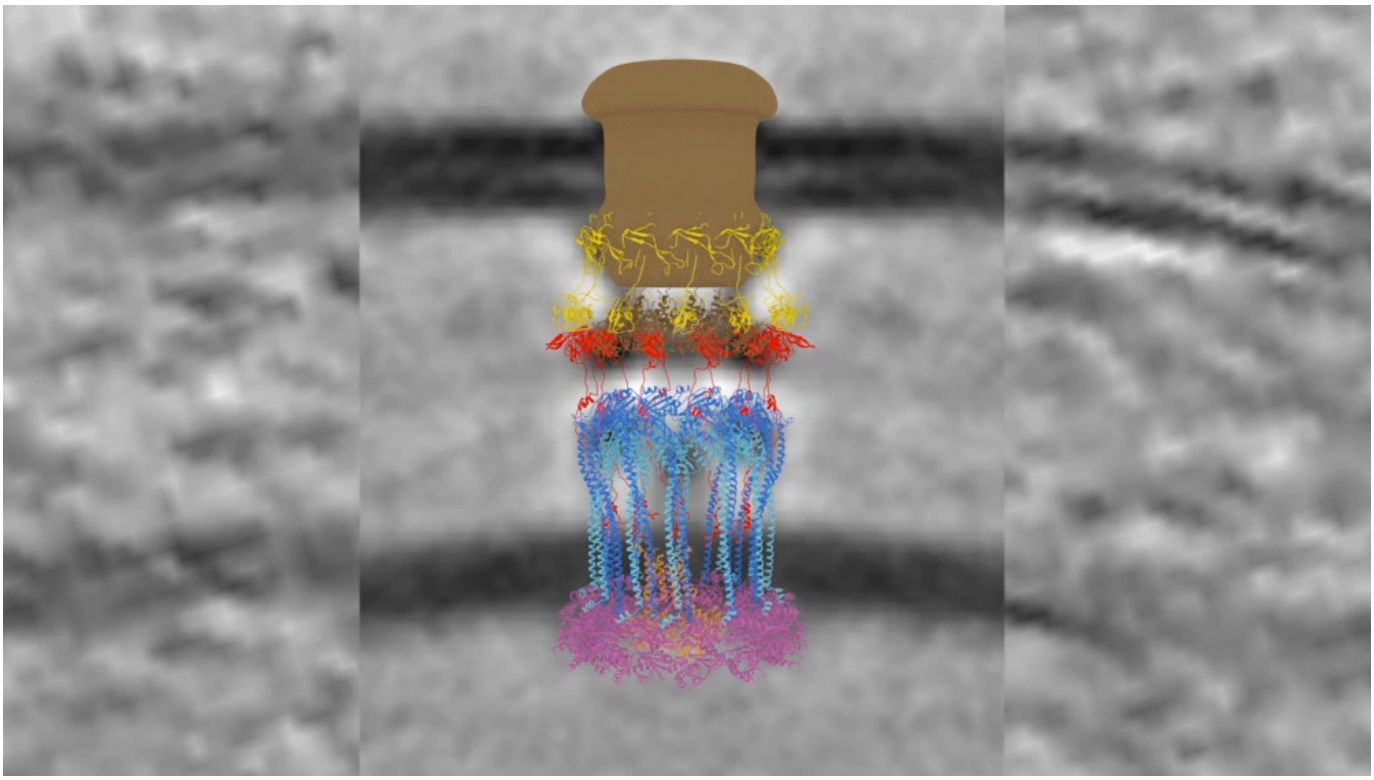

100 nm

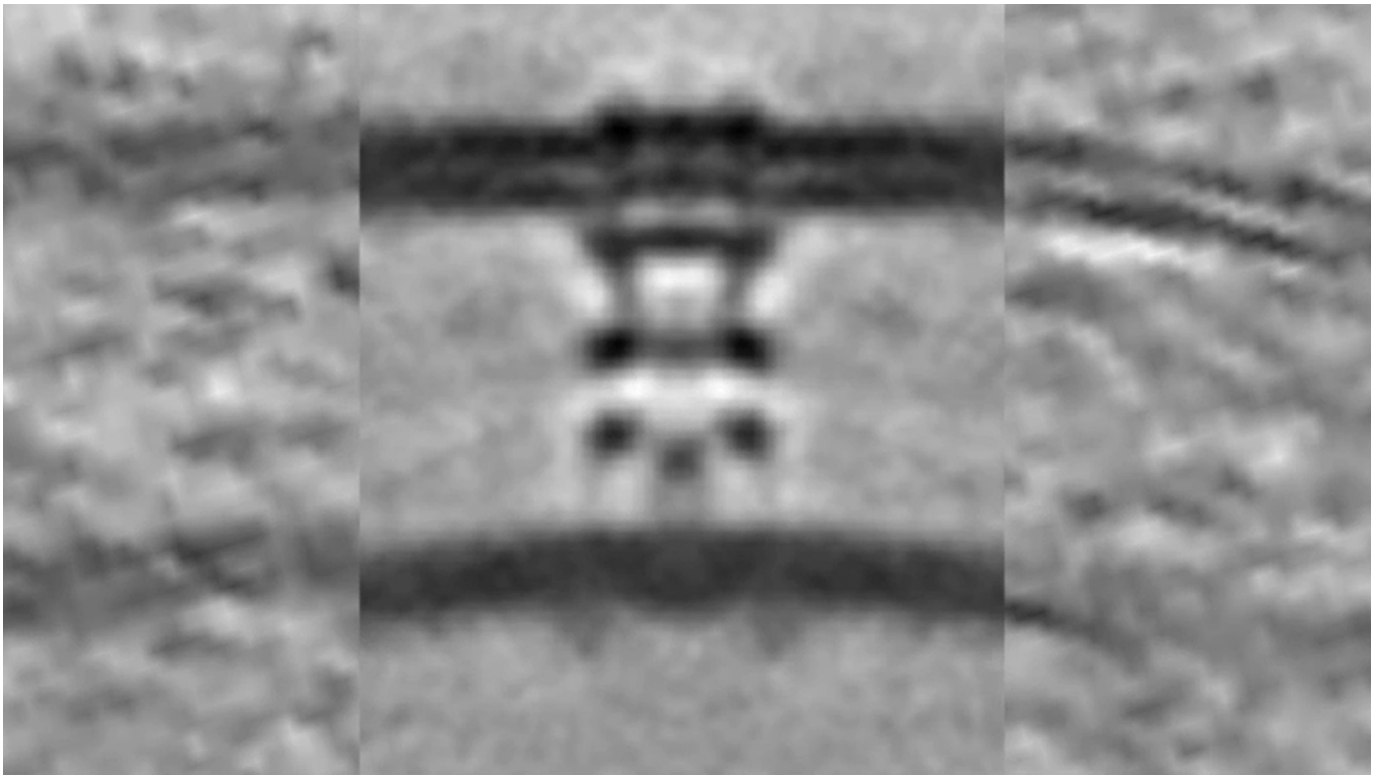
10. Type IV Pilus

If your cell lives on a surface, what is the best way to get around? How about using a grappling hook? Some bacteria, like this *Myxococcus xanthus*, use a type II secretion system related to the archaellar motor to pull themselves around their environment. As you can see, the structure looks familiar: a motor embedded in the envelope attached to a long extracellular filament. In this case the filament is called a **pilus** ("hair" in Latin). Bacteria and archaea make many kinds of pili (also generically called **fimbriae** ("fringe")) and you will see some of their other functions in later chapters. The *M. xanthus* pili, classified as **type IV pili**, function not as propellers like flagella or archaella, but rather extend linearly, stick to a surface at the tip, and then retract to pull the cell toward the attachment point (*Learn More* on the next page). (You can watch a video of Pseudomonas aeruginosa moving with type IV pili on Howard Berg's website: http://www.rowland.harvard.edu/labs/bacteria/movies/pseudo.php.) The pilus motors are the strongest known motors in nature, and can retract pili at up to 1 μm/s; the combined action of multiple pili leads to extremely rapid "twitching" motility of the cell over a surface. The motor structure, or basal body, remains intact even when no pilus is assembled. These rod-shaped cells have many basal bodies at both cell poles; to switch direction, the cell simply disassembles the pili on one end and builds new pili from the machines waiting on the other.

In addition to attaching to a surface, the pili can also stick to other *M. xanthus* cells. This enables the cells to move over surfaces en masse. Combined with their practice of eating other bacteria, this property has led them to be compared to packs of wolves hunting down their prey.

Myxococcus xanthus Collected by: Yi-Wei Chang DOI: 10.22002/D1.1534



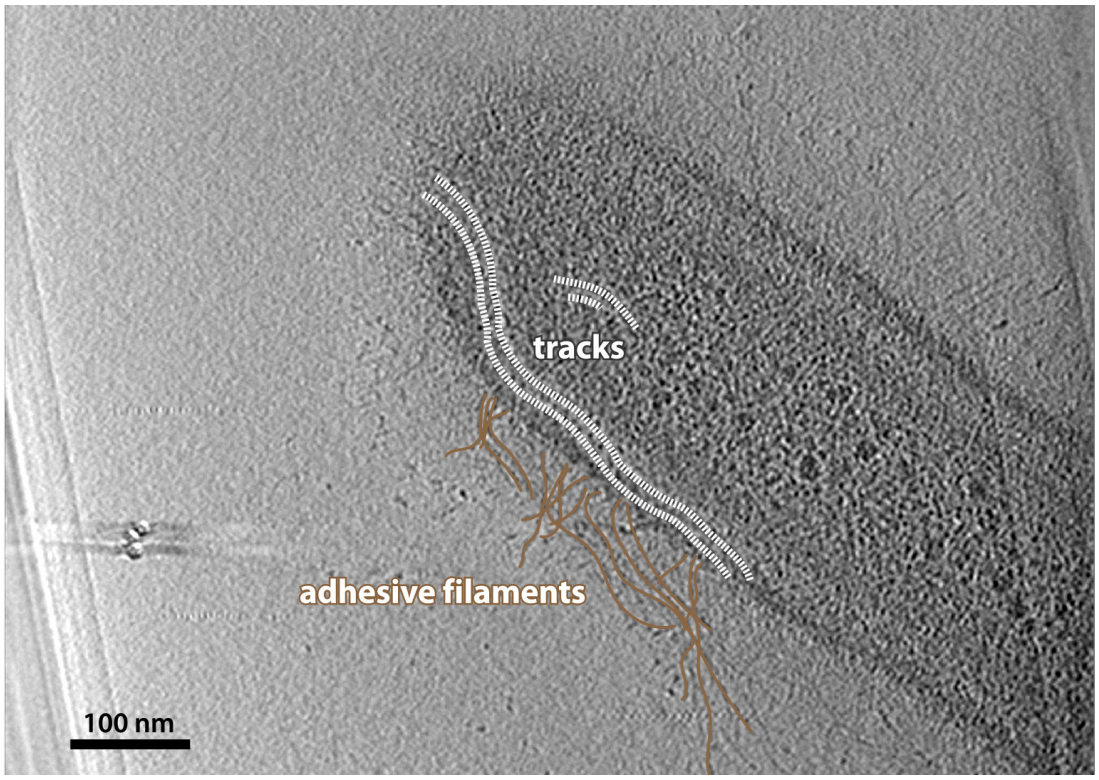


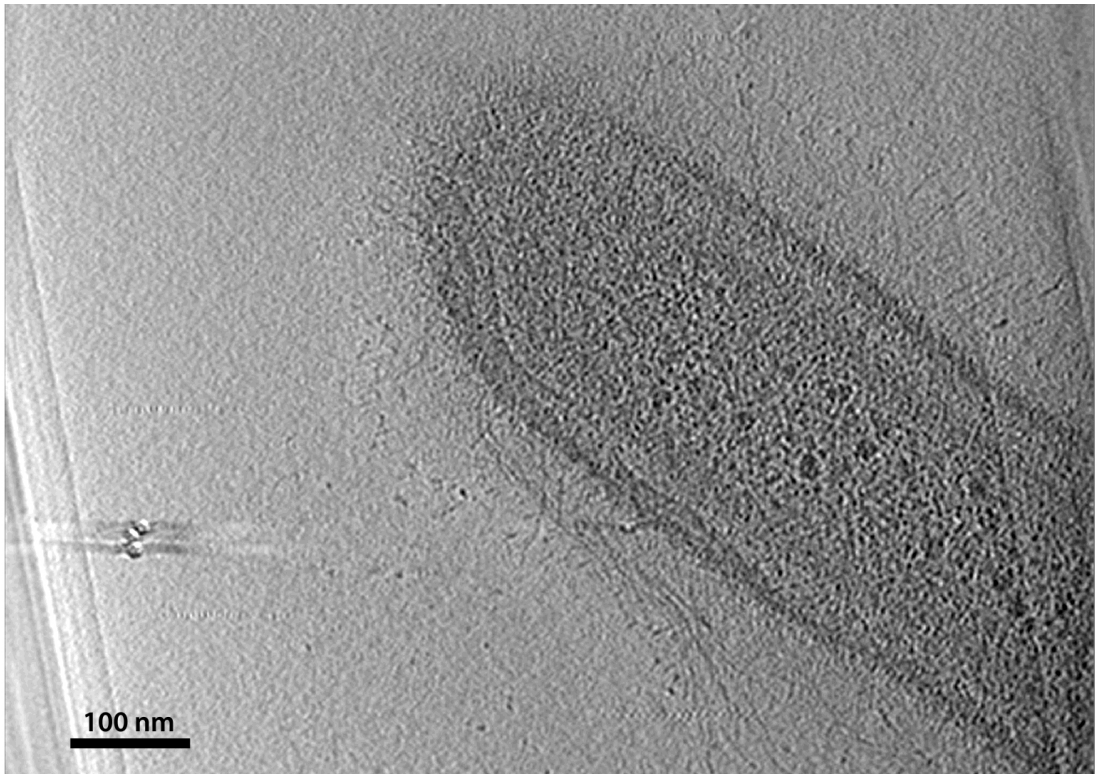
Learn More: Structure

A series of rings anchors the type IV pilus basal body in the cell envelope. This rigid structure provides leverage for an ATPase at the base to rotate an adaptor in the inner membrane. We think that when it spins in one direction, the adaptor rotates to lock pilin monomers that diffuse from the inner membrane into the assembling pilus [61]. Once the pilus has reached its target, attachment is sensed by the basal body (we do not yet know how). As a result, the assembly ATPase dissociates and a second, homologous ATPase takes its place. This disassembly ATPase spins the adaptor in the opposite direction, ushering pilin monomers back into the inner membrane, ready to join the next growing pilus. (You can watch the full video on YouTube: https://youtu.be/HGvnrWrudpA.) Animation by Janet Iwasa.

Source: Chang et al. (2016)

Structure: EMD-3247, PDB 3JC8

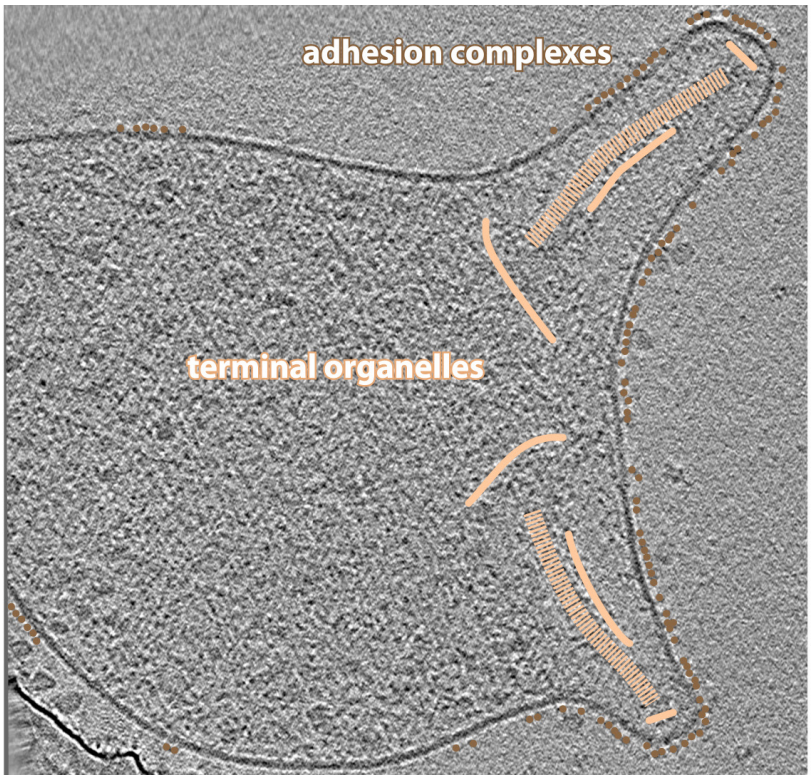

tracks
adhesive filaments
100 nm


100 nm

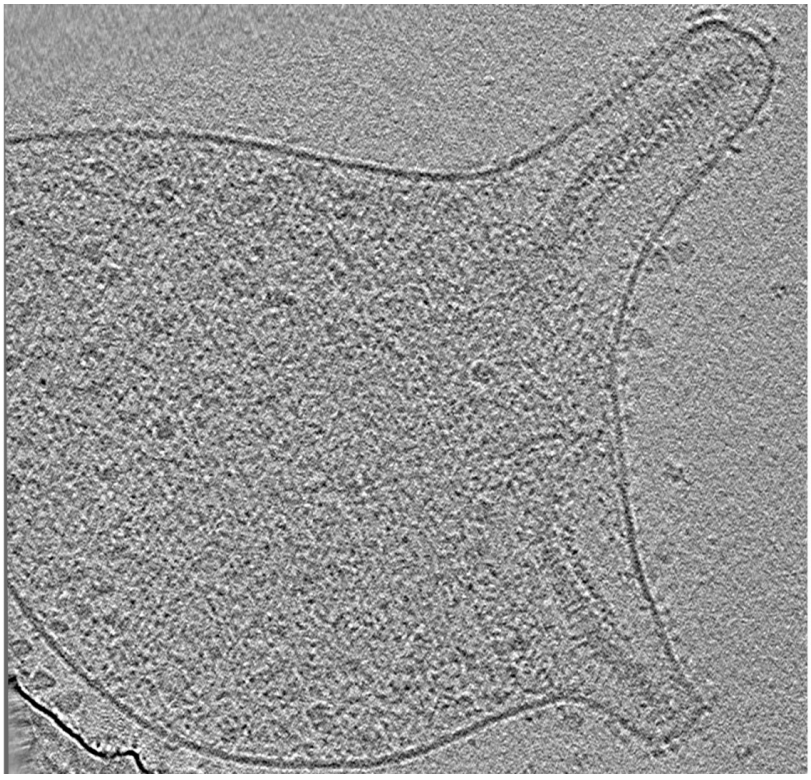
11. Type IX Secretion System

Other bacteria use different machinery to move over surfaces. *Flavobacterium johnsoniae* like this cell use a **type IX secretion system** to secrete adhesive filaments. These filaments move on a helical track around the cell, and thereby the cell moves forward on a surface (see Howard Berg's website for a video of the cells moving: http://www.rowland.harvard.edu/labs/bacteria/movies/flavobacterium.php). The power for this gliding movement is thought to come from rotary motors anchored in the cell wall that propel the adhesive filaments along their track in a rack-and-pinion fashion, but we still do not understand the mechanism in detail.

Flavobacterium johnsoniae Collected by: Gregory Henderson DOI: 10.22002/D1.1535



100 nm



100 nm

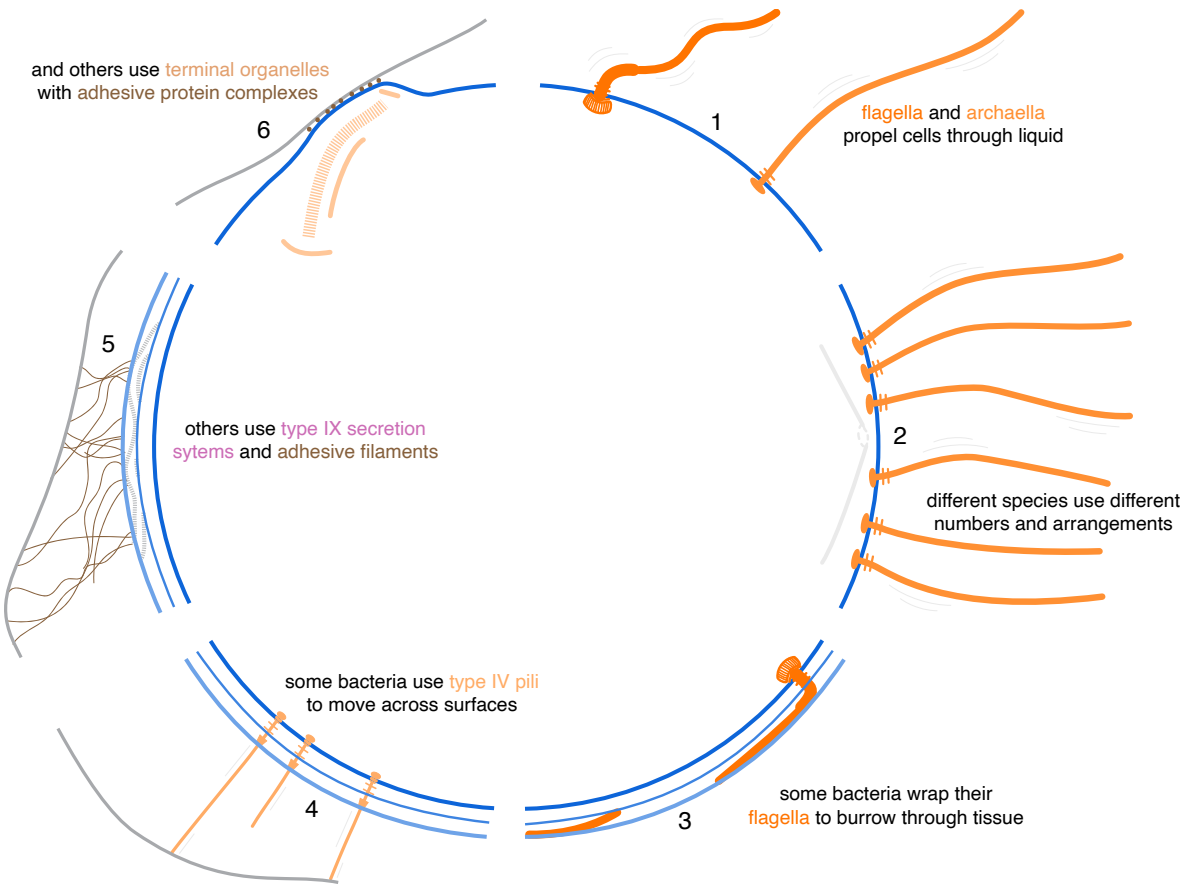
12. Terminal Organelle

Another, familiar, way to get across a surface is to crawl. For this to work, though, you need to be able to change the conformation of your body. This is possible if your cell lacks a cell wall or surface layer, like this *Mycoplasma pneumoniae*. Like the *Mycoplasma genitalium* you saw in Chapter 2.1, these cells are intracellular pathogens, so they do not need to buttress their membrane against differences in osmolarity. As a result, they are soft and flexible. This may allow them to use a leg-like internal structure called a **terminal organelle** to crawl, or "glide," across a surface. The exact mechanism is still unclear, but one possibility is that a hinge-like conformational change in the terminal organelle extends and contracts the back of the cell with respect to the front, similar to the movement of an inchworm (but less exaggerated). Combined with adhesion proteins on the cell surface, this might propel the cell forward. (You can watch a video of *Mycoplasma mobile* gliding on Howard Berg's website: http://www.rowland.harvard.edu/labs/bacteria/movies/mycoplasma.php.)

The skeleton-like terminal organelle gives these cells their characteristic flask shape. In combination with their minimal cell envelope, it can also give rise to an unusual method of cell division. In species (or genetically engineered strains) that lack the division protein FtsZ, *Mycoplasma* cells still manage to divide. They replicate their terminal organelle normally, as you can see this cell has done, and then the two copies simply walk away from each other, stretching the mother cell between them until the membrane pinches off to produce two daughters.

Keep in mind that these are simply *some* of the ways we know bacteria and archaea get around, and we continue to discover new ones.

Mycoplasma pneumoniae Collected by: Gregory Henderson DOI: 10.22002/D1.1536


1
flagella and archaella
propel cells through liquid
2
different species use different
numbers and arrangements
3
some bacteria wrap their
flagella to burrow through tissue
4
some bacteria use type IV pili
to move across surfaces
5
others use type IX secretion
sytems and adhesive filaments
6
and others use terminal organelles
with adhesive protein complexes

Summary

Concept Check Questions

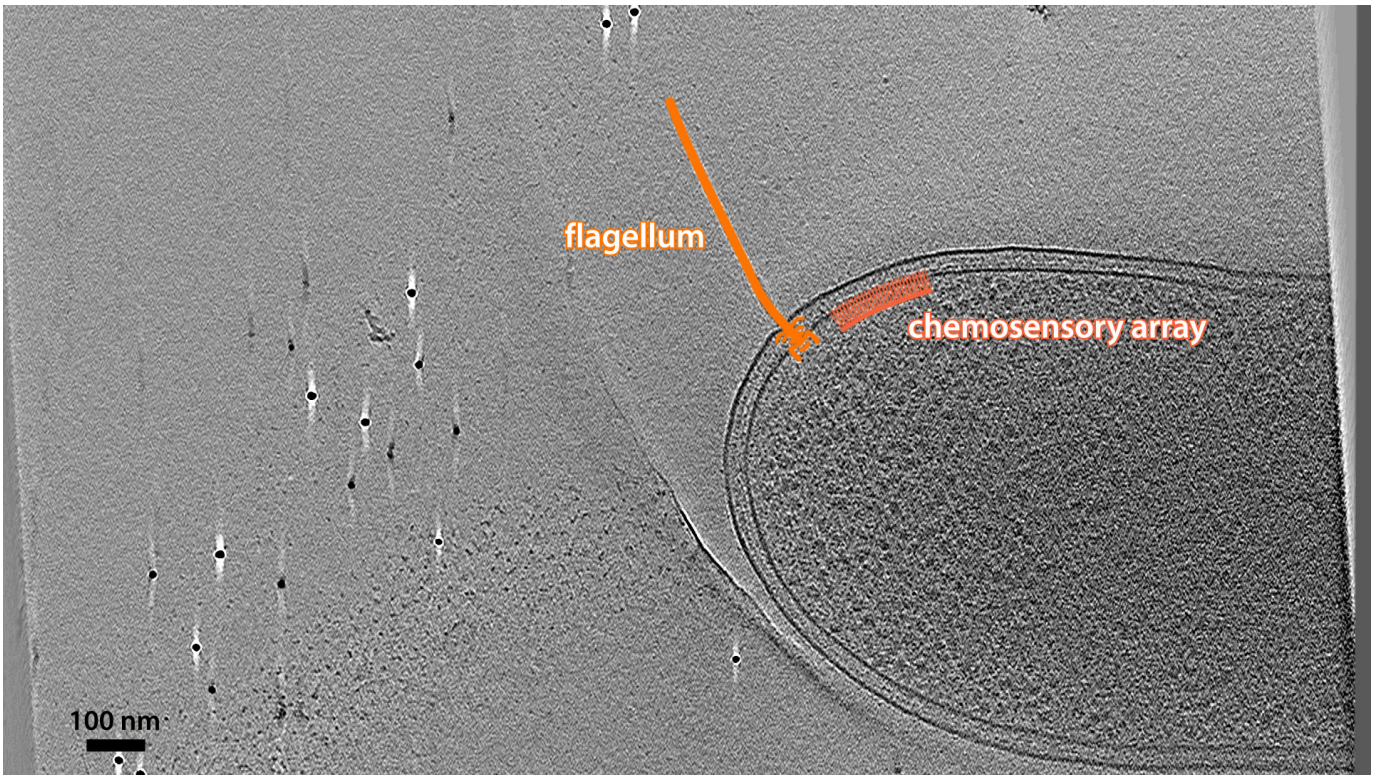
- Which works better in high viscosity: an oar or a propeller?
- What other motility machinery is genetically related to the archaellum?
- What motility machinery works like a grappling hook?
- How can Mycoplasma divide if they lose FtsZ?

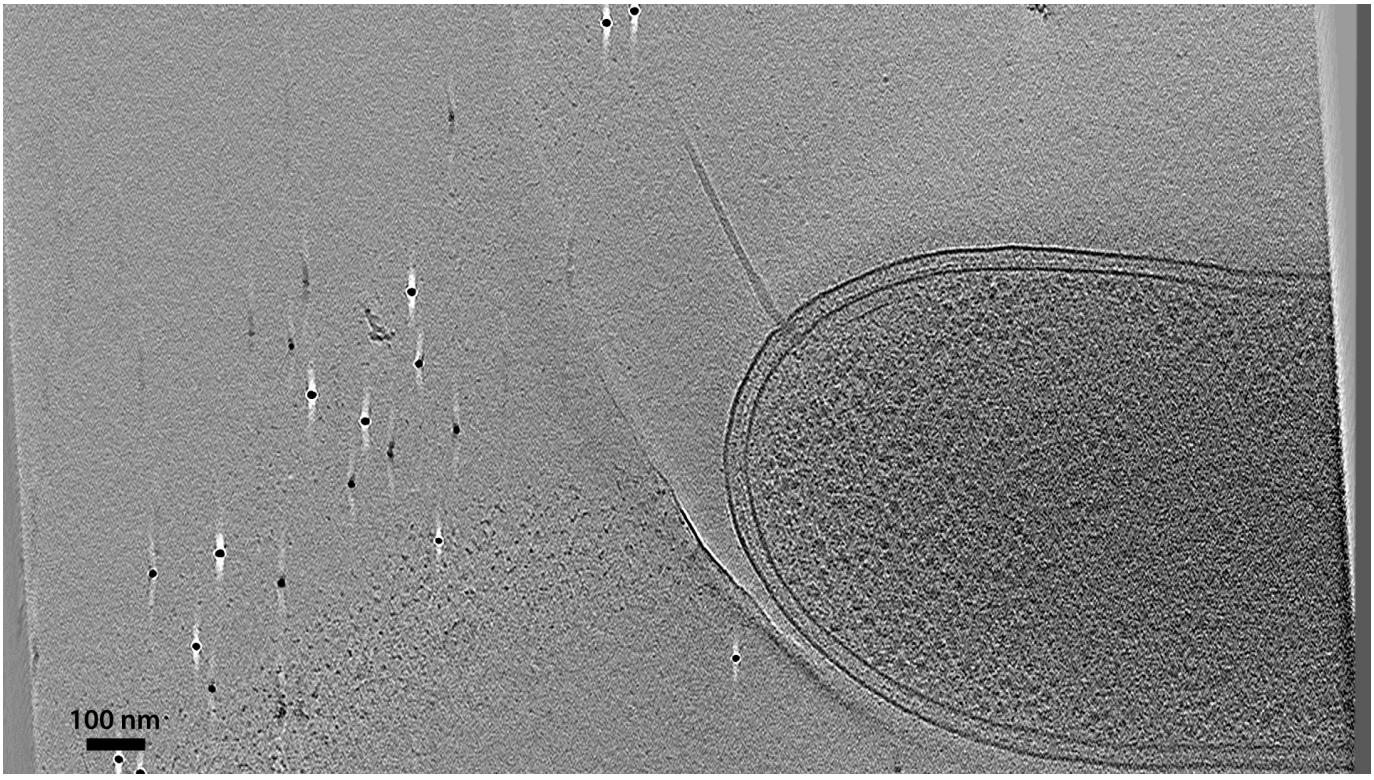
Further Reading

- Albers and Jarrell (2018). *The archaellum: An update on the unique archaeal motility structure* [62].
- Armbruster and Mobley (2012). *Merging mythology and morphology: The multifaceted lifestyle of Proteus mirabilis* [63].
- Berg (2003). *The rotary motor of bacterial flagella* [64].
- Jarrell and McBride (2008). *The surprisingly diverse ways that prokaryotes move* [65].
- Muñoz-Dorado et al. (2016). *Myxobacteria: Moving, killing, feeding, and surviving together* [66].
- Shrivastava and Berg (2015). *Towards a model for Flavobacterium gliding* [67].

7
Navigation

"E. coli *forgets where it is going in about 10 seconds.*"
- Howard Berg [50]


flagellum
chemosensory array
100 nm


100 nm

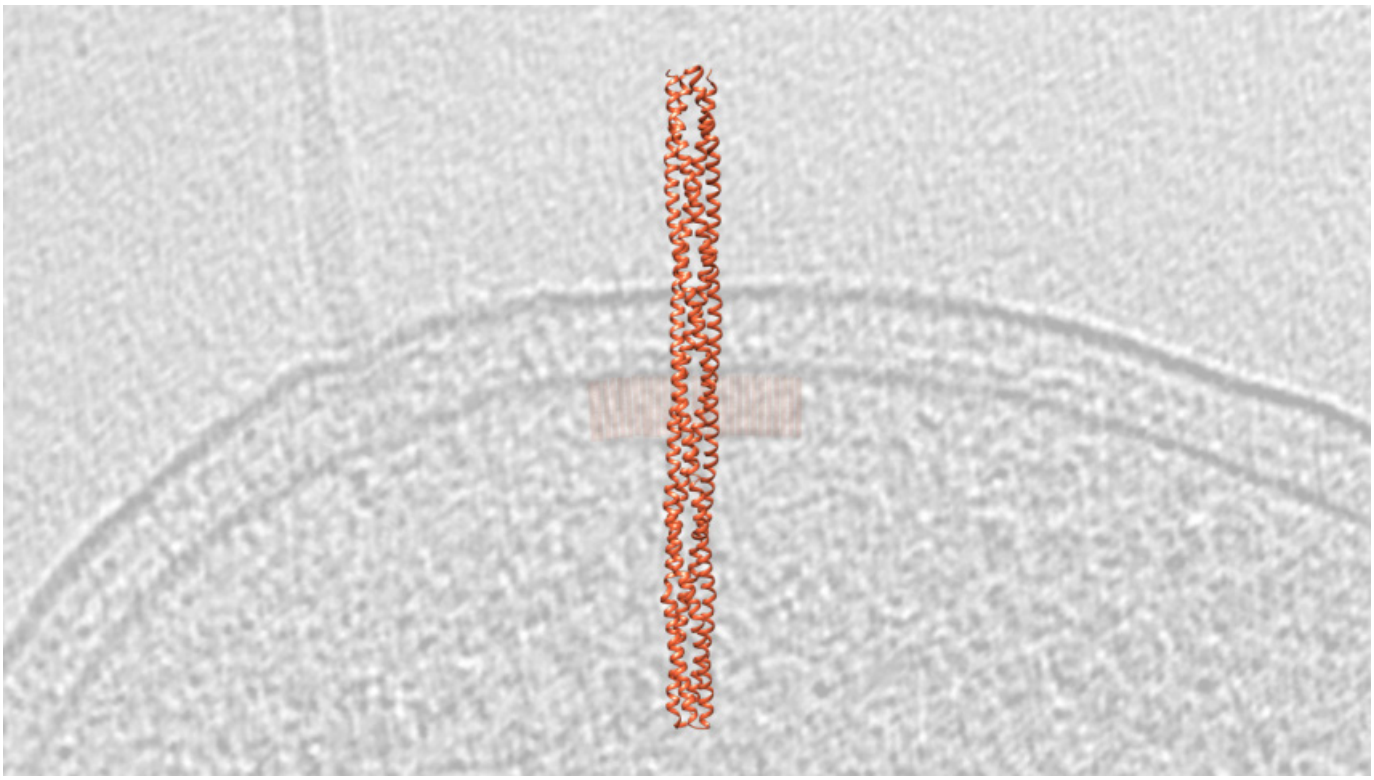
1. Chemotaxis

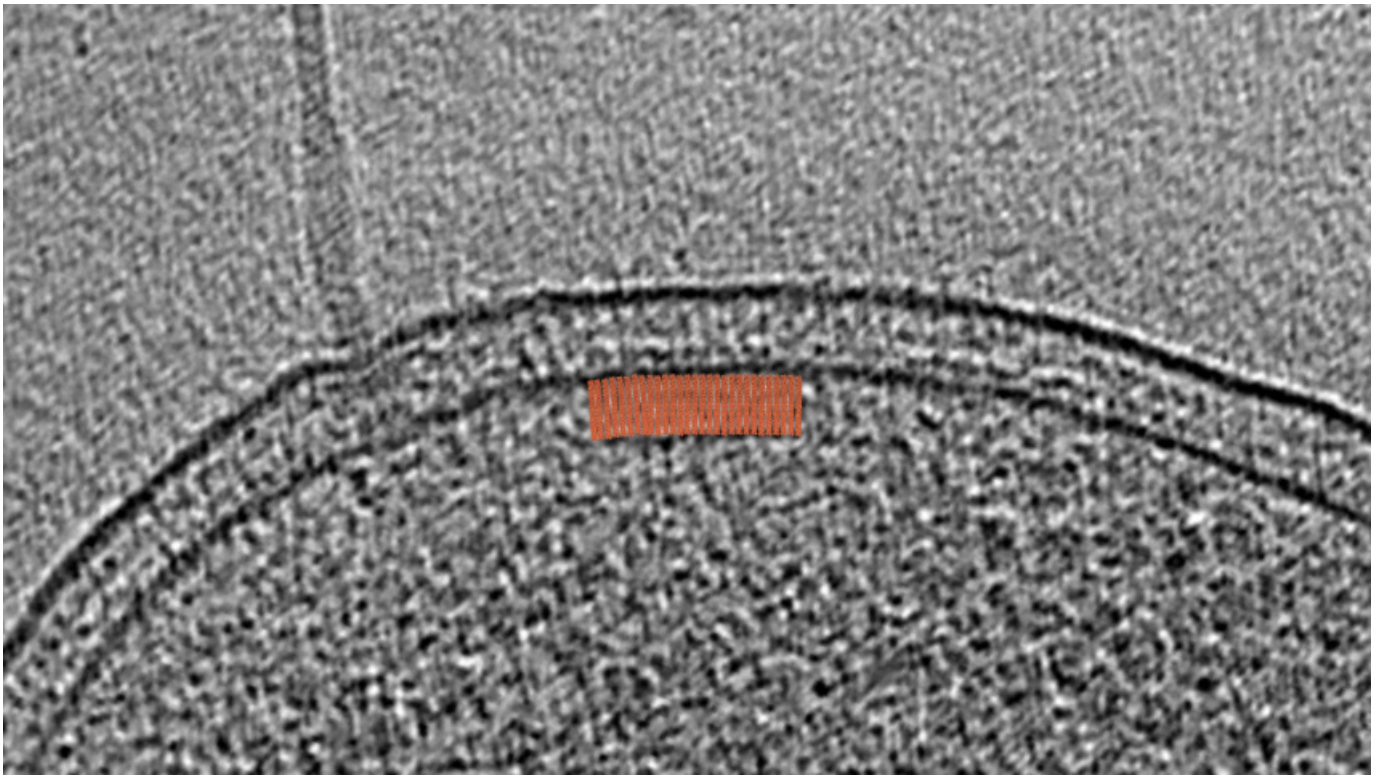
Now that your cell can move, it needs to figure out which way to go. Perhaps it should take a sniff. **Chemosensory systems** are ancient (they were already present in the common ancestor of bacteria and archaea) and widespread, reflecting their great utility. Chemosensory systems are two-component signaling systems. The first component is a **chemoreceptor** that binds a specific chemical, such as a sugar or amino acid. The binding results in a conformational change that propagates down the long receptor (*Learn More* on the next page), turning off a kinase bound at the other end that controls the state of the second component: a **response regulator**. These response regulator proteins then carry the signal elsewhere in the cell by diffusion.

For **chemotaxis** ("taxis," or ordered movement, in response to chemicals) the signal is carried to the cell's motility machinery, type IV pili or flagellar motors. Phosphorylated response regulators bind the flagellar motor, switching the direction of rotation. This produces different results depending on the pattern of flagella on the cell. If there is a single flagellum, as on this *Shewanella oneidensis*, it switches the flagellum between pushing and pulling the cell body, reorienting the cell in the process. In peritrichously-flagellated cells like *Escherichia coli*, it brings the flagella into and out of a bundle. Remember that bundled flagella drive the cell forward in straight "runs" and dissociated flagella "tumble" the cell to try a new direction. (You can watch videos of this on Howard Berg's website: http://www.rowland.harvard.edu/labs/bacteria/movies/ecoli.php.) Constant feedback from the chemosensory system switches the balance of phosphorylated/unphosphorylated response regulators and therefore keeps the cell heading in the general direction of an attractant chemical cue, or away from a repellant.

Chemosensory systems form arrays containing many copies of the proteins. As in this *S. oneidensis* cell, arrays are usually located near the flagella they control, with the tips of the chemoreceptors sticking through the membrane into the periplasm or extracellular space where they can detect signals from the environment. At the other end of the array, the associated kinases interact with the response regulators. These kinases, along with an additional structural protein that helps organize the array, form a layer that we see as a dense line in the cytoplasm.

Shewanella oneidensis Collected by: Mohammed Kaplan DOI: 10.22002/D1.1545

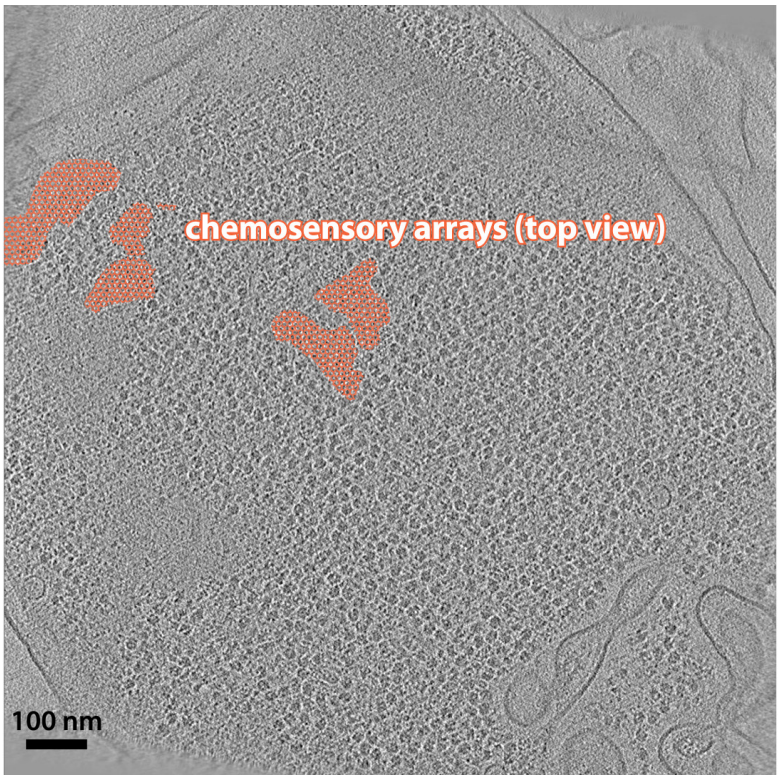


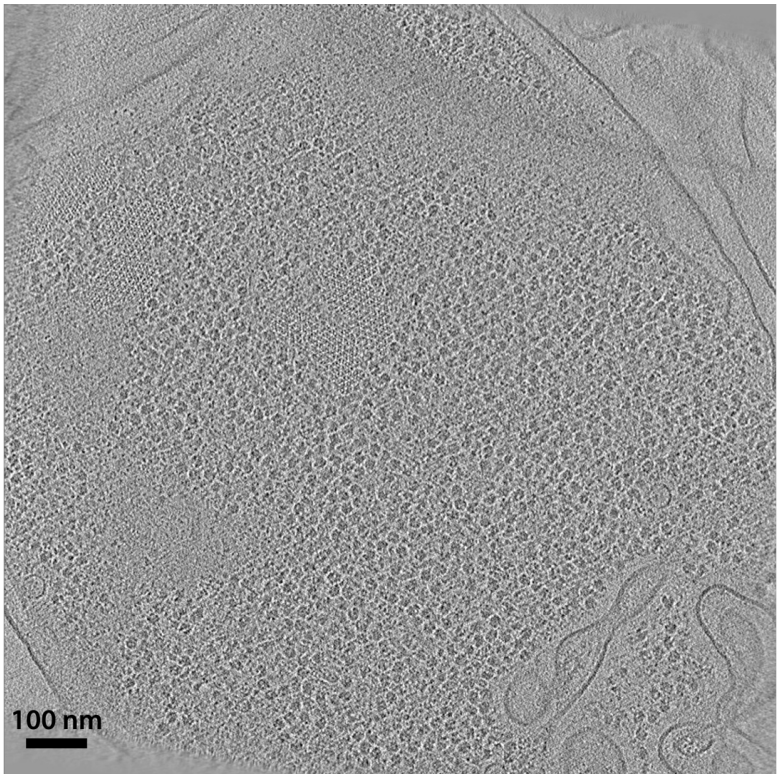


Learn More: Chemoreceptor

Chemoreceptors take the form of long rods. A single protein zips back along itself, and then joins together with a second copy, forming a rigid bundle of four intertwining helices (two from each member of the dimer), as you can see in this dimer of receptors from Thermotoga maritima *[68]. Only the cytoplasmic portion is shown here; in the cell, the receptors would also have a membrane-embedded anchor at the top and, beyond that, a small domain in the periplasm (or outside the cell in a monoderm) to bind the chemical of interest. Once a chemical binds, the signal is transmitted down the length of the receptor to a kinase waiting at the distal tip.*

Source: Park et al. (2006) *Structure: PDB 2CH7*

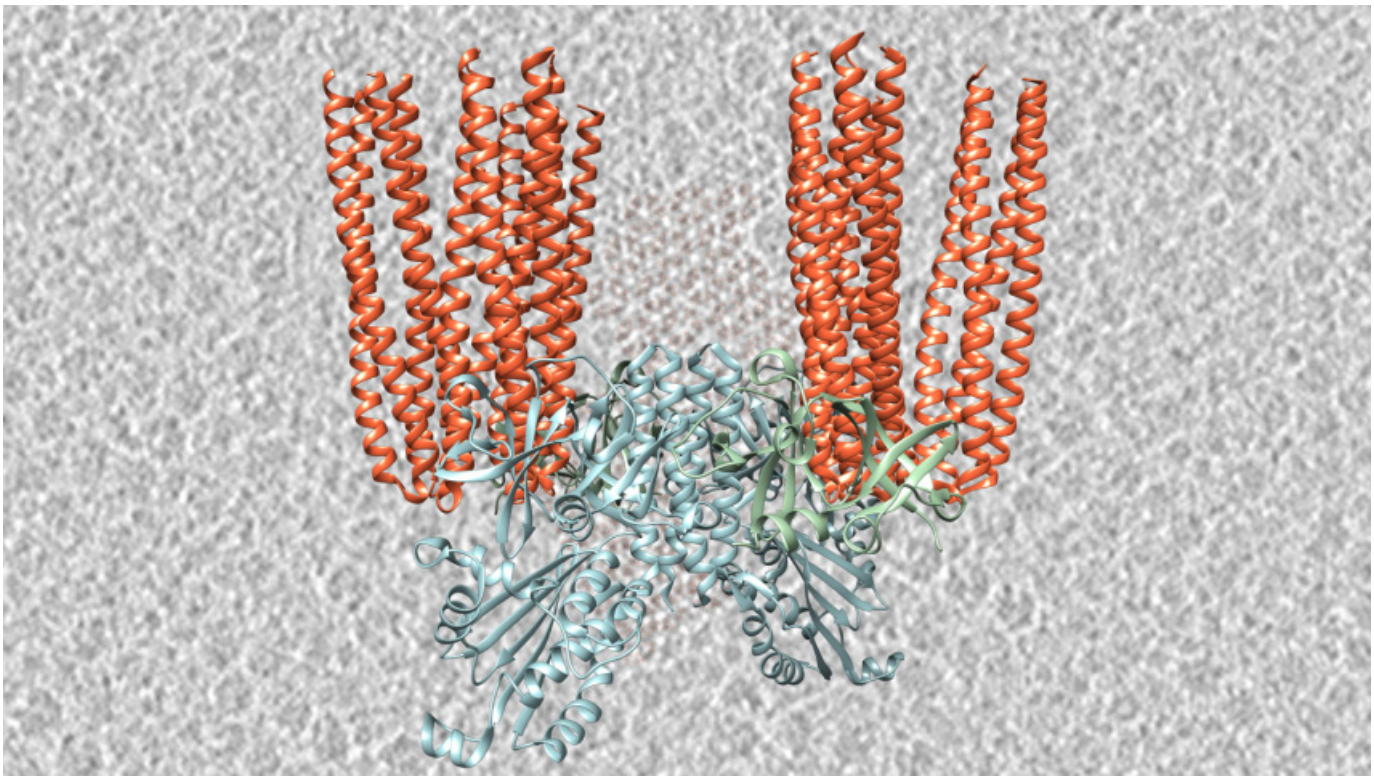

chemosensory arrays (top view)
100 nm

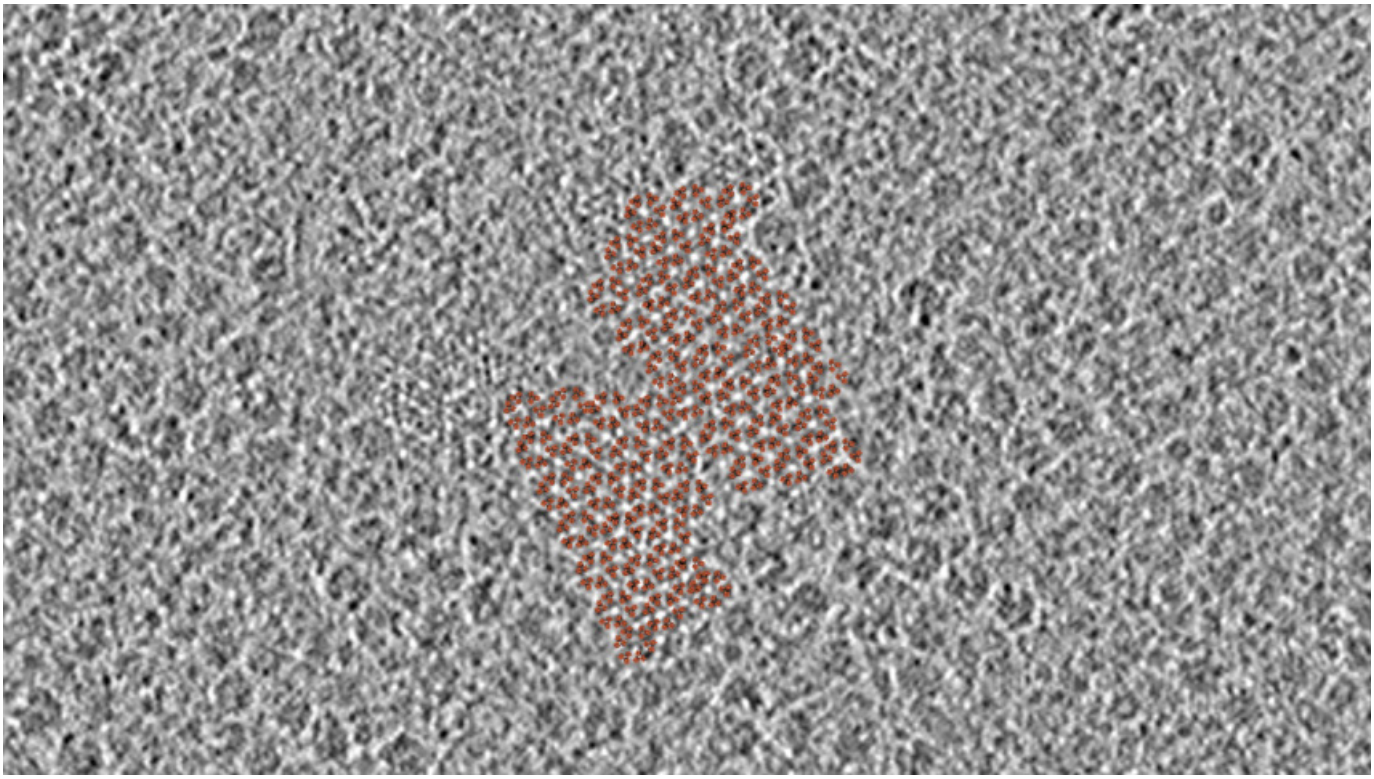

100 nm

2. Chemosensory Arrays

Chemosensory arrays are highly ordered, as you can best see from a bird's-eye view, as in this lysed *Salmonella typhimurium*. Chemoreceptors come together as dimers, which in turn organize into trimers, which are further packed into the extensive hexagonal honeycombed array you see here. The hexagonal arrangement comes from the baseplate, where the kinases and coupling proteins bind into an ordered array (*Learn More* on the next page). In what should be a familiar theme by now, organization provides a great benefit. Bacteria and archaea have a tremendous sense of smell, responding to as few as one or two molecules of an attractant, or many more. In fact, the range of chemical concentrations they can discriminate among extends over 5 orders of magnitude. But how can a single receptor transmit a signal efficiently? Perhaps it should share the message with its neighbors. The interlocking network of chemoreceptors enables just this kind of amplification; a target binding to one receptor may translate into activation of 36 adjacent receptors, enormously boosting the gain of the signal.

Salmonella typhimurium Collected by: Morgan Beeby DOI: 10.22002/D1.1546

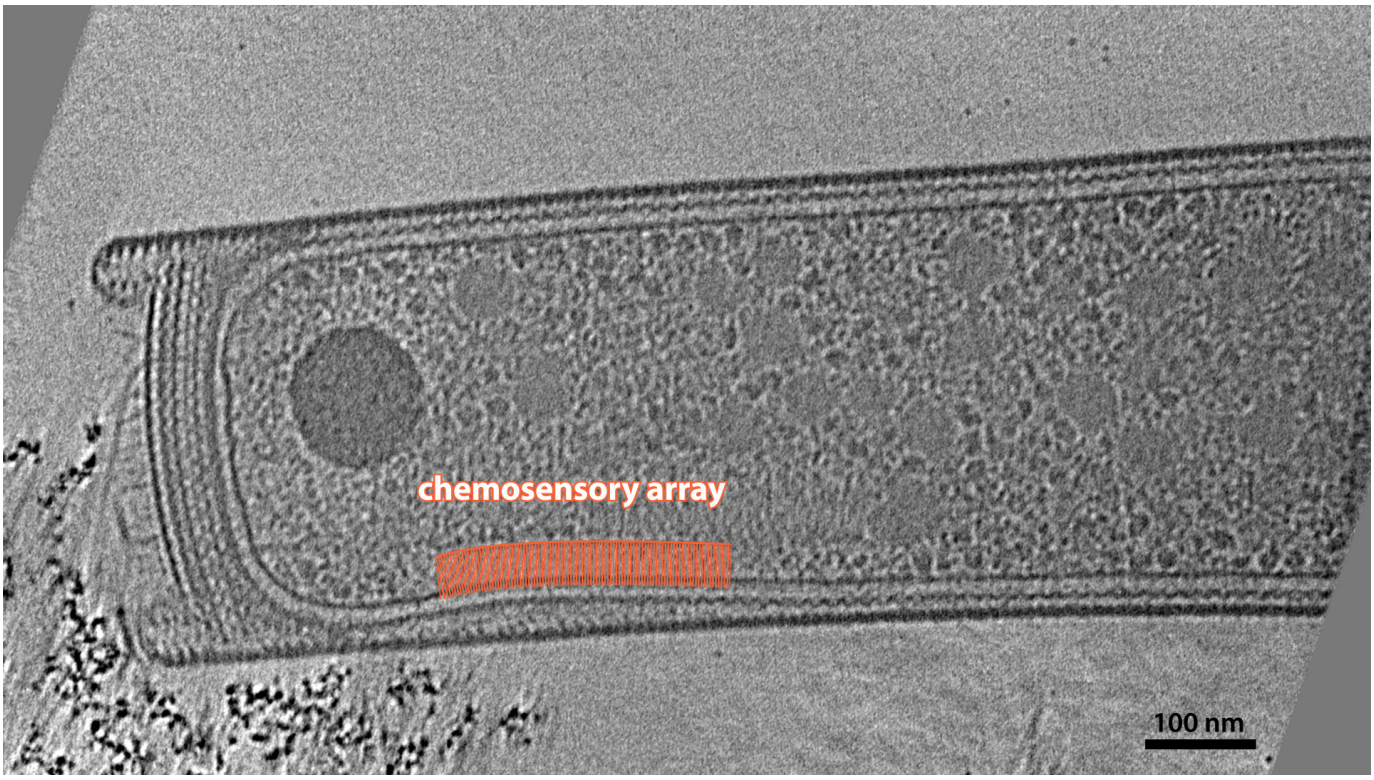


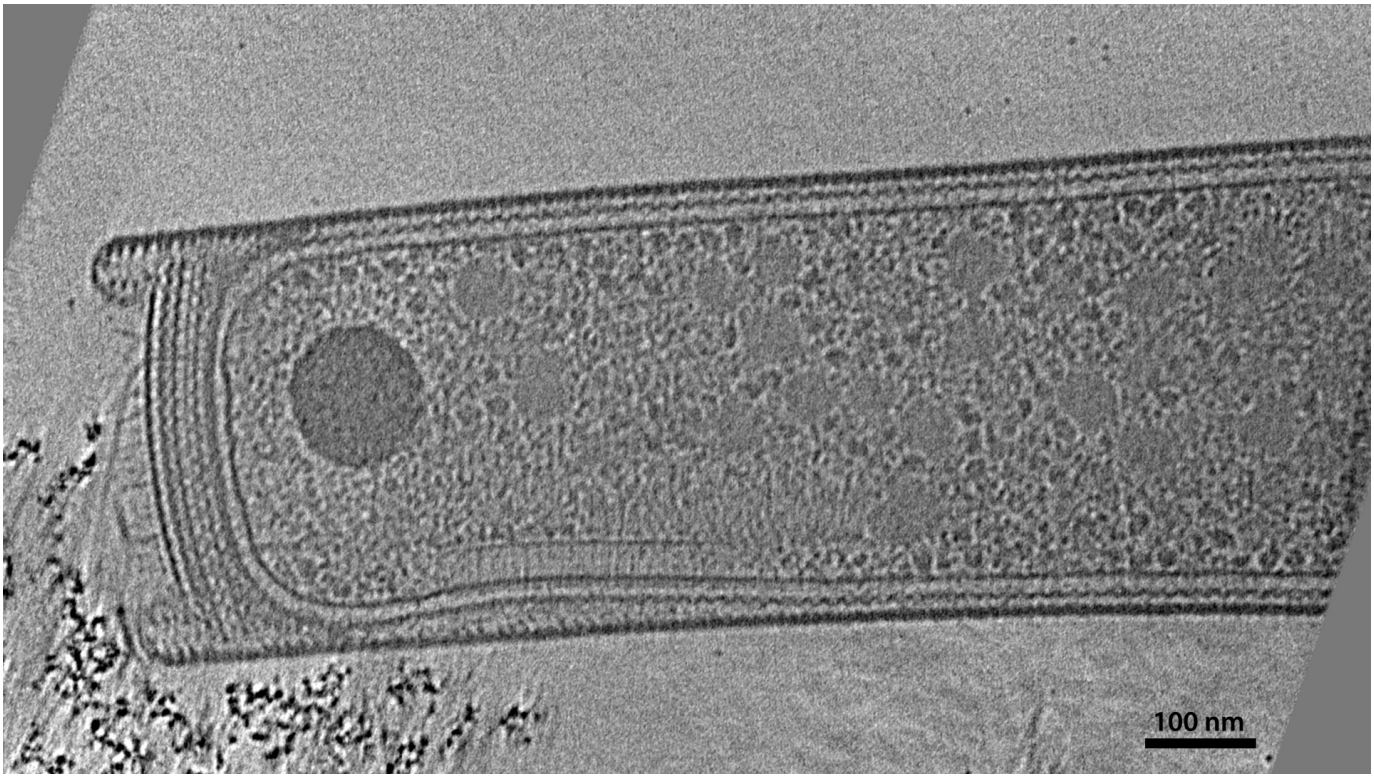


Learn More: Architecture

Here you see two of the basic units of a chemosensory array from Escherichia coli *[69]. Each unit consists of a trimer of chemoreceptor dimers (a section of which is shown in orange), a kinase (in blue), and a coupling protein (in green). In the cell, these two units would further associate with four more into a rosette of six units, then with other rosettes, forming the extensive hexagonal array. Also keep in mind that these are just the stable components; additional proteins (including the response regulators) interact transiently with the receptors and kinases.*

Source: Cassidy et al. (2020) *Structure: PDB 6S1K*

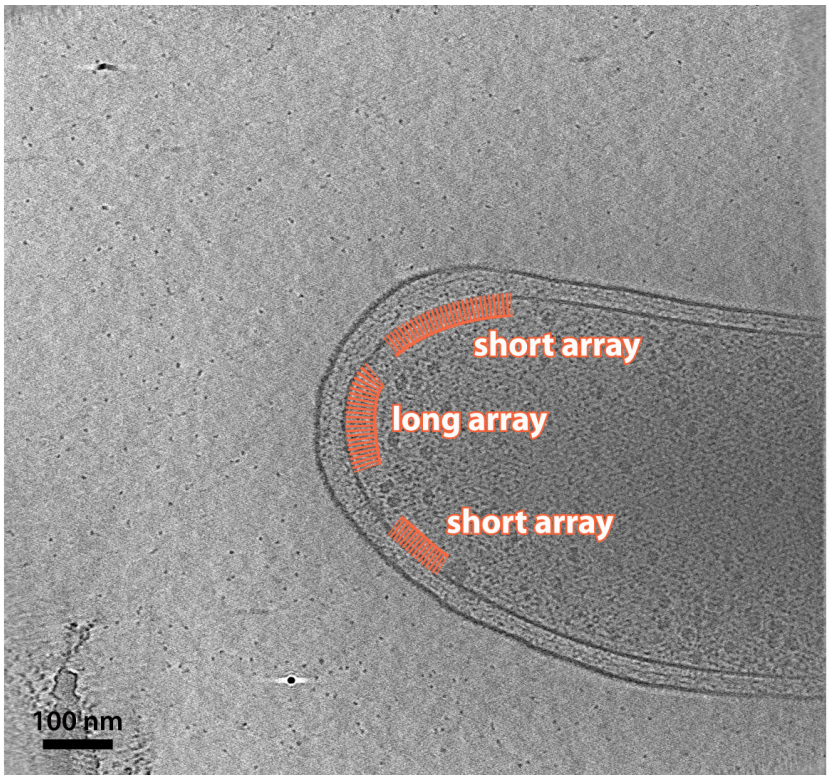

chemosensory array
100 nm

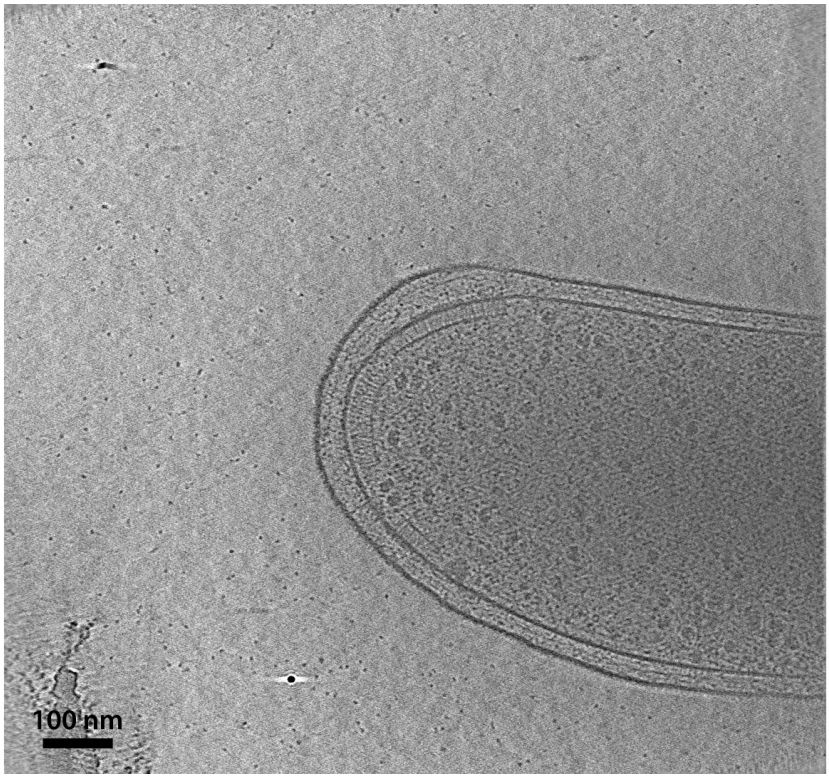

100 nm

3. Chemosensory Array Conservation

The hexagonal array structure of chemosensory systems is invariant across species, and even across domains. As you can see in this *Methanospirillum hungatei*, the arrays have the same architecture in archaea as in bacteria, with 12 nm center-to-center spacing between the hexagons. This strong conservation indicates the importance of chemotaxis for cells' fitness, and suggests that evolution had already honed it to a relatively optimal form in the common ancestor of all these cells.

Methanospirillum hungatei Collected by: Ariane Briegel DOI: 10.22002/D1.1547

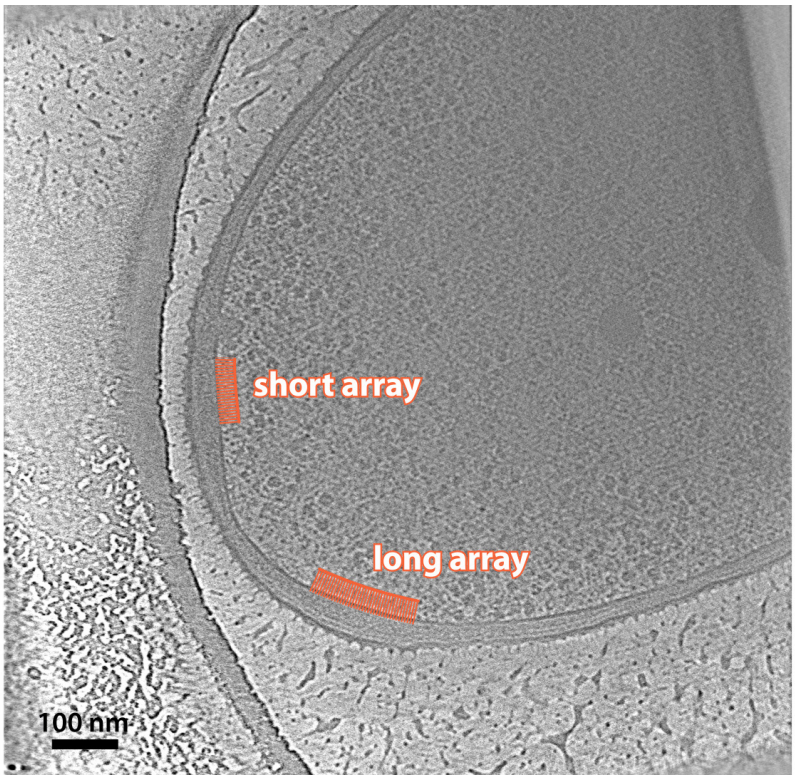

short array
long array
short array
100 nm

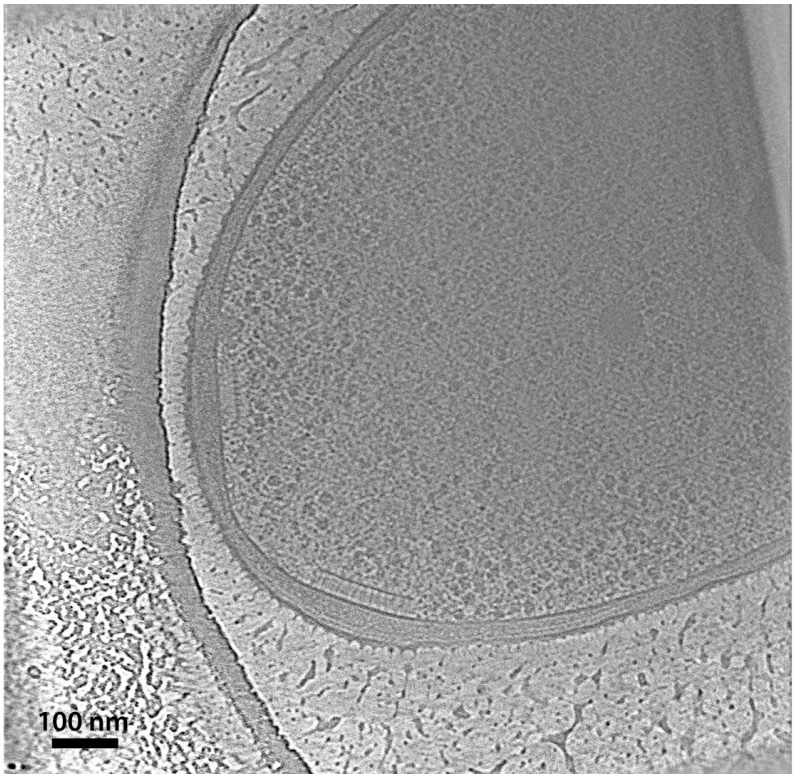

100 nm

4. Chemoreceptor Variety

While there is only one architectural style for chemosensory systems, there are many different building materials. Each chemoreceptor senses one, or perhaps two related, types of chemicals. This means that if your cell wants to sense multiple chemicals in its environment, it needs a matching assortment of chemoreceptors. This can quickly get complicated. Some signals need to go to the flagella, some to the pili, and others to the transcriptional machinery to turn on or off genes. How can you keep the wires from getting crossed? The simplest approach is just to separate them, and that seems to be exactly what cells do. Chemoreceptors that signal to different systems have different lengths, which sorts them into separate arrays in the cell, as you can see in this *Vibrio cholerae*. The shorter arrays signal to the flagellar motor, and the longer array signals to a different target we have not yet identified. Even when multiple chemosensory systems signal to the same target, arrays that respond to different environmental cues are kept separate by length differences in the receptors (*Learn More* on the next page).

Vibrio cholerae Collected by: Ariane Briegel DOI: 10.22002/D1.1548

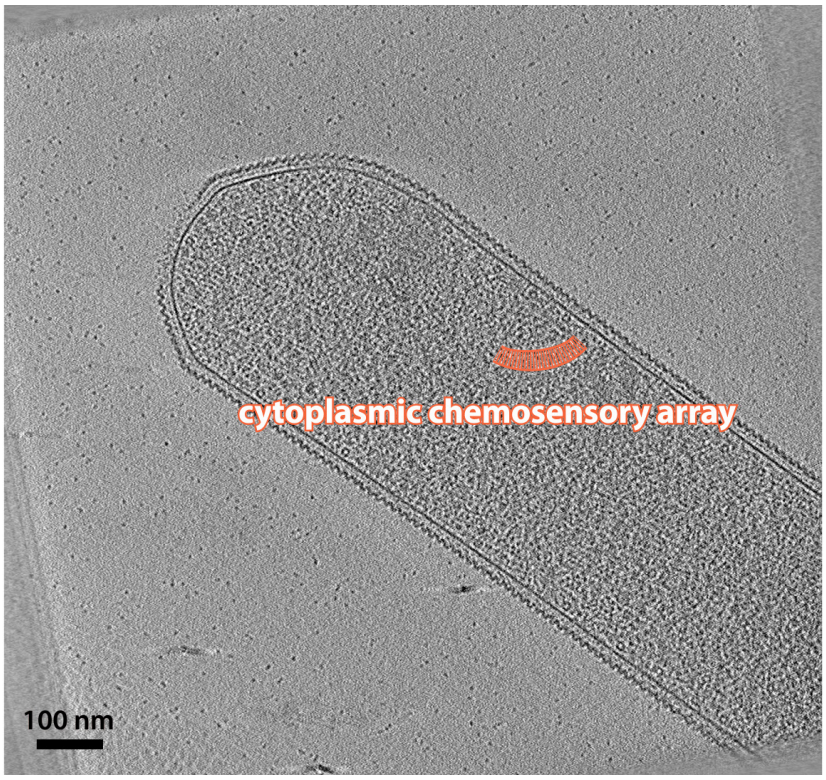

short array
long array
100 nm

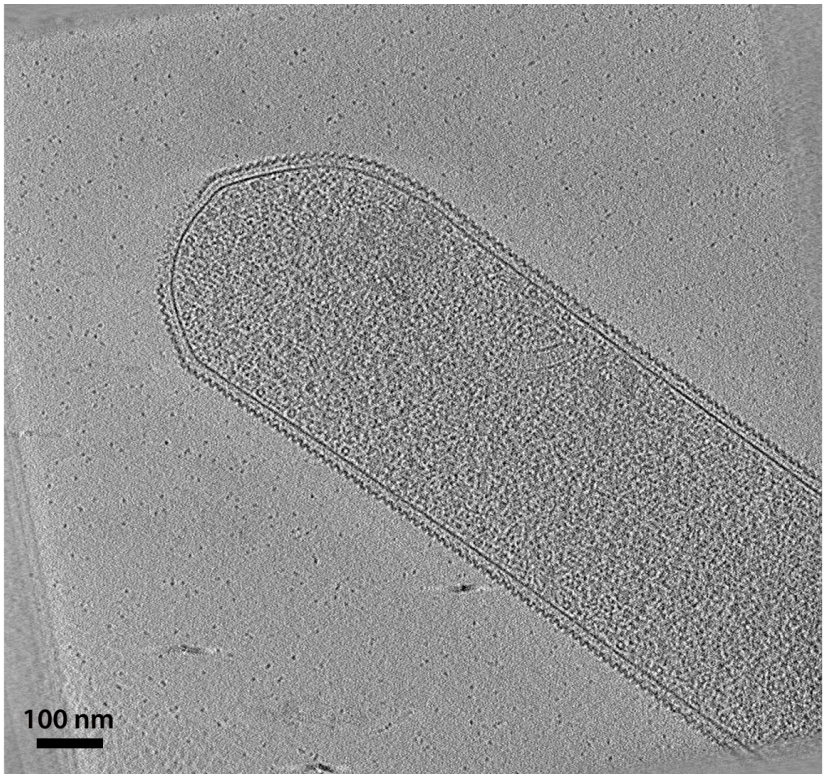

100 nm

Learn More: Aerotaxis

A length difference of as little as 2 nm is enough to separate receptors, like these in Azospirillum brasilense *that sense oxygen (28 nm array) and sources of energy like malate (30 nm array). Both receptors send signals to the single flagellar motor, promoting runs or direction switches to guide the bacterium through a combination of chemotaxis and* ***aerotaxis*** *(ordered movement in an oxygen gradient) to its target: plant roots.* A. brasilense *fixes nitrogen, boosting the growth of plants it colonizes.*

Azospirillum brasilense *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1551*

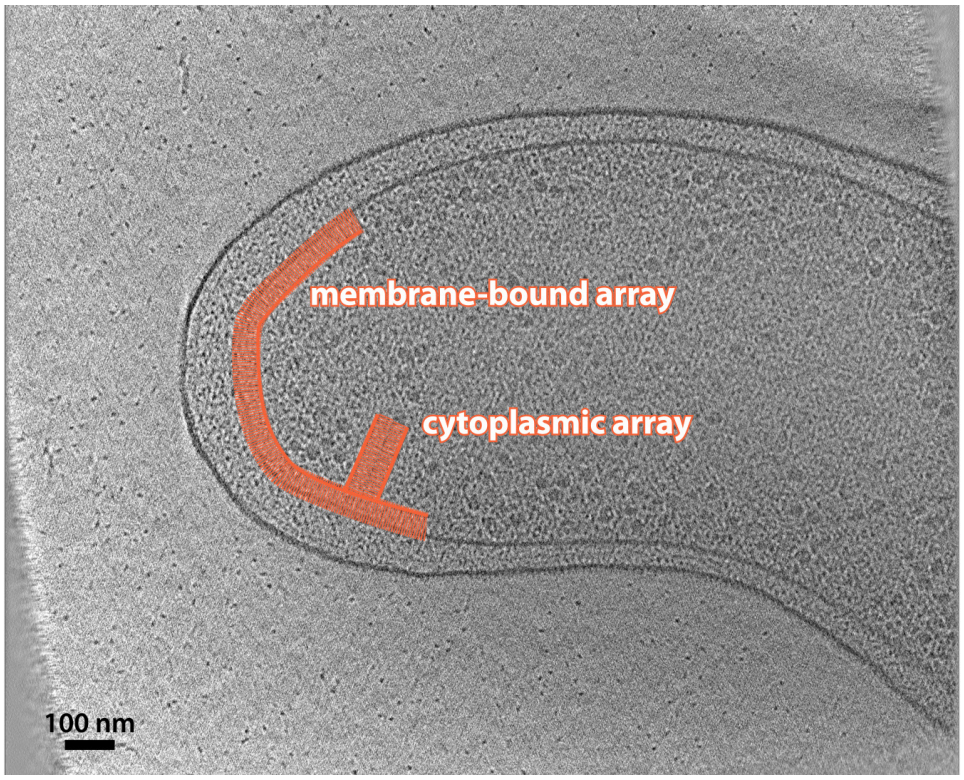

cytoplasmic chemosensory array
100 nm

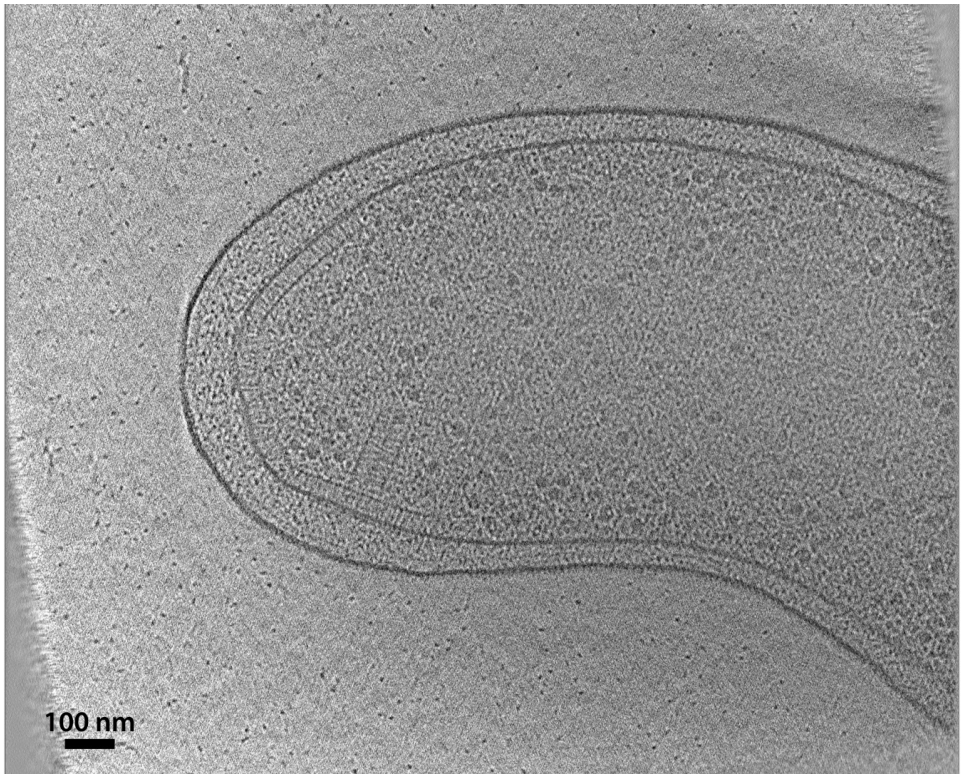

100 nm

5. Cytoplasmic Chemosensory Arrays

In addition to their extensive repertoire of membrane-embedded chemoreceptors, cells may also have another type. Many species of bacteria and archaea, like this *Methanoregula formicica*, contain **cytoplasmic chemosensory arrays**. The receptors lack membrane insertion patches. Instead, they interact end-to-end with each other at their chemical-sensing tips, zippering into a sandwich with a baseplate of kinases and coupling proteins on either side. In some species, the arrays are curved like this; in others, they are straight. What these systems sense remains a mystery. One idea is that they monitor the internal state of the cell (e.g. the levels of various metabolites) to fine-tune signaling responses to meet the current needs of the cell. They are often found along with, and often quite close to, membrane-embedded chemosensory arrays (*Learn More* on the next page).

Methanoregula formicica Collected by: Ariane Briegel DOI: 10.22002/D1.1549

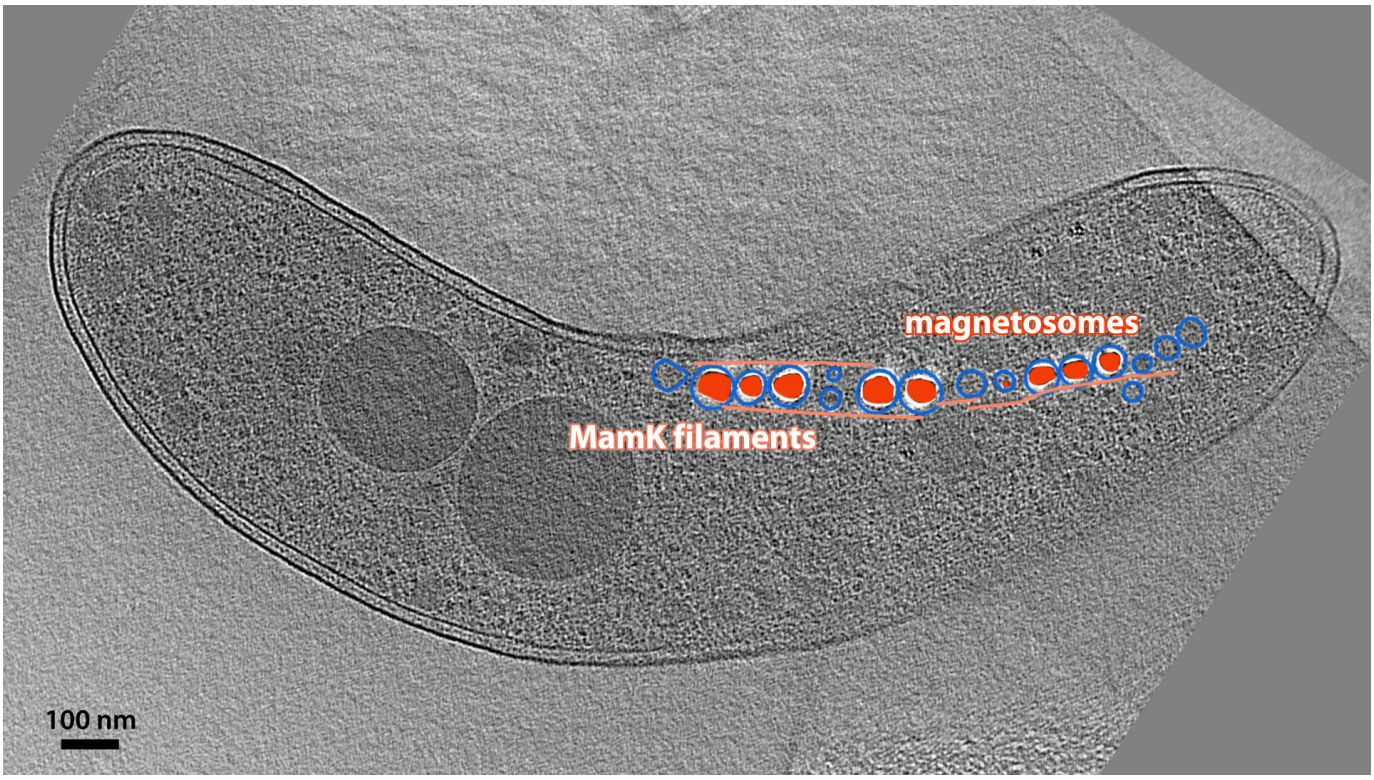

membrane-bound array
cytoplasmic array
100 nm

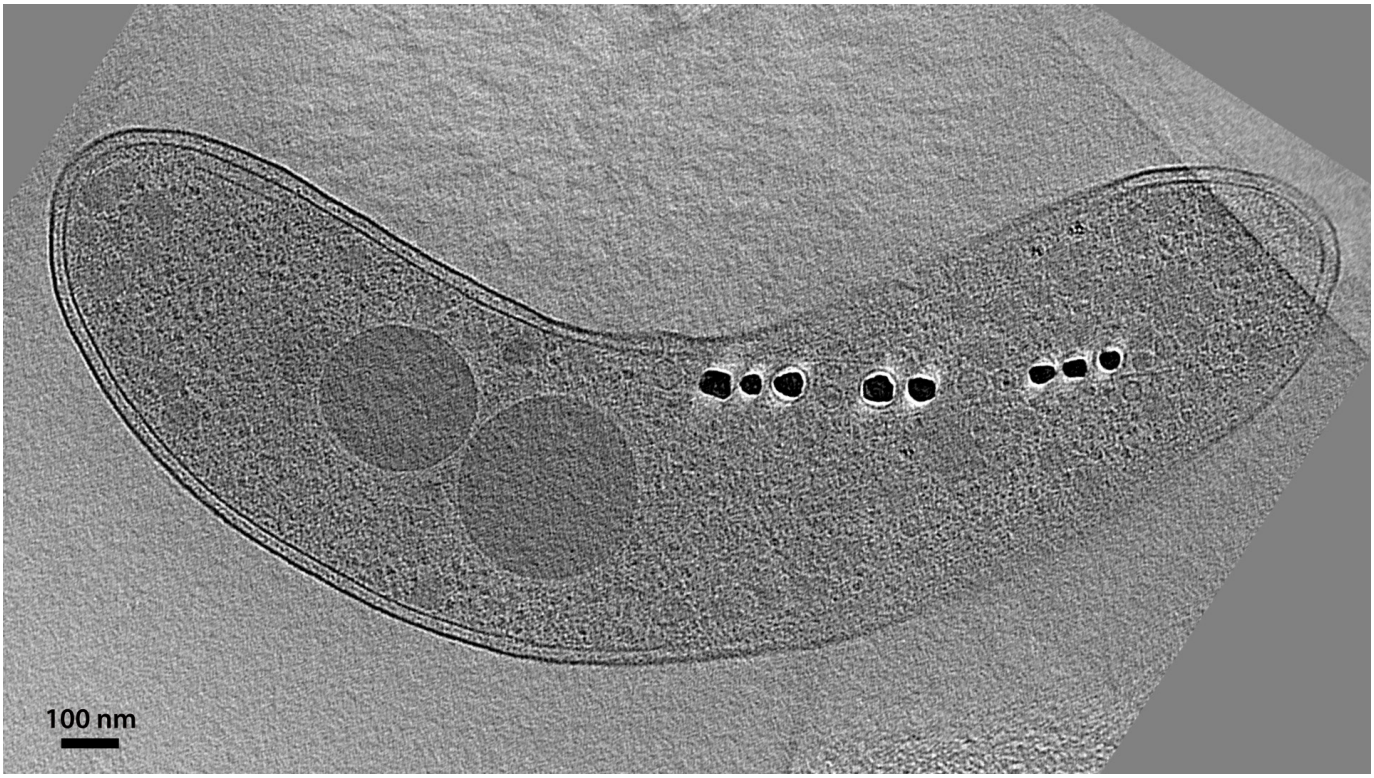

100 nm

Learn More: Multiple Arrays

This Vibrio cholerae *cell showcases a full complement of chemosensory systems: an extensive membrane-embedded array (signaling to the flagellar motor) and a cytoplasmic array (signaling to a still-unknown target). In each case, the basic architecture is identical: hexagonally-arranged trimers of chemoreceptor dimers.*

Vibrio cholerae *Collected by: Ariane Briegel* *DOI: 10.22002/D1.1552*


magnetosomes
MamK filaments
100 nm


100 nm

6. Magnetotaxis

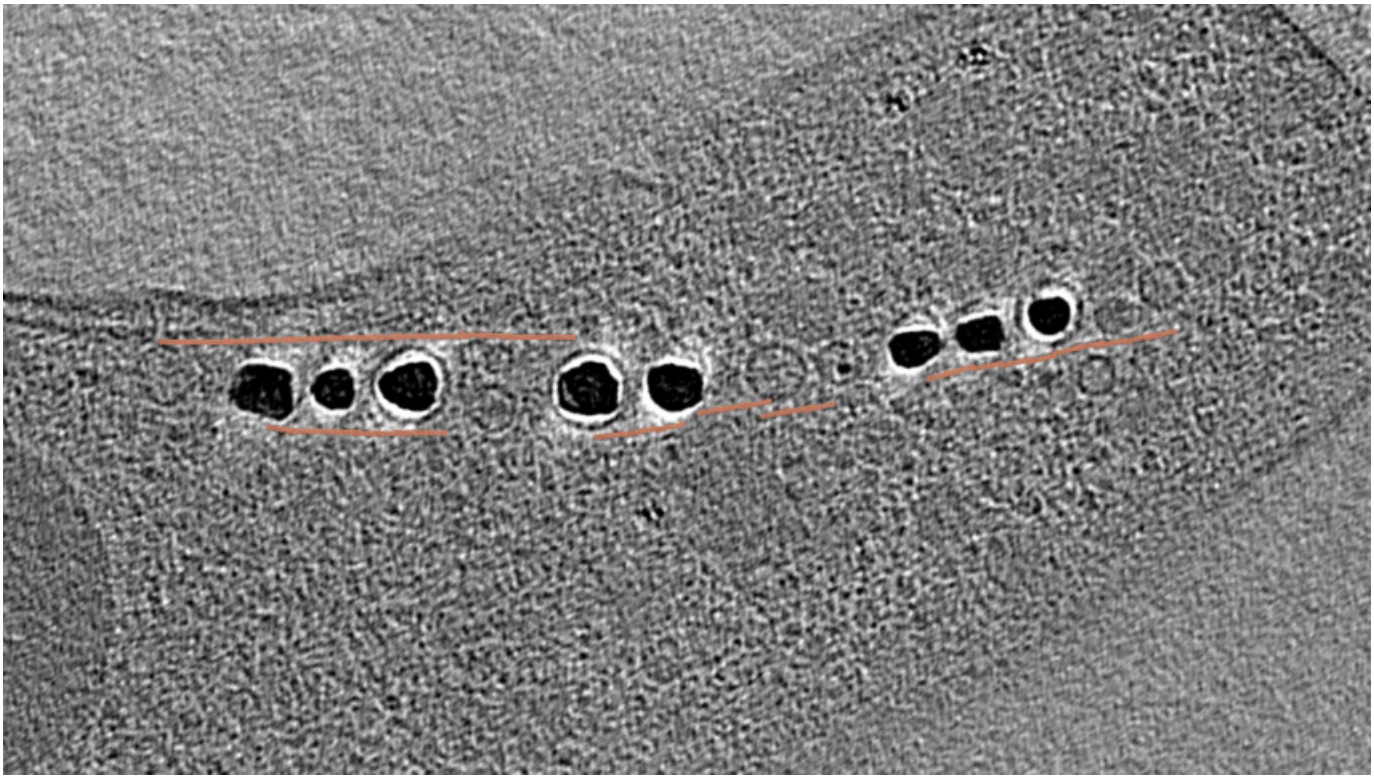
Chemotaxis is not the only mode of navigation available to your cell. For a clue to others, think about how we orient ourselves in the world. In addition to our nose sniffing out food, we also use our eyes to sense light. Similarly, some photosynthetic bacteria have evolved **phototaxis** (ordered movement in response to light).

We may also use an external tool to navigate unfamiliar surroundings: a compass. Believe it or not, some bacteria have one, too. Magnetotactic species like this *Magnetospirillum magneticum* have evolved specialized structures called **magnetosomes**. They are pockets of inner membrane filled with crystals of a magnetic iron mineral like magnetite. The cell organizes the magnetosomes into a line using filaments of a cytoskeletal protein called **MamK** (named for its association with magnetosome membranes) (*Learn More* on the next page), which is related to eukaryotic actin. The linear chain of magnetosomes functions like the needle of a compass, aligning the cell in a magnetic field.

Magnetosomes first form with the creation of a pocket of inner membrane, as you can see in this cell. Multiple short chains of magnetosomes may form, which are then organized into a single chain by MamK filaments. As mineralization begins, the pockets enlarge to accommodate the growing crystals, ultimately reaching ~60 nm in diameter. Magnetosome chains are inherited by daughter cells through division. Segregation is relatively easy: the chain spans the division plane so that the inward-growing cell wall simply splits the chain between magnetosomes, delivering half the chain to each daughter.

Much remains mysterious about these structures. For instance, what forms the membrane pockets? An even bigger question is how cells use their compasses. One theory is that magnetosomes guide the aquatic bacteria vertically to the optimal height in the water column for e.g. a certain oxygen level. However, these species are also found at the magnetic equator, where the Earth's field is horizontal. Another hypothesis is that magnetic orientation improves the efficiency of navigation by buffering the cell against Brownian motion that would knock it off course.

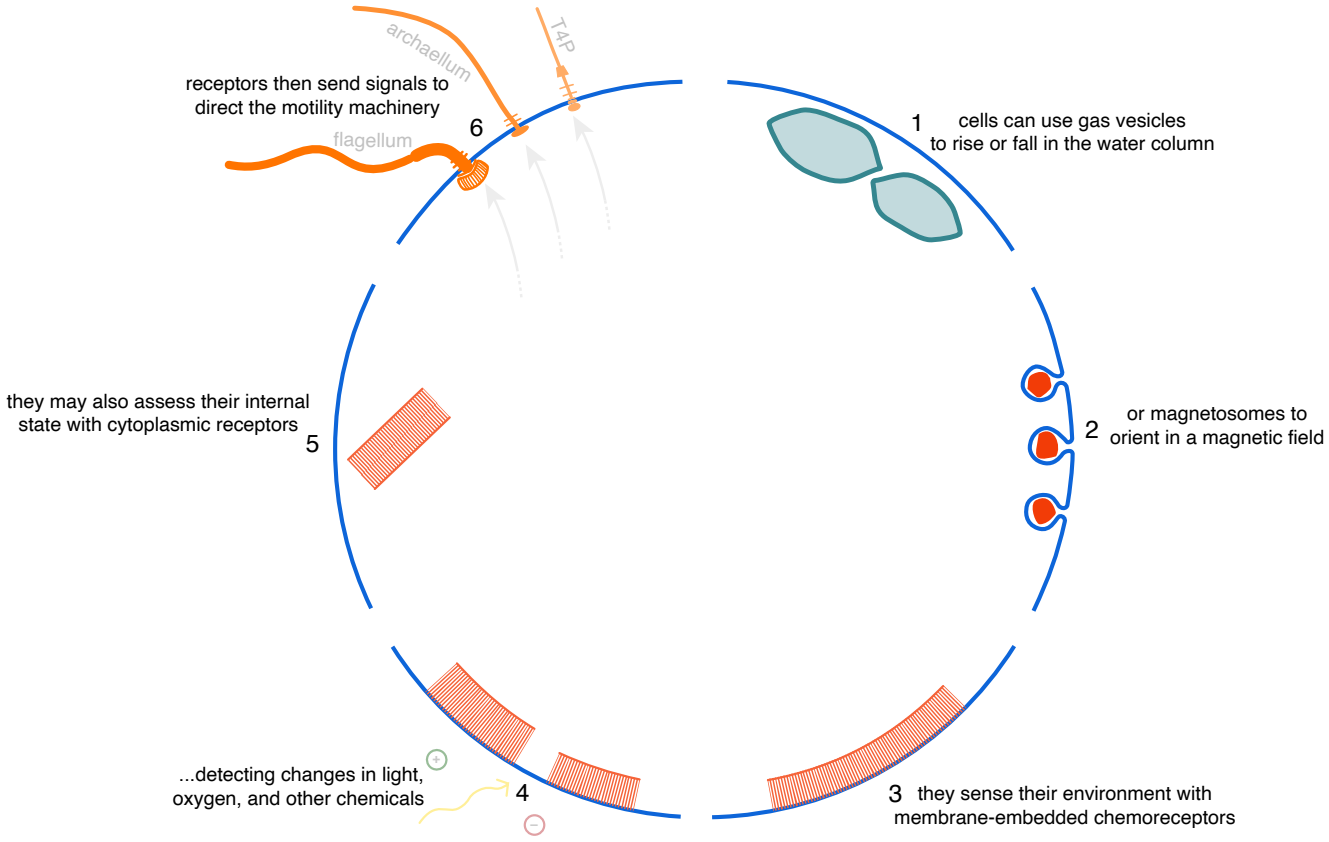
Magnetospirillum magneticum Collected by: Zhuo Li DOI: 10.22002/D1.1550



Learn More: MamK

MamK, like its eukaryotic homologue, actin, polymerizes into double-stranded filaments like this one from Magnetospirillum magneticum *[70]. Unlike eukaryotic actin, though, the two strands are aligned in register, rather than being staggered so that the bumps and grooves would pack more tightly.*

Source: Bergeron et al. (2017) *Structure: PDB 5JYG*


archaellum
T4P
receptors then send signals to direct the motility machinery
6
flagellum
1 cells can use gas vesicles to rise or fall in the water column
2 or magnetosomes to orient in a magnetic field
they may also assess their internal state with cytoplasmic receptors
5
...detecting changes in light, oxygen, and other chemicals
4
3 they sense their environment with membrane-embedded chemoreceptors

Summary

Concept Check Questions

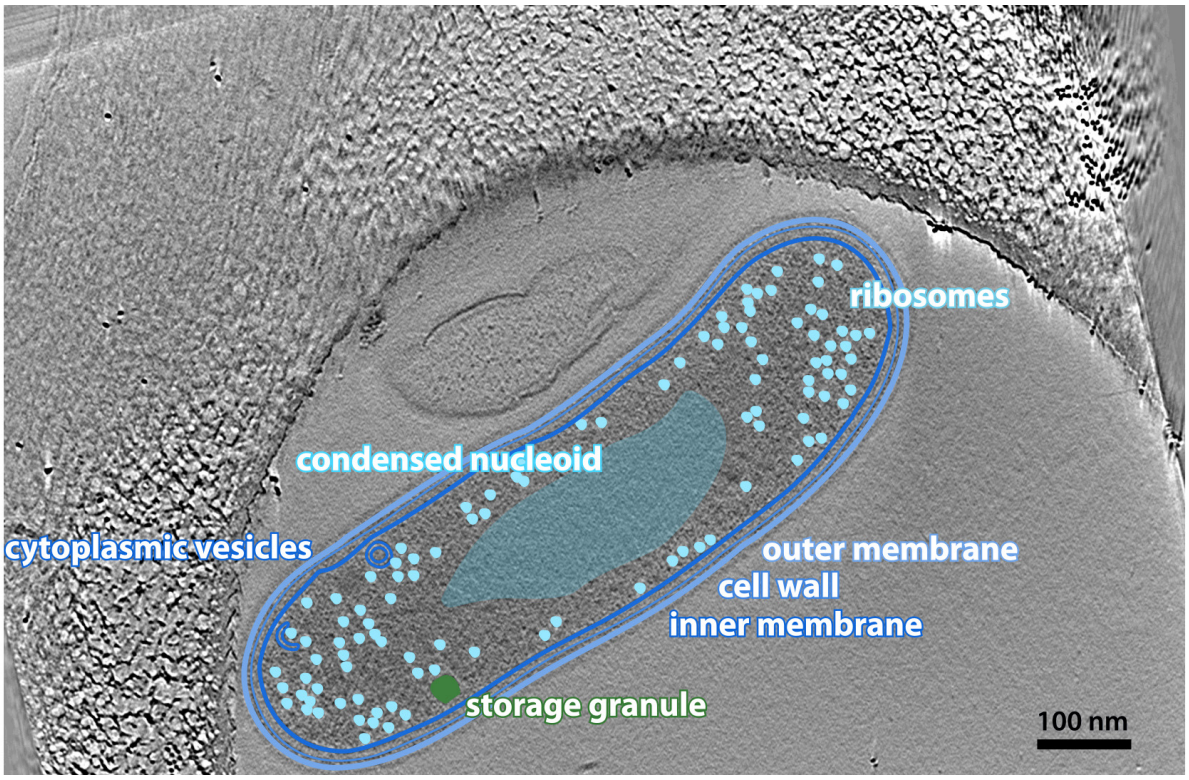
- What is the lattice arrangement of chemoreceptors in a sensory array?
- Which proteins make up the baseplate of a chemosensory array?
- What happens to the flagellar motor when a response regulator binds?
- What cytoskeletal element organizes magnetosomes into a chain?

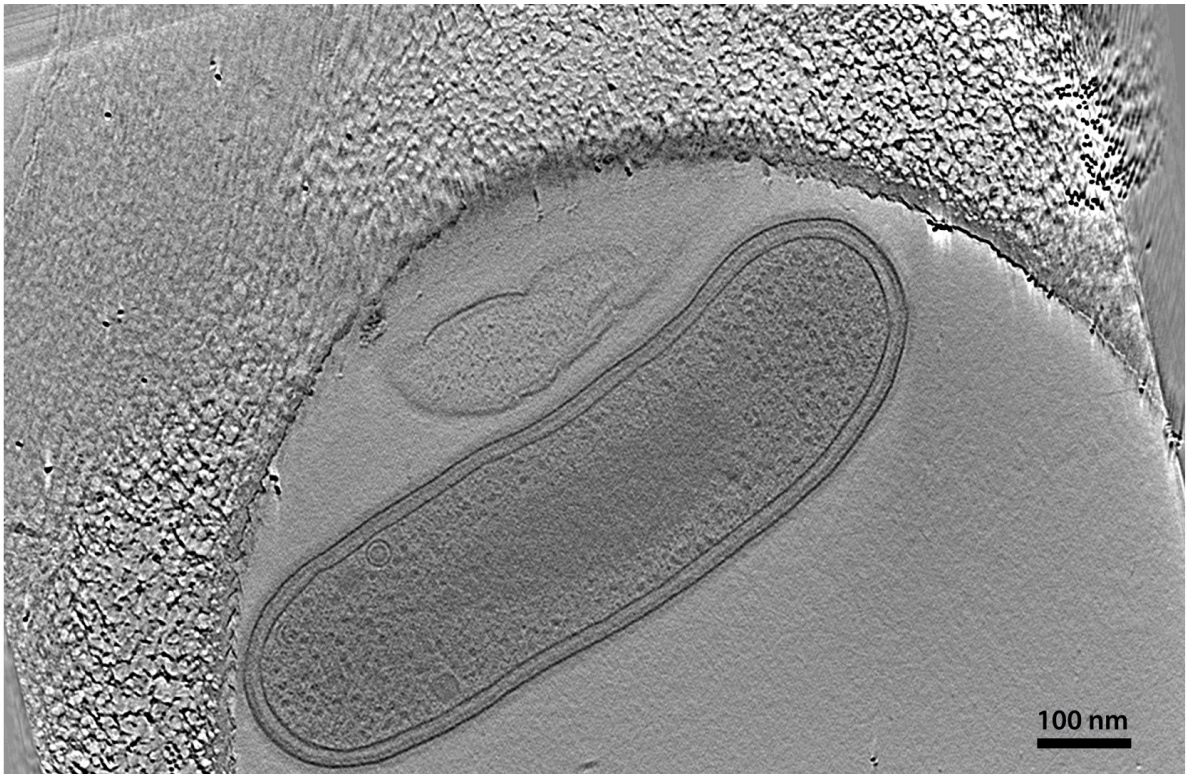
Further Reading

- Berg (1988). *A physicist looks at bacterial chemotaxis* [50].
- Hazelbauer et al. (2008). *Bacterial chemoreceptors: High-performance signaling in networked arrays* [71].
- Lower and Bazylinski (2013). *The bacterial magnetosome: A unique prokaryotic organelle* [72].
- Schuergers et al. (2016). *Cyanobacteria use micro-optics to sense light direction* [73].

8
Lifecycle

"…the microbial world consists almost entirely of bacteria in various degrees of starvation."
- John Postgate [74]

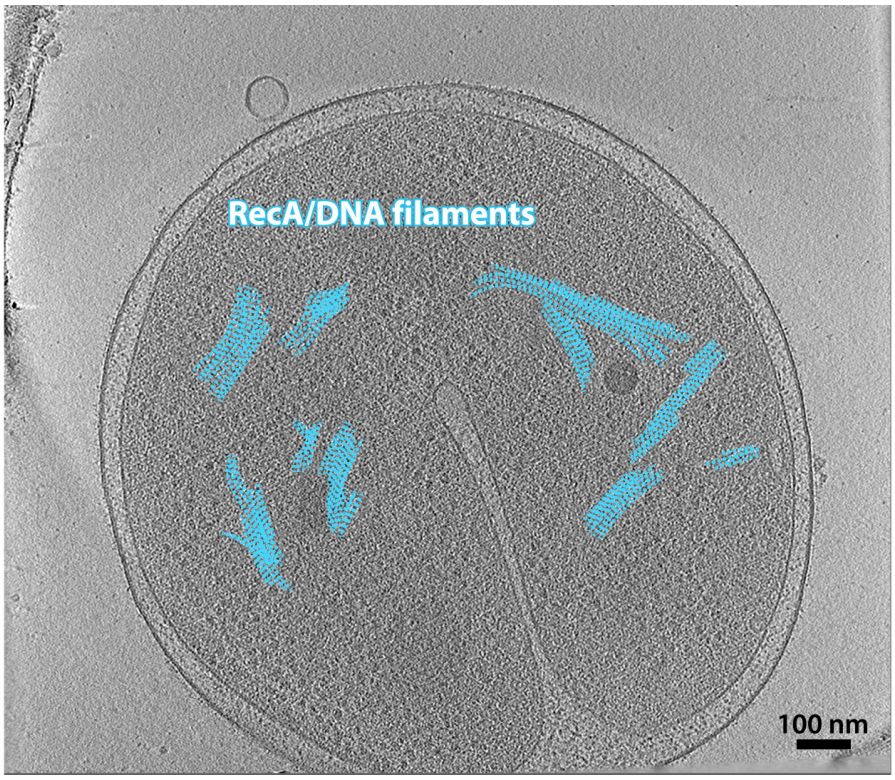

ribosomes
condensed nucleoid
cytoplasmic vesicles
outer membrane
cell wall
inner membrane
storage granule
100 nm

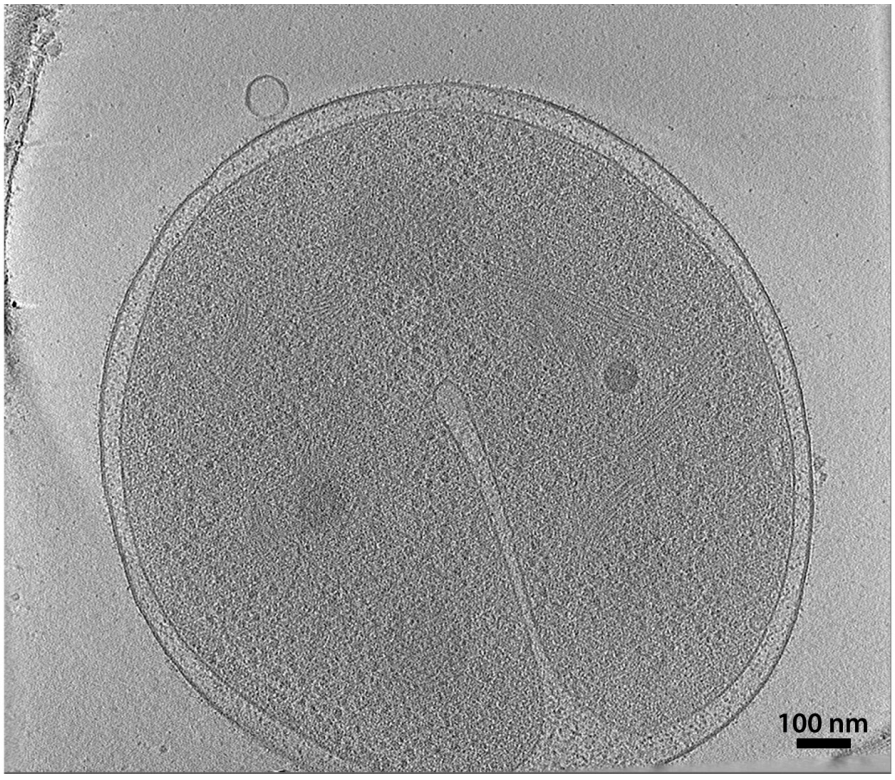

100 nm

1. Stationary Phase

Your cell is fairly well optimized now. It can grow, divide, and find fuel to repeat the process. But what happens when there is no food to be found? Natural environments offer famine more often than feast. And conditions can become harsh (high temperature, low pH, little water). For minor shortages of carbon or phosphate, your cell can fall back on its storage granules. But what about more prolonged starvation or stress? To outlast these conditions, your cell would do well to hunker down and conserve its remaining energy. Cells have evolved just such a mechanism: a **stationary** (non-growing) state. When conditions are no longer optimal for growth, cells shrink their cytoplasm either by dividing at a smaller size, or by digesting nonessential components and cinching down their membrane (stressed cells often contain more cytoplasmic vesicles). Some diderm bacteria, including *Caulobacter crescentus* like this, also remodel their cell walls and outer membranes (releasing extracellular vesicles). Others, like *Escherichia coli*, do not, resulting in a larger periplasm. Either way, a smaller cell size increases surface area relative to volume, facilitating uptake of scarce nutrients. Eventually, if conditions remain tough, the cell largely stops protein production and compacts its nucleoid to protect the DNA from damage, as you can see in this cell. Nonessential protein assemblies are lost; note the missing surface layer on this cell. When cells no longer have energy to spare to power motility, they also lose their flagella (see them floating next to these cells) and dismantle their chemosensory arrays. The cell now plays a waiting game. If the environment changes, your cell can resume active growth. Or the death of a neighboring cell, like the one you see here, might release a burst of nutrients that your cell can use for one more round of growth and division, producing progeny that might find a better life. Unlike in a well-maintained laboratory culture, in nature bacteria and archaea spend most of their time in stationary phase, awaiting fleeting opportunities for growth.

Caulobacter crescentus Collected by: Prabha Dias DOI: 10.22002/D1.1553


RecA/DNA filaments
100 nm

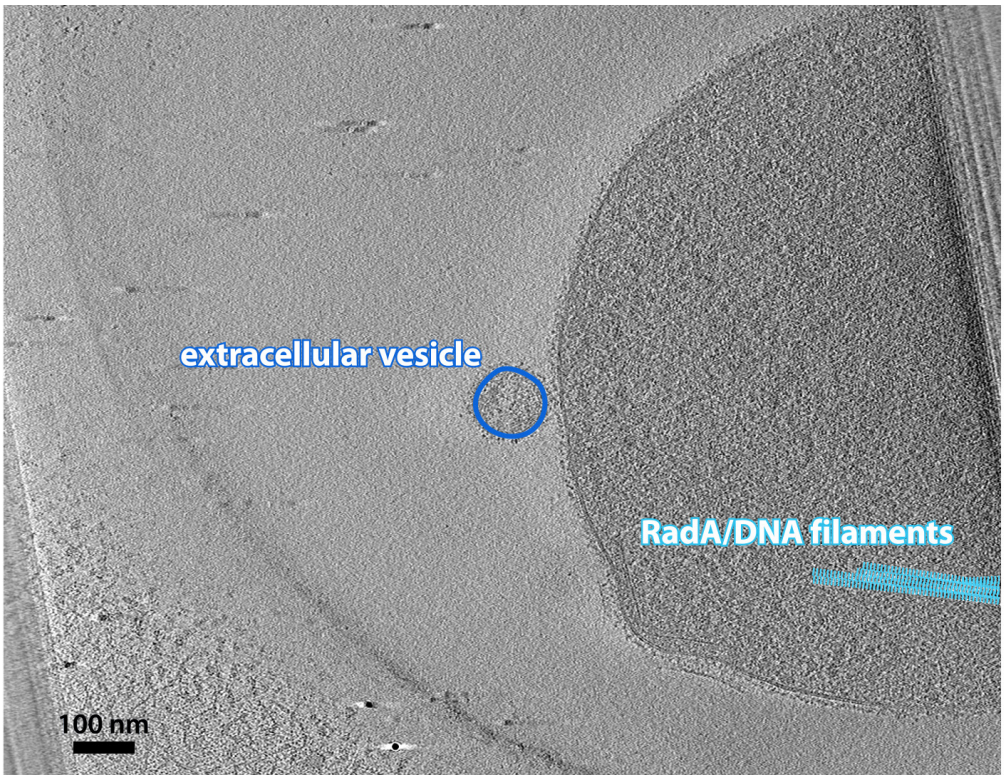

100 nm

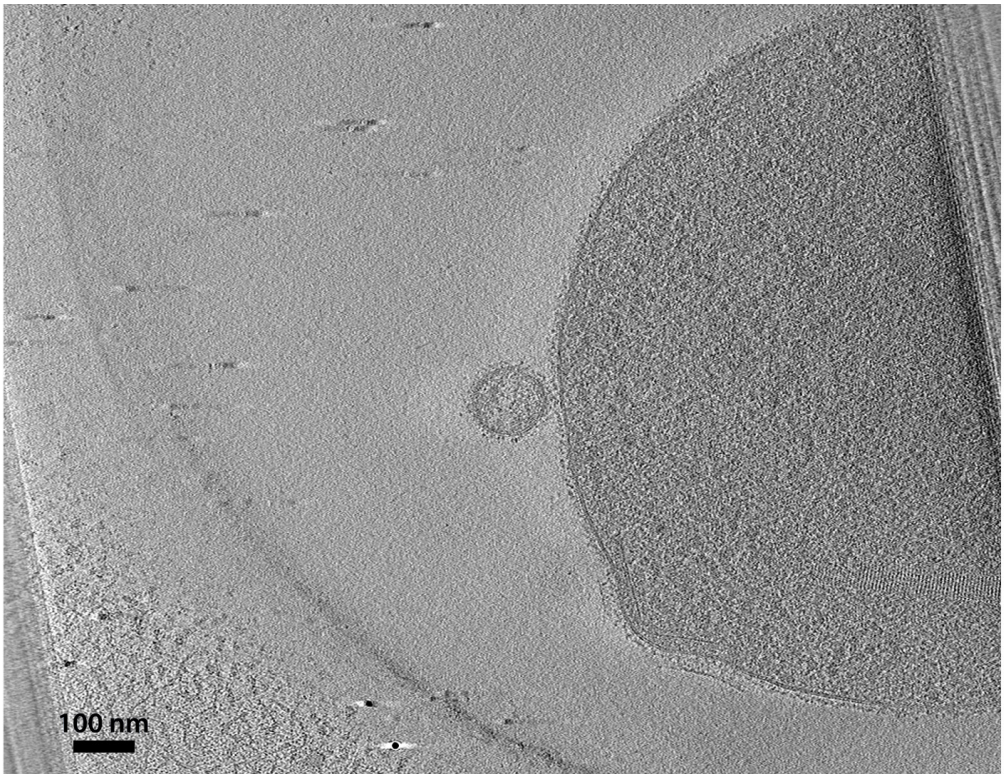
2. Genome Protection

Remember that the most critical part of your cell is its instruction manual–the genome. Ensuring that the instructions remain intact is paramount to your cell in stationary phase. To do this, all cells have proteins (**RecA** in bacteria, **RadA** in archaea, and **Rad51** in eukaryotes) that repair DNA damage caused by reactive oxygen species, UV (or other) radiation, heat, or other sources. RecA (and related) proteins form complexes with the DNA they protect. These ordered arrays can become quite extensive in stationary phase cells, like this *Helicobacter pylori*.

This cell exhibits other hallmarks of stationary phase: its shrinking membranes have produced vesicles and it has shed its flagella. It has not yet (at least fully) digested its chemosensory array. Note also the relatively weak association of the outer membrane on this pathogenic diderm, allowing the cell to curl up inside.

Helicobacter pylori Collected by: Yi-Wei Chang DOI: 10.22002/D1.1554

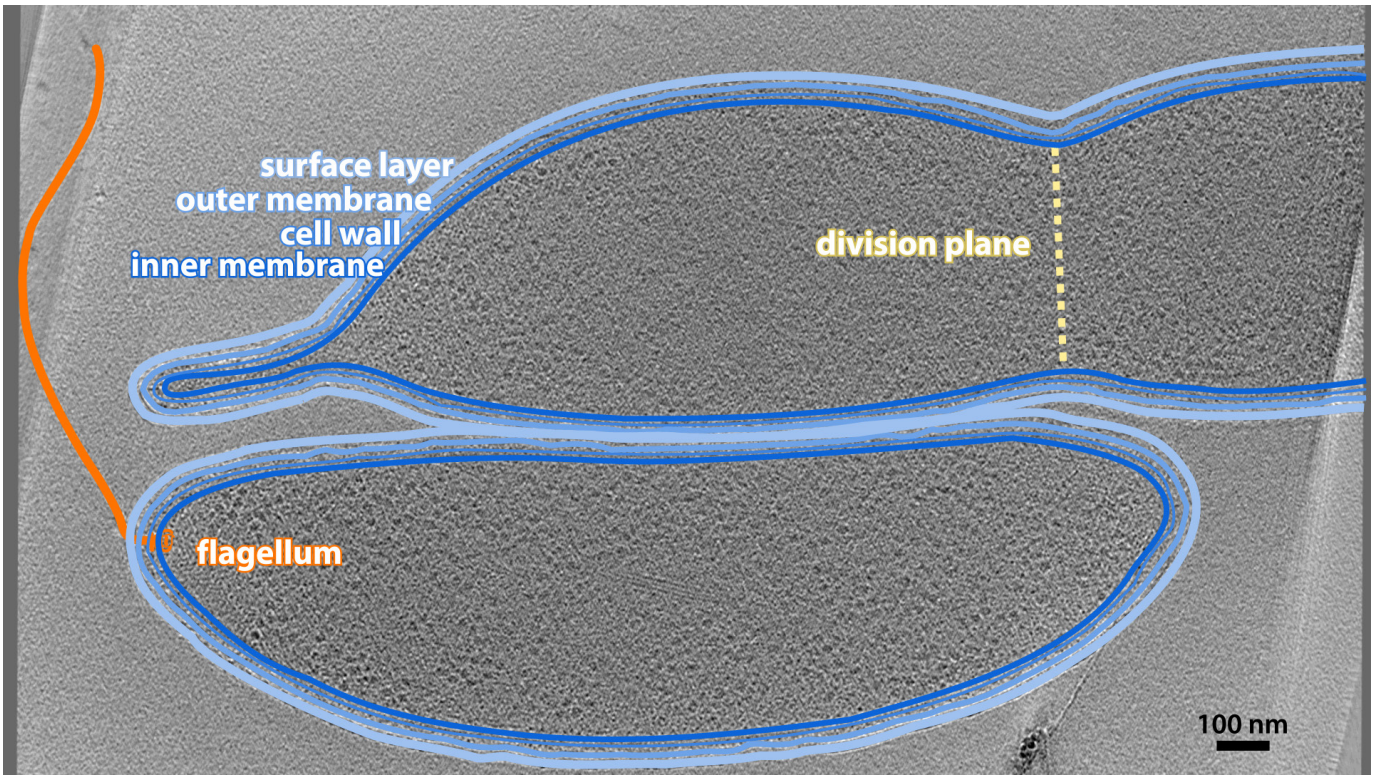

extracellular vesicle
RadA/DNA filaments
100 nm

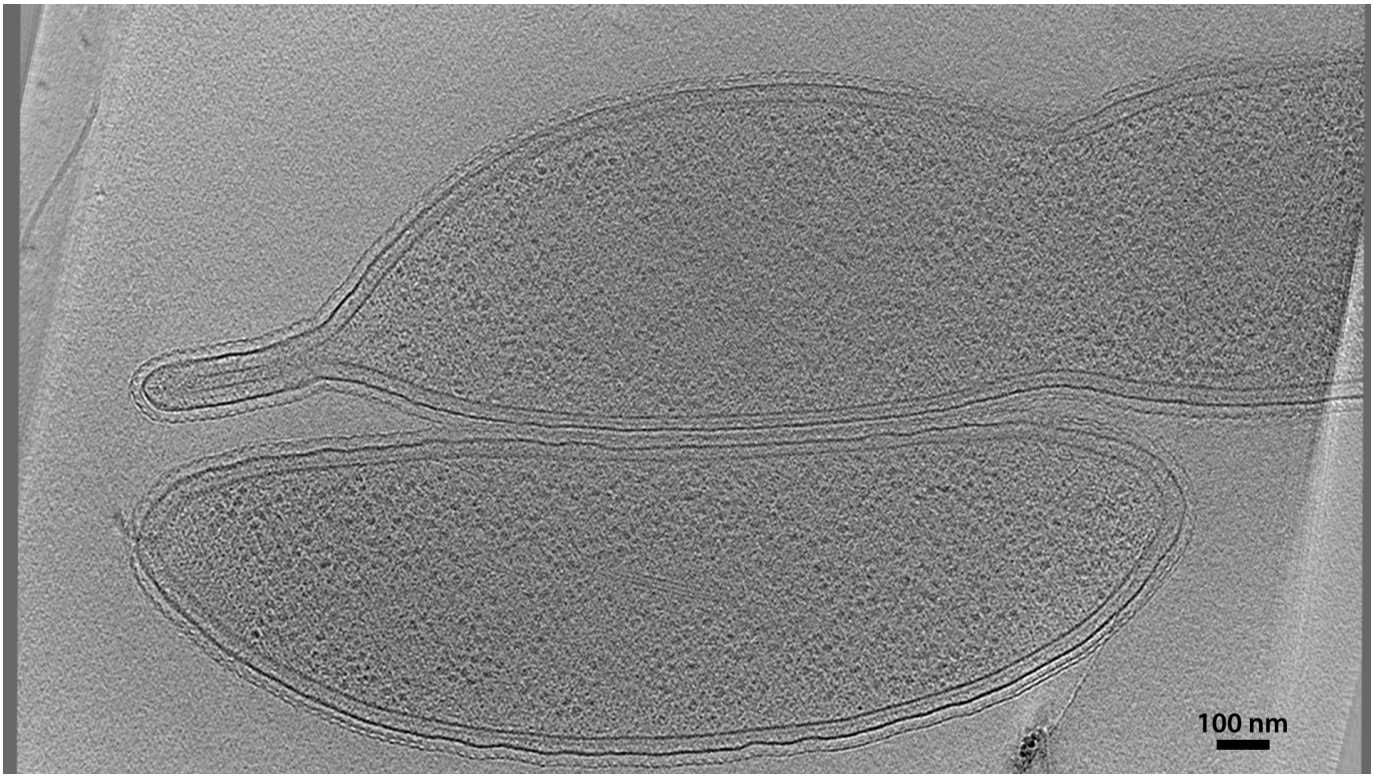

100 nm

3. Archaeal Stationary Phase

Stationary phase in archaea looks much the same as in bacteria, as you can see in this *Thermococcus kodakaraensis*. The cell has shrunk to about half its size in active growth, partly by shedding vesicles, and much of the surface layer has been dismantled. It has lost its archaella (though the conical plate in the cytoplasm remains) and degraded its chemosensory array, and is protecting its genome with RadA filaments. Now it waits for things to get better.

Thermococcus kodakaraensis Collected by: Debnath Ghosal DOI: 10.22002/D1.1555

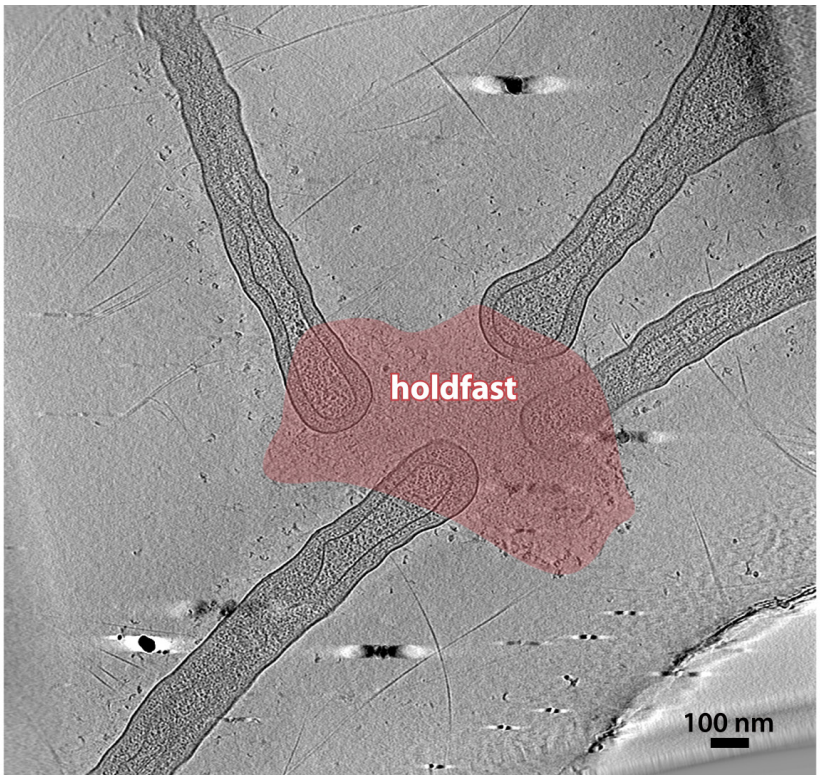

surface layer
outer membrane
cell wall
inner membrane
division plane
flagellum
100 nm

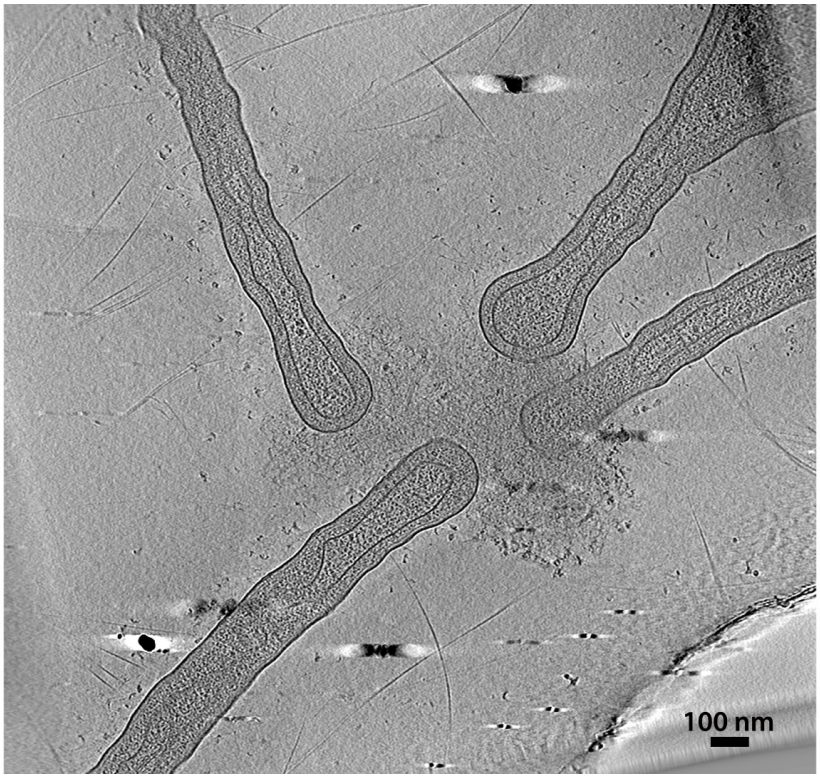

100 nm

4. Differentiation

While all cells go through cycles of growth and quiescence in response to changes in their environment, some species have gone a step further to evolve a programmed set of states–a **lifecycle**. In some cases, as you will see in the next chapter, a lifecycle allows bacteria to prey efficiently on other cells, including ours. In other cases, such as these *Caulobacter crescentus*, it allows the bacterium to thrive in an environment with low nutrient levels. *C. crescentus* has a **dimorphic** ("two form") lifecycle. Newborn cells start out life as **swarmers**, with a polar flagellum. They swim through the environment (either freshwater or saltwater) until they (hopefully) reach a favorable spot to put down roots, which they do by first transiently attaching to a surface with pili, then making the attachment permanent with a polymer of a sticky protein called "**holdfast**," which currently holds the record for the strongest known biological adhesive (*Learn More* on the next page). They shed their flagellum and grow a stalk in its place. Only after growing a stalk, completing the process of differentiation, will the cell begin to divide. The **stalked cell** will remain attached to the surface for the rest of its life, dividing asymmetrically to produce new swarmer cells, which swim away to try their luck elsewhere. Here you see a swarmer cell on the bottom, and a stalked cell on the top, in the process of dividing to produce another swarmer that would swim off to the right. You saw a similar dimorphic lifecycle in Chapter 5, in sessile (non-motile) *Hyphomonas neptunium* that bud to produce motile daughters. This kind of lifecycle helps prevent related cells from competing with one another for scarce local nutrients.

Caulobacter crescentus Collected by: Zhuo Li DOI: 10.22002/D1.1556

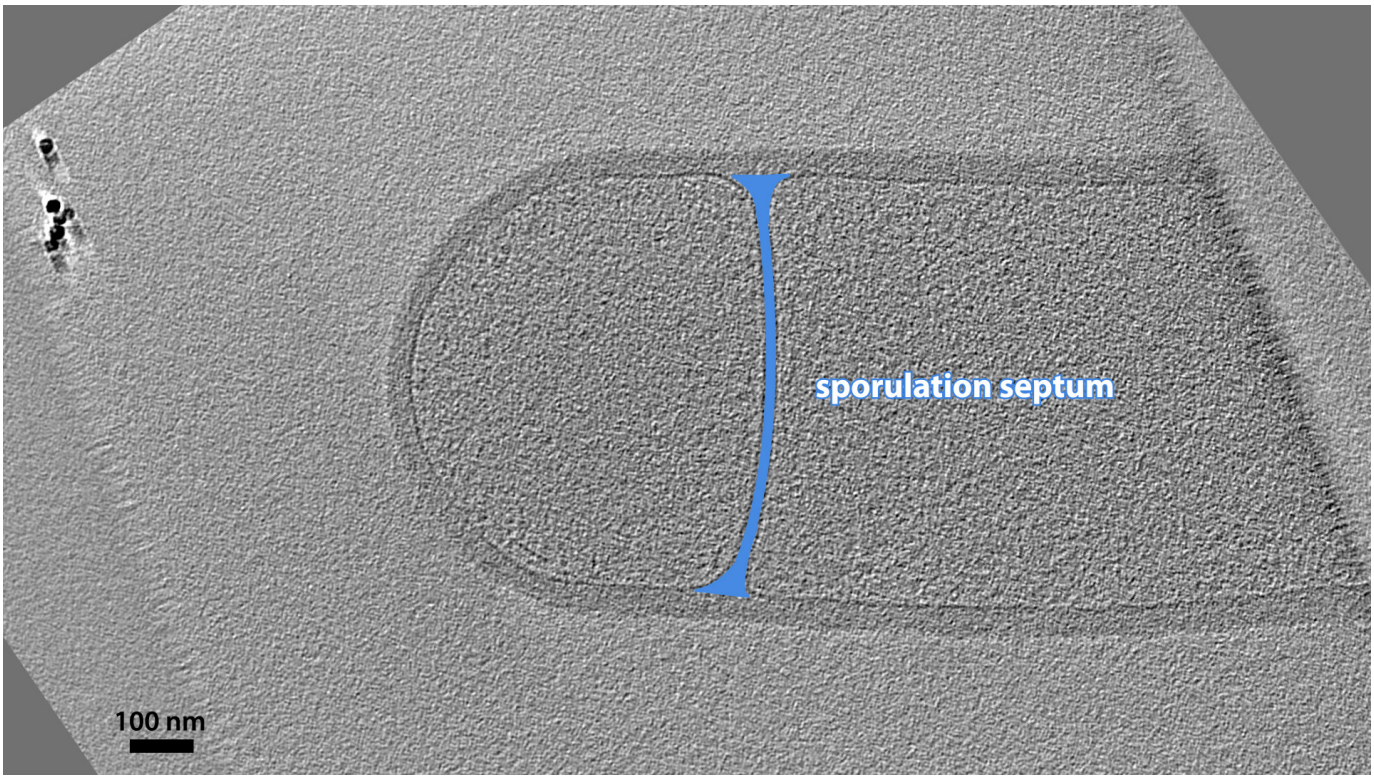

holdfast
100 nm

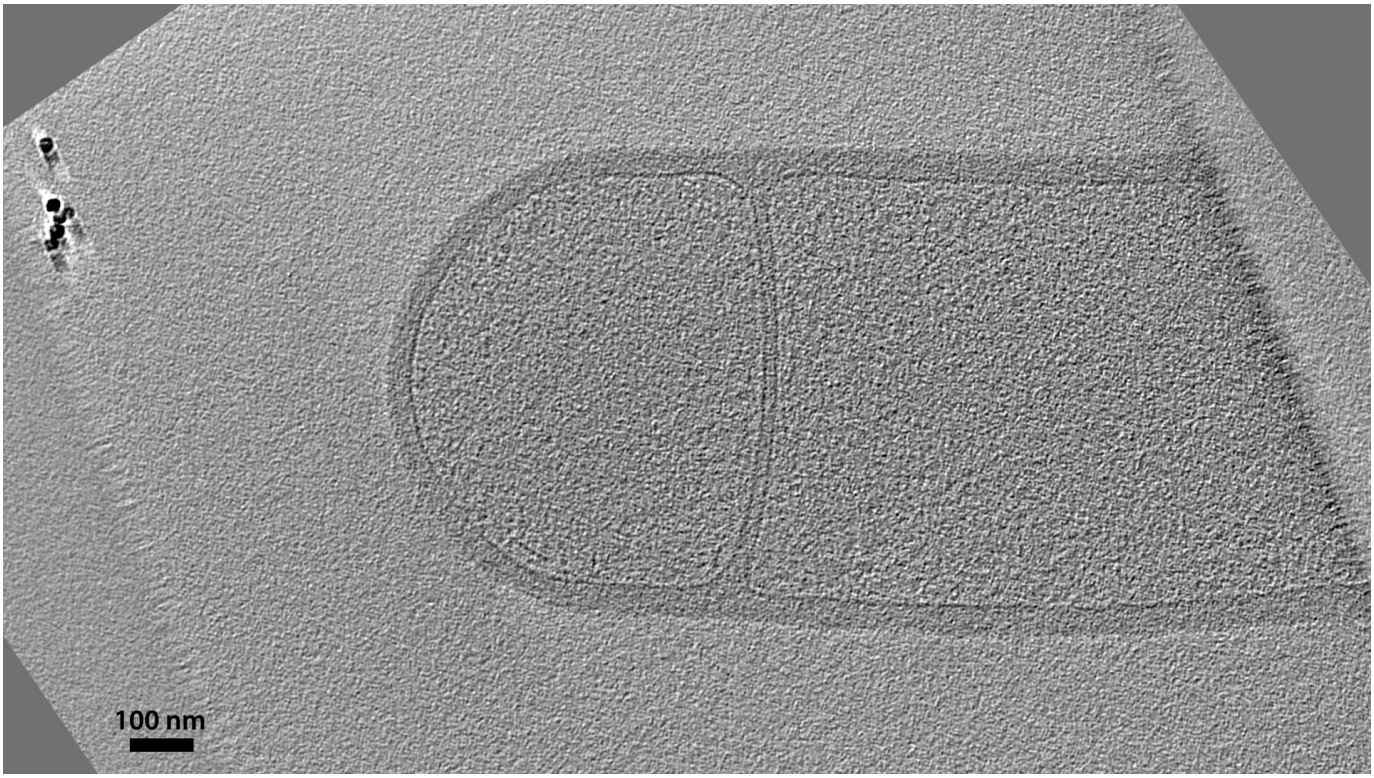

100 nm

Learn More: Holdfast

*P*rosthecobacter vanneervenii *is a stalked bacterium like* Caulobacter crescentus*, and attaches to surfaces by the same mechanism: a sticky ball of holdfast at the tip of its stalk. Here you see four cells whose stalks have attached to one another in the process of sample preparation. Unlike its cousin* C. crescentus*, however,* P. vanneervenii *is non-motile (no flagellum) and does not have a dimorphic life cycle. It divides symmetrically.*

Prosthecobacter vanneervenii *Collected by: Martin Pilhofer* *DOI: 10.22002/D1.1564*


sporulation septum
100 nm


100 nm

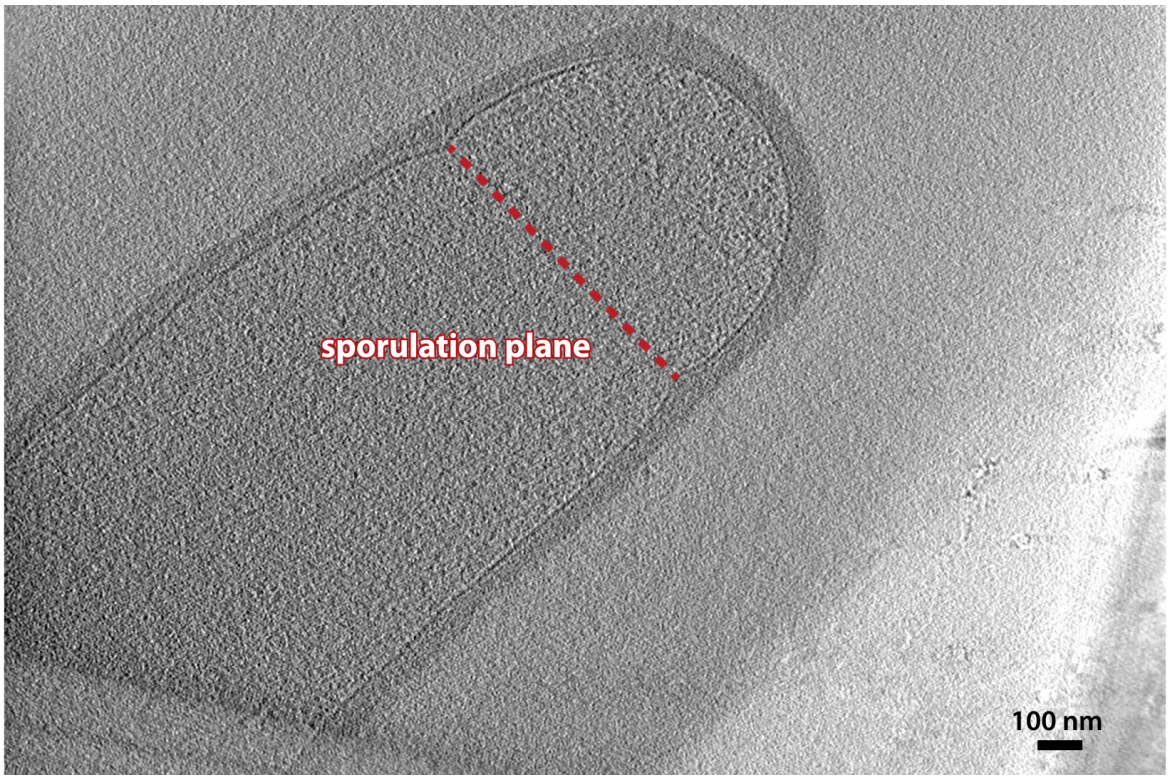
5. Monoderm Sporulation

The strategies we just discussed can help your cell deal with lean times. But what if things get *really* bad? Maybe you need to build your cell a survivalist bunker. Some species of bacteria do just this, using a modified cell division program to create a special reinforced daughter cell called a **spore**. Spores are extremely resistant to dehydration, acid, heat and other dangers. They are a purely dormant cell form, neither growing nor dividing, but simply waiting for improved conditions.

Some bacterial species form **exospores**, which are released directly to the environment as in a normal division. Others, like monoderm *Bacillus subtilis*, form **endospores**, which mature inside the protective envelope of the mother cell. As you can see in this cell, the first step resembles an asymmetric (closer to one pole) but otherwise normal cell division, with a septum of cell wall separating the compartment that will become the spore. Unlike in regular monoderm division, though, in which the septum is the same thickness as the rest of the cell wall, the **sporulation septum** is thinner, containing fewer layers of peptidoglycan (*Learn More* on the next page).

Note: This cell (and the ones on the next two pages) is narrower than normal B. subtilis*; a cell wall remodeling enzyme has been removed from its genome, leaving essential functions like cell wall growth and division (and sporulation) intact, but making the cells narrower and therefore better to image by cryo-ET.*

Bacillus subtilis Collected by: Elitza Tocheva DOI: 10.22002/D1.1557

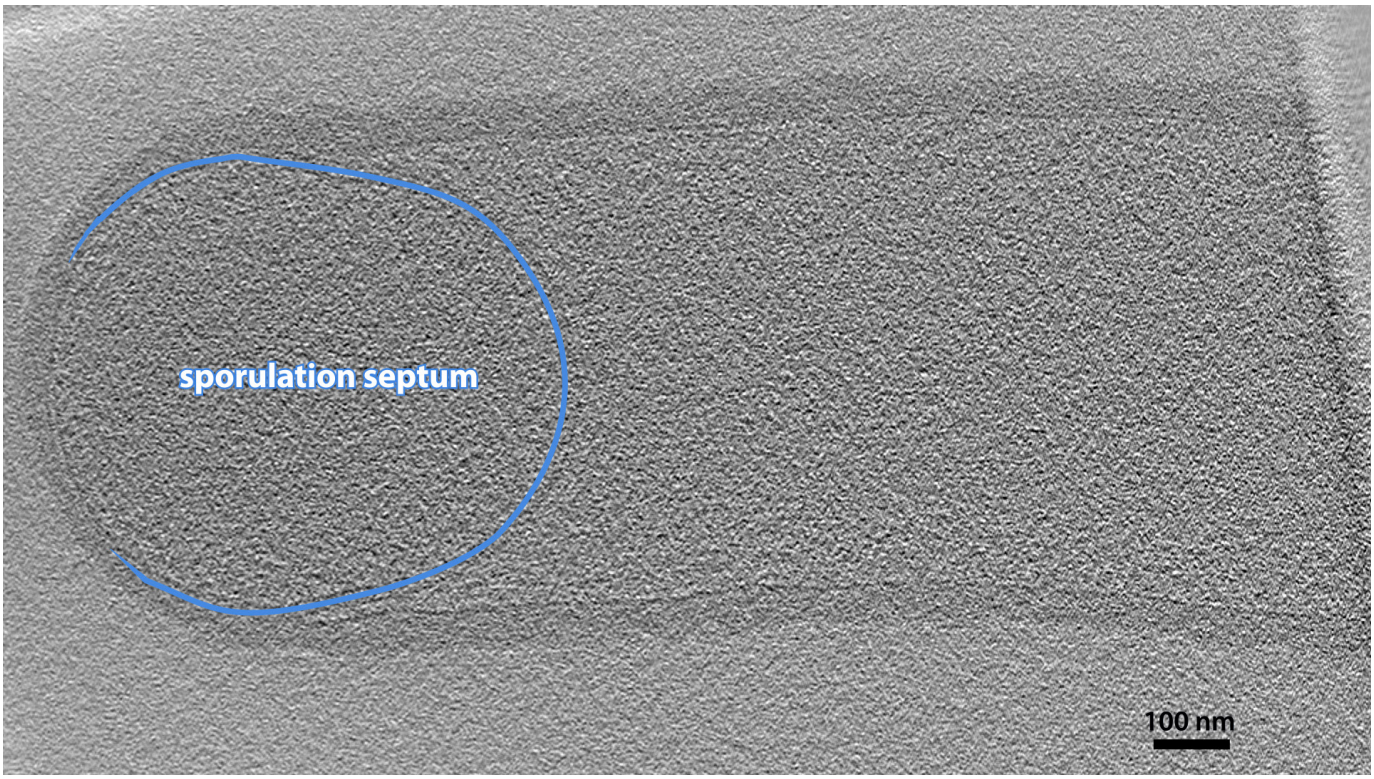

sporulation plane
100 nm

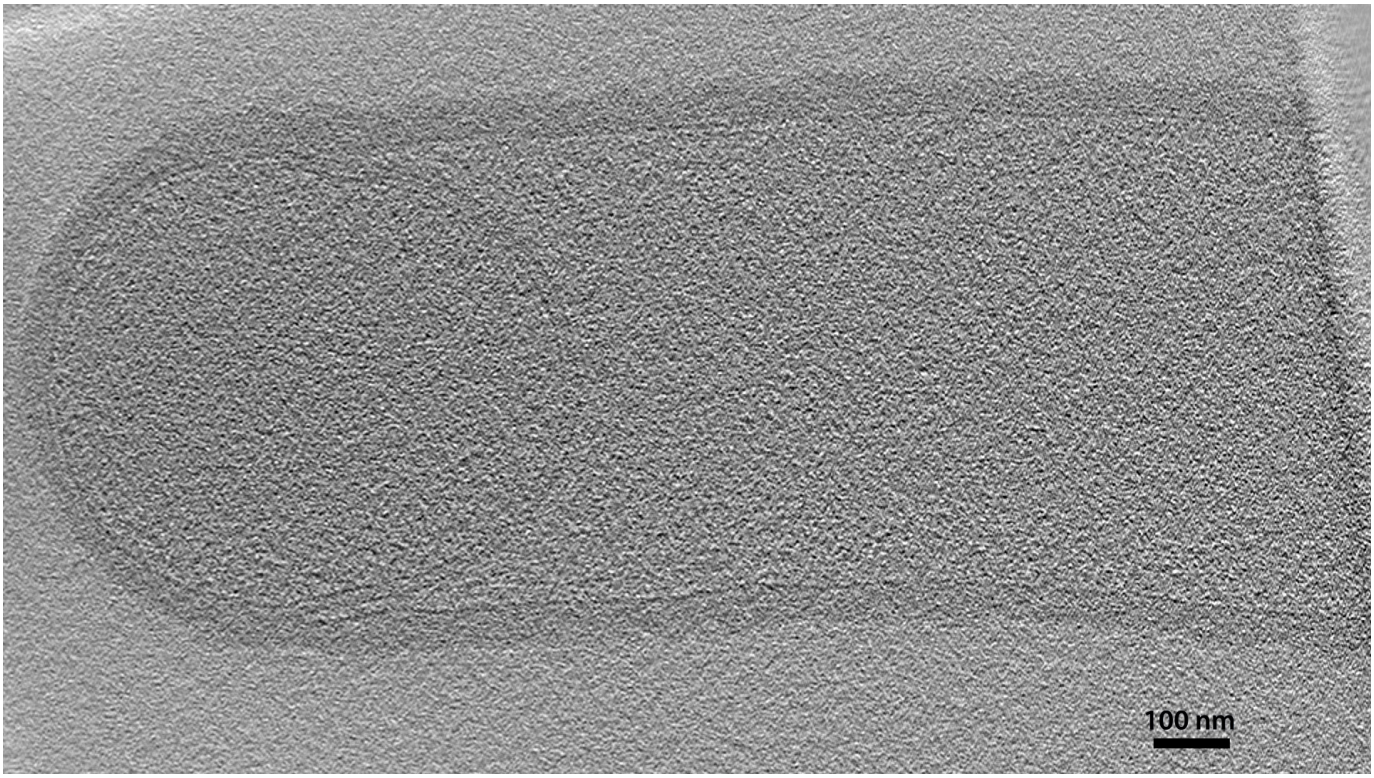
100 nm

Learn More: Septum Formation

The sporulation septum forms much like the division septum, as you can see in this Bacillus subtilis *at a very early stage of the process; FtsZ draws in the membrane and directs cell wall to be built behind it. The arrangement of FtsZ is slightly different, though, with filaments forming on only one face of the division plane (facing the larger compartment of the cell). A sporulation-specific protein excludes them from the other side (facing the smaller compartment that will become the spore). This asymmetry likely explains why sporulation septa are thinner, with fewer layers of peptidoglycan, than division septa.*

Bacillus subtilis *Collected by: Elitza Tocheva* *DOI: 10.22002/D1.1565*


sporulation septum
100 nm

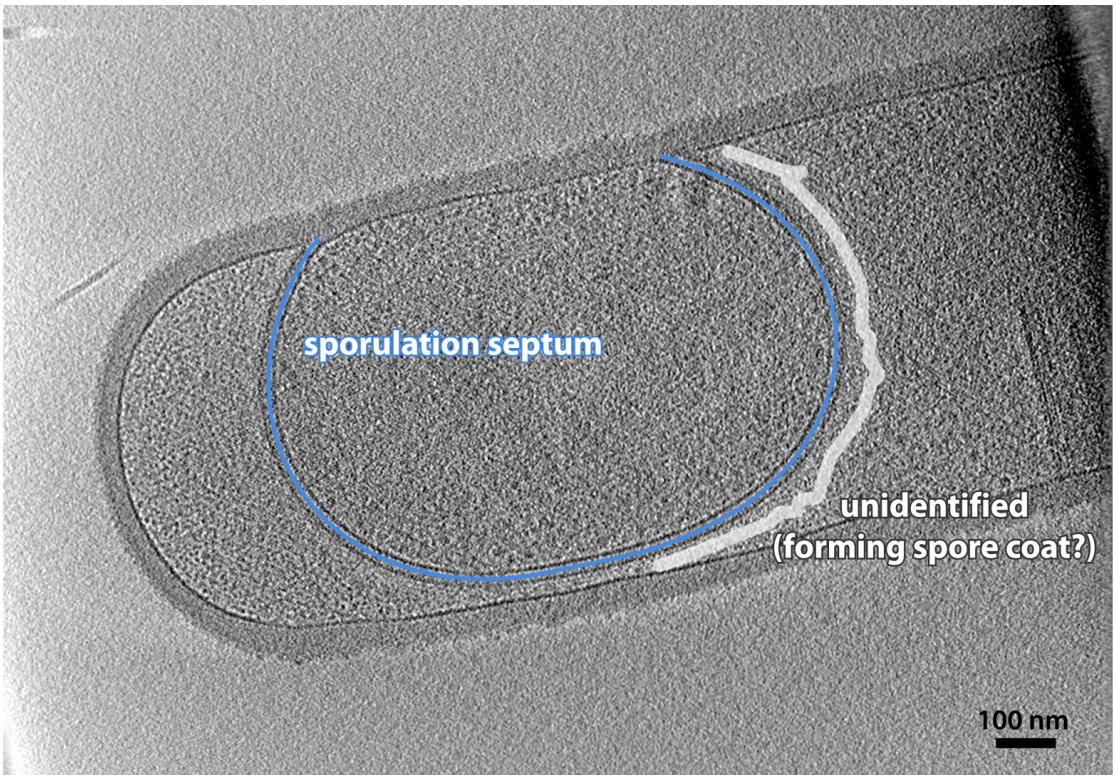

100 nm

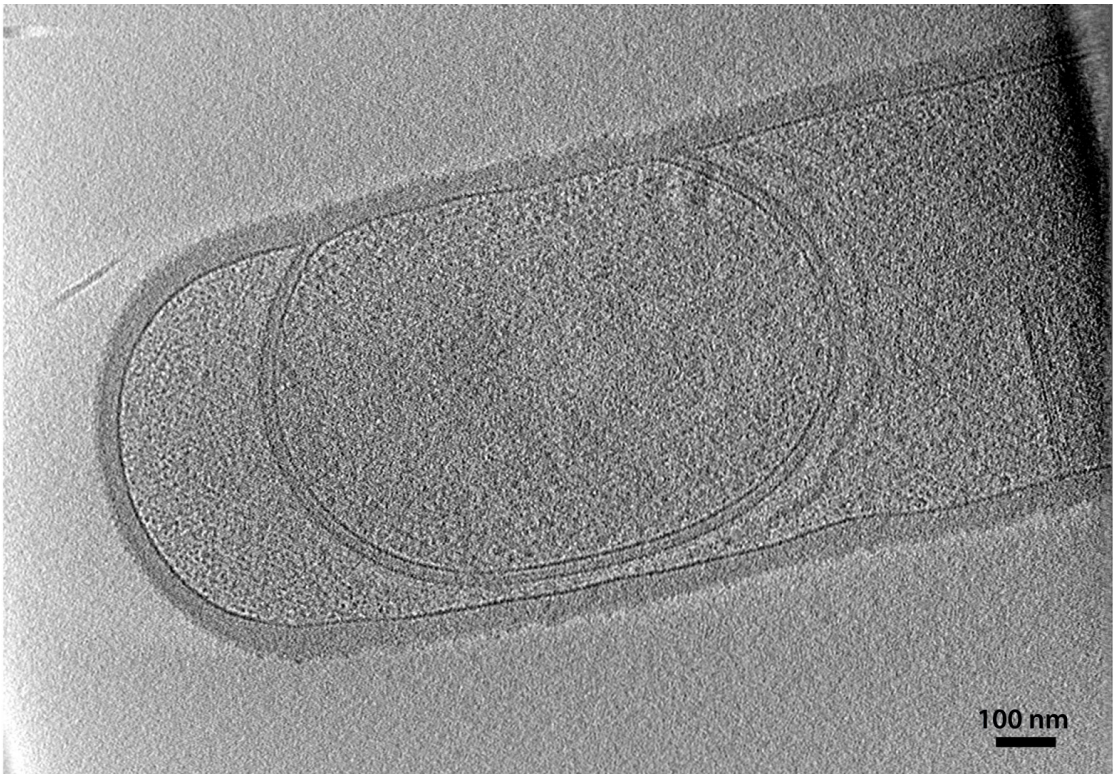
6. Monoderm Spore Engulfment

Once the septum is in place, it is then extended, as you can see in this *Bacillus subtilis*, enlarging the nascent spore and ultimately separating it from the mother cell envelope. Note that the monoderm mother is producing a diderm spore, surrounded by two layers of its mother's membrane. As this occurs, a copy of the genome is pumped through a specialized protein nanomachine. The increased turgor pressure from the highly-concentrated DNA helps the **forespore** round and expand. Usually, the mother cell membrane finishes closing off at the cell pole, but occasionally this occurs instead on the side of the mother cell (*Learn More* on the next page).

When the process of **engulfment** is finished, the forespore's cell wall is reinforced and expanded into a tough "**cortex**," and an extra protein **coat** is added to the outside, completing the sturdy envelope that will protect the spore from harsh environments (*Learn More* on following pages). Finally, when the spore is ready, the mother cell lyses, releasing the time capsule to its fate.

Bacillus subtilis Collected by: Elitza Tocheva DOI: 10.22002/D1.1558

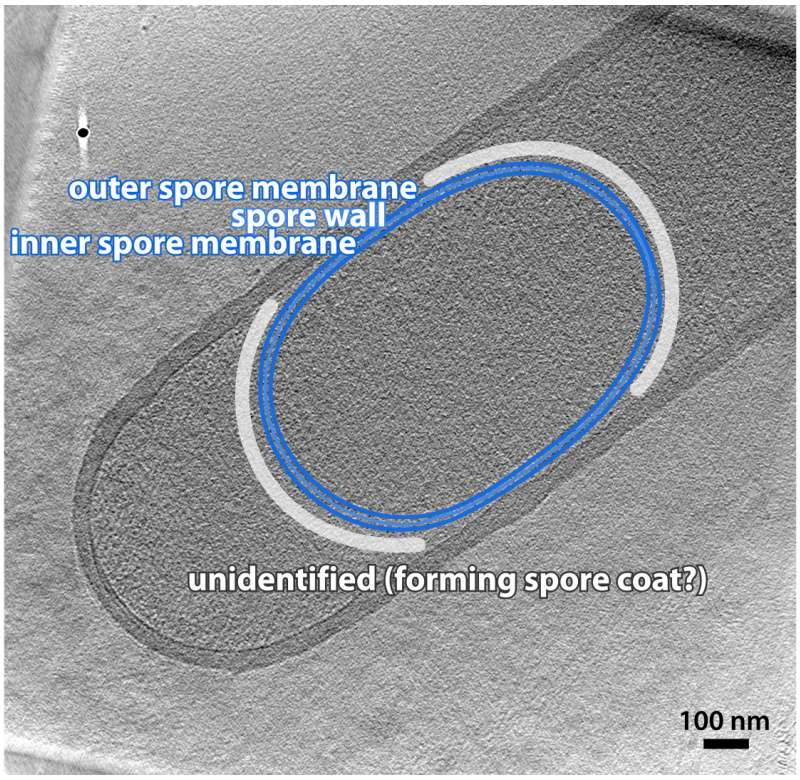

sporulation septum
unidentified
(forming spore coat?)
100 nm

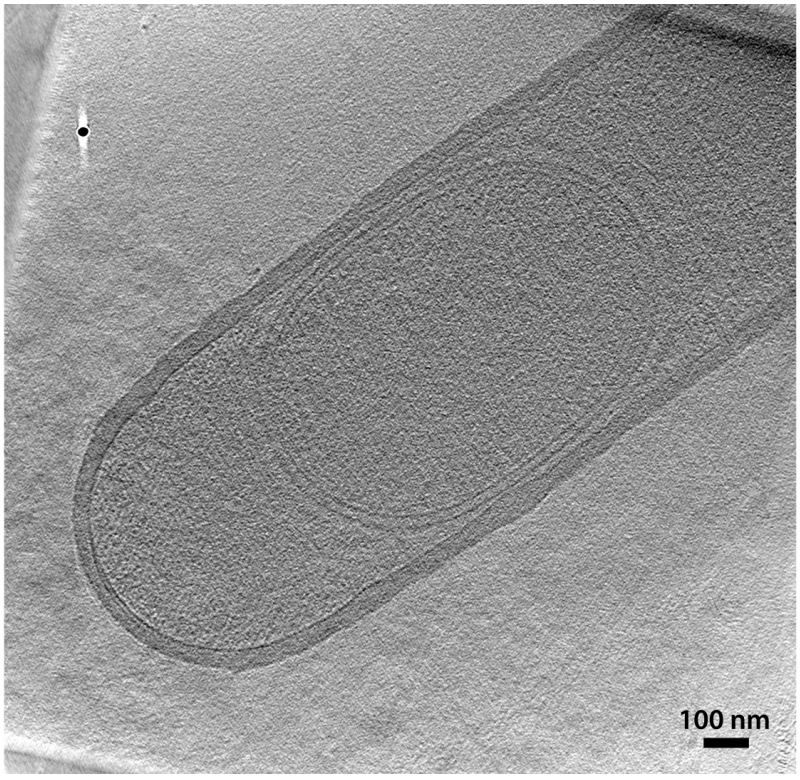

100 nm

Learn More: Engulfment

The process of forespore engulfment by the mother cell membrane culminates in closure of the membranes, creating an outer spore membrane and an inner spore membrane on either side of the sporulation septum. The final point of separation is usually at the center of the cell pole, but not always, as you can see in this Bacillus subtilis *forespore being engulfed from the side of the mother cell. The layer to the right of the developing spore may be a precursor of the coat (*Learn More *on the next page).*

Bacillus subtilis *Collected by: Elitza Tocheva* *DOI: 10.22002/D1.1566*

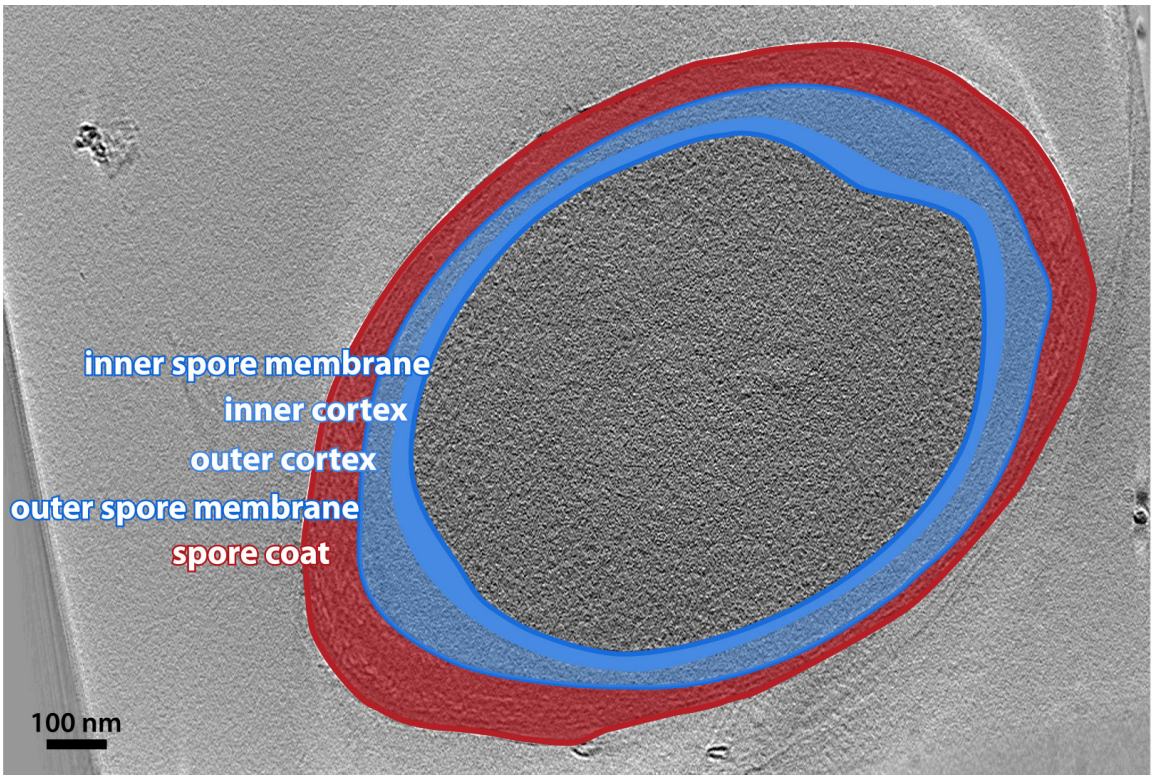

outer spore membrane
spore wall
inner spore membrane
unidentified (forming spore coat?)
100 nm

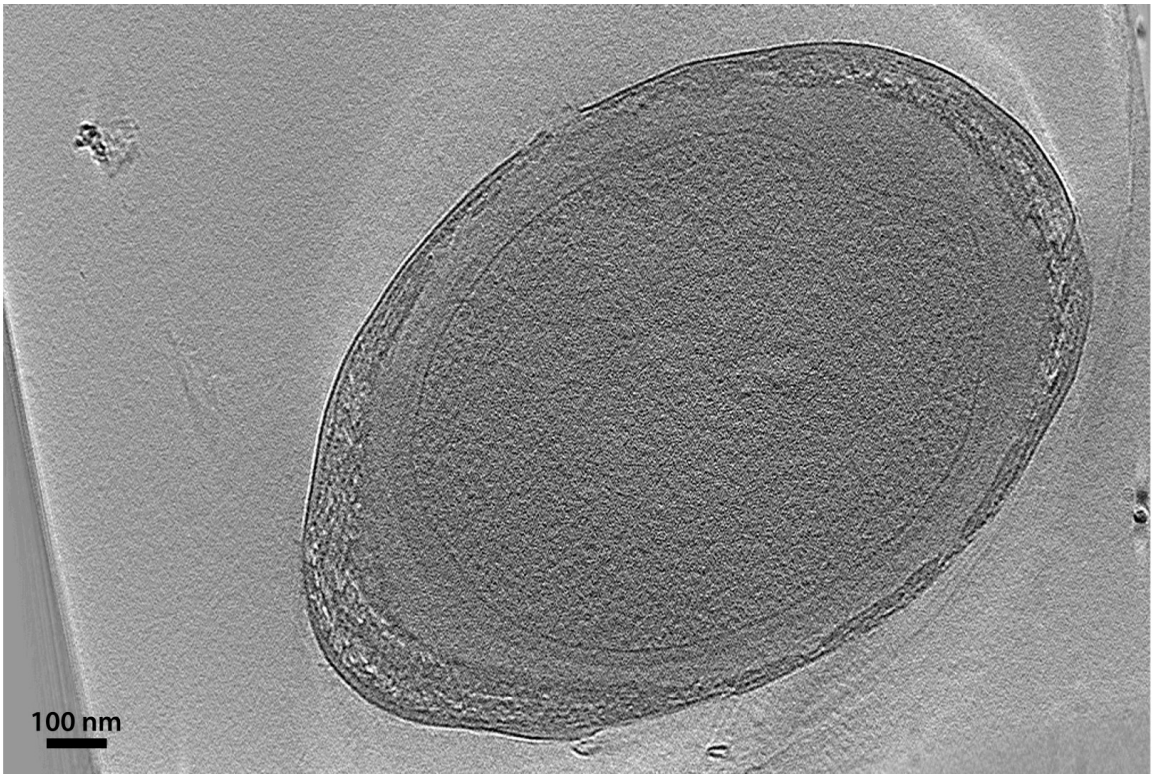

100 nm

Learn More: Separation

In this later stage of sporulation, you can see that the Bacillus subtilis *forespore has now separated completely from the mother cell envelope. It has also moved away from the cell pole. The layer flanking the forespore may be the assembling spore coat.*

Bacillus subtilis *Collected by: Elitza Tocheva* *DOI: 10.22002/D1.1567*


inner spore membrane
inner cortex
outer cortex
outer spore membrane
spore coat
100 nm

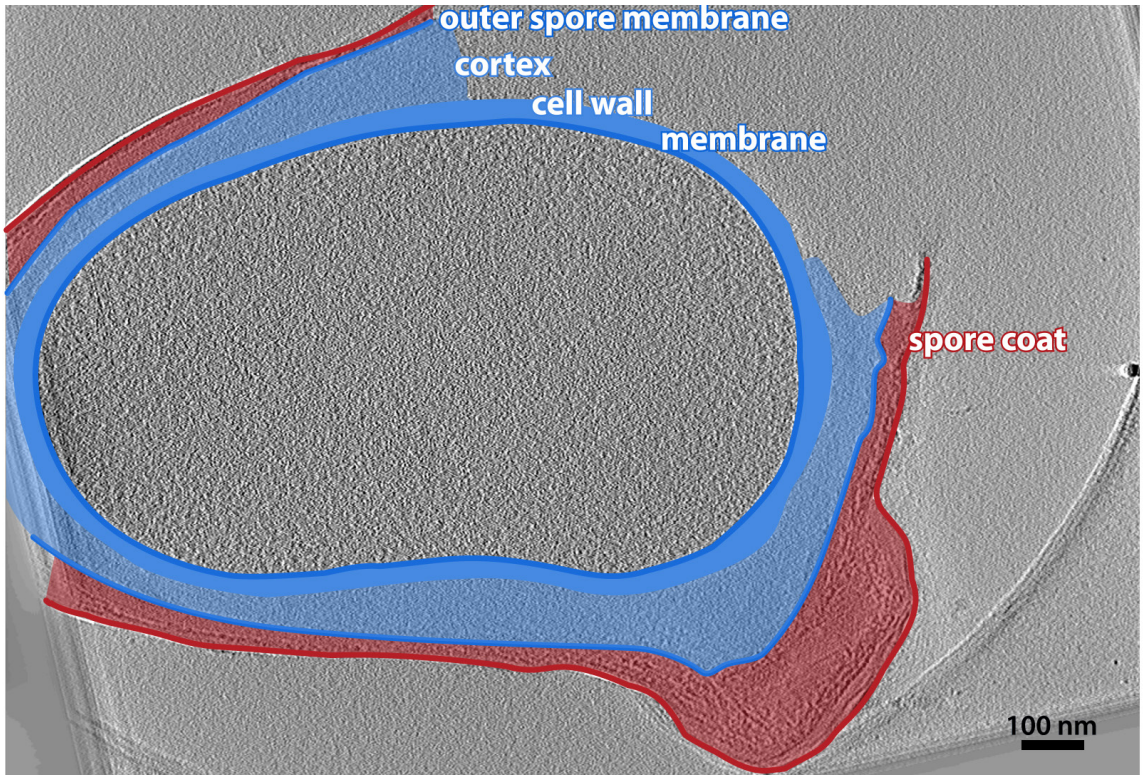

100 nm

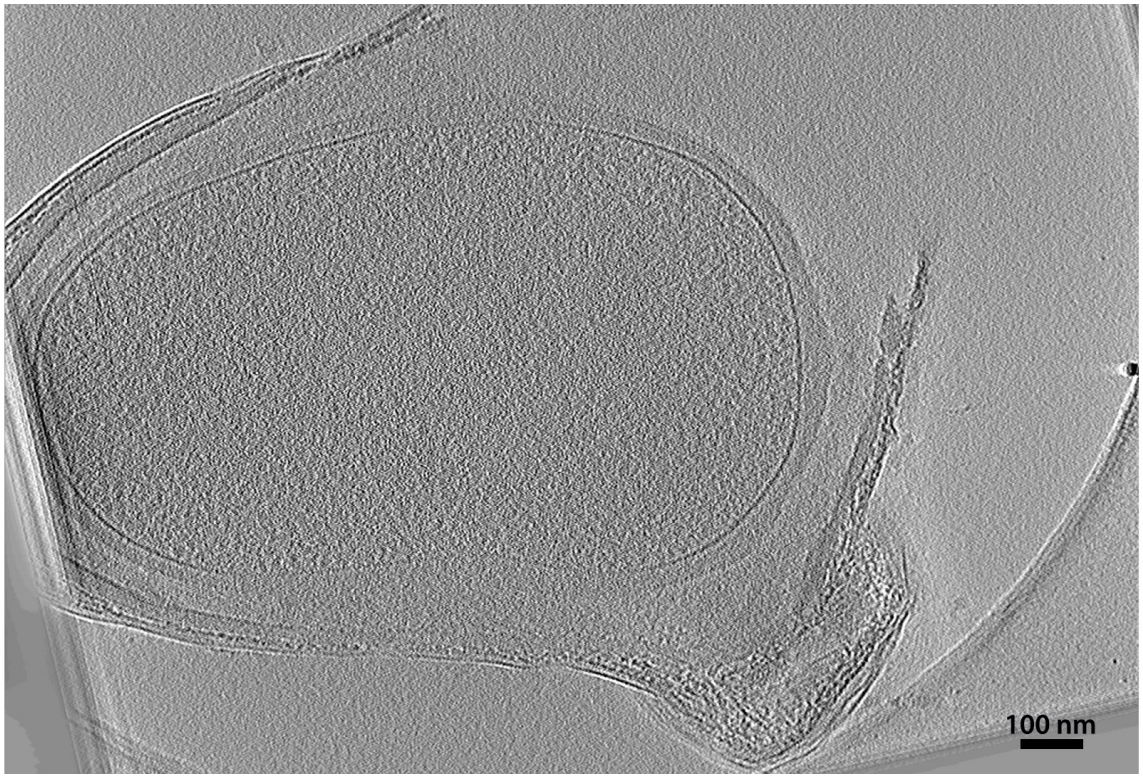
7. Monoderm Spores

Fully mature *Bacillus subtilis* spores like this one are ovoid, and swollen with armor, including the thick cortex and multilayered coat. This dense shield protects the cargo within from drying out, or denaturing in heat or acid. (It also makes it hard to make out much internal detail in images.)

Once released, the spore will remain dormant as long as necessary, protected by its carbohydrate and protein armor, waiting for optimal conditions of water, temperature and pH to germinate. The wait can be long, sometimes very, very, *very* long. In one case, *Bacillus subtilis* spores were revived from a salt crystal thought to have formed 250 million years ago, about 20 million years before the first dinosaurs appeared.

Bacillus subtilis Collected by: Elitza Tocheva DOI: 10.22002/D1.1559


outer spore membrane
cortex
cell wall
membrane
spore coat
100 nm

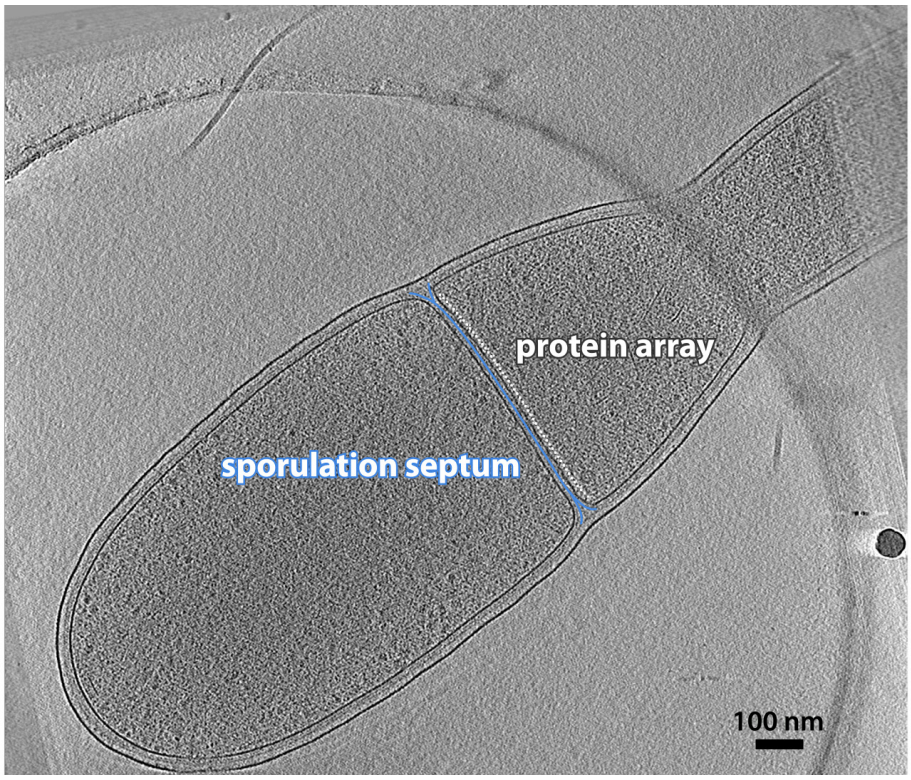

100 nm

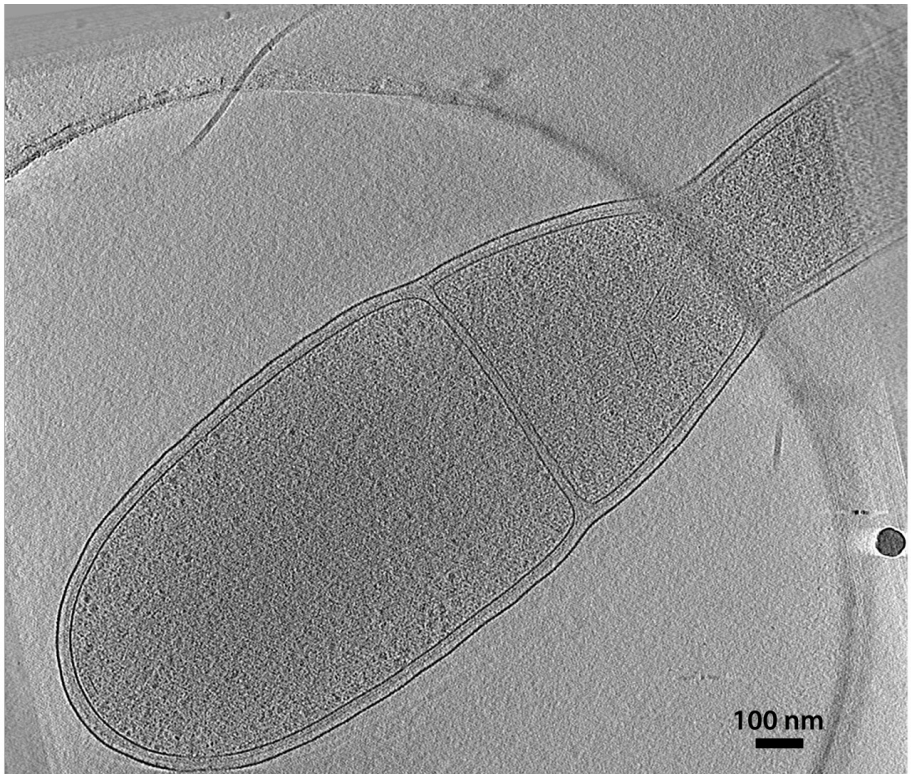
8. Monoderm Germination

When conditions become conducive to growth, the spore will return to an active state, shedding its protein coat and outer membrane and dismantling its cortex to allow outgrowth of the reviving cell, as you see in this **germinating** *Bacillus subtilis* spore.

The ready transition between a monoderm cell with a thick cell wall and a diderm spore with a thin septal wall may provide a hint to the origin of diderm bacterial cells. Perhaps spores, released prematurely from their mothers before their septum had thickened into a full cortex, kept growing and dividing with both membranes and a thin cell wall. Over many generations, evolution could then tinker with the second membrane, specializing it into the modern bacterial outer membrane.

Bacillus subtilis Collected by: Elitza Tocheva DOI: 10.22002/D1.1560

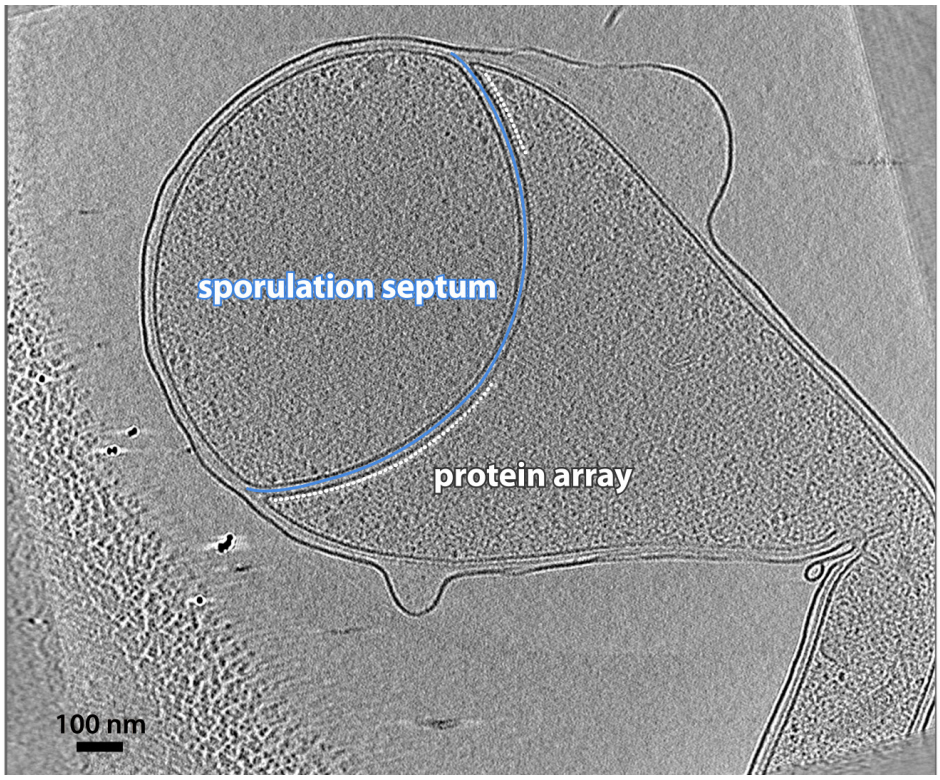

protein array
sporulation septum
100 nm

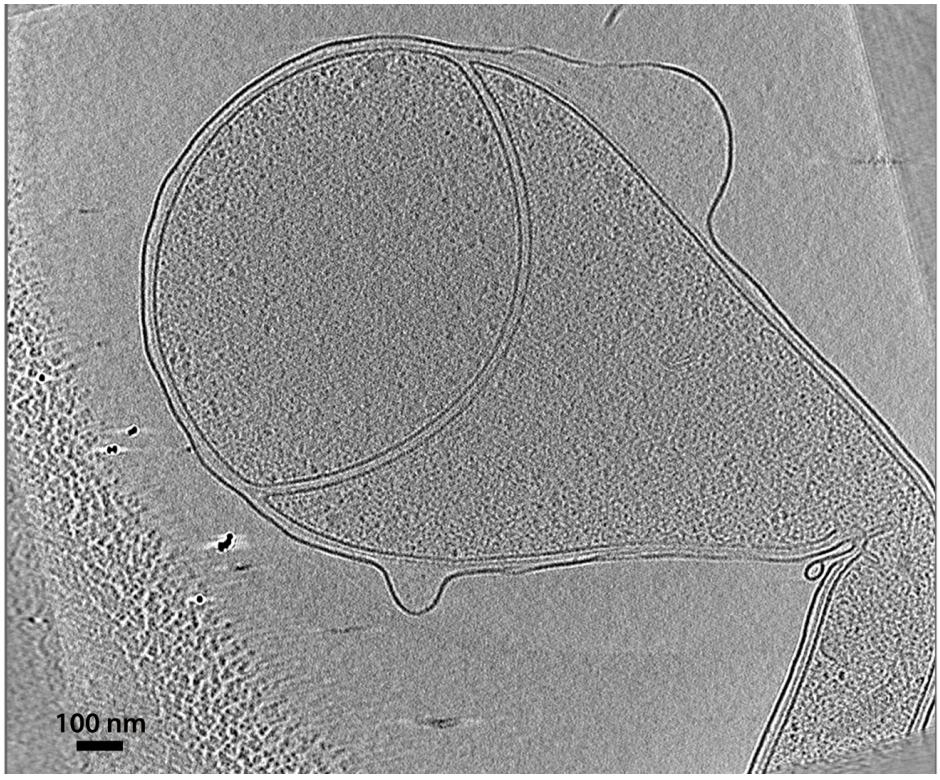

100 nm

9. Diderm Sporulation

Sporulation is not limited to monoderms. A few species of diderm bacteria also form spores (some exospores and others endospores). The process is much the same as in monoderms, despite the different cell envelope structure. It begins with an asymmetric septum close to one pole of the cell, as you can see in this *Acetonema longum*. As in monoderms, the septum contains a thin layer of peptidoglycan (remember that diderms have a thin cell wall, though, so the thickness is about the same as in the rest of the cell). Note the array of densities lining the septal membrane on the right (facing the mother cell's cytoplasm). This is likely a protein involved in making the spore coat.

Acetonema longum Collected by: Elitza Tocheva DOI: 10.22002/D1.1561


sporulation septum
protein array
100 nm

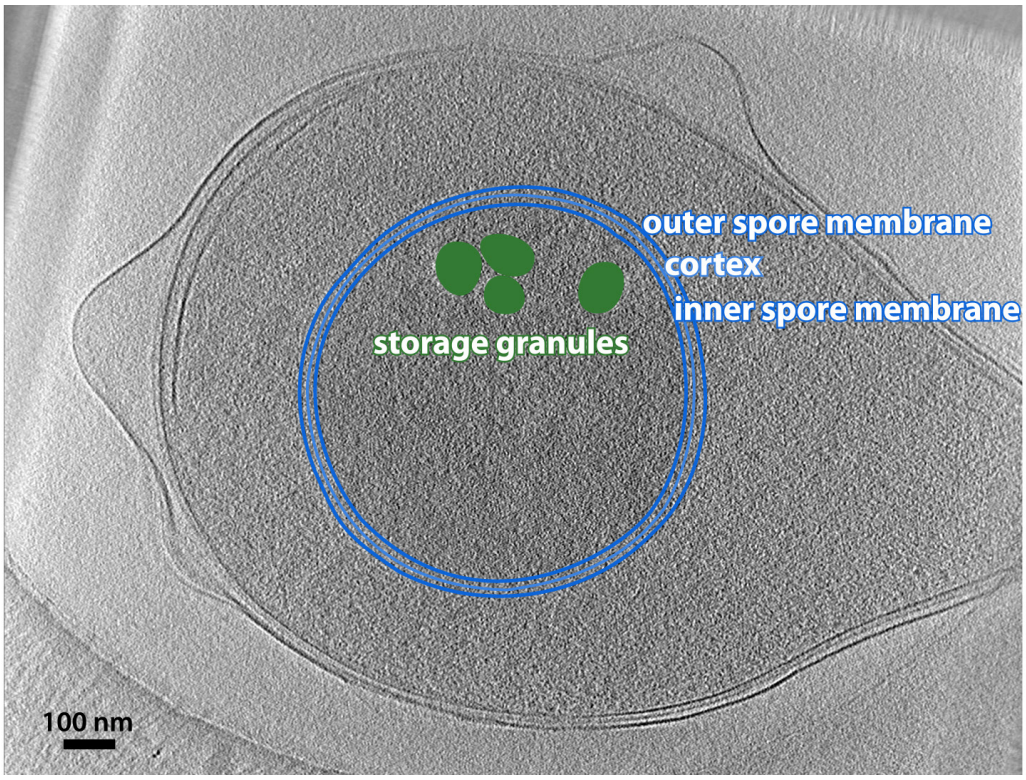

100 nm

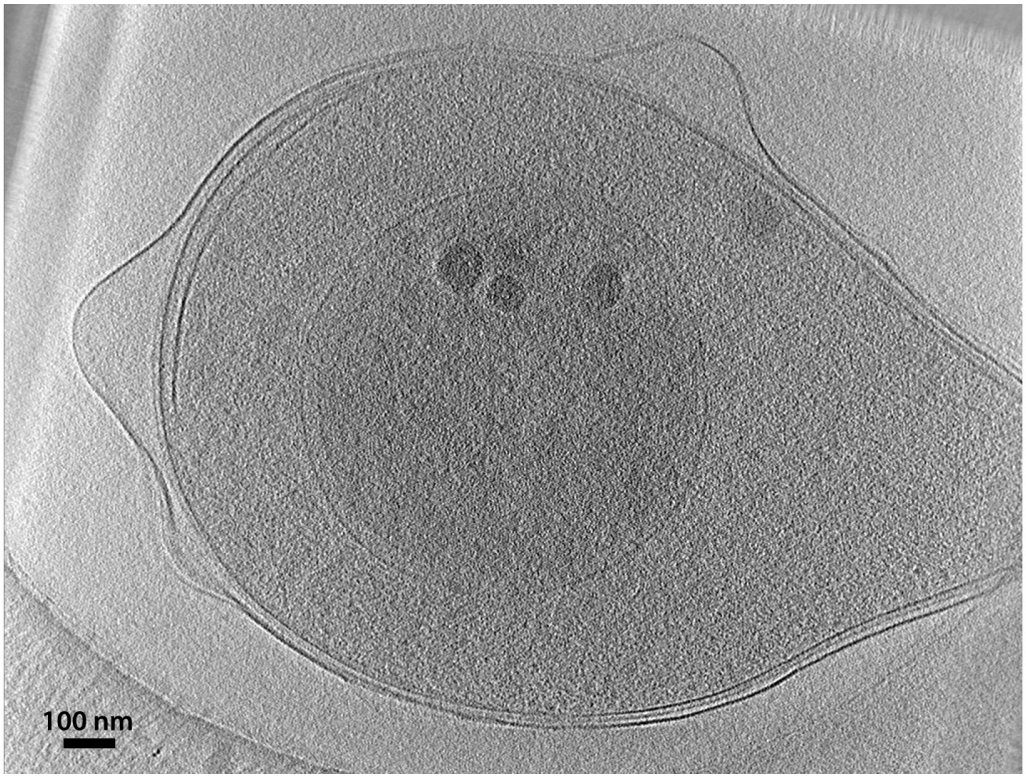
10. Diderm Spore Engulfment

Engulfment also proceeds similarly, as you can see in this *Acetonema longum* cell, with the septum expanding and moving to cinch off at the pole of the mother cell (*Learn More* on the next page). As the cell wall is remodeled to accomplish this, the rod shape of the mother is lost. Note also how the outer membrane of the mother cell ruffles and expands, apparently having detached from the cell wall. Compared to a daughter cell produced by division, there is something quite interesting about the envelope of the endospore. Look carefully at the membranes. Both the inner and outer membranes of the spore derive from the inner membrane of the mother cell. This implies a surprising interchangeability of the two membrane types, and lends further credence to the idea that the outer membrane of some or all diderm bacteria originally came from a monoderm spore that failed to shed its second membrane.

As you saw in *Bacillus subtilis*, once engulfment is complete, the thin septal wall will be expanded and reinforced into a thick cortex and a protective protein coat will be added before the mature spore is released by lysis of the mother cell (*Learn More* on following pages). *A. longum* spores are round, but other rod-shaped diderms form ovoid spores (*Learn More* on following pages).

Acetonema longum Collected by: Elitza Tocheva DOI: 10.22002/D1.1562

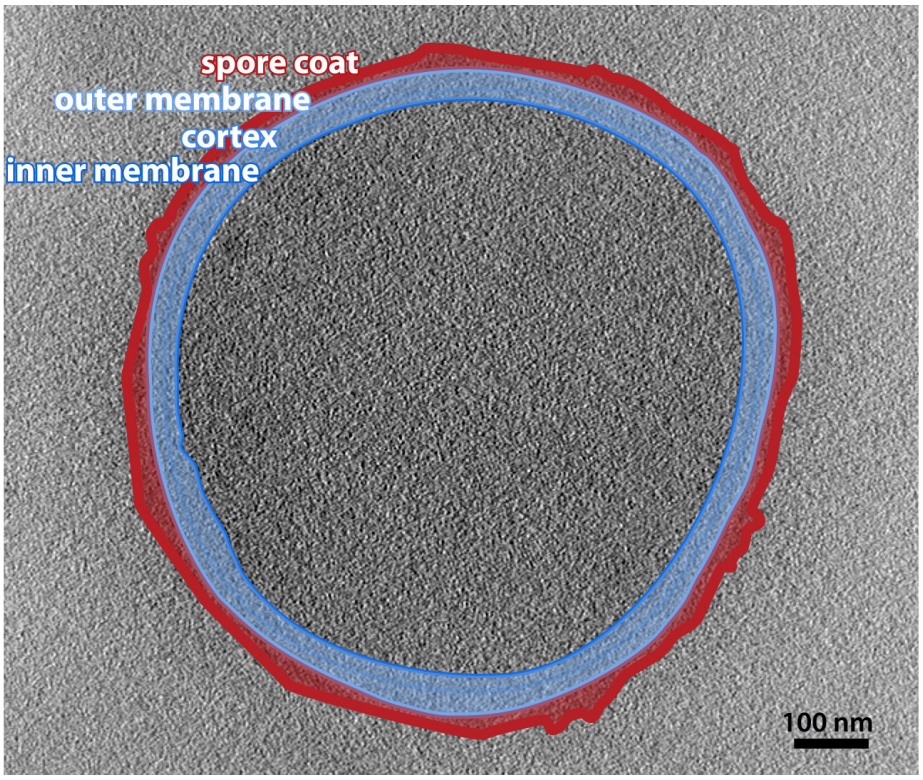

outer spore membrane
cortex
inner spore membrane
storage granules
100 nm

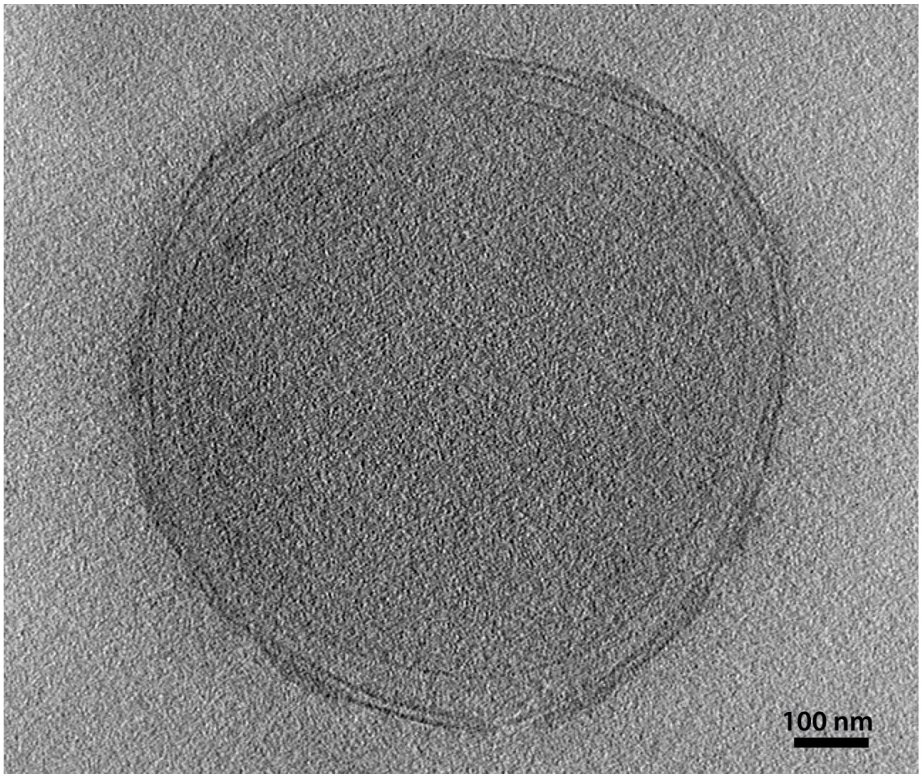

100 nm

Learn More: Forespore

This Acetonema longum *cell has completed engulfment of the forespore.* A. longum *forespores (and mature spores) typically contain several granules, likely storing poly-phosphate. These may help power outgrowth when the spore germinates. Spores from other species, though, like* Bacillus subtilis, *do not contain obvious storage granules, so they may serve another, species-specific role.*

Acetonema longum *Collected by: Elitza Tocheva* *DOI: 10.22002/D1.1568*

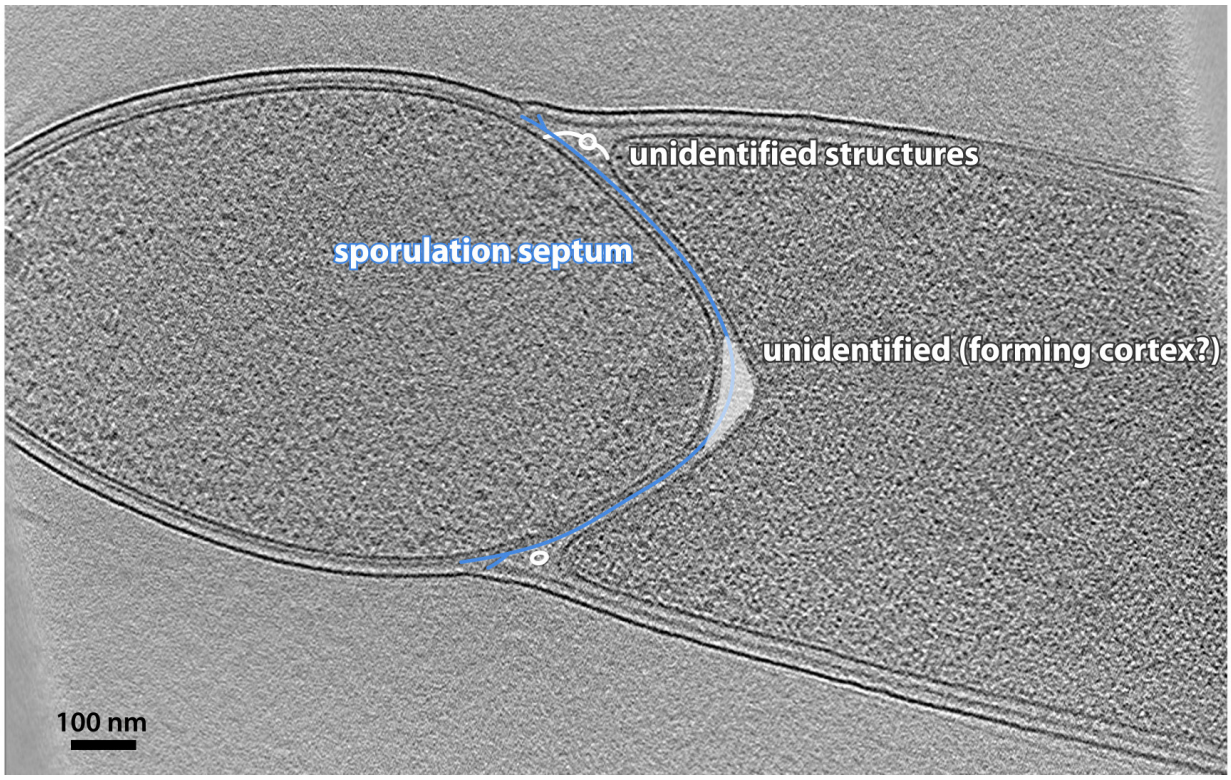

spore coat
outer membrane
cortex
inner membrane
100 nm

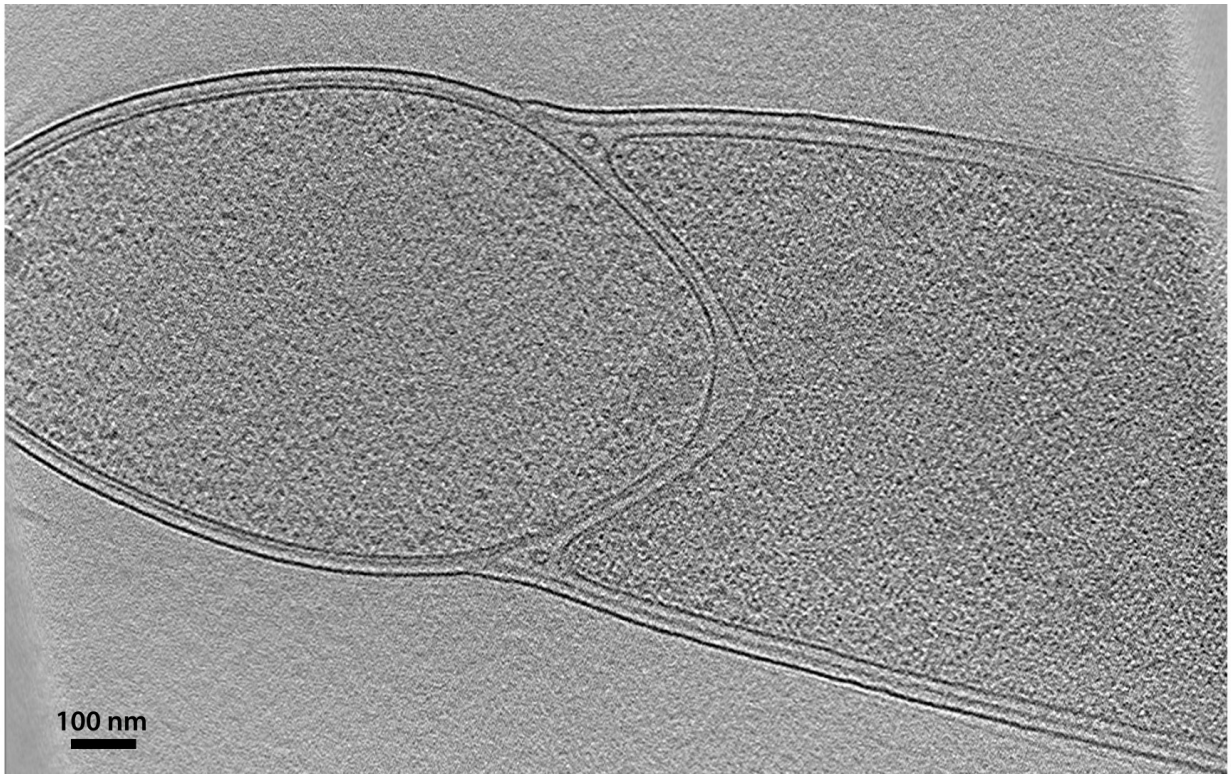

100 nm

Learn More: Spore

This mature Acetonema longum *spore has been released by lysis of its mother cell. Note its extensive armor: a thick cortex between the membranes, and a multilayered protein coat.*

Acetonema longum *Collected by: Elitza Tocheva* *DOI: 10.22002/D1.1569*

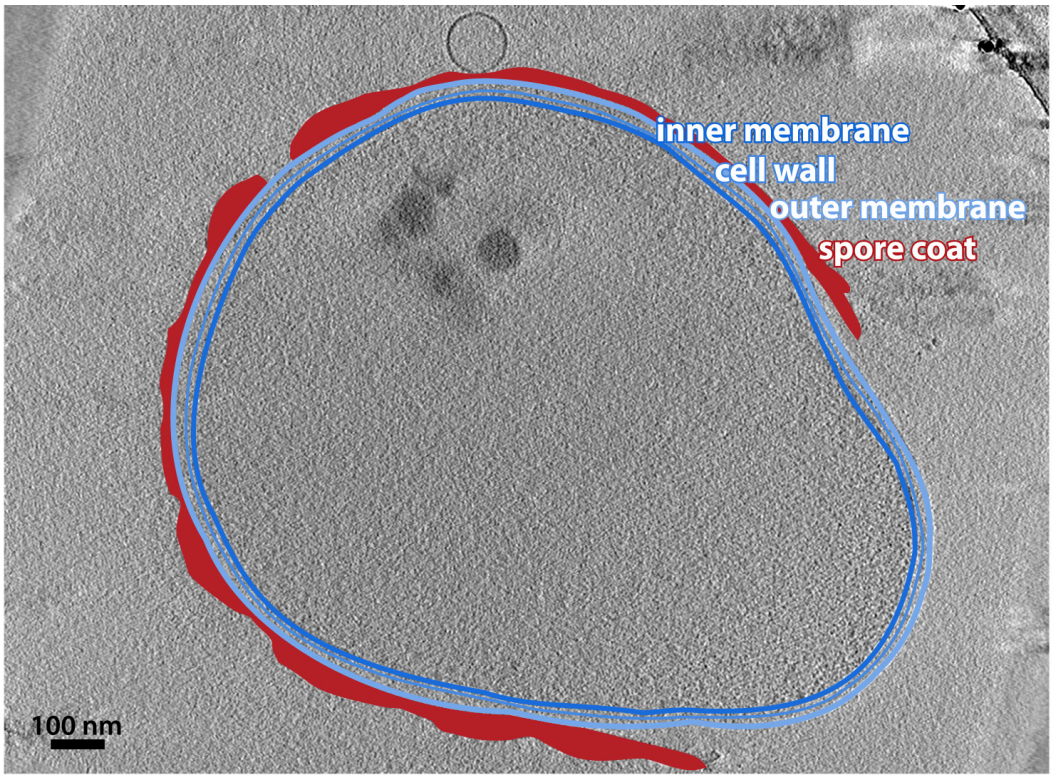

unidentified structures
sporulation septum
unidentified (forming cortex?)
100 nm


100 nm

Learn More: Ovoid

This diderm Sporomusa acidovorans *cell is in the process of forming an ovoid endospore. Note also what may be an early stage of cortex formation at the end of the forespore, as well as novel structures at the points of cell wall remodeling. We do not know what they are, but they may be involved in engulfment.*

Sporomusa acidovorans *Collected by: Elitza Tocheva* *DOI: 10.22002/D1.1570*


inner membrane
cell wall
outer membrane
spore coat
100 nm

100 nm

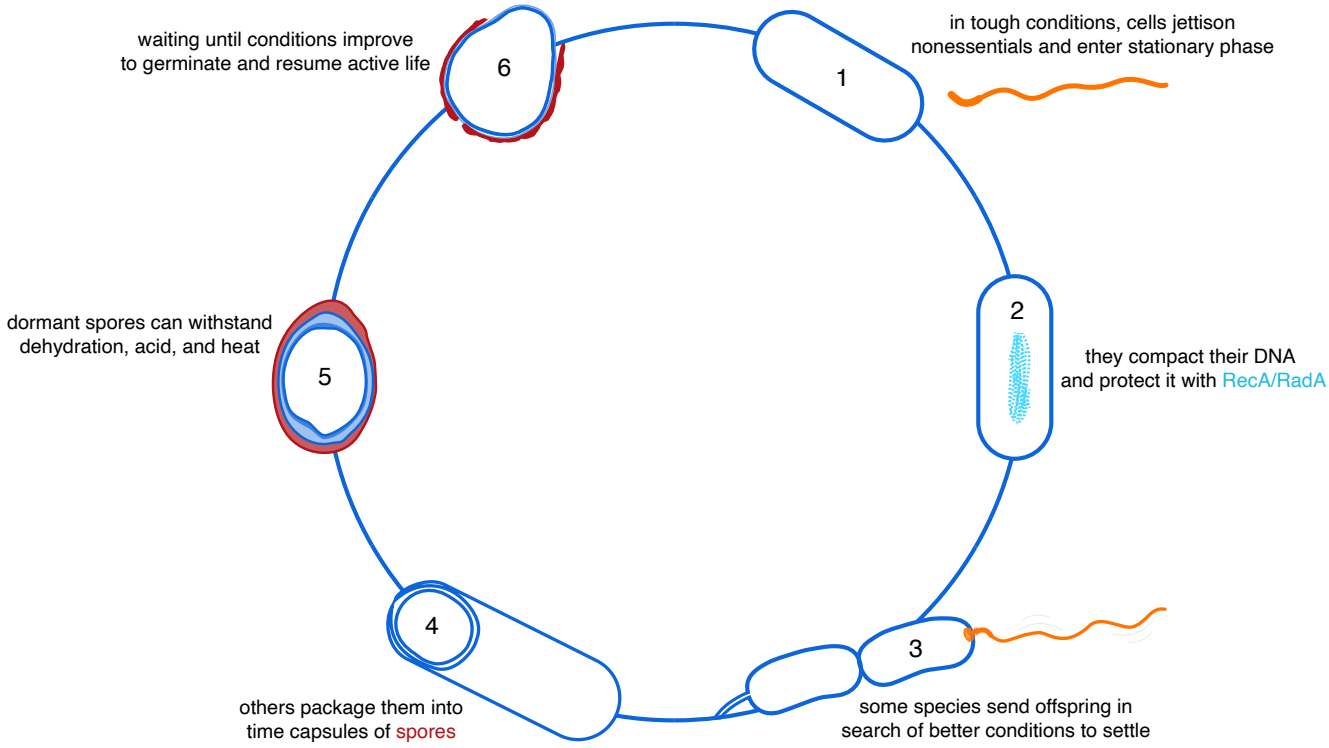
11. Diderm Germination

Again, when the spore encounters favorable conditions, it will germinate, as this *Acetonema longum* spore is doing. Unlike the *Bacillus subtilis* you saw earlier, germinating diderm spores do not shed their outer membrane. Instead, they remodel their cortex back into a thin cell wall (compare to the mature spore on the previous page). This process is a good reminder of the similarity of monoderm and diderm cell walls; they may look very different, but their fundamental architecture is the same, just with more sheets of material in monoderms (and spores). After the remodeling, the cell sheds its protein coat, as you see here, and starts to grow.

Some archaea also have resistant forms similar to bacterial spores. For instance, when water levels drop in its salty environment, rod-shaped *Halobacterium salinarum* uses a variant division process to produce three or four hardy spherical cells that can survive dormant in halite crystals for tens of thousands of years, awaiting water to revive.

Sporulation may seem like a highly specialized function practiced by relatively few species, but there is reason to think that it may have evolved long ago and been key to the success of life on Earth. Conditions today are extremely clement compared to what they likely were a few billion years ago, when the last common ancestor of all modern cells (called **LUCA**, for Last Universal Common Ancestor) may have lived. It is quite possible that LUCA was a spore, the only form of life hardy enough to survive conditions volatile and violent enough to kill off the spore's ancestors. If so, most modern lineages of bacteria and archaea simply lost the ability to form spores when it was no longer essential to their survival. It is further possible that a LUCA spore was formed by a cell somewhere else in the solar system and delivered to Earth on one of the asteroids that bombarded the planet in its infancy. We may never know, but the possibilities are fun to consider.

Acetonema longum Collected by: Elitza Tocheva DOI: 10.22002/D1.1563


waiting until conditions improve
to germinate and resume active life
6
1
in tough conditions, cells jettison
nonessentials and enter stationary phase
2
they compact their DNA
and protect it with RecA/RadA
dormant spores can withstand
dehydration, acid, and heat
5
4
others package them into
time capsules of spores
3
some species send offspring in
search of better conditions to settle

Summary

Concept Check Questions

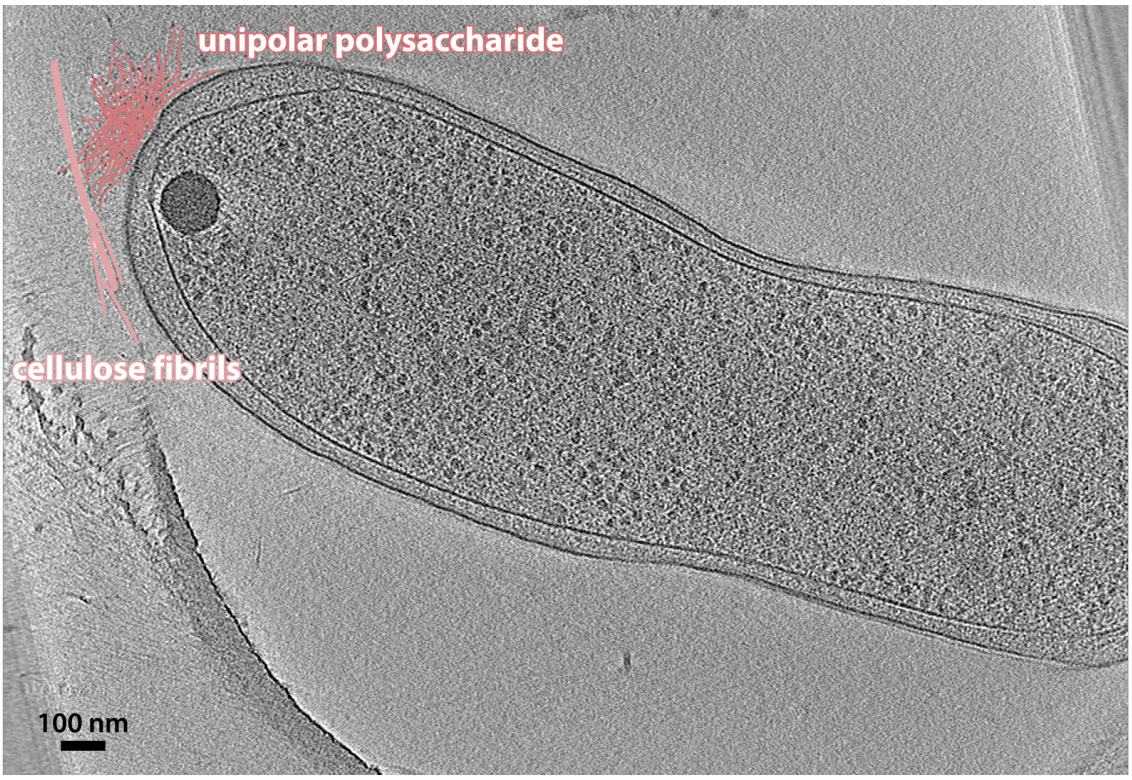
- What is the function of RecA and RadA?
- What is the difference between the two daughters produced by Caulobacter crescentus division?
- What kind of macromolecule makes up the spore cortex? The spore coat?
- In diderms, which membrane forms the inner spore membrane? The outer spore membrane?

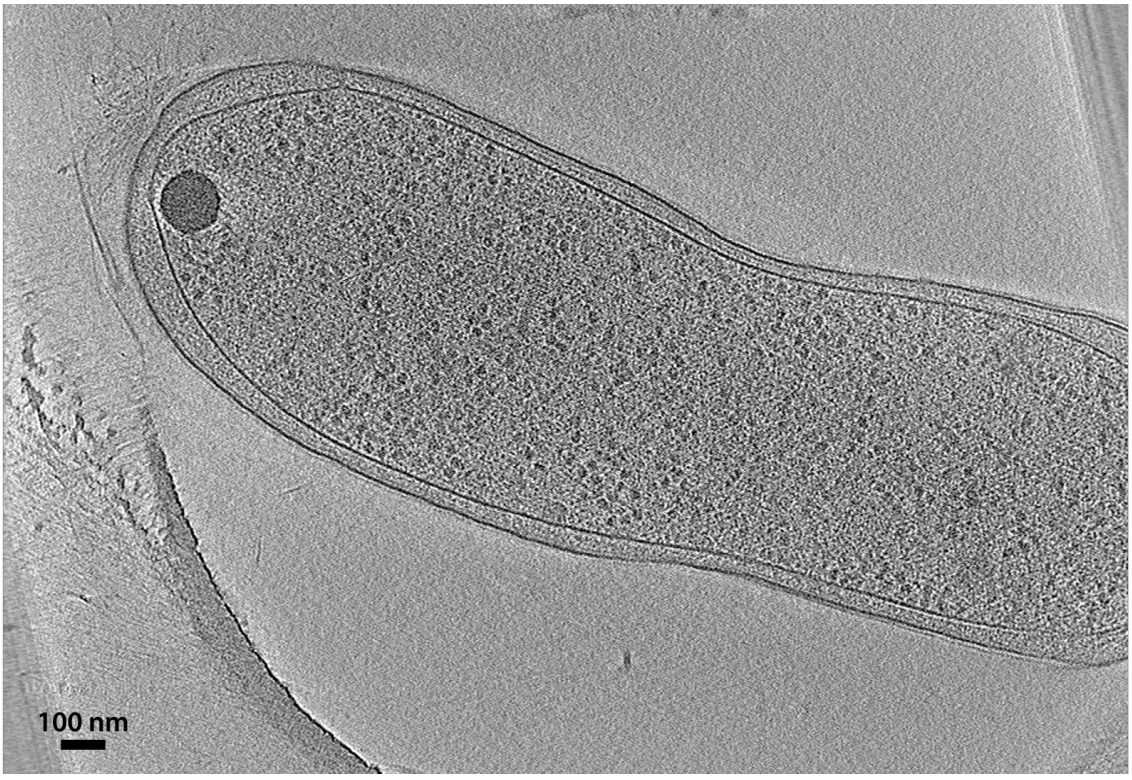
Further Reading

- Fendrihan et al. (2006). *Extremely halophilic archaea and the issue of long-term microbial survival* [75].
- Pletnev et al. (2015). *Survival guide: Escherichia coli in the stationary phase* [76].
- Tocheva et al. (2016). *Sporulation, bacterial cell envelopes and the origin of life* [77].
- Vreeland et al. (2000). *Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal* [78].

9
Interaction

"We do not have solitary beings. Every creature is, in some sense, connected to and dependent on the rest."
- Lewis Thomas [79]


unipolar polysaccharide
cellulose fibrils
100 nm

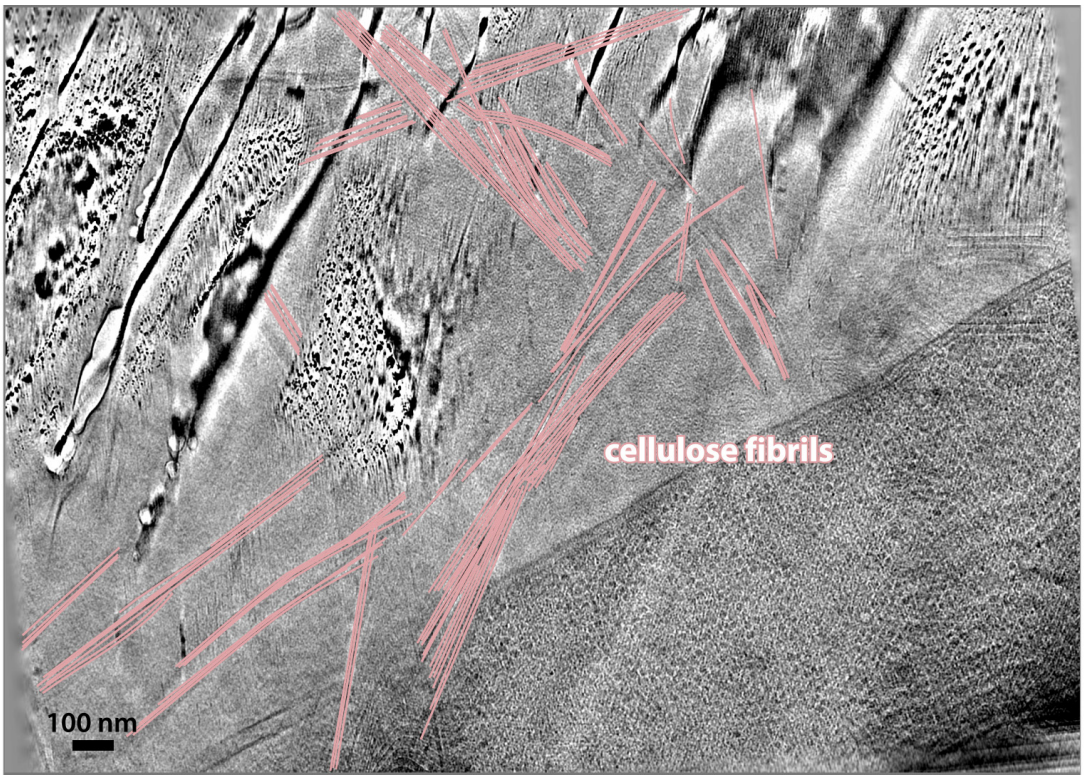

100 nm

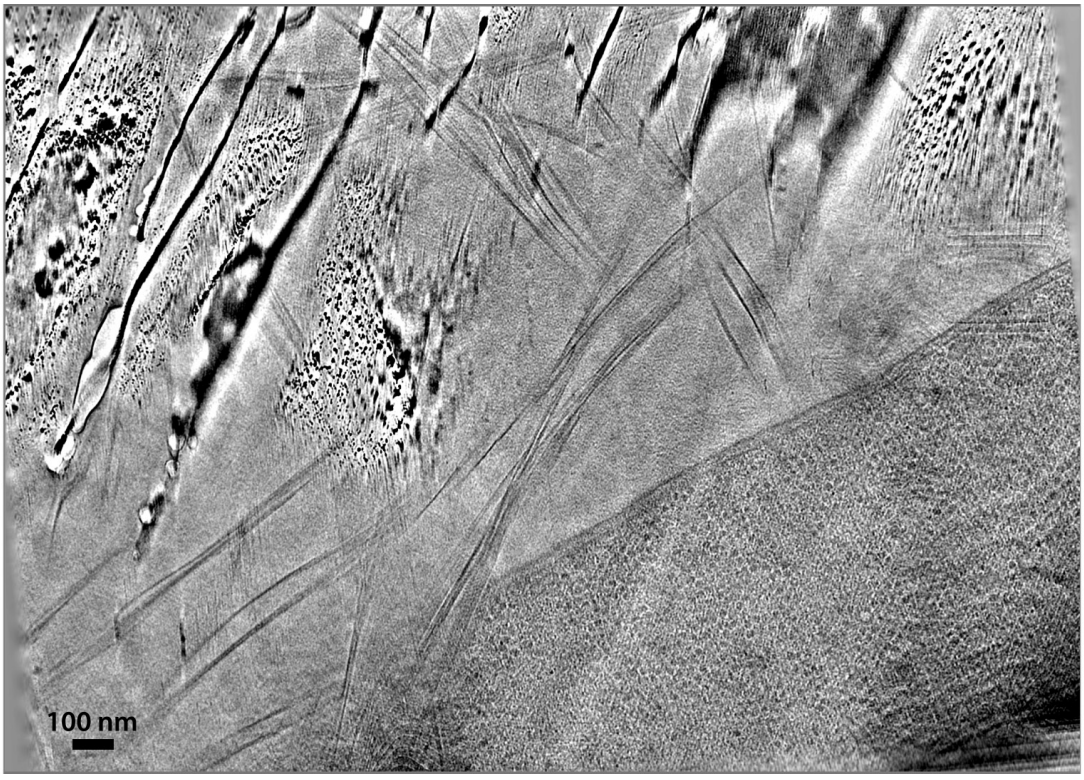
1. Biofilm

So far we have largely ignored a major part of a cell's environment: other organisms. Your cell is far from alone out there. It shares space and resources with cells of the same species (some its own mothers/sisters/daughters), other species, and even other domains. Think about strategies that could help your cell thrive in such a crowded world. For starters, why can't we all just get along? Cooperation is common in nature, often in the form of **biofilms**–communal groups of microorganisms. Biofilms offer advantages to their members, shielding cells on the interior from harsh conditions or antibiotics, and ensuring that nutrients produced by cells' metabolism or released by their death are readily available to others. Biofilms should be familiar to you; think of the scum on your teeth, or the nearest pond. In fact, the earliest physical evidence we have of life on earth is in the form of stromatolites ("layered rocks"), which are meter-scale fossilized biofilms of cyanobacteria (you can see still-growing stromatolites in Shark Bay, Australia).

To attach to a biofilm, cells use strategies we have already discussed, including pili and holdfast. *Agrobacterium tumefaciens* like this one use a holdfast-like polymer called Unipolar Polysaccharide, or **UPP**. Some bacteria, including this one, also contribute to the superstructure of the biofilm by secreting **cellulose** fibers that help form the non-living matrix of the community.

Agrobacterium tumefaciens Collected by: Elitza Tocheva DOI: 10.22002/D1.1571

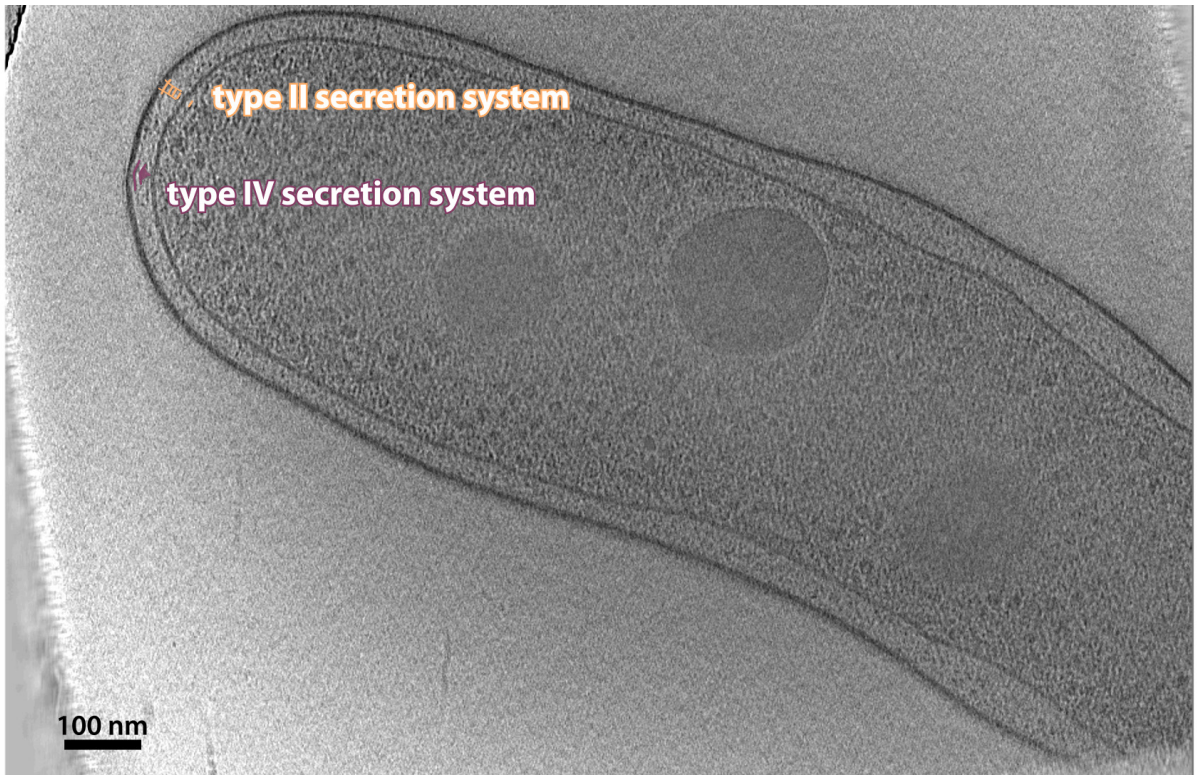

cellulose fibrils
100 nm

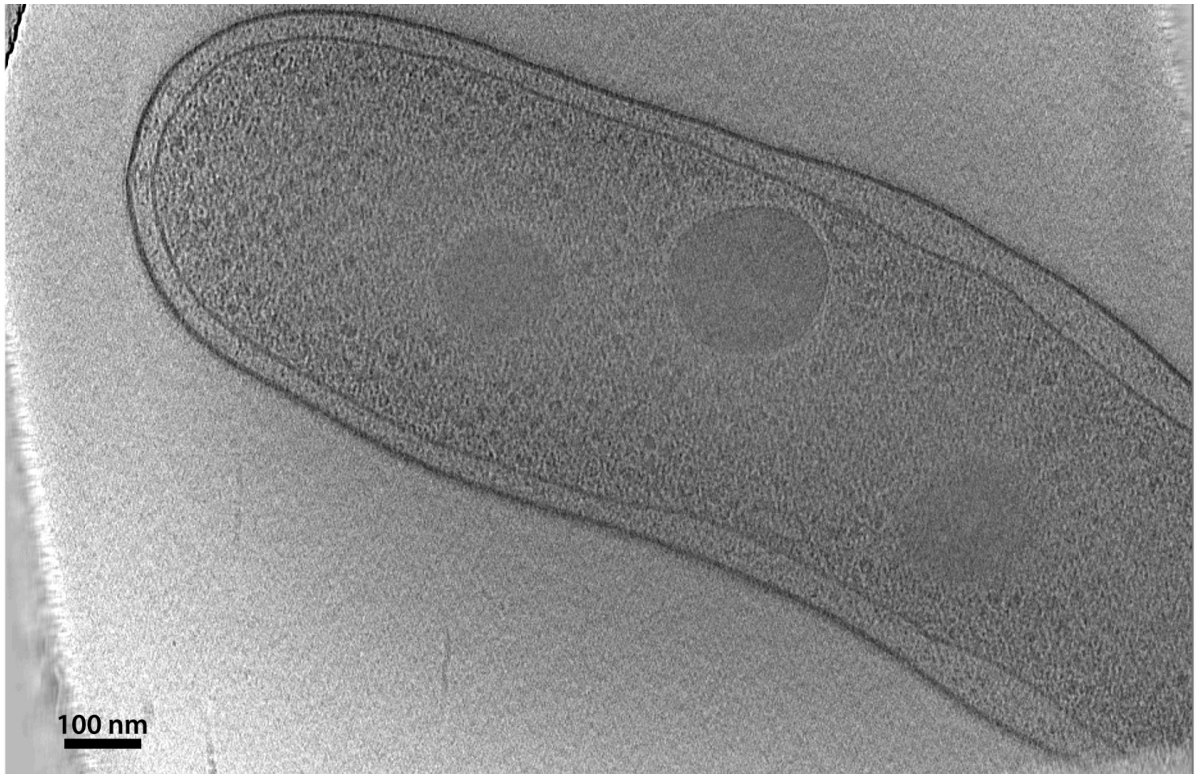

100 nm

2. Biofilm Matrix

Individual cellulose fibers associate into bundles to form a 3D matrix for the cells, as you can see in this thin slice through a *Gluconacetobacter hansenii* biofilm. Not all biofilms contain cellulose, but all have an **extracellular matrix**, typically made of proteins secreted by the component cells. These proteins have properties that help cells aggregate, like the unipolar polysaccharide you just saw. They may also have other properties useful to the community; for instance, *Bacillus subtilis* secretes a hydrophobic protein that forms a resistant coat around the biofilm. Matrices also contain lipids and extracellular DNA, and dead cells contribute material to the superstructure. Keep in mind that the lab-grown biofilm you see here consists of a single species, but in nature a biofilm often contains multiple species.

Gluconacetobacter hansenii Collected by: William Nicolas DOI: 10.22002/D1.1572


type II secretion system
type IV secretion system
100 nm


100 nm

3. Type II and Type IV Secretion

How else might your cell deal with the crowd? Well, if you can't join them, maybe you should try to beat (and eat) them. Bacteria have evolved an impressive arsenal of molecular weaponry. In fact, most of the antibiotics we use were invented by bacteria. In many cases, cells simply release these antibiotics to the environment, either directly or in membrane vesicles, which may travel further. For more specific targeting, though, cells employ a varied array of **secretion systems**. You already saw some of these nanomachines in Chapter 6: a type III secretion system assembles the flagellum and a type II secretion system assembles the archaellum. Another type II secretion system makes (and unmakes) type IV pili. Other family members have evolved more militaristic functions.

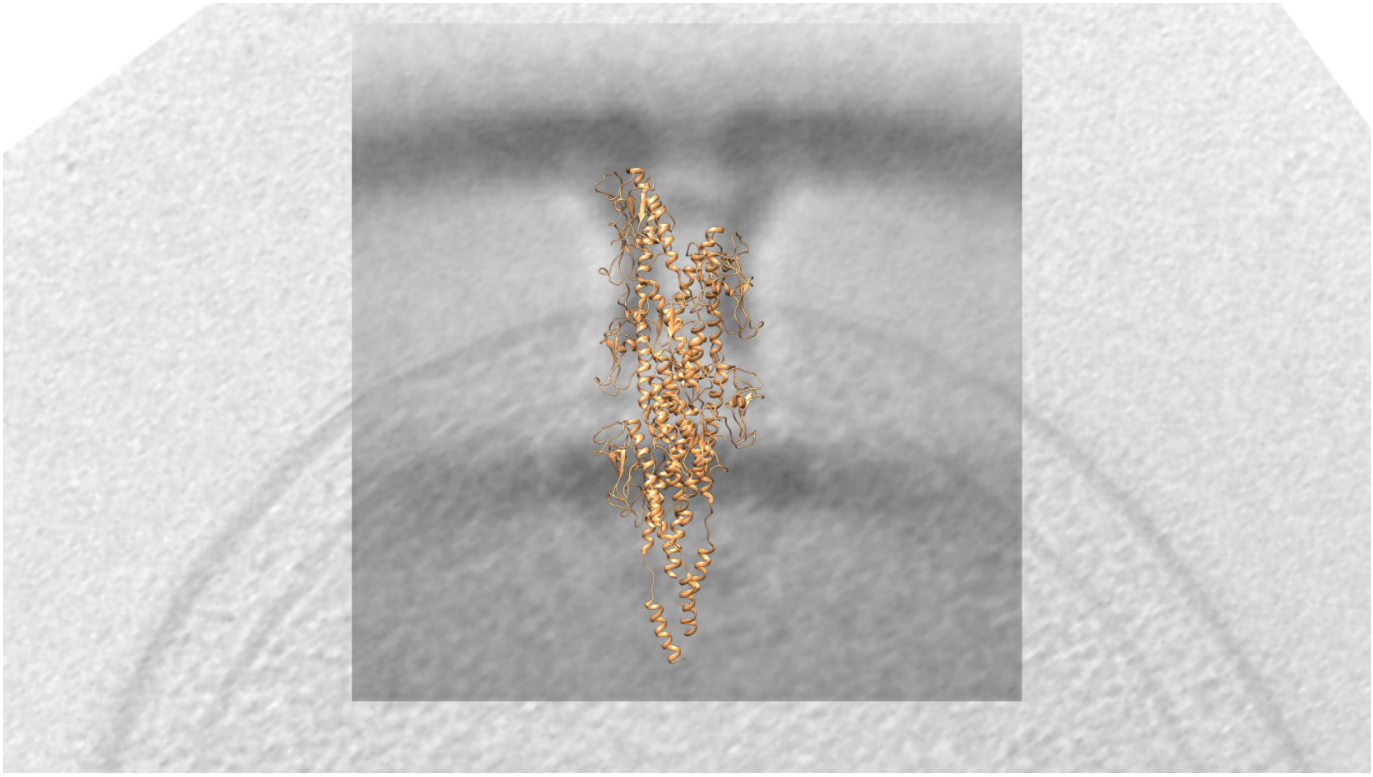
Type II secretion systems (**T2SSs**) in *Legionella pneumophila* like this pump out effector proteins that enable a complex pathogenic lifecycle. *L. pneumophila* live in several environments: inside amoebae, outside cells (but sometimes with others in biofilms), and inside *our* cells, where they cause the severe pneumonia known as Legionnaires' disease. In each environment, the T2SS pumps out proteins that facilitate growth there. For instance, once we inhale *L. pneumophila* with water droplets, they are internalized by our macrophages. They then use their T2SSs to secrete proteins that prevent their pockets in the cell (called *Legionella*-containing vacuoles) from being degraded, and suppress our innate immune response. This gives the bacteria time to replicate and establish a persistent colony. The T2SSs also secrete a toxin protein that breaks down lung tissue.

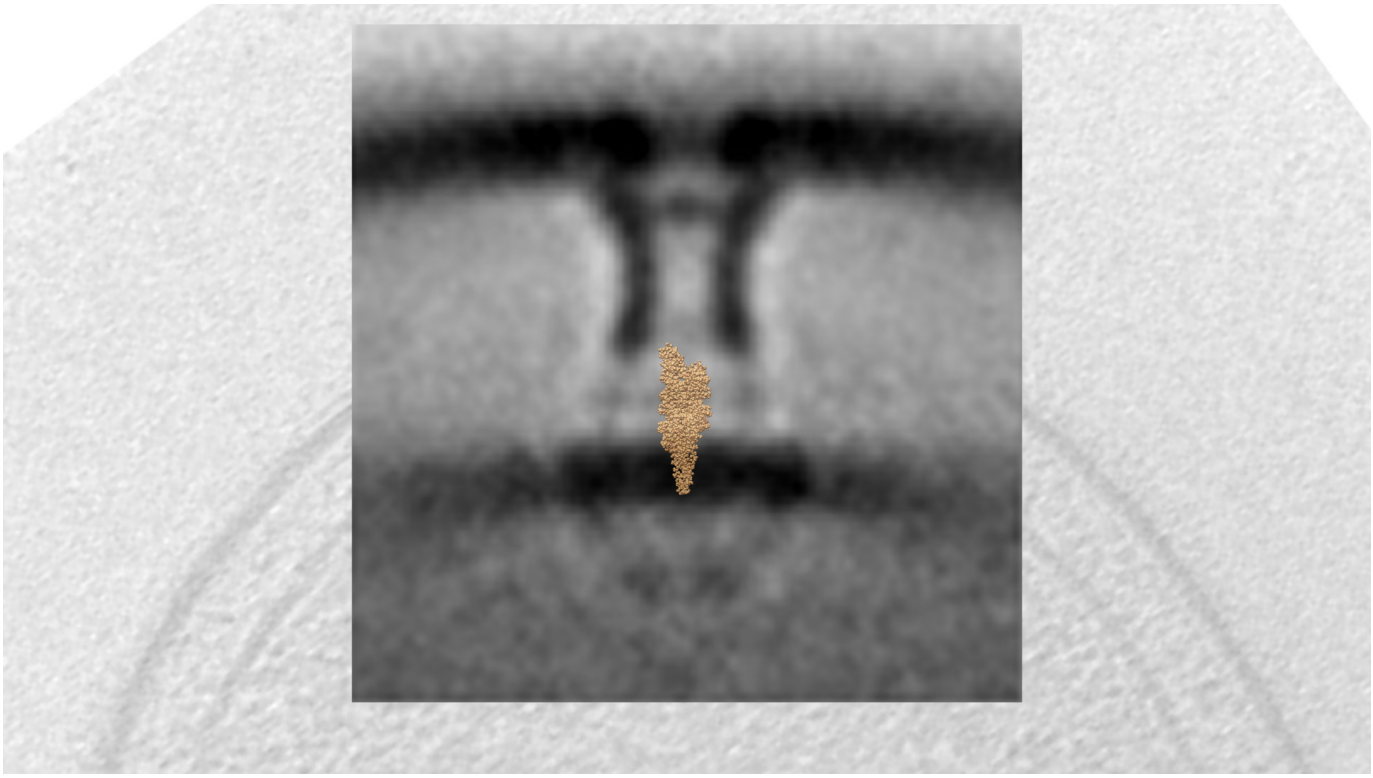
Unlike the secretion systems in Chapter 6, the T2SS does not build an extracellular appendage. Instead, it is thought to work by pumping proteins through the envelope channel using a short piston-like "pseudo-pilus" (*Learn More* on the next page), pushing the molecules into the extracellular space. (Keep in mind that for these intracellular pathogens, their extracellular space is the interior of the host cell.)

L. pneumophila also use another, unrelated machine, a **type IV secretion system** (**T4SS**), to deliver more than 400(!) different kinds of effector proteins into host cells to promote infection. You can see multiple inactive T4SSs in this cell (*Learn More* on following pages). We do not yet know what they look like during active secretion, so the mechanism remains unclear. In another human pathogen, *Helicobacter pylori*, inactive T4SSs are sometimes seen next to mysterious extracellular tubes, raising the intriguing possibility that the structures are somehow related (*Learn More* on following pages).

This *L. pneumophila* cell is not unusual; it is common for pathogenic bacteria to employ multiple types of secretion systems. The bristling arsenal of bacteria is a fearsome thing.

Legionella pneumophila Collected by: Debnath Ghosal DOI: 10.22002/D1.1573



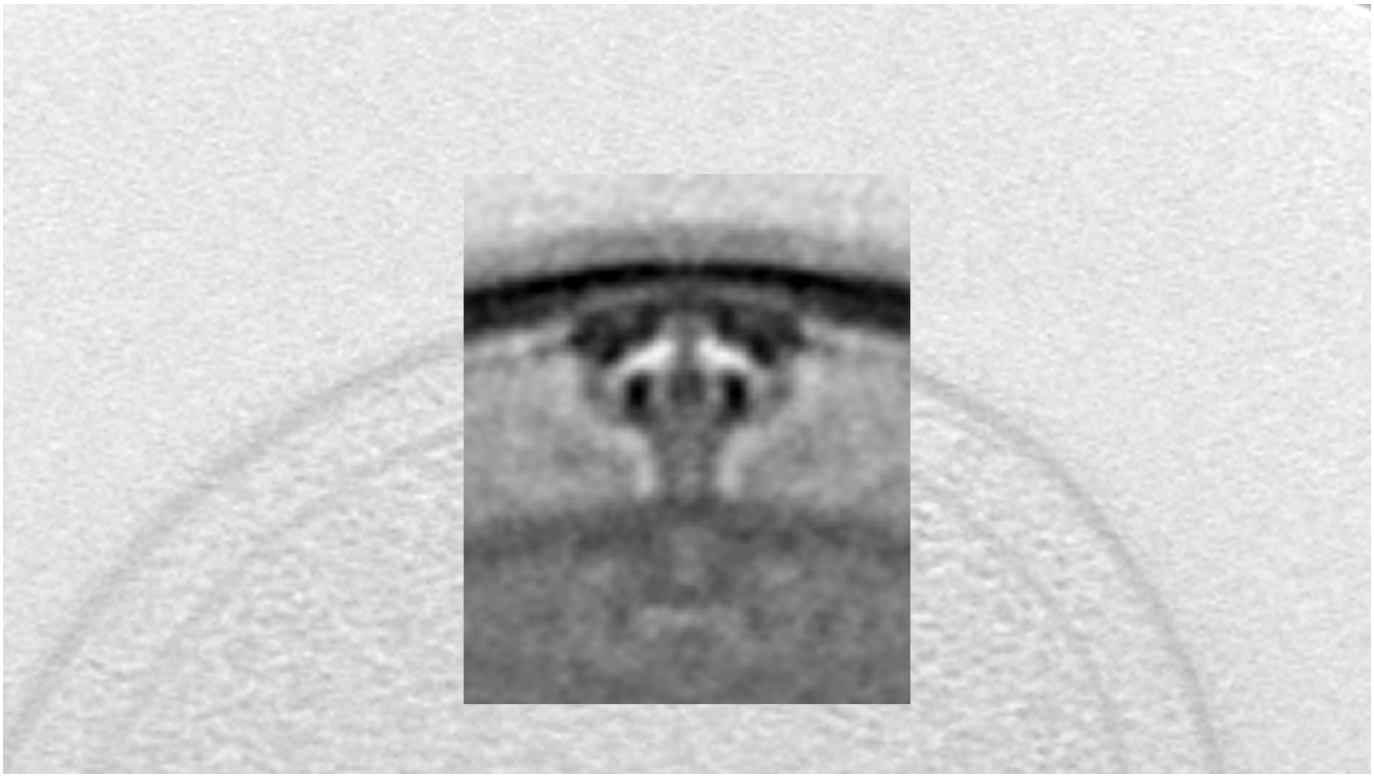


Learn More: T2SS

This average of type II secretion systems from many Legionella pneumophila *cells shows the overall structure: a channel through the cell envelope, gated near the outer membrane [80]. The pseudo-pilus, whose structure is shown here, can be seen in individual T2SSs, extending from the inner membrane to the bottom of the main channel. It gets washed out in the average, though, indicating that it is relatively flexible (i.e. not always in the same position). We still need to figure out exactly how the system works, including whether the pseudo-pilus does act like a piston, and how the gate opens.*

Source: Ghosal et al. (2019)

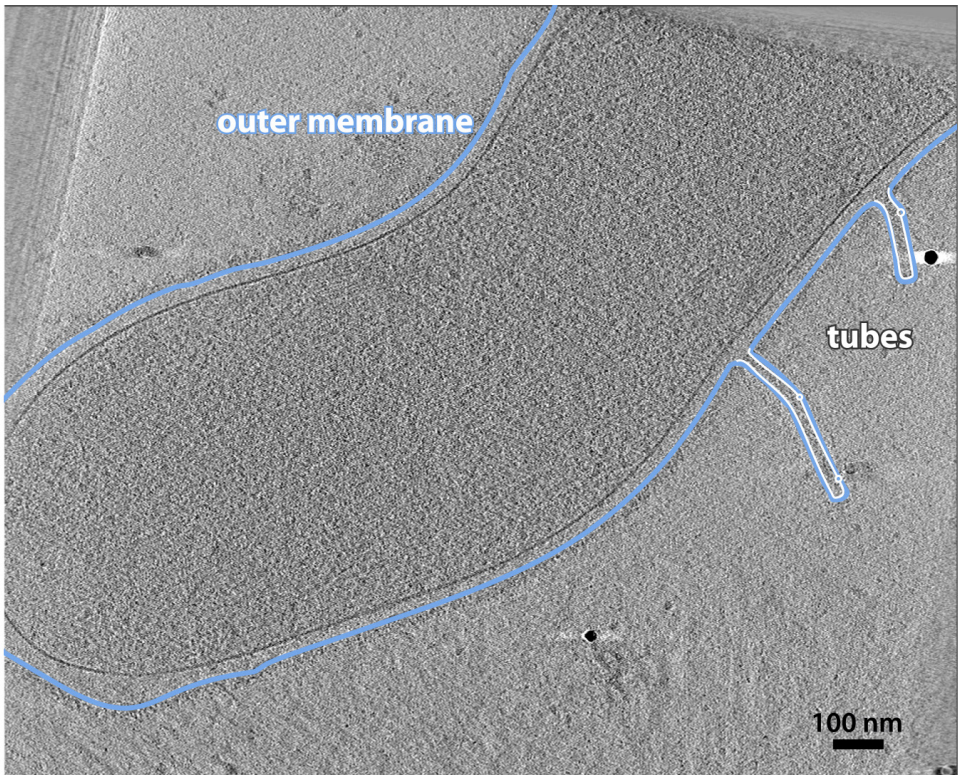
Structure: EMD-20712, EMD-20713, PDB 5WDA

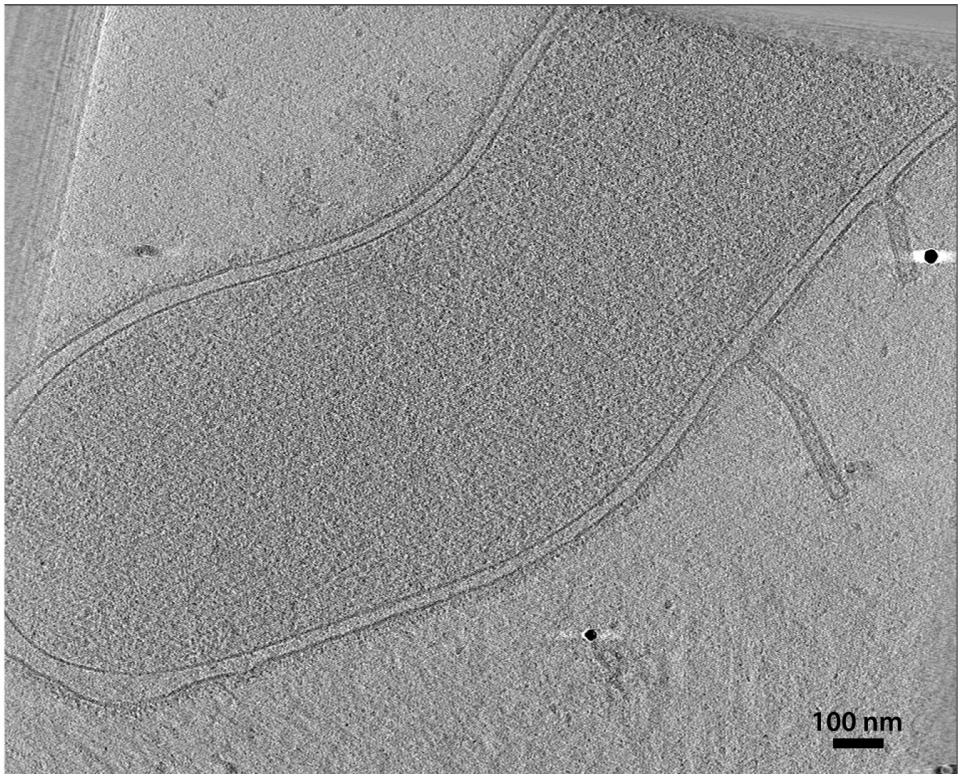


Learn More: T4SS

This average of type IV secretion systems from many Legionella pneumophila *cells shows the overall structure [81]. (You can watch a more detailed version of the animation on YouTube: https://youtu.be/mJk9xNdoTzQ.) We do not yet know how secretion works, but one possibility is that effectors are transported either straight through the channel from the cytoplasm and/or first into the periplasm and from there into the windowed chamber just inside the outer membrane before finally being escorted out of the cell.* Animation by Janet Iwasa.

Source: Ghosal et al. (2019) *Structure: EMD-0566*

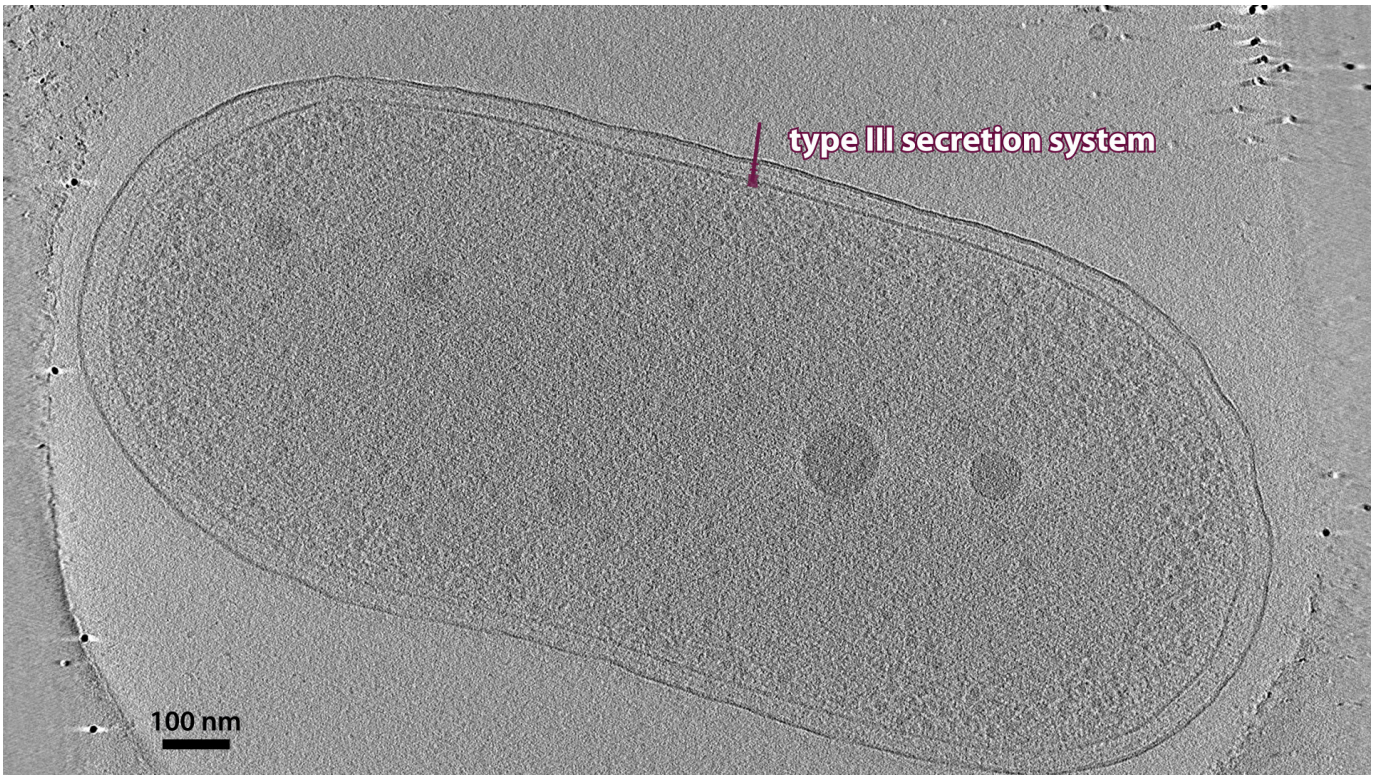

outer membrane
tubes
100 nm

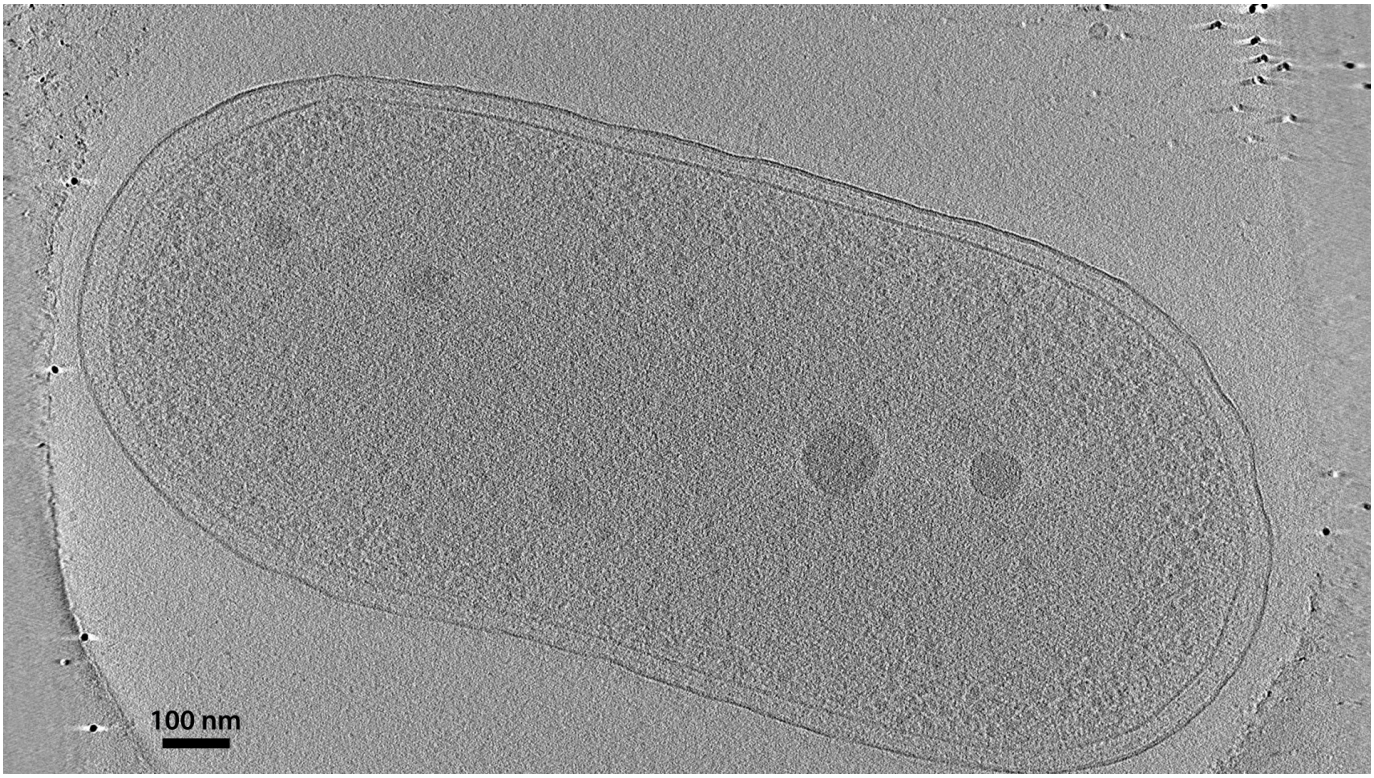

100 nm

Learn More: Tubes

This Helicobacter pylori *cell was cultured together with human gastric epithelial cells, its target for infection. It contains inactive type IV secretion systems, as well as several mysterious tubes. The tubes are formed from the outer membrane, but we do not know what scaffolds them (they have a consistent diameter of 37 nm and a helical protein scaffold inside), or what makes the portals along their length. The fact that we sometimes see tubes near inactive T4SSs suggests a relationship, but the details are unclear. Much remains mysterious about this structure and its potential role in infection.*

Helicobacter pylori *Collected by: Yi-Wei Chang* *DOI: 10.22002/D1.1582*


type III secretion system
100 nm


100 nm

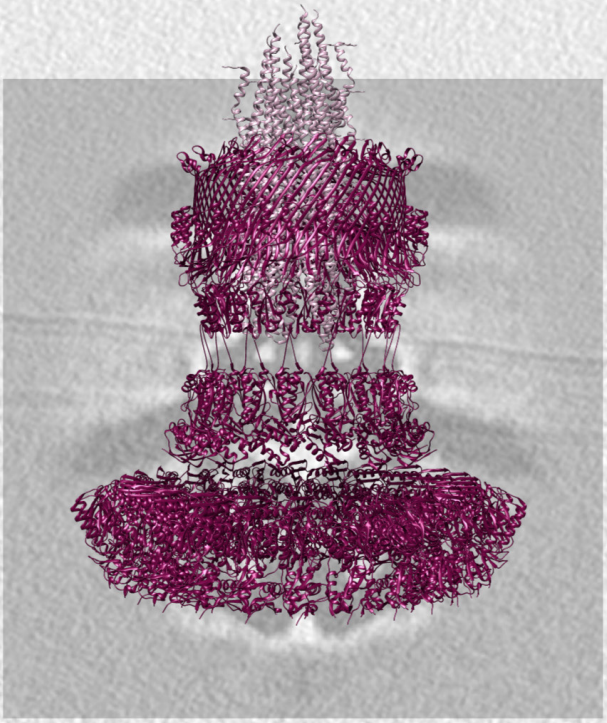
4. Type III Secretion

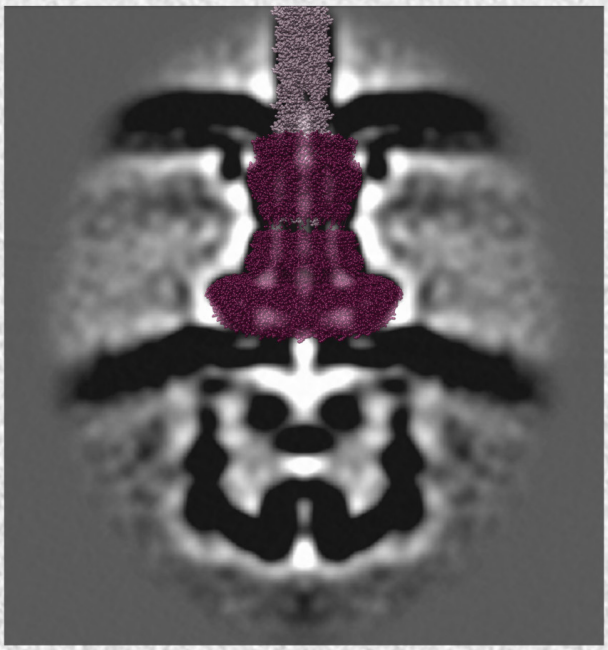
What if your cell wanted to go a step further and deliver toxins directly into its target? Perhaps it needs a syringe. One of the most common, and best-studied, pathogenic secretion systems is the type III secretion system (**T3SS**) that assembles a needle-like toxin delivery system called the **injectisome**. T3SS injectisomes and flagellar motors are closely related, and the basal machines have similar structures (*Learn More* on the next page). Rather than assembling a long helical filament for motility, though, injectisomes form a shorter needle, which you can see on this *Pseudomonas aeruginosa*. The needle can penetrate a eukaryotic cell, delivering effectors directly into its cytoplasm that block the immune response of the host, and lead to cell lysis. (You can watch an animation of this process on YouTube: https://youtu.be/OBf64TEo7gA.)

Note: It would be wasteful, or detrimental, for your cell to secrete toxins all the time. Pathogens typically have multi-stage lifecycles, often passing through an intermediate host and/or the environment. Pathogenic machinery like secretion systems are typically only produced when conditions signal that the cell has reached its target (e.g. increased temperature or salt concentration). Until then, the genes encoding the protein components are turned off. This cell is missing a protein (ExsD) that turns off expression of these injectisome components. As a result, the strain makes injectisomes even outside the host, enabling us to image their structure.

T3SSs are closely related to type II secretion systems (T2SSs) and share a common element: the channel embedded in the outer membrane, formed by many copies of a protein called a **Secretin** (*Learn More* on following pages). The rest of the machine looks very different, though, reflecting their evolution into separate mechanisms.

Pseudomonas aeruginosa Collected by: Steven Wang DOI: 10.22002/D1.1574



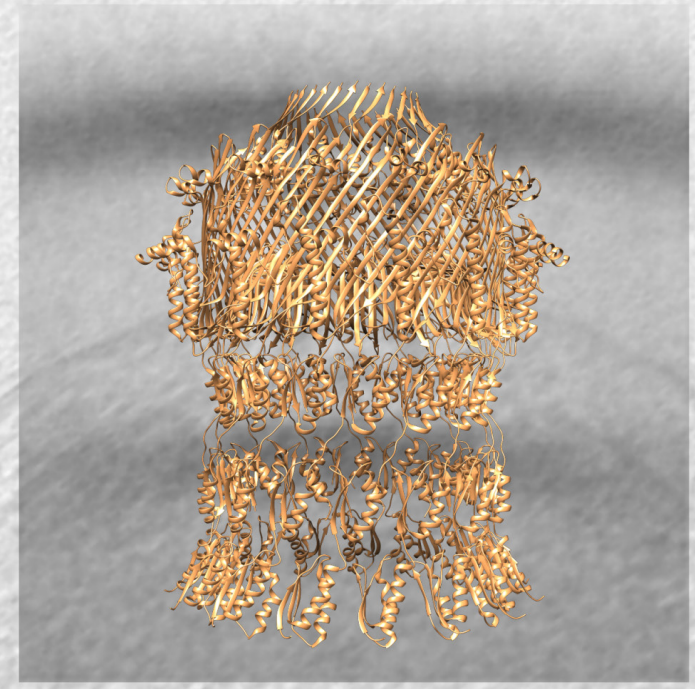


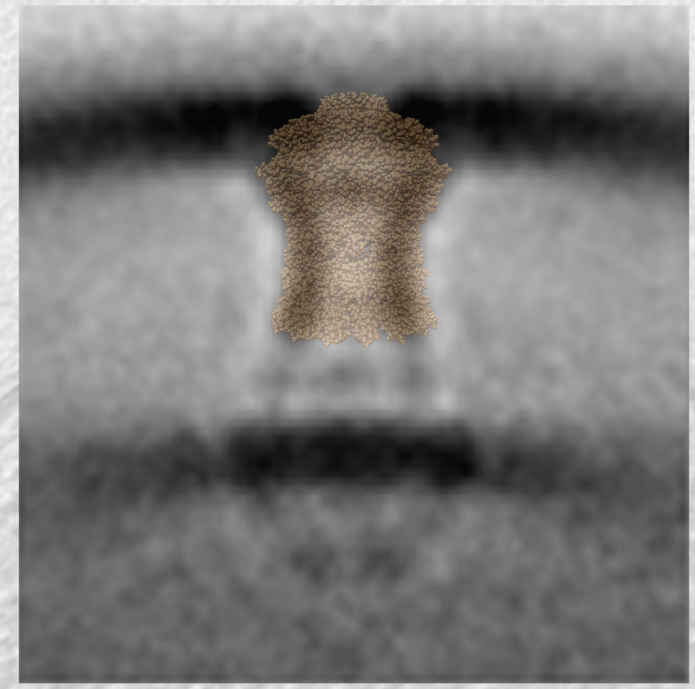
Learn More: Structure

This average of T3SS injectisomes from many Shigella flexneri *cells shows the overall structure, including the large "sorting platform" in the cytoplasm which selects proteins for secretion through the channel of the needle, whose atomic structure you see here [82].*

Source: Tachiyama et al. (2019)

Structure: EMD-20611, PDB 5TCR



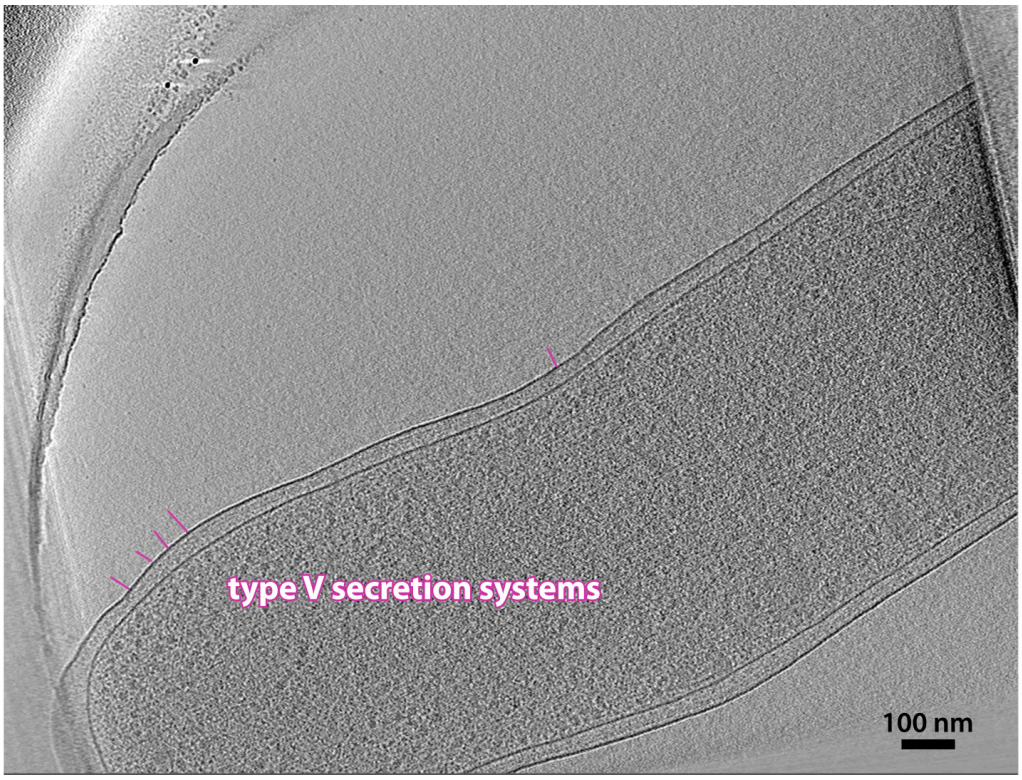


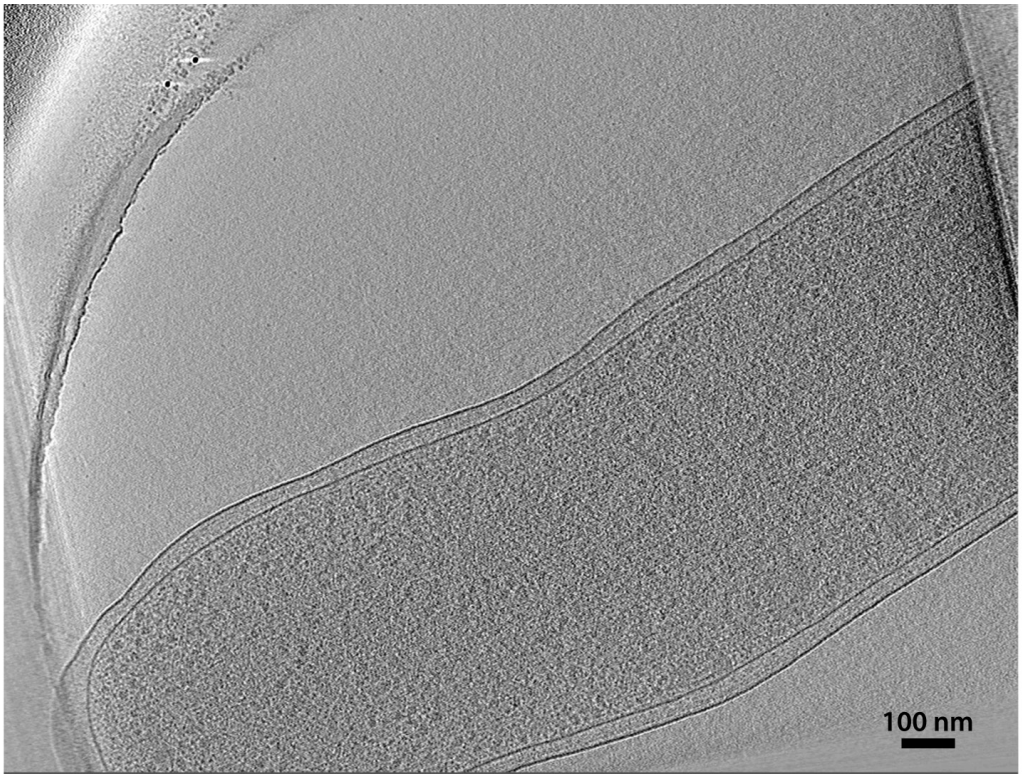
Learn More: Secretin Channel

Many copies of a Secretin protein come together to form a channel, as in this one from a Vibrio cholerae *type II secretion system [83]. In addition to the main channel embedded in the outer membrane, additional domains of the protein form a series of rings in the periplasm that interact with other components of the system. The Secretin channel forms the core of both type II and type III secretion systems, showing that they evolved from the same ancestral machine, adding and repurposing other components over time to serve their different functions.*

Source: Yan et al. (2017)

Structure: PDB 5WQ8


type V secretion systems
100 nm


100 nm

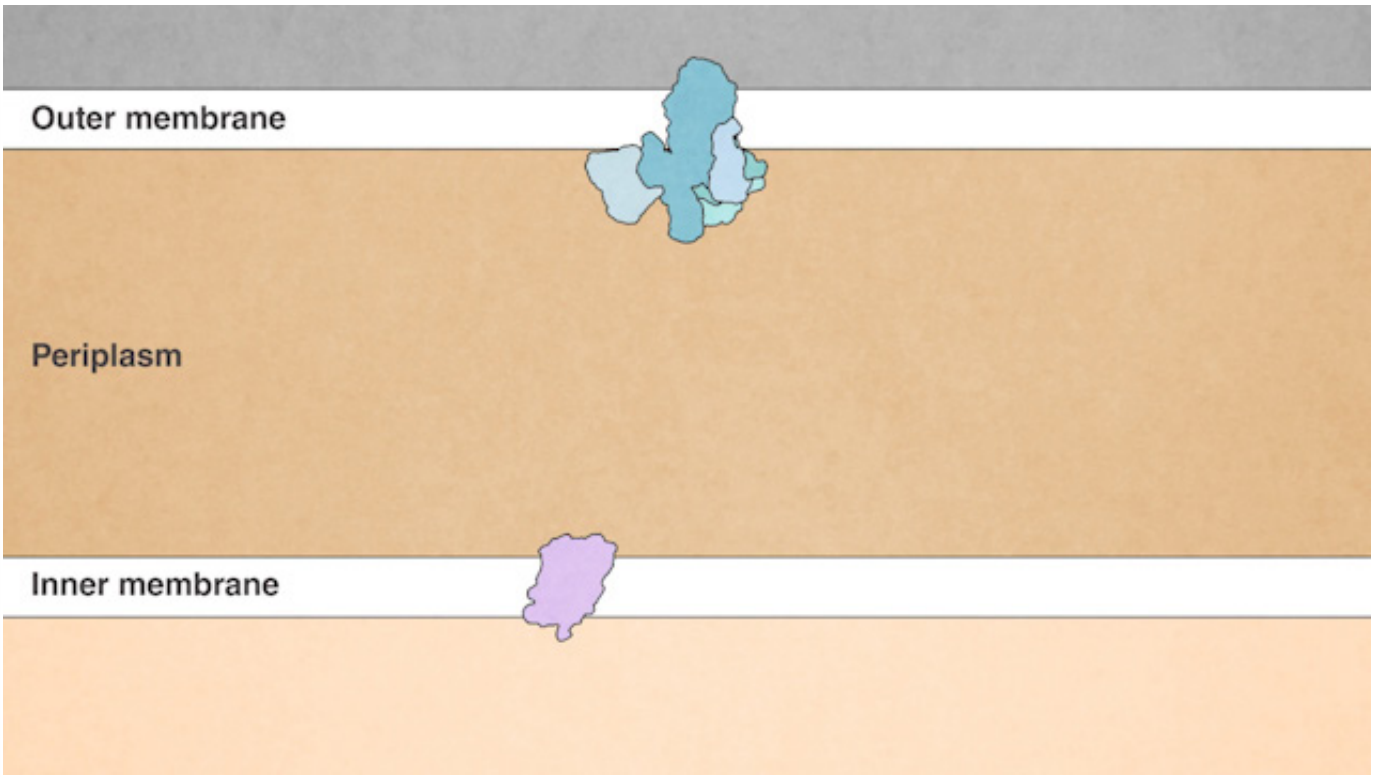
5. Type V Secretion

Other secretion systems independently evolved similar mechanisms, though using very different structures. Some strains of *Escherichia coli* have an ability, called "Contact-Dependent Inhibition" or **CDI**, to inhibit the growth of neighboring diderm bacterial cells. They do this by coopting a pore in the outer membrane of the target cell to introduce a toxin that halts its growth. To safeguard themselves, they make an antitoxin that binds to the toxin and renders it harmless. Such antidote systems are common for microbe-produced toxins.

To deliver the toxin to the target, the cells use **type V secretion systems** (**T5SSs**) like the ones on this *Escherichia coli*. In contrast to the nanomachines you have been seeing so far, which contain dozens of unique components, this system is elegantly spare, using just two proteins and a mechanism reminiscent of a medieval ball-and-chain flail (*Learn More* on the next page).

Note: This cell belongs to a strain that is normally incapable of CDI, but has been genetically engineered to produce the T5SS components. Once again, this lets us image the machines more easily; in this case, the CDI-practicing strain has a thicker layer of extracellular proteins, which would obscure the needles in a visual haystack.

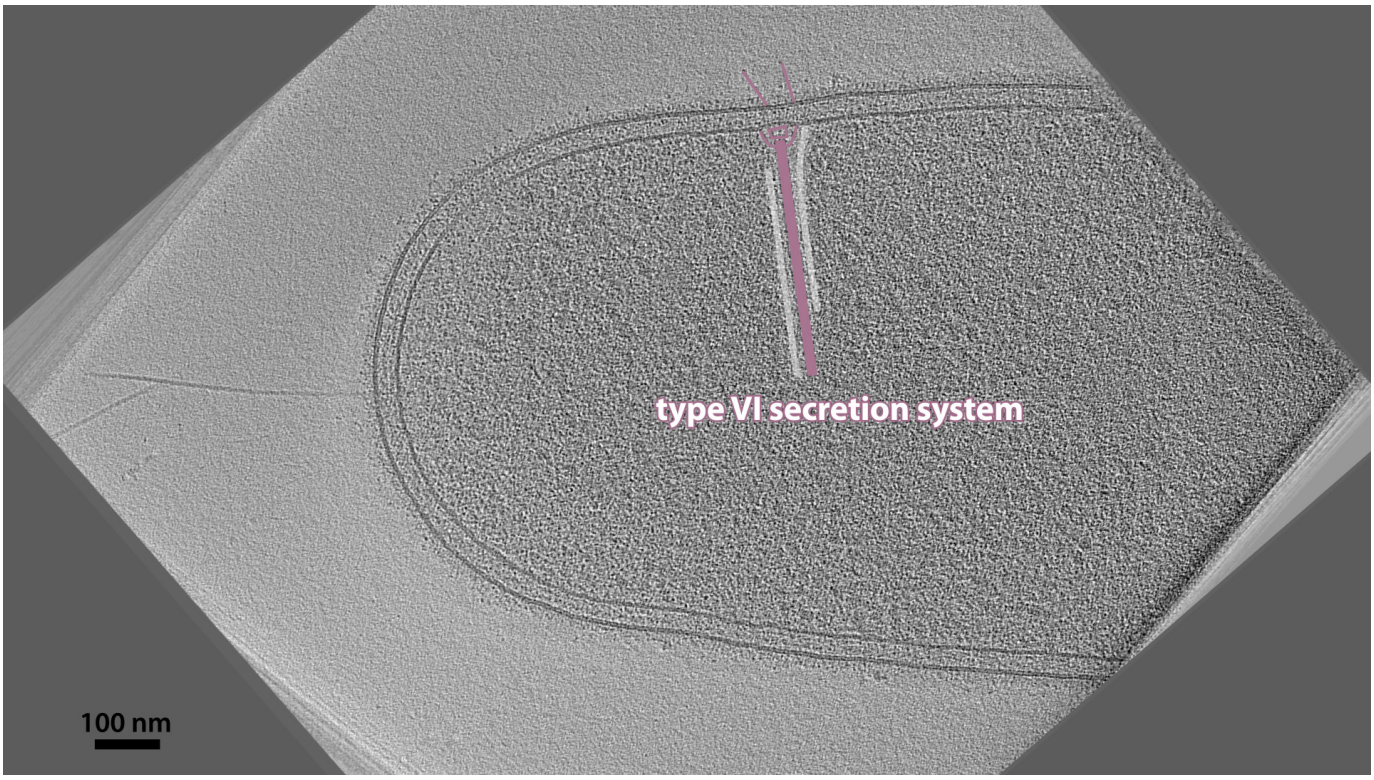
Escherichia coli Collected by: Poorna Subramanian DOI: 10.22002/D1.1575

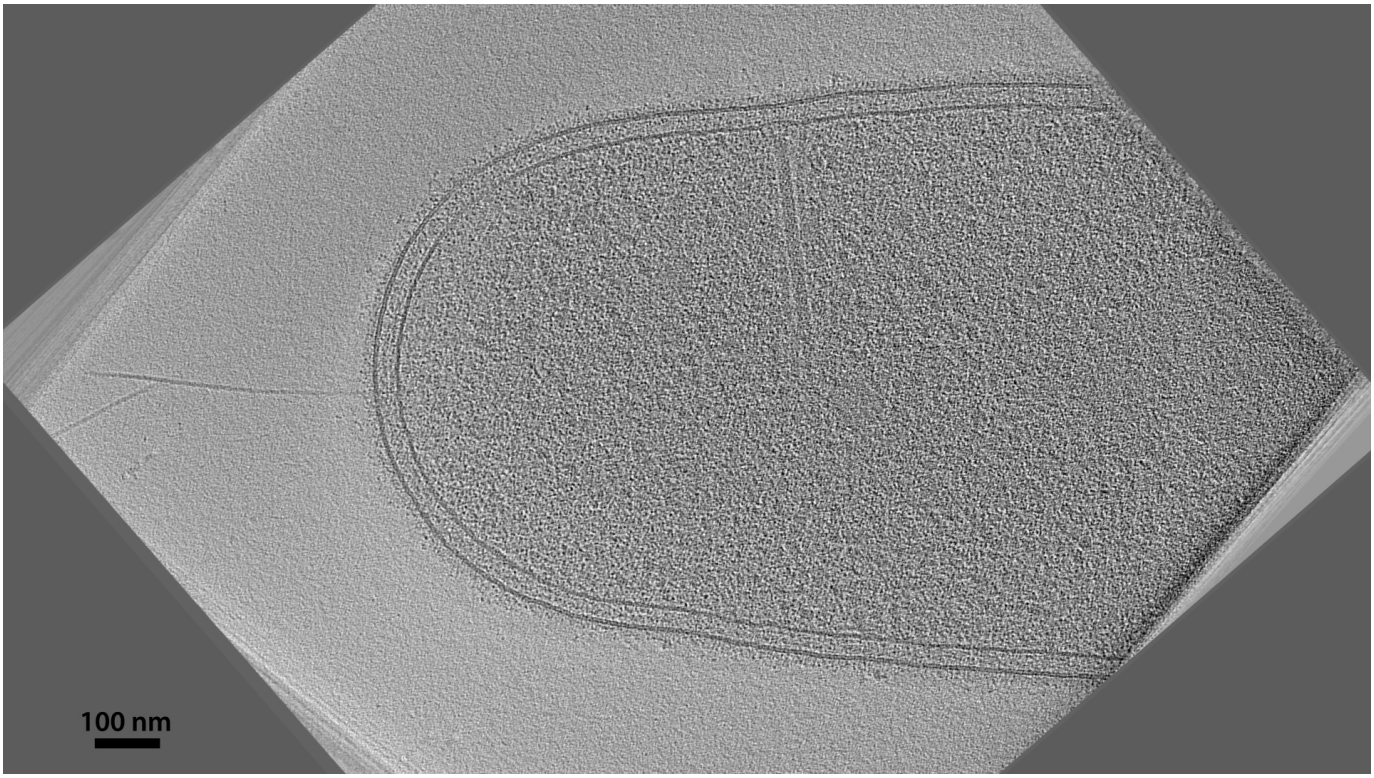

Outer membrane
Periplasm
Inner membrane

Learn More: Mechanism

The type V secretion system is a minimal machine: a channel formed by several copies of one protein (CdiB) in the outer membrane secretes another protein, CdiA. Or rather, it secretes part of it. The first half of CdiA forms the rigid stick you see extending from the cell. The second half loops back to keep the toxin domain at the end of the protein sequestered in the periplasm, awaiting secretion. The tip of the stick (which is the middle of the protein) contains the domain that recognizes and docks to the receptor pore on the target. When it bumps into a neighboring cell and locks on to the receptor, the rest of CdiA is secreted and delivers the toxin into the other cell like a tetherball [84]. (You can watch the full animation of this process on YouTube: https://youtu.be/KomlYC7Q11w). Animation by Janet Iwasa.

Source: Ruhe et al. (2018)


type VI secretion system
100 nm

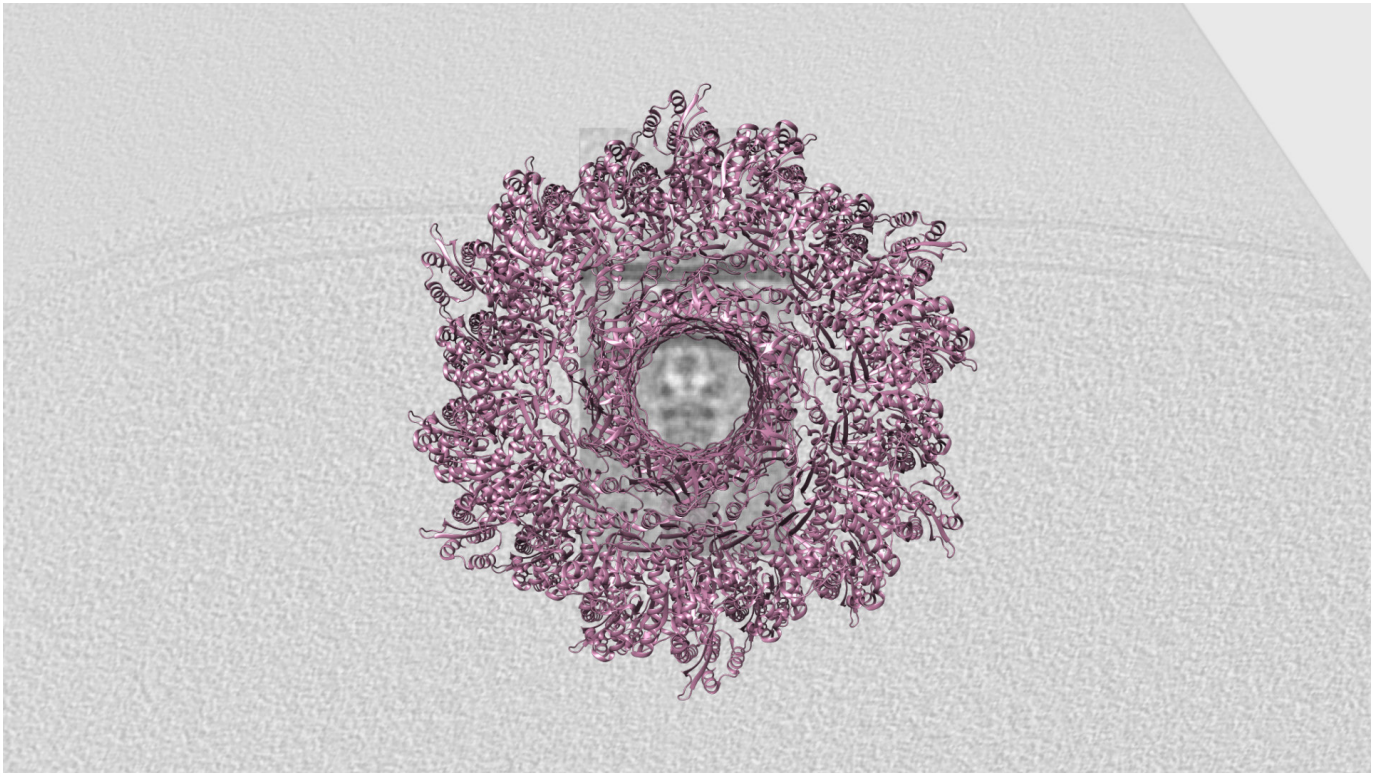

100 nm

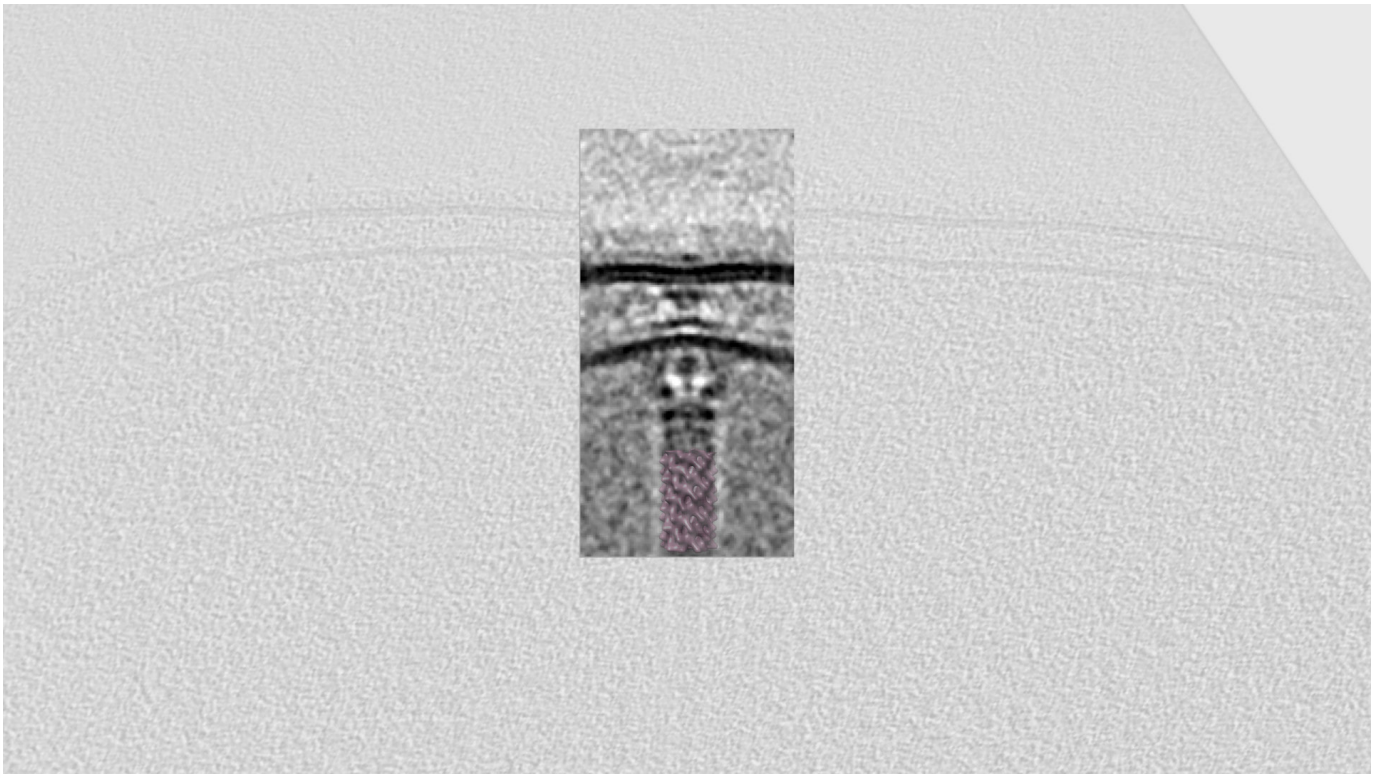
6. Type VI Secretion

How else could your cell deliver a toxin to a nearby competitor? As usual, nature has provided another, even more entertaining, option: the poison dart gun. Many diderm bacteria, including *Myxococcus xanthus* like this, use **type VI secretion systems** (**T6SSs**) to launch effector proteins into neighboring cells. The effectors vary, from toxins that cause lysis to factors that promote biofilm formation. The target range of the T6SS is similarly broad, including monoderm and diderm bacteria and eukaryotic cells.

This loaded T6SS consists of a hollow outer tube, with a narrower dart inside (*Learn More* on the next page). The effector protein is loaded at the tip of the dart, where the machine is anchored in the cell envelope. In a few cells, including this one, we see additional filaments flanking the primed T6SS. They are lacking in other cells even of the same species, and their identity and function remain unknown.

Myxococcus xanthus Collected by: Yi-Wei Chang DOI: 10.22002/D1.1576



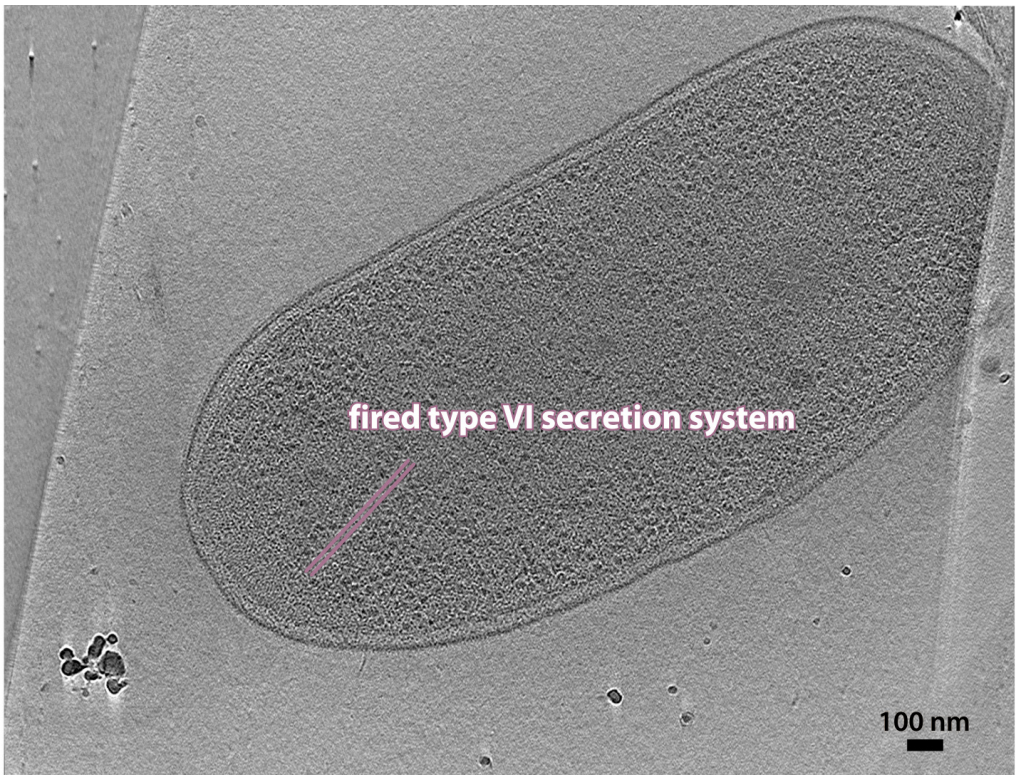


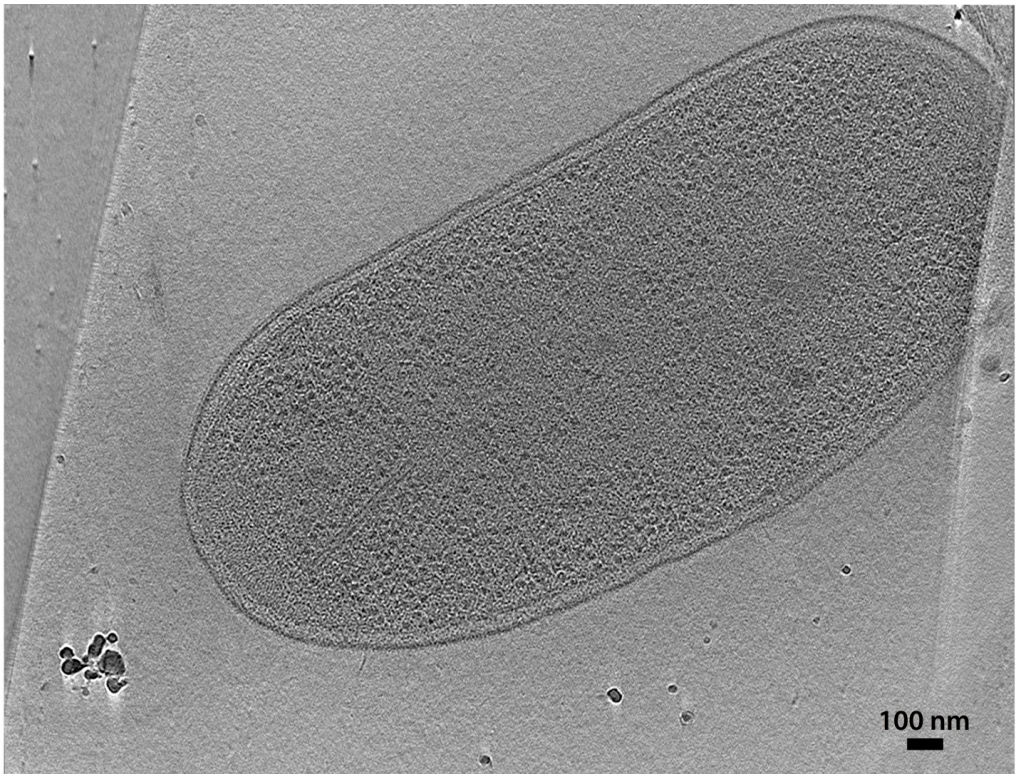
Learn More: Loaded T6SS

In a loaded T6SS, the projectile rod nests inside the contractile sheath, as in this section of a T6SS from Myxococcus xanthus *[85].*

Source: Chang et al. (2017)

Structure: PDB 5URW


fired type VI secretion system
100 nm

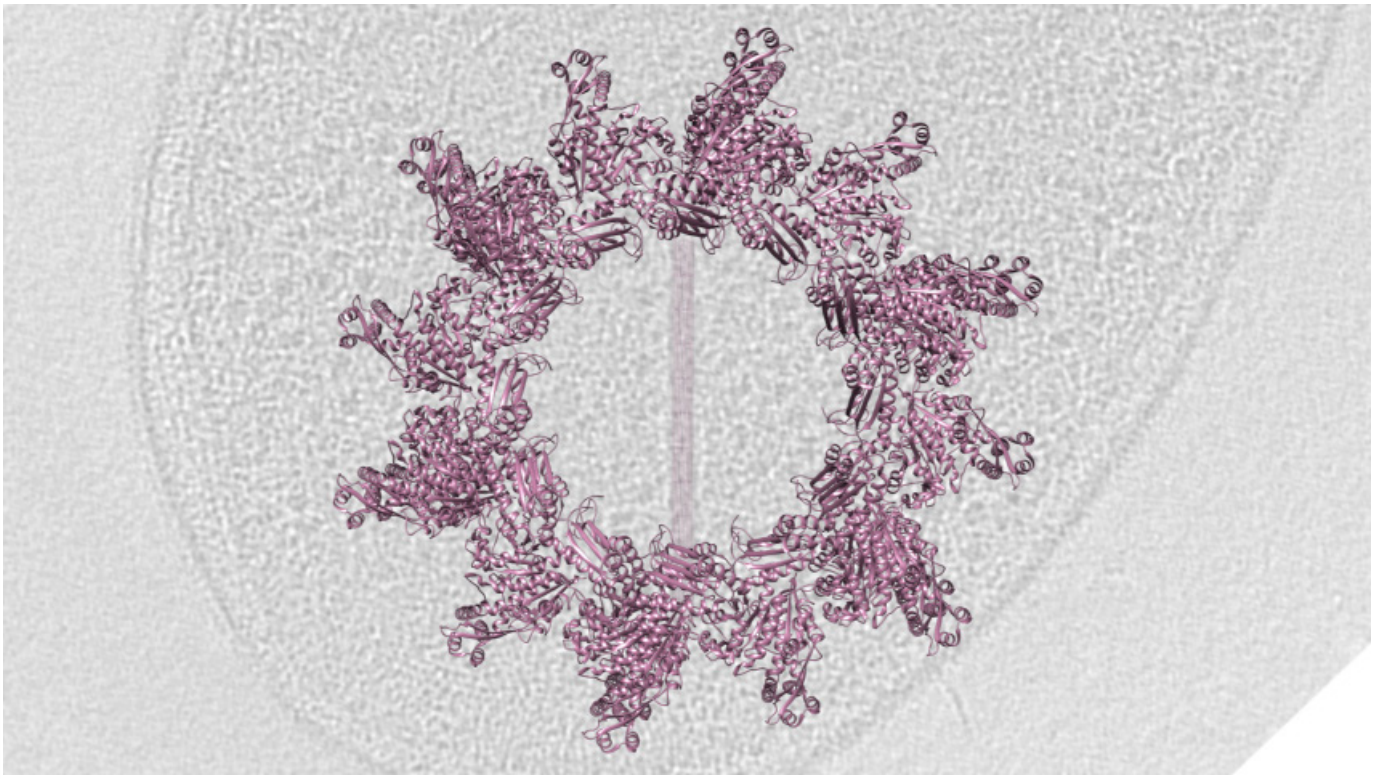

100 nm

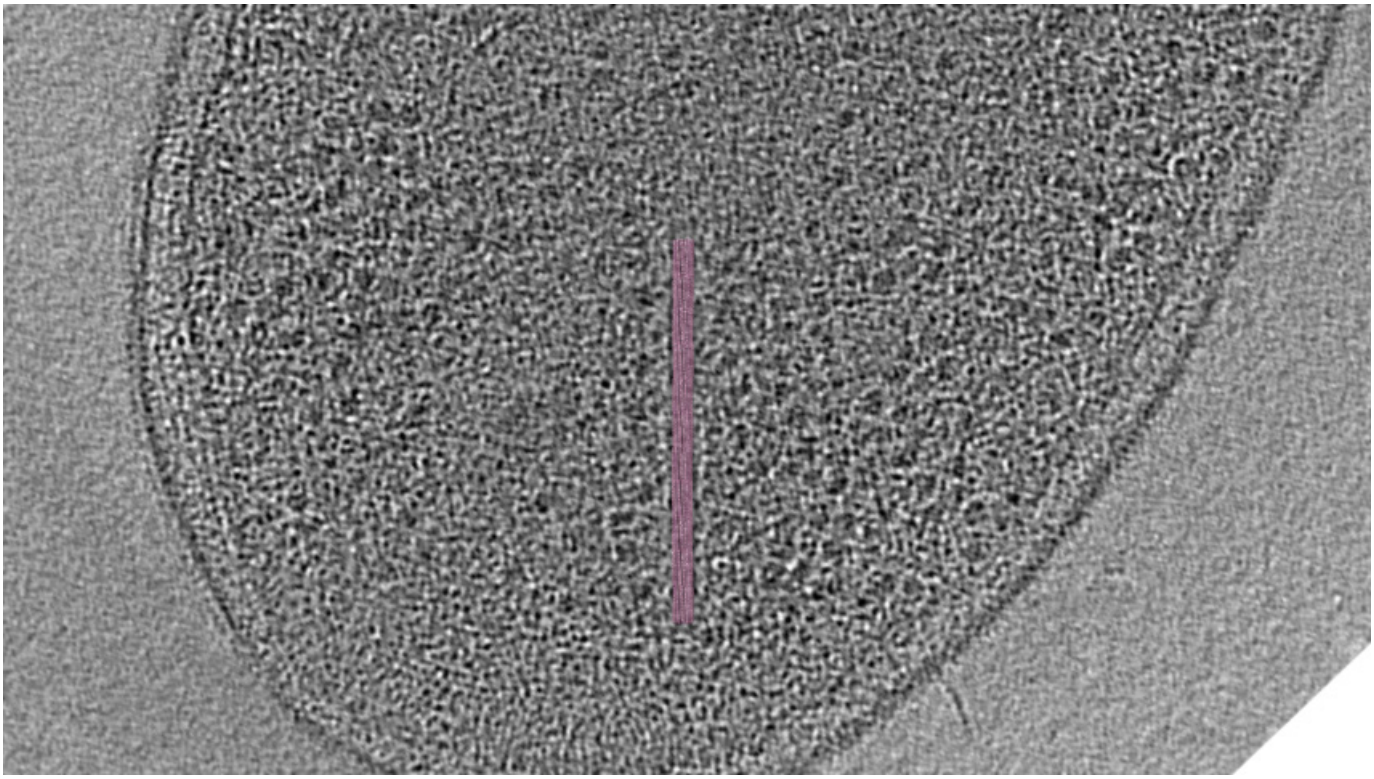
7. Contractile Weapons

When the T6SS fires, the helical outer sheath twists into a shorter, wider form, a conformational change that provides the energy to explosively propel the inner dart, cargo-first, out and into its target (*Learn More* on the next page). In some species, like this *Vibrio cholerae*, the outer sheath will then be recycled, broken down into building blocks that can be used to rapidly assemble a new machine. Other species build again from scratch, translating new protein building blocks. Warfare is dynamic, with cells rapidly assembling and firing T6SSs. Cells have even been observed to engage in T6SS duels, with an attacked cell building and firing a T6SS back in the direction from which it was hit. (You can see fluorescence microscopy of these duels on YouTube: https://youtu.be/nP4Ou2eoq4c.) Some species assemble batteries of parallel T6SSs (*Learn More* on following pages).

These contractile weapons evolved from the same ancestor as the "tail" structure viruses called phage use to inject their genetic material into bacteria and archaea (more on that in the next chapter). Some related bacterial weapons are even closer to the phage tail, in that they are released to the environment in a primed state, waiting to bump into a target cell to fire. The target is recognized by filamentous proteins called "tail fibers," the same identification mechanism used by phage. Other members of this evolutionary family are even stranger, like the Morphogenesis-Associated Contractile structures (**MACs**) released by a marine bacterium (*Learn More* on following pages).

Vibrio cholerae Collected by: Martin Pilhofer DOI: 10.22002/D1.1577



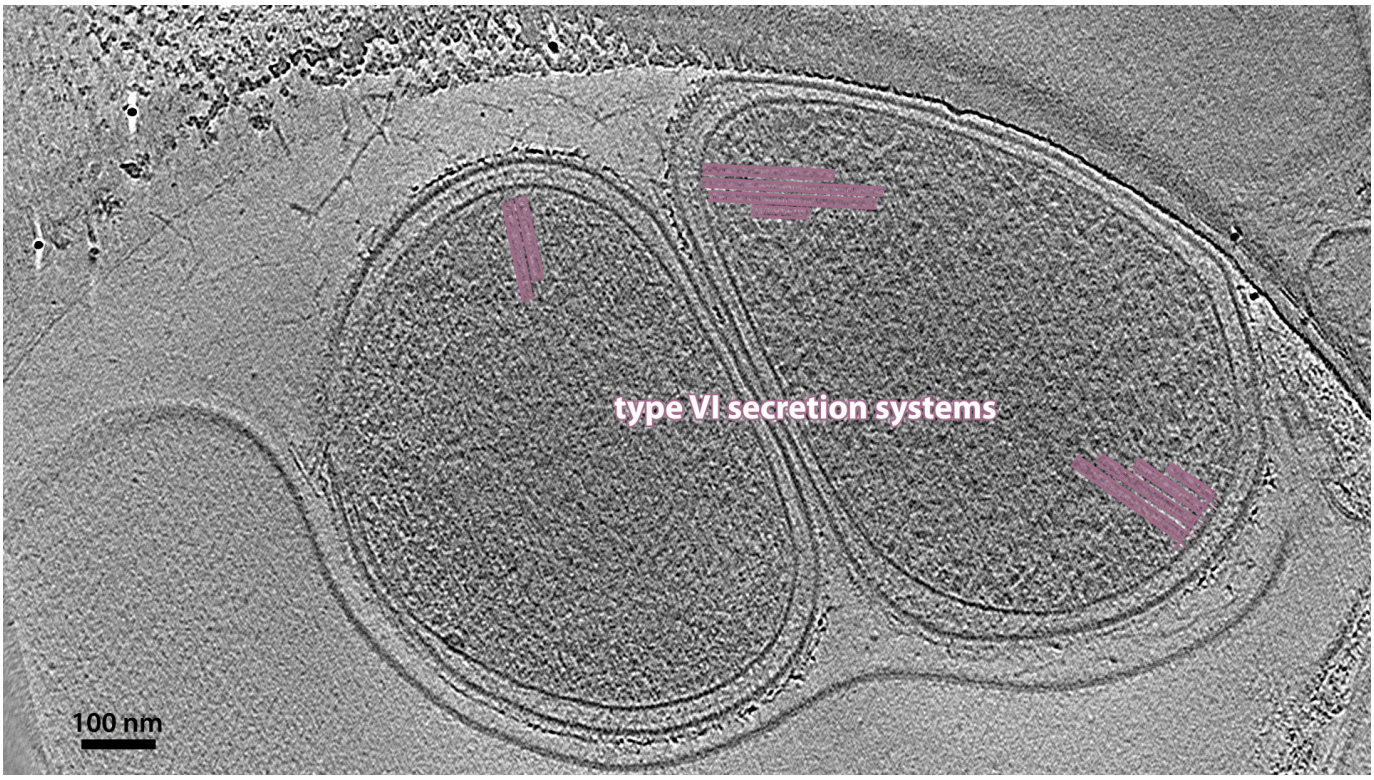


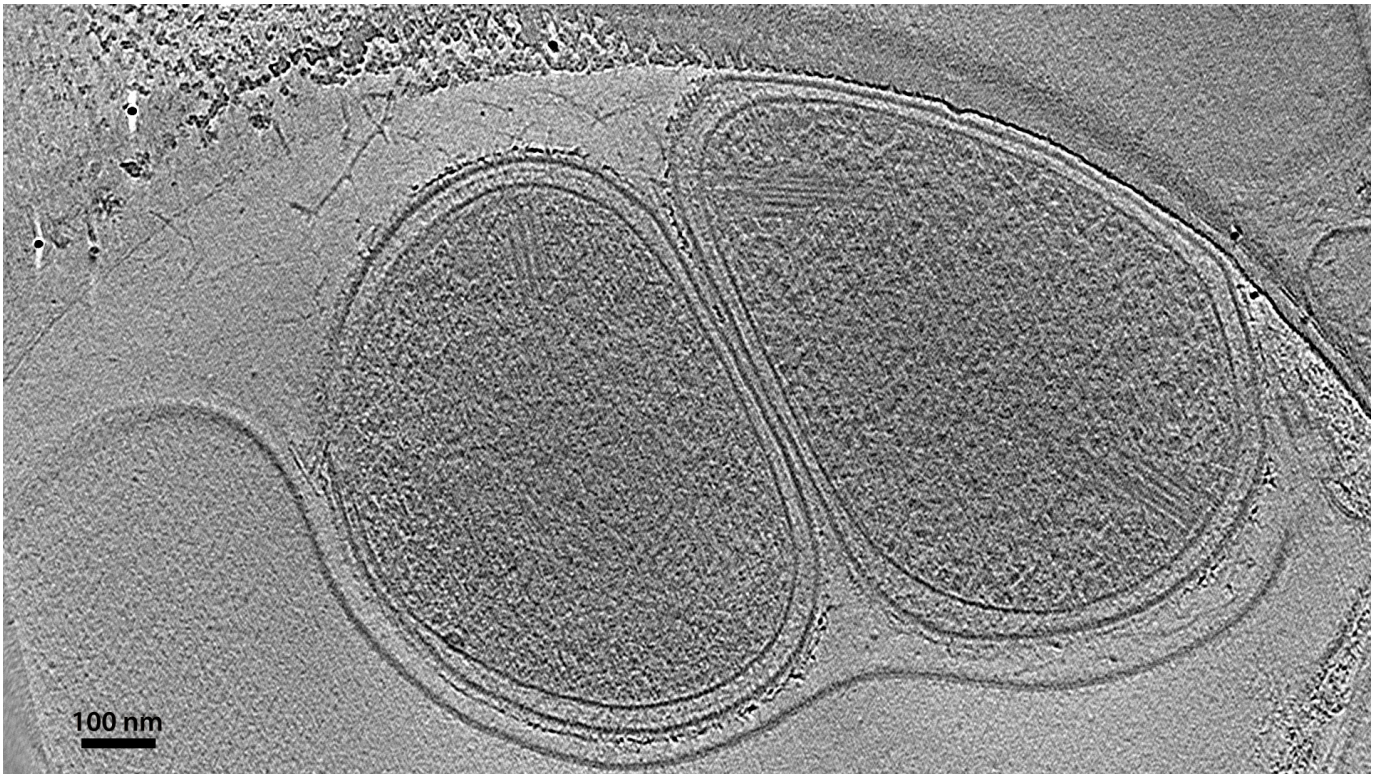
Learn More: Fired T6SS

A conformational change in the T6SS sheath provides the energy to fire the inner rod toward its target. The remaining sheath is shorter and wider than in its primed state, as in this section of a fired T6SS from Myxococcus xanthus *[85]. (You can watch an animation of the firing process on YouTube: https://youtu.be/Xk7rxv6HtKo.)*

Source: Chang et al. (2017)

Structure: PDB 5URX

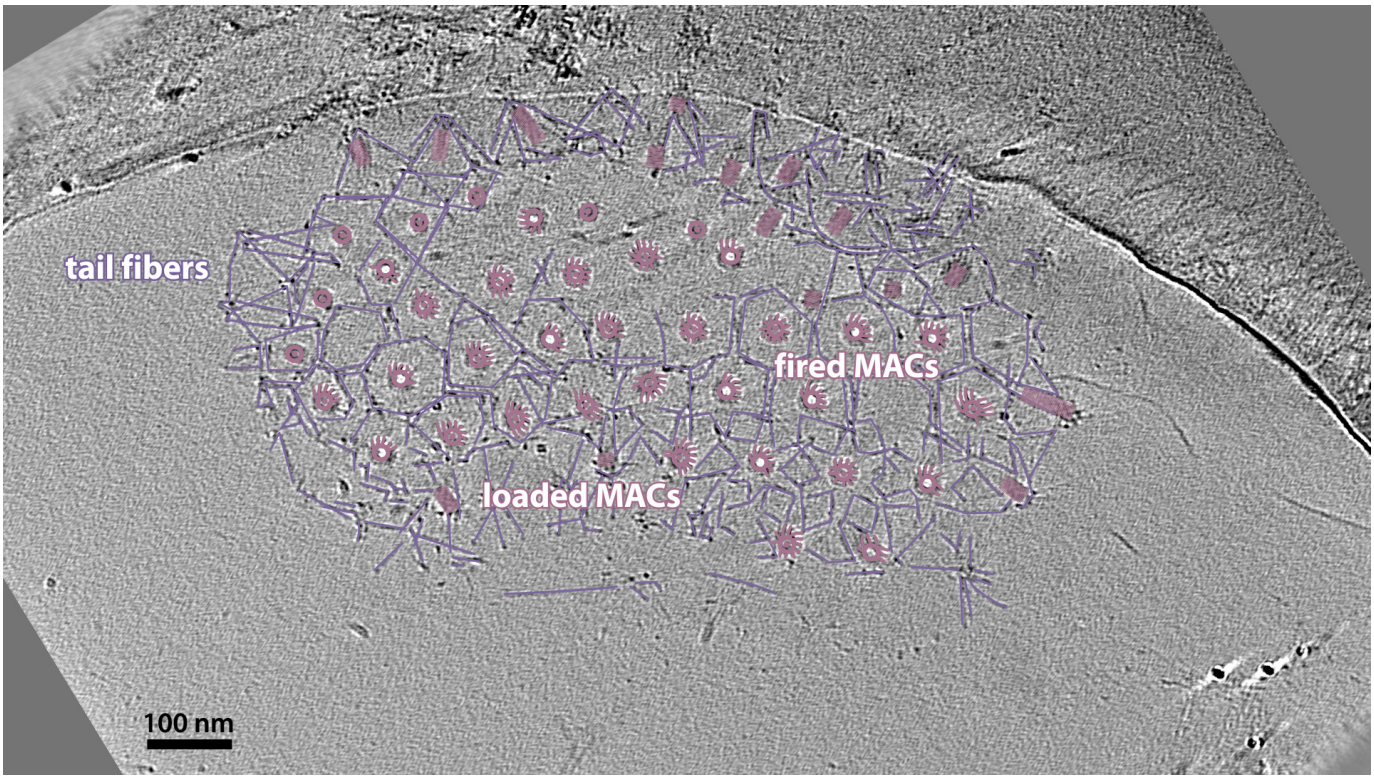

type VI secretion systems
100 nm

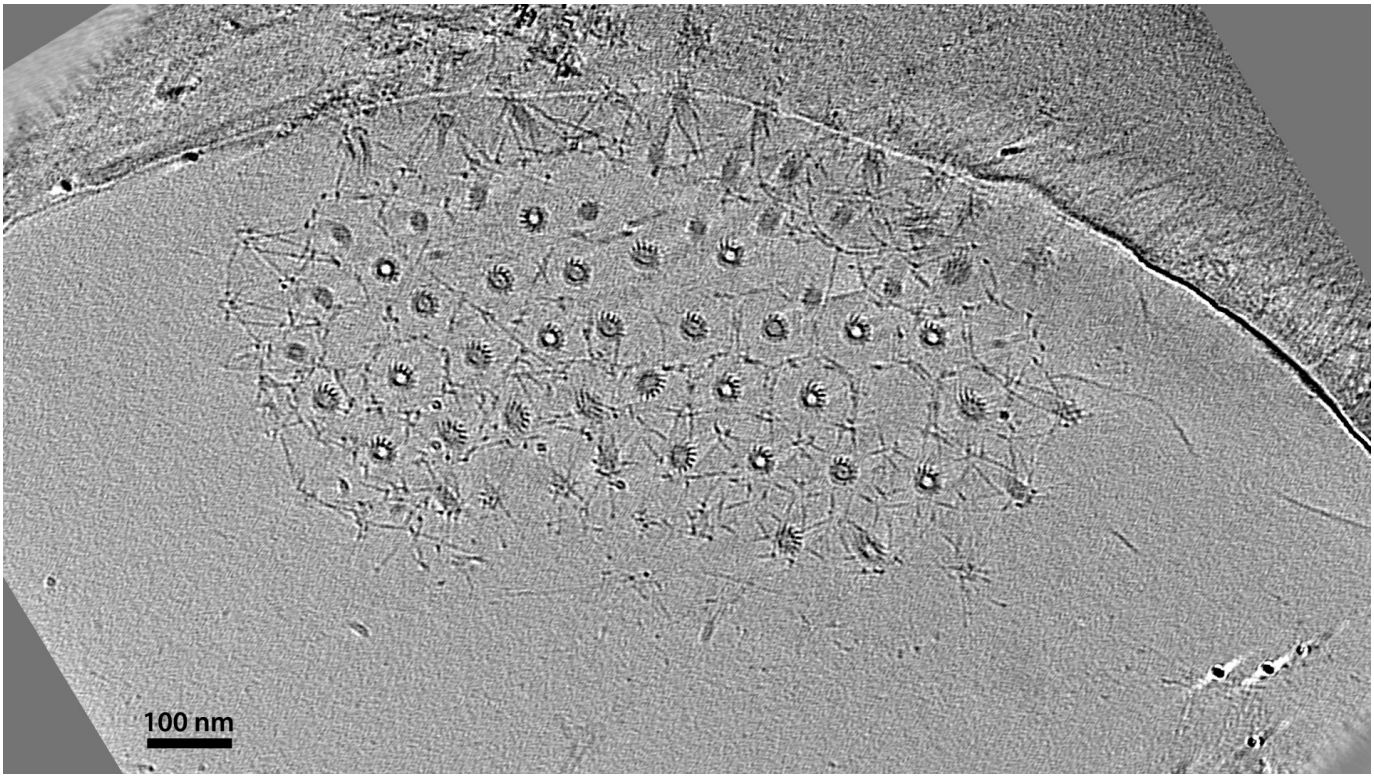

100 nm

Learn More: Battery

Some cells assemble a few, scattered type VI secretion systems. Other species, like this Amoebophilus asiaticus *build ordered arrays of parallel machines. These diderm bacteria live symbiotically inside amoebae. Their T6SS batteries help them colonize their host, perhaps by rupturing the membrane of the host phagosome that encloses them after they are engulfed, releasing them into the host cytoplasm. The number of T6SSs in each array varies, but can reach a few dozen.*

Amoebophilus asiaticus *Collected by: Martin Pilhofer* *DOI: 10.22002/D1.1583*


tail fibers
fired MACs
loaded MACs
100 nm

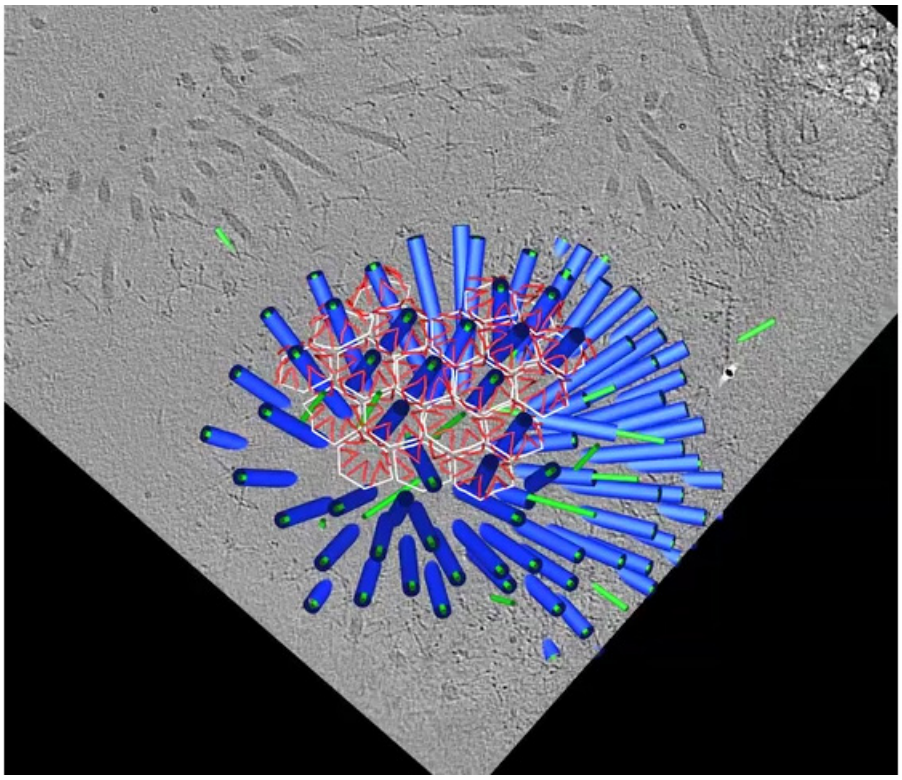

100 nm

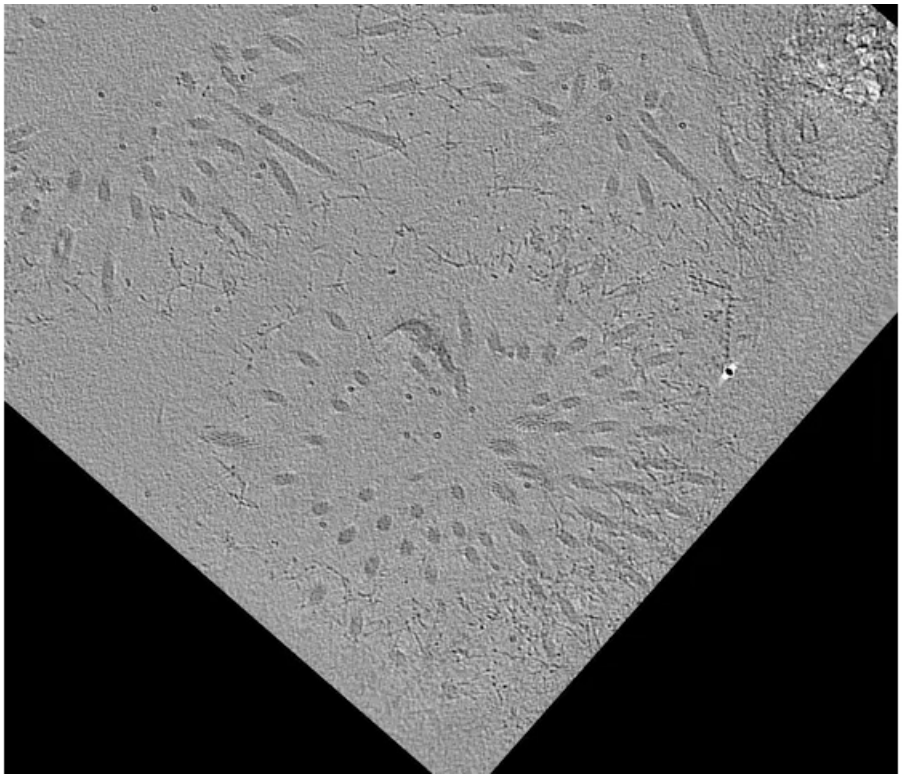
Learn More: MACs

Pseudoalteromonas luteoviolacea *produce mysterious, massive arrays of MACs, contractile machines related to T6SSs. Cells lyse to release the arrays, like the one you see here, which can contain 100 individual MACs and extend more than 1 μm across. The arrays are highly ordered, with the tail fibers of the MACs networked into a hexagonal array that may help synchronize firing (*Learn More *on the next page). MAC arrays serve an important function for larvae of the marine tubeworm* Hydroides elegans*, and likely the larvae of other invertebrates such as sea urchins and corals as well. They signal to the larva that it is the right place to settle down and differentiate into a sessile (surface-attached) adult. The function of MAC arrays for the bacterium is less clear. It may be that the invertebrates benefit the lysed cell's surviving relatives in the biofilm. Or perhaps the MACs play another, completely unrelated role.*

Note: This MAC array was produced by a cell lacking genes encoding other contractile weaponry (type VI secretion systems and a related machine called a bacteriocin). This was done to make it easier to identify the MACs.

Pseudoalteromonas luteoviolacea *Collected by: Martin Pilhofer* *DOI: 10.22002/D1.1584*

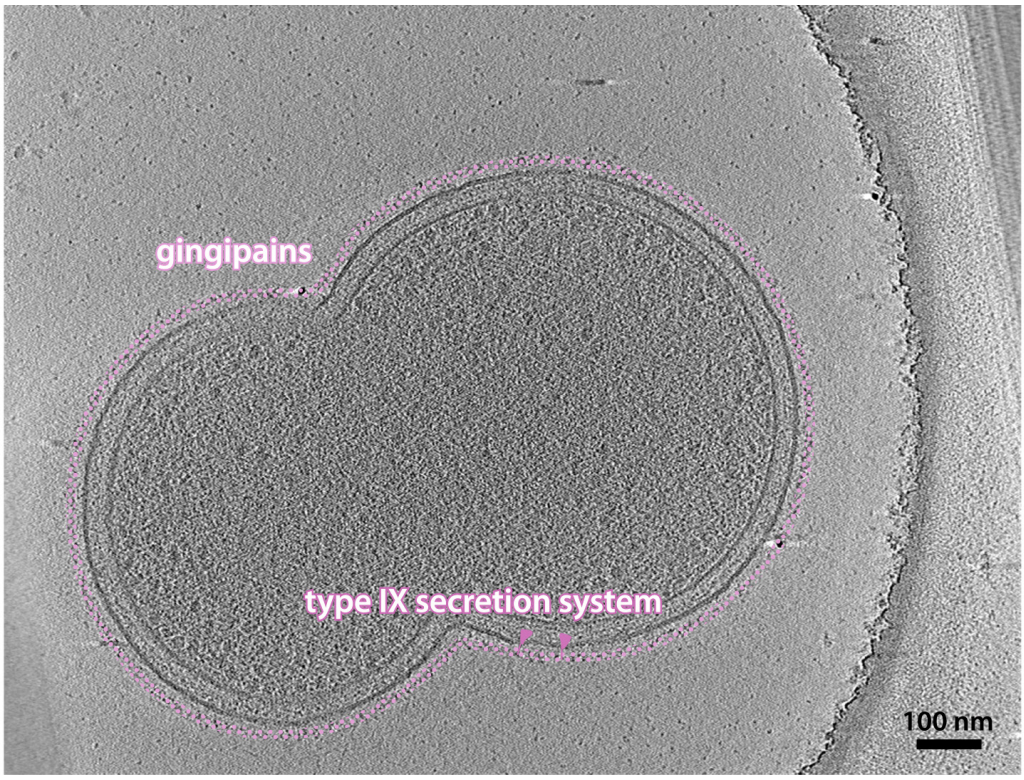


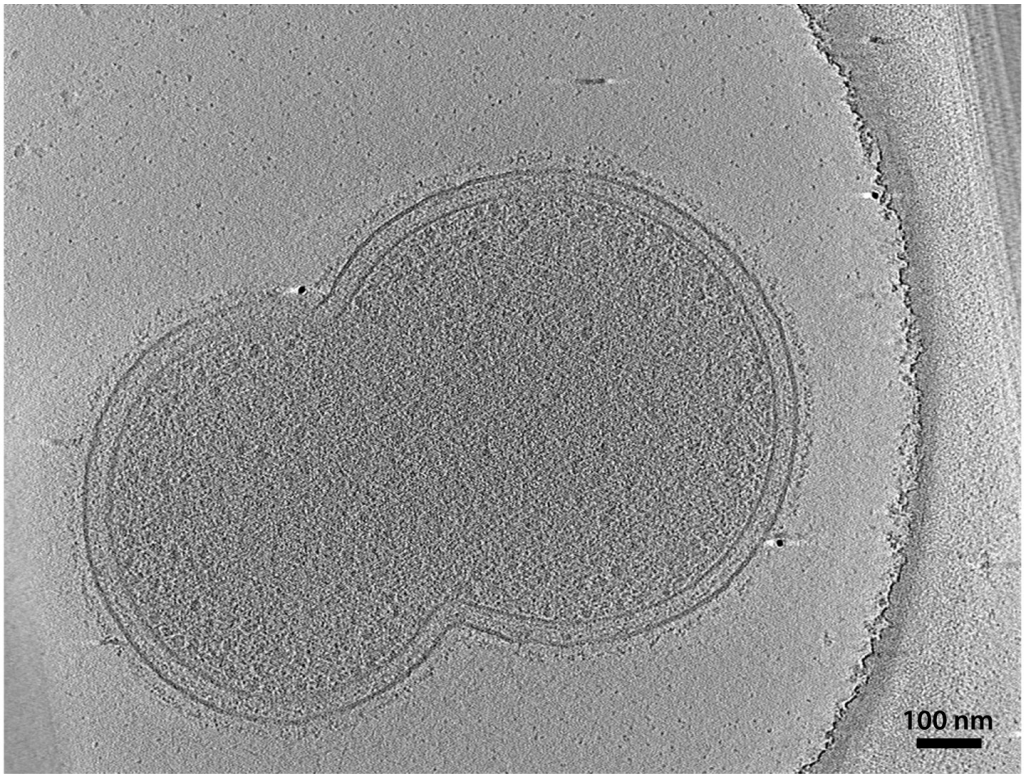


Learn More: Arrays

This digital segmentation of a MAC array shows the overall order of the structure [86]. Outer sheaths are in blue, inner rods in green, tail fibers in orange, and networking fibers in white.

Source: Shikuma et al. (2014)


gingipains
type IX secretion system
100 nm

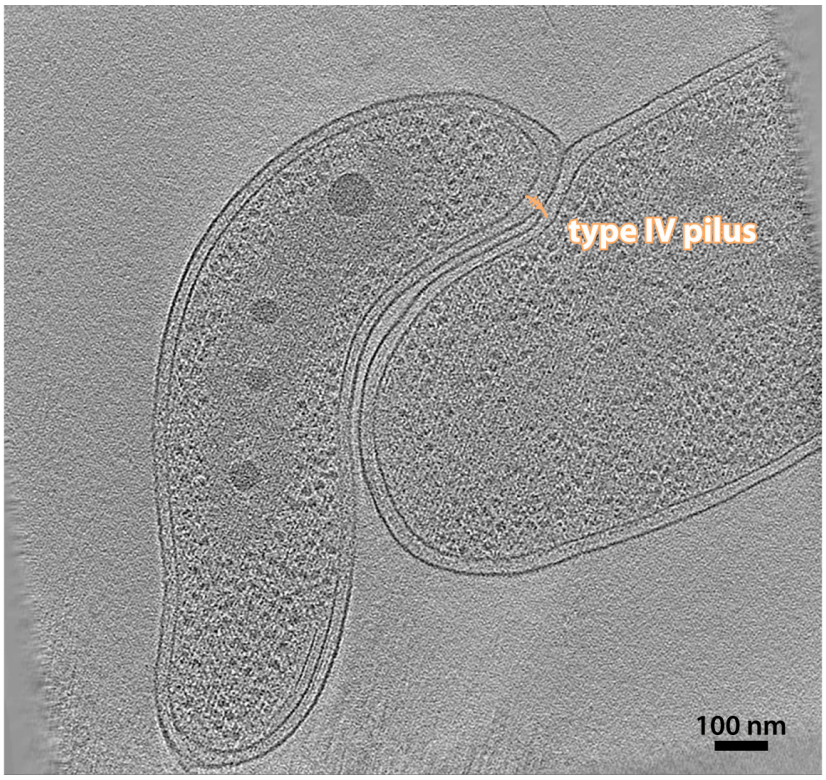

100 nm

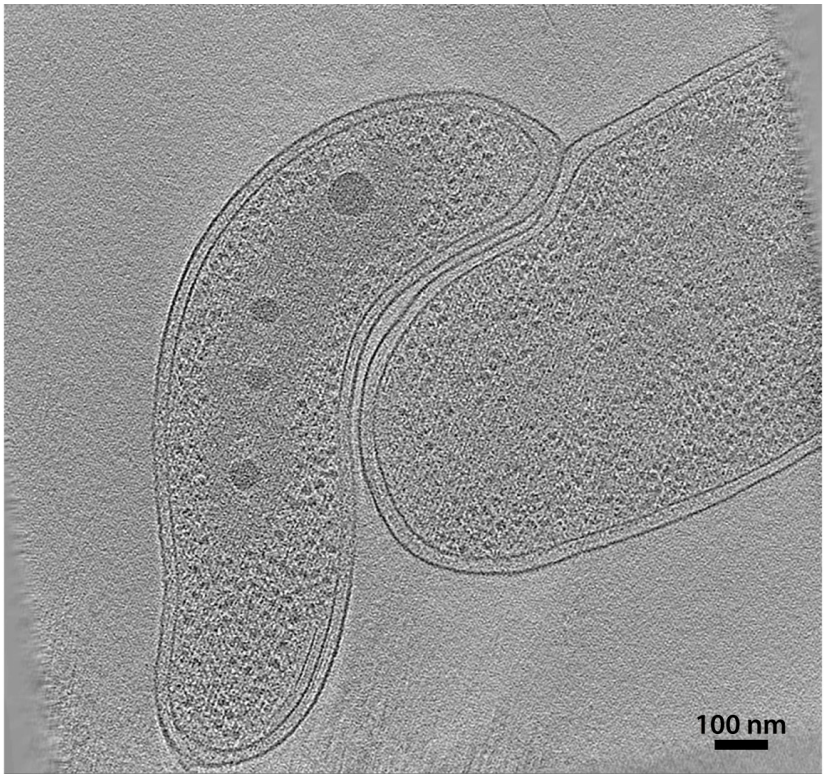
8. Type IX Secretion

Instead of going to all the trouble of launching a weapon, why not just turn yourself into the weapon? *Porphyromonas gingivalis* like this one use yet another secretion system, the type IX secretion system (**T9SS**), to secrete proteins called **gingipains** to the cell surface, where they are anchored to the outer membrane. Gingipains are peptidases, enzymes that chop up other proteins. In their lifestyle as human oral pathogens, the cells use gingipains to block host immune system signals and digest host biomolecules for food, causing the tissue damage of periodontitis.

In Chapter 6 you saw another T9SS with a very different function, in the gliding motility of *Flavobacterium johnsoniae*. In that case, instead of gingipains, the machine secretes adhesive filaments which are also anchored to the cell surface.

Porphyromonas gingivalis Collected by: Poorna Subramanian DOI: 10.22002/D1.1578


type IV pilus
100 nm


100 nm

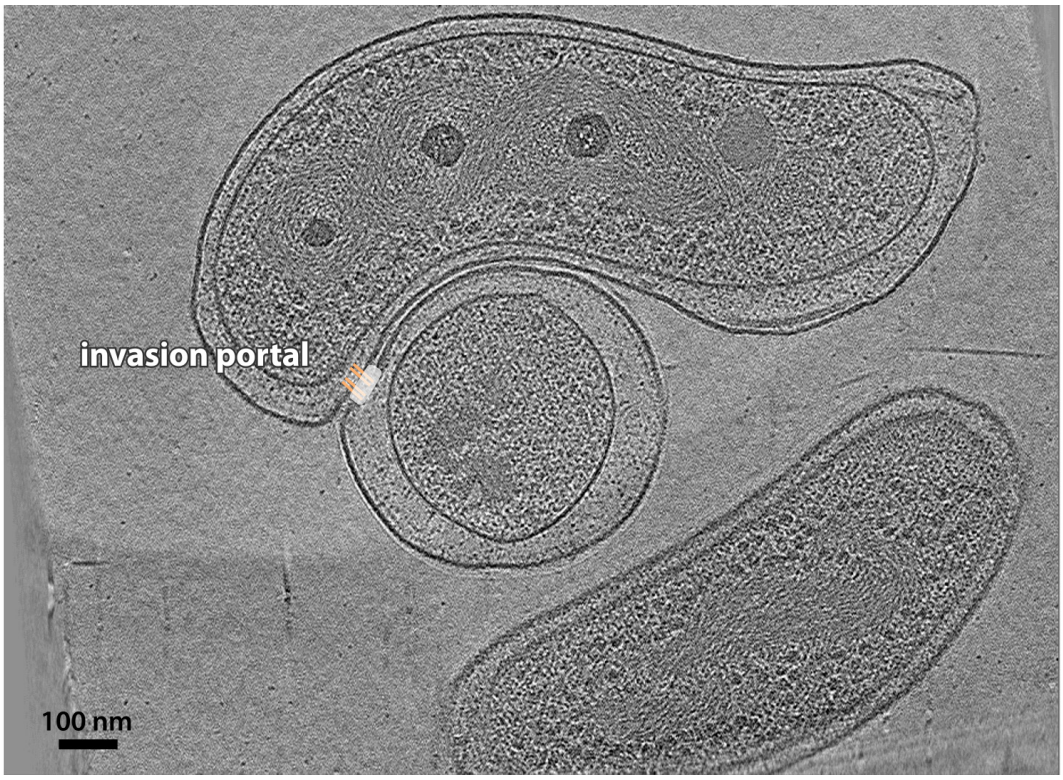
9. Predation

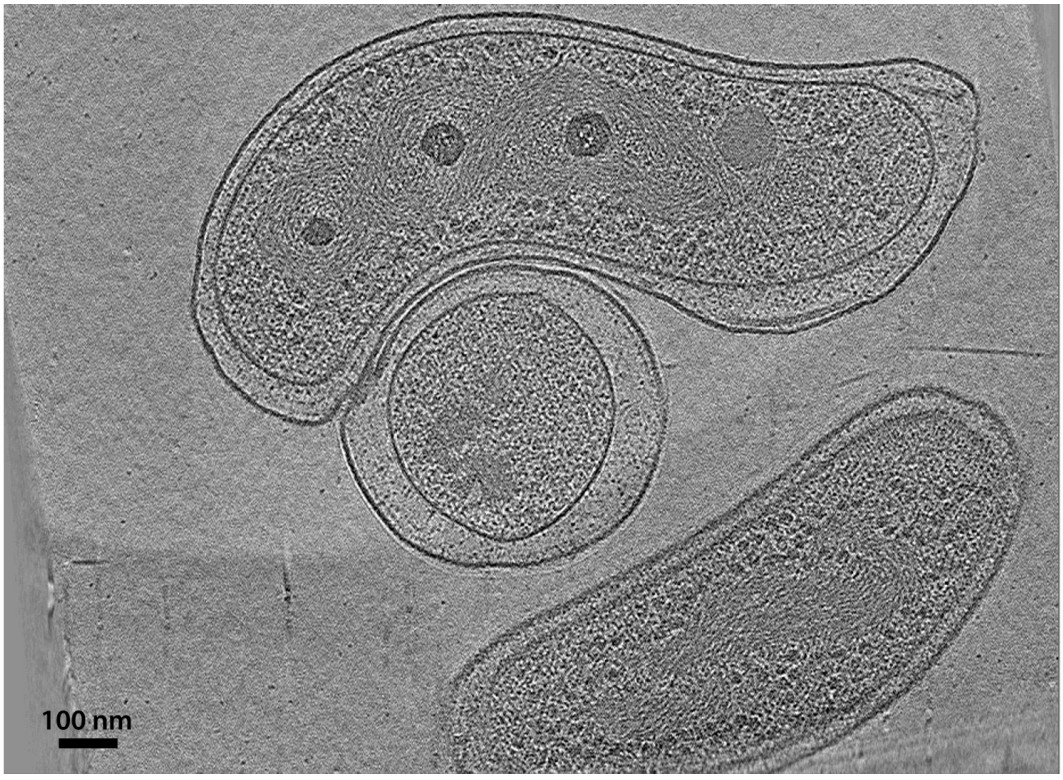
Some bacteria take the strategy of eating the neighbors to a whole new level. Remember the pack-based hunting of *Myxococcus xanthus* in Chapter 6 (chasing their prey with type IV pilus-based twitching motility)? There are also solitary hunters, like *Bdellovibrio bacteriovorus*, which has evolved an elaborate vampiric lifestyle centered around its prey. You have already seen examples of *B. bacteriovorus* cells on the prowl, in what is called "attack phase." Their bodies are ideally shaped and sized for rapid flagellum-propelled swimming and all superfluous functions like growth and division are halted (remember the highly condensed nucleoid you saw at the end of Chapter 2). When a cell finds a diderm bacterium–*Vibrio cholerae* in this case–it uses its type IV pili to lock on, as you can see here.

Bdellovibrio bacteriovorus / *Escherichia coli*

Collected by: Yi-Wei Chang

DOI: 10.22002/D1.1579


invasion portal
100 nm


100 nm

10. Predator Entry

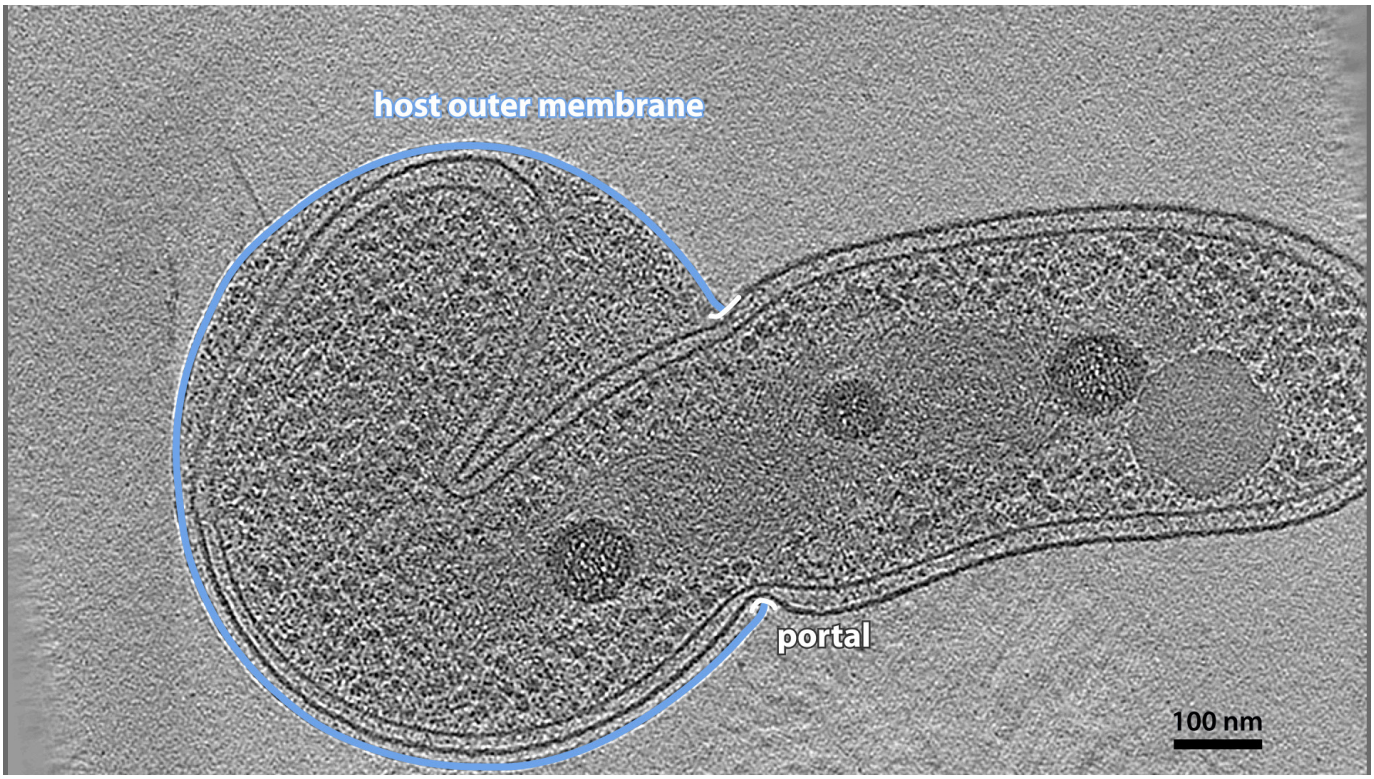
Once a *Bdellovibrio bacteriovorus* like this one has latched onto its unfortunate host, it opens a portal in the host's outer membrane, secreting a cocktail of enzymes that alter the host cell wall, causing the cell to round up and enlarge the periplasmic space. The *B. bacteriovorus* quickly slides into the host periplasm through the portal (*Learn More* on the next page) and seals the door behind it. In the process of preparing for entry, the cell dismantles equipment that is no longer necessary, including its chemosensory array and flagellum. As you can see here, the sheathed flagellum dislocates from the motor and is pulled into the periplasm, where the filament is degraded.

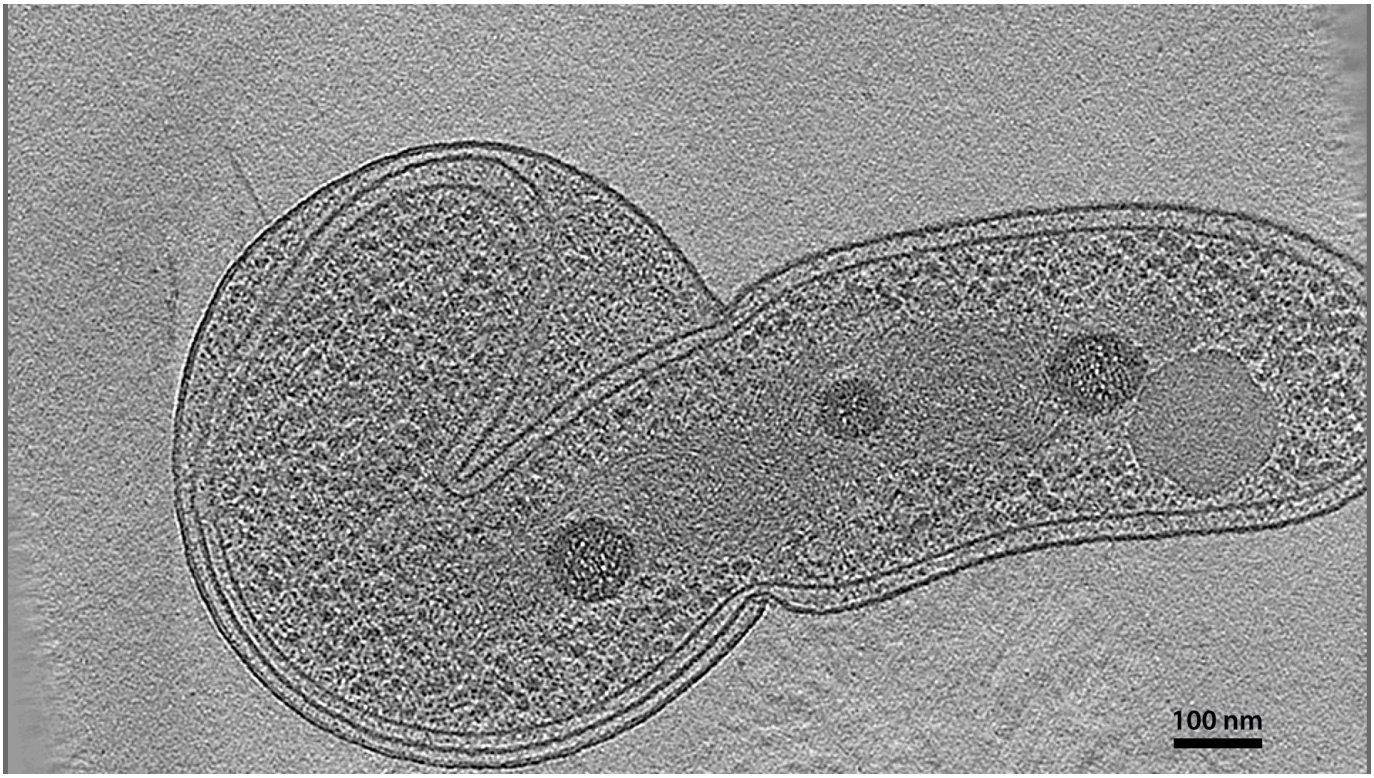
Note: The prey here–the small, round cell–comes from a mutant strain of Escherichia coli *that has an altered form of the shape-regulating protein MreB, which results in thinner cells. It is also lacking the Min system that positions the division plane at midcell. As a result, division occurs randomly along the length of the cell. The cells that divide near the pole produce "**minicells**" like this one. Minicells do not contain a genome and are not viable cells, but they can be useful for experiments. In this case, the reduced thickness of the sample improves the quality of cryoET imaging. It also lets us capture intermediates in the* B. bacteriovorus *entry process.*

Bdellovibrio bacteriovorus / Escherichia coli

Collected by: Yi-Wei Chang

DOI: 10.22002/D1.1580


host outer membrane
portal
100 nm


100 nm

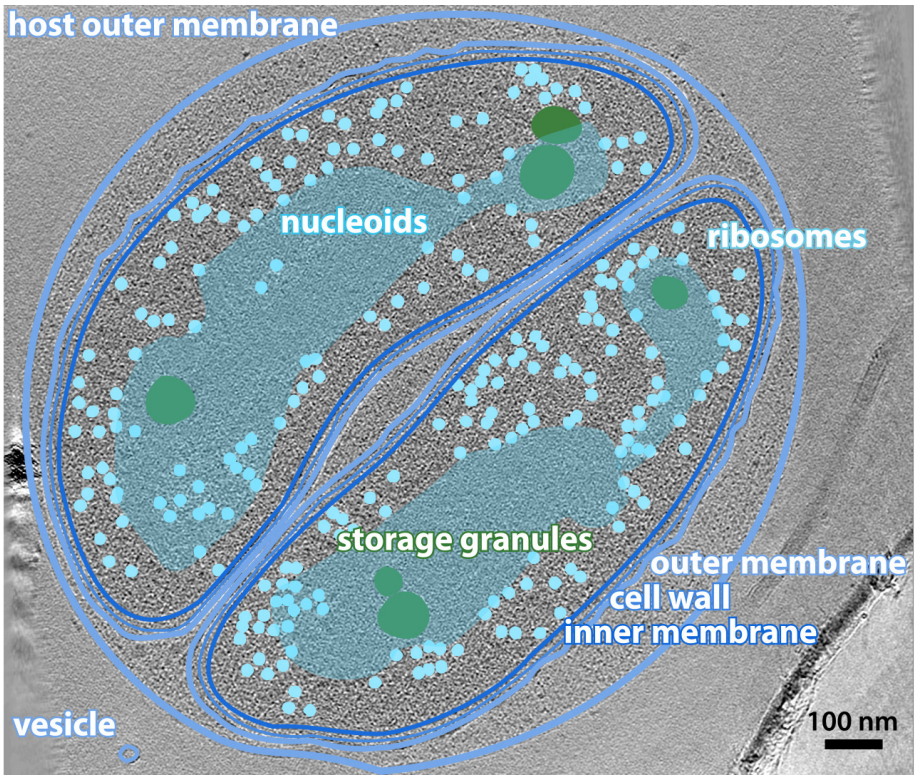
Learn More: Entry

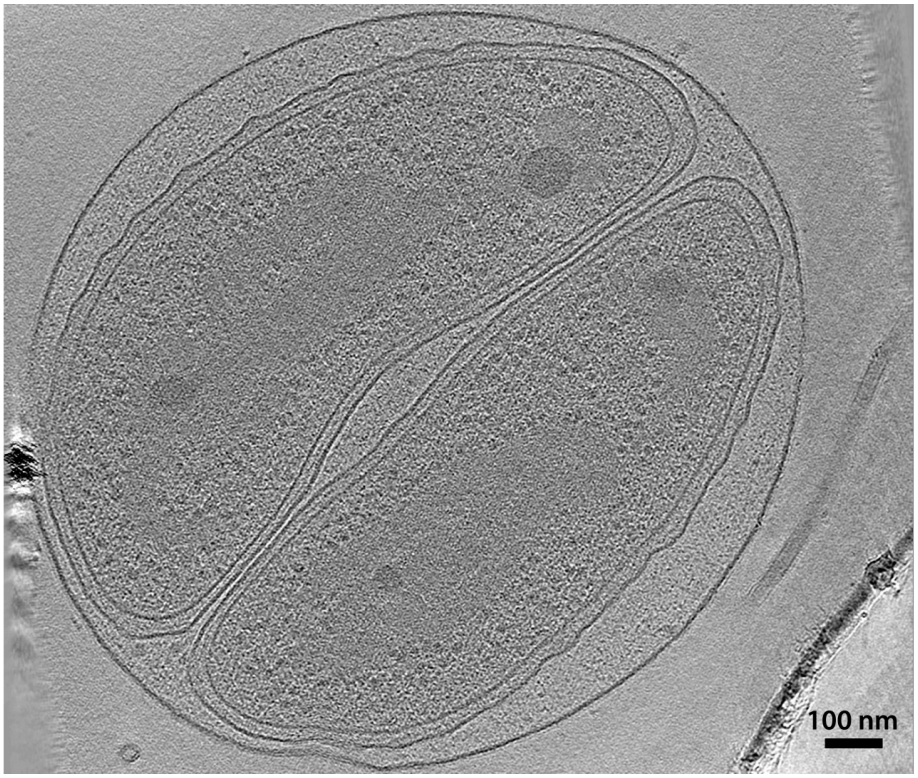
*We still do not know exactly how B*dellovibrio bacteriovorus *enters its host, but the process is extremely rapid. In order to catch a snapshot, we mixed* B. bacteriovorus *with miniaturized prey (see details on the main page). As the* B. bacteriovorus *cell cannot fit inside the prey, it remains stuck half-in and half-out. Note how the portal holds the host outer membrane tight around the invader. How this seal is maintained throughout invasion remains a mystery.*

Bdellovibrio bacteriovorus / Escherichia coli

Collected by: Yi-Wei Chang

DOI: 10.22002/D1.1585


host outer membrane
nucleoids
ribosomes
storage granules
outer membrane
cell wall
inner membrane
vesicle
100 nm


100 nm

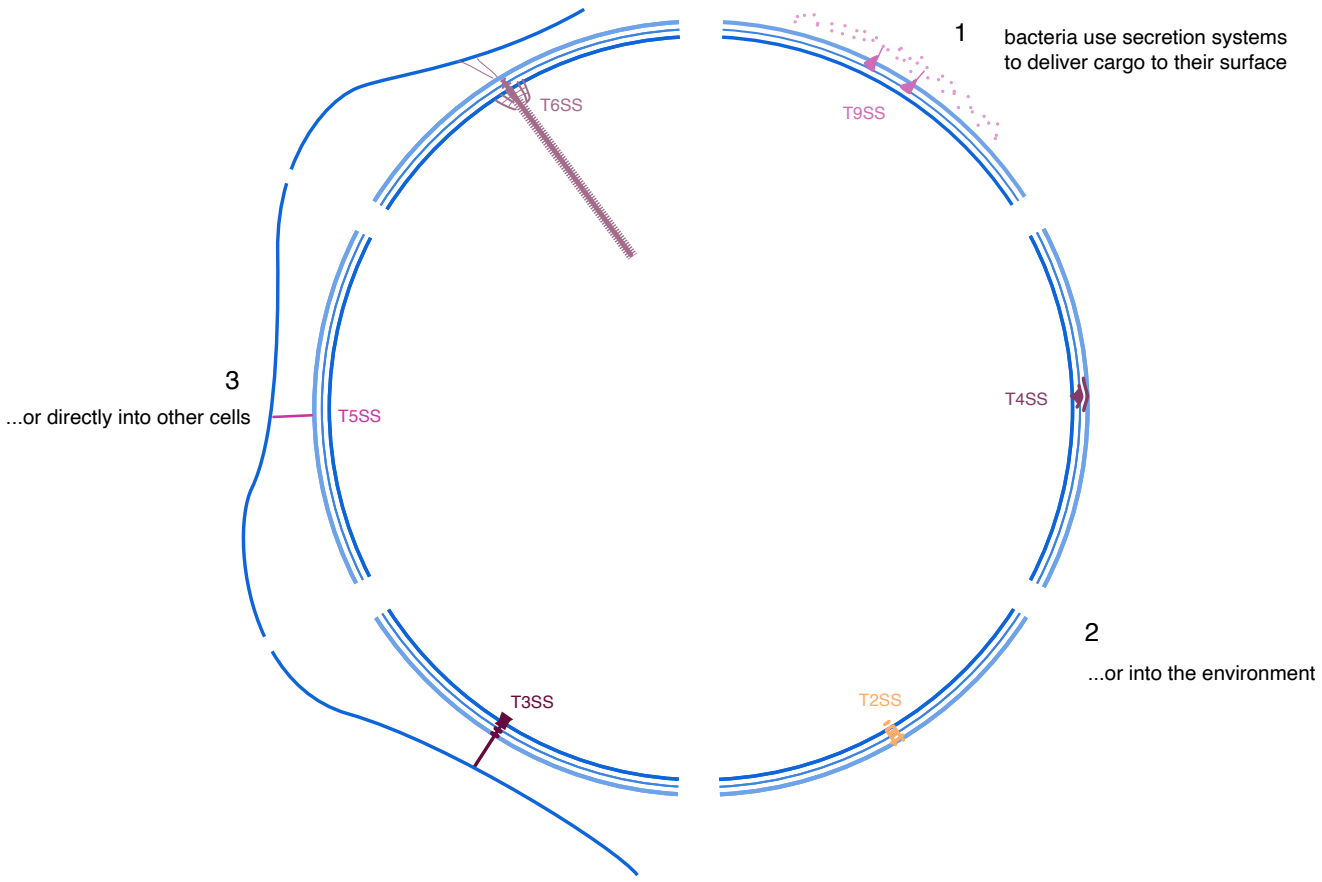
11. Predator Replication

Once inside its host, *Bdellovibrio bacteriovorus* has the leisure to grow and replicate. From its position in the periplasm, the *B. bacteriovorus* cell proceeds to digest the contents of the host cytoplasm, which shrinks down to a dense ball of material. This meal provides the fuel necessary for the *B. bacteriovorus* to grow to several times its original length and undergo a synchronous division to produce several (usually between 2 and 7) daughters. The number of progeny depends on the size of the host cell: two in the case of this relatively small *Vibrio cholerae*. The progeny, reset to the attack phase (note the condensed nucleoids), finally lyse what is left of the host to swim off in search of their next victims.

Bdellovibrio bacteriovorus / *Vibrio cholerae*

Collected by: Yi-Wei Chang

DOI: 10.22002/D1.1581


1
bacteria use secretion systems
to deliver cargo to their surface
T9SS
T6SS
T4SS
3
...or directly into other cells
T5SS
2
...or into the environment
T3SS
T2SS

Summary

Concept Check Questions

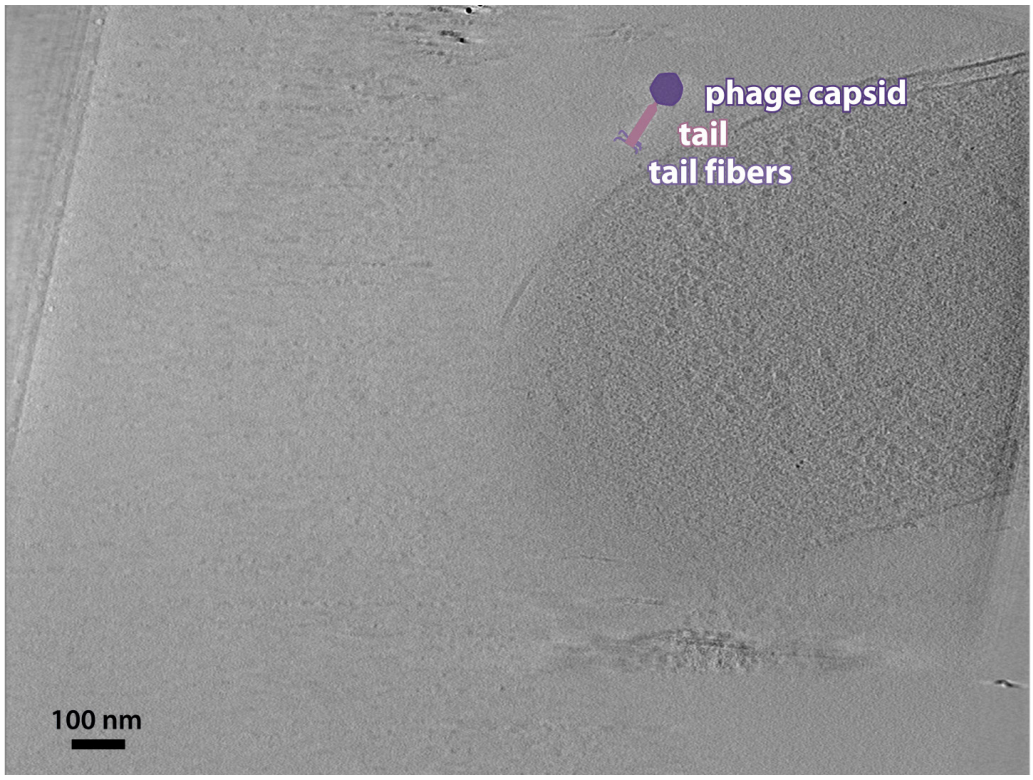
- What are two structures bacteria use to attach to a biofilm?
- Which two motility machineries are related to the type II secretion system?
- Which bacterial weapon is related to the flagellar motor?
- What does Bdellovibrio bacteriovorus use to latch onto its prey?

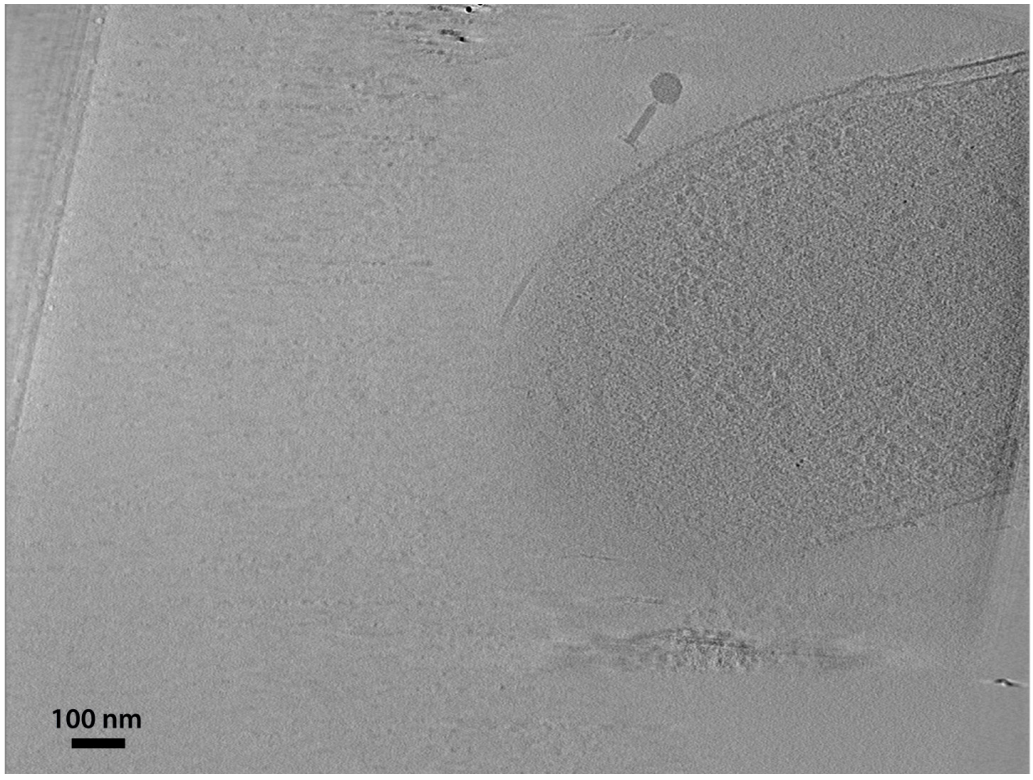
Further Reading

- Christie (2019). *The rich tapestry of bacterial protein translocation systems* [87].
- Flemming et al. (2016). *Biofilms: An emergent form of bacterial life* [88].
- Patz et al. (2019). *Phage tail-like particles are versatile bacterial nanomachines* [89].
- Sockett (2009). *Predatory lifestyle of Bdellovibrio bacteriovorus* [90].

10
Viruses

"Phages are the winners in the game of life."
- Forest Rohwer [91]


phage capsid
tail
tail fibers
100 nm

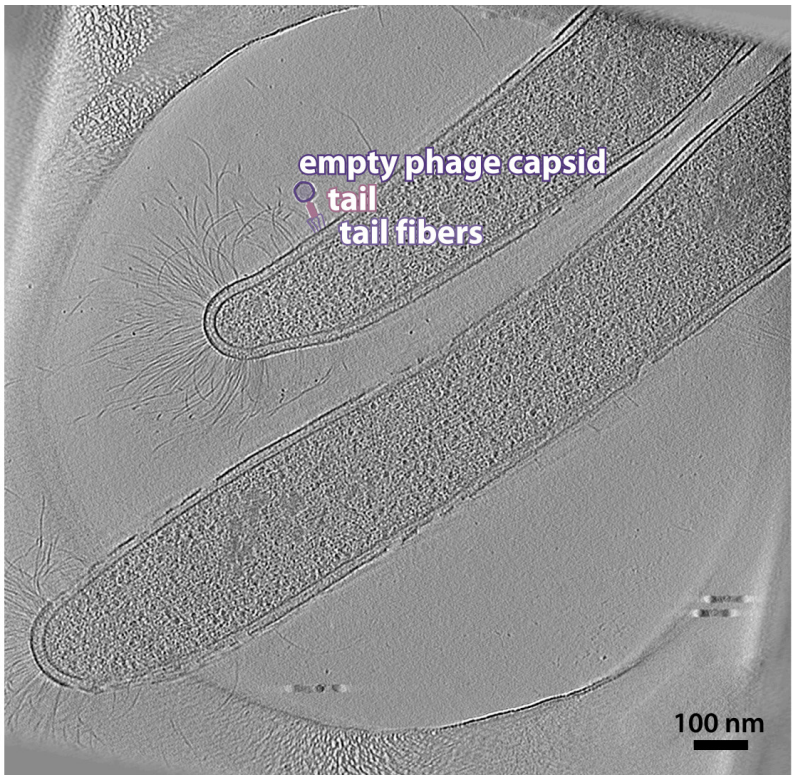

100 nm

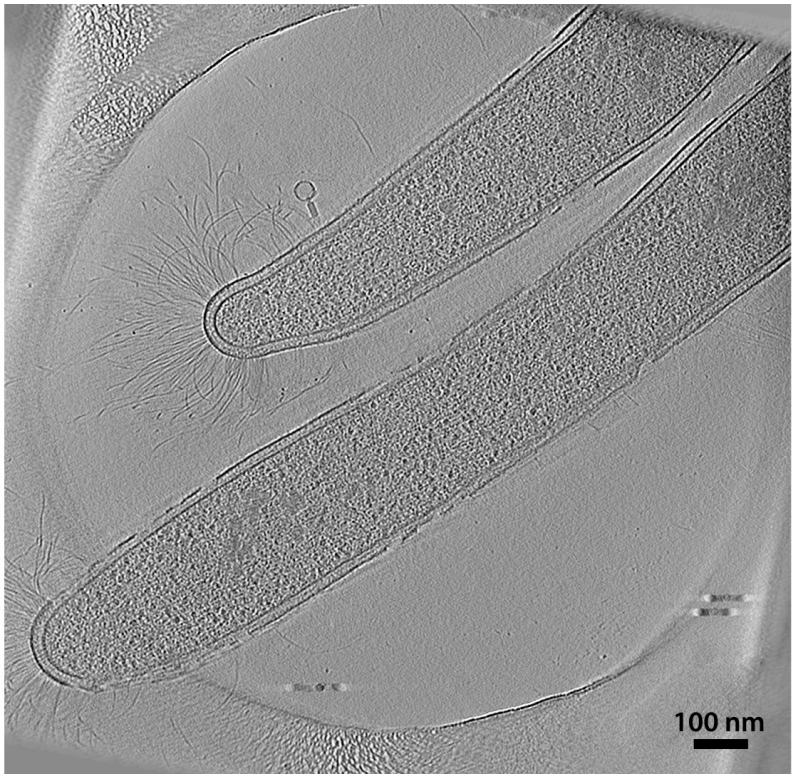
1. Phage

Life is a battlefield for your cell, and other cells are not the only enemy. Attack can come from another front: viruses called **phage** (short for bacteriophage, or "bacterium eaters" because they were first identified infecting and killing bacteria). Like all viruses, phage use cells as vehicles for their replication. And, just like eukaryotic viruses, there is an army of different phage that prey on bacteria and archaea. No species is known to be immune.

The basic strategy of a phage is to inject its genome into a host cell, use the cell's machinery to replicate the genome and manufacture the proteins it encodes, then use these materials to build the next generation of phage. Since the phage is dependent on the cell's machinery for replication, it does not fulfill the criteria for a living organism. (Despite this distinction, we still talk about the viral "lifecycle.") By cleverly outsourcing, phage can be highly streamlined structurally, little more than a genome in an envelope en route to its next destination. All phage have a genome (either double or single-stranded DNA or RNA), tightly packed inside a protective envelope of either protein (forming a "**capsid**") or lipid, or both. The genome-containing shell is called the **head** of the phage. Many phage also have a **tail**, like this myophage attacking *Shewanella oneidensis*. **Tail fibers** at the tip recognize features on their target host and allow the phage to dock.

Shewanella oneidensis Collected by: Poorna Subramanian DOI: 10.22002/D1.1586

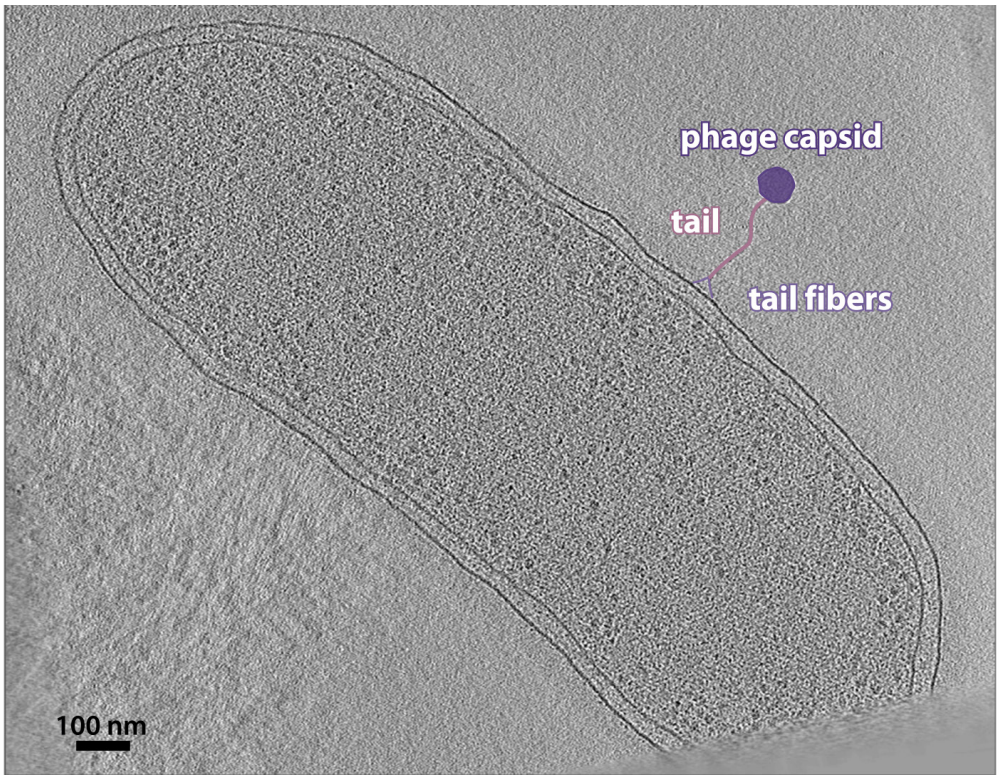

empty phage capsid
tail
tail fibers
100 nm

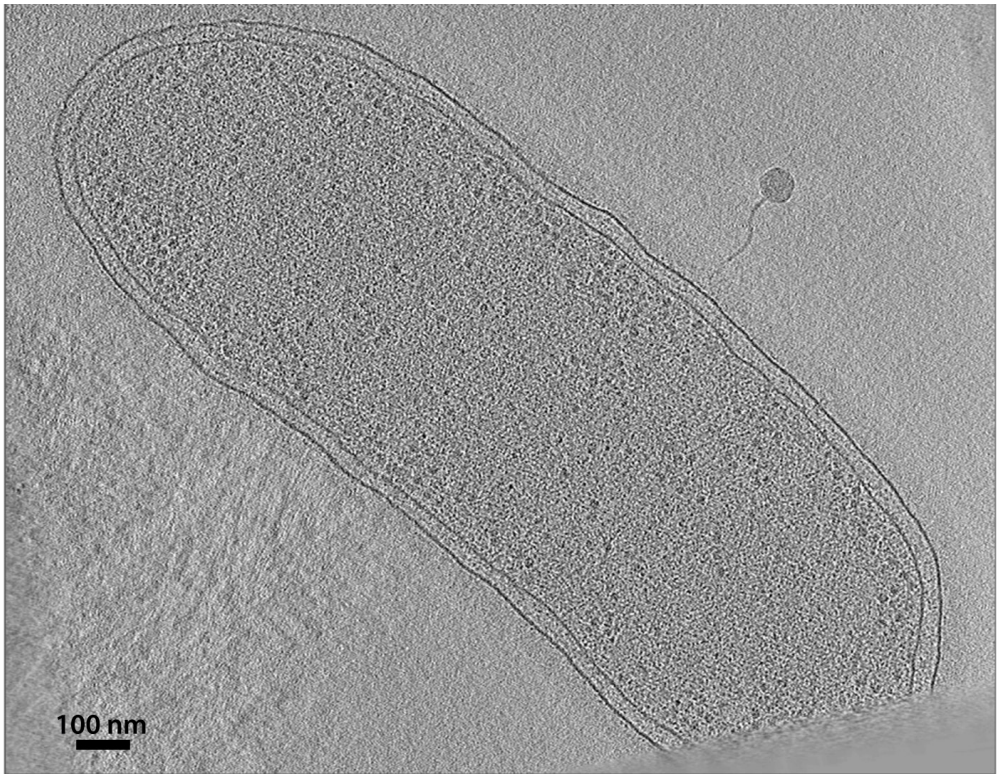

100 nm

2. Contractile Tails

Once docked, the phage delivers its cargo. The tails of some, but not all, phage are contractile, punching a channel through the cell's envelope to inject the genome, emptying the capsid as you see in this myophage infecting a *Bacteroidetes* cell. This syringe-like mechanism should be familiar from the related contractile weapons discussed in the last chapter. (You can watch an animation of a contractile myophage tail in action on YouTube: https://www.youtube.com/watch?v=h_CnXmoZwbI.)

Bacteroidetes JT5 Collected by: Elitza Tocheva DOI: 10.22002/D1.1587


phage capsid
tail
tail fibers
100 nm

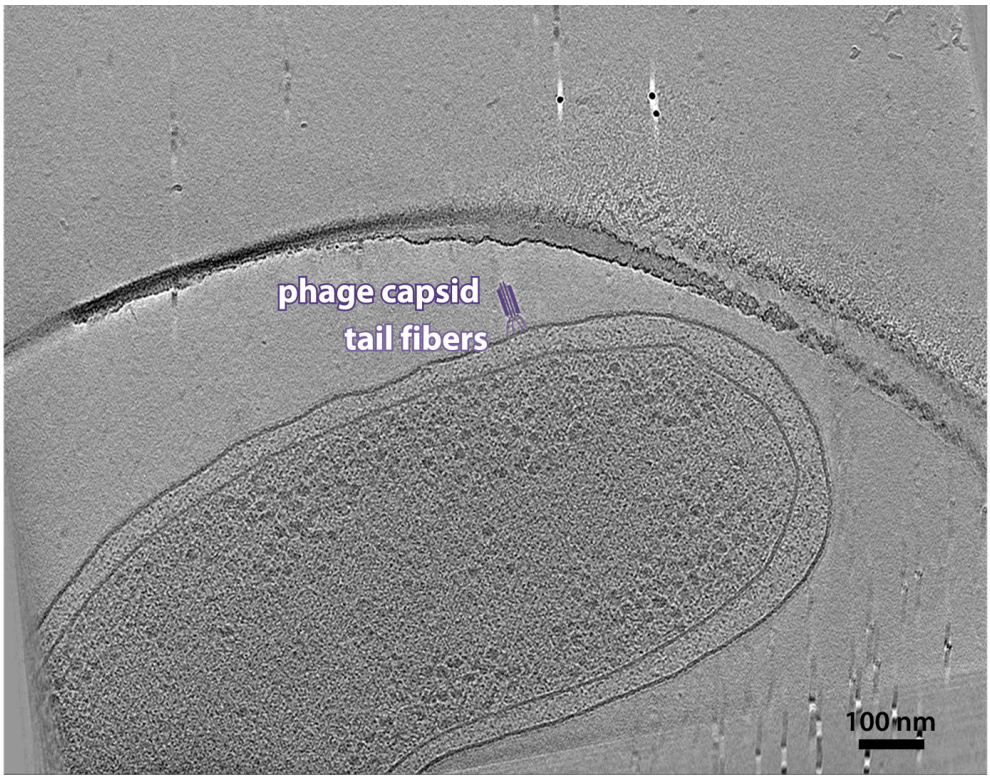

100 nm

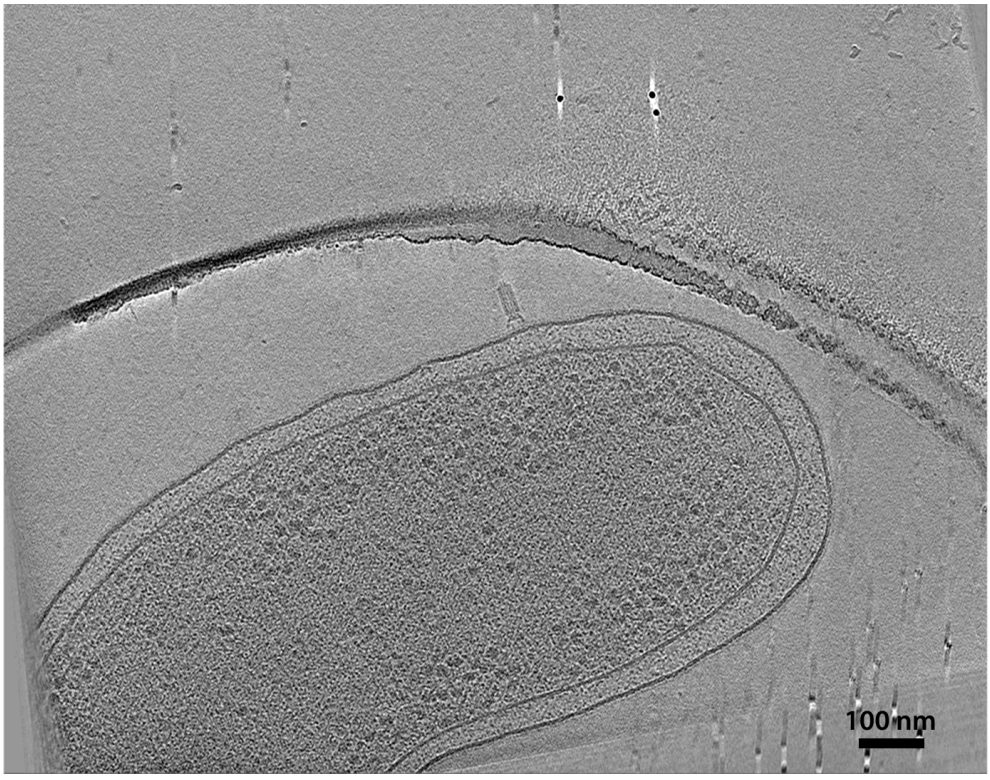
3. Noncontractile Tails

Some phage have tails that are not contractile, like this siphophage attacking a *Shewanella oneidensis* cell. The long, flexible tail still serves the essential function of crossing the barrier of the cell's envelope, providing a conduit for the genome to enter the cell.

Note that you already saw a different type of phage attacking *S. oneidensis*. While most phage are adapted to infect one or a few closely-related species of bacteria or archaea, there are often multiple different types of phage adapted to attack each species. The diversity of phage is staggering, and they have diverged considerably through evolution. One lacks a single gene with detectable homology to any other in any known virus or cell.

Shewanella oneidensis Collected by: Poorna Subramanian DOI: 10.22002/D1.1588


phage capsid
tail fibers
100 nm

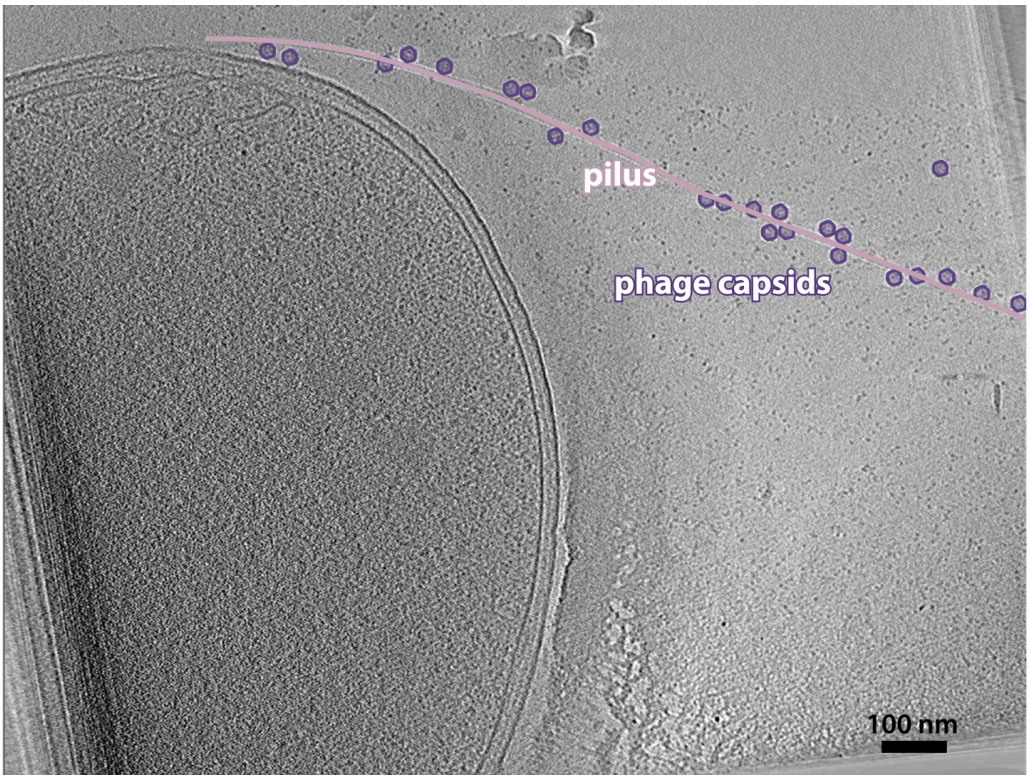

100 nm

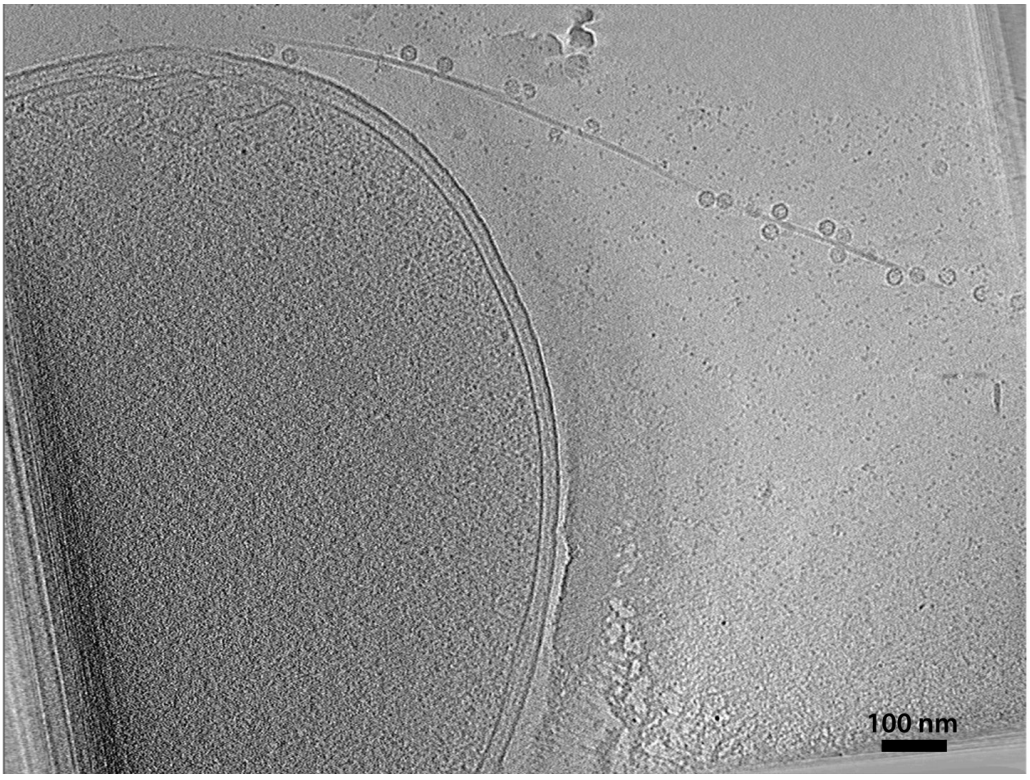
4. Structural Variation

Not all phage tails are so long. Podophage (which this unidentified attacker of a *Vibrio cholerae* may be) have short tails that extend after docking to cross the host envelope. Other phage lack tails entirely.

Similarly, heads can look very different. The unique rocket-ship shape of this phage's capsid highlights the diversity of phage morphology. Some, mostly infecting archaea in extreme environments, have heads shaped like bottles or lemons. Others take the form of long, thin filaments.

Vibrio cholerae Collected by: Yi-Wei Chang DOI: 10.22002/D1.1589


pilus
phage capsids
100 nm

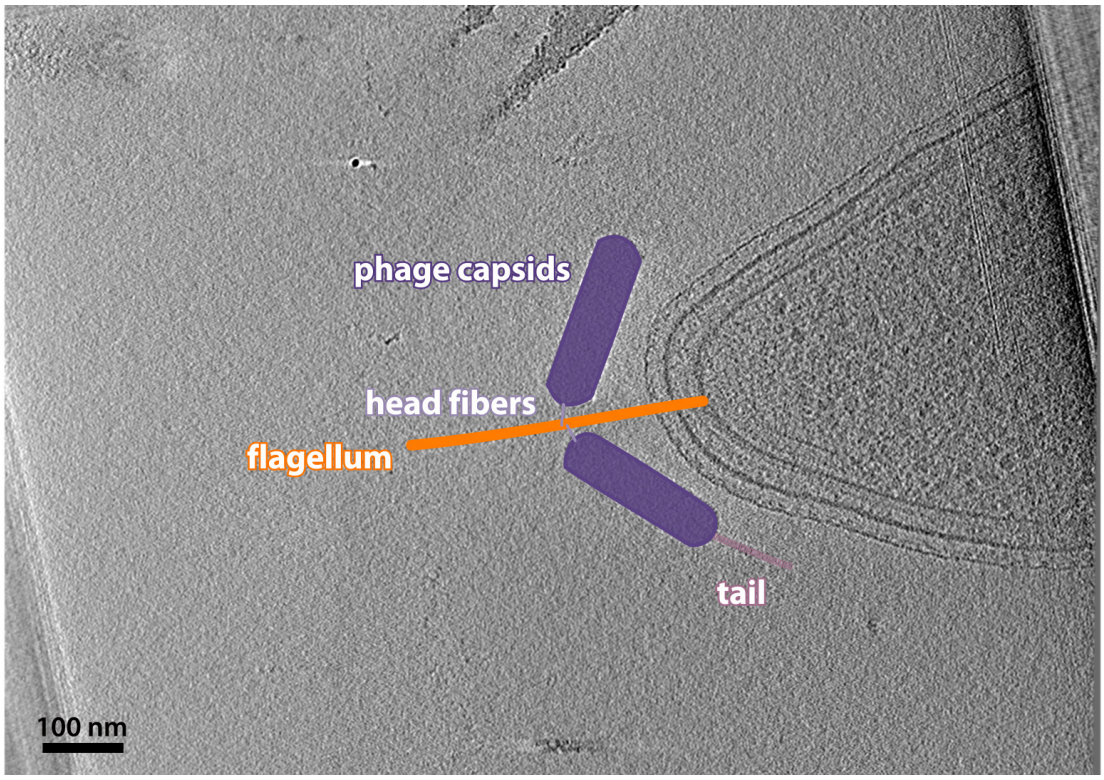

100 nm

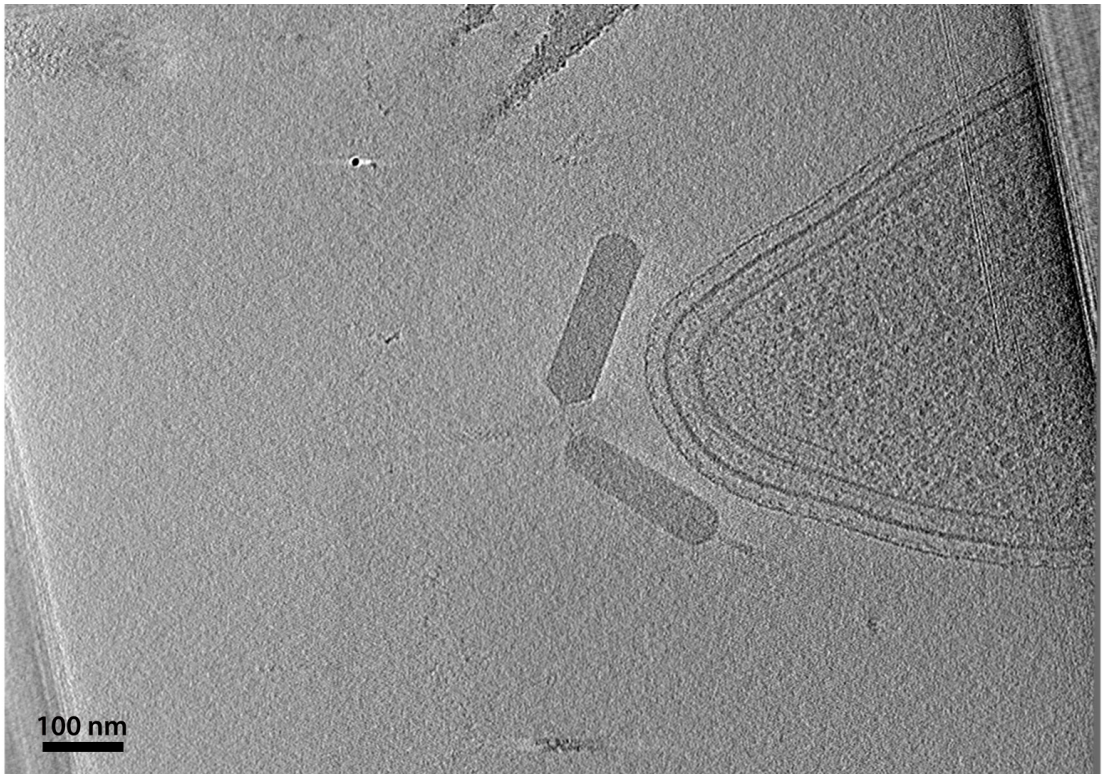
5. Targeting

Unlike your cell, phage cannot direct their movement. They simply ride the currents of Brownian motion, waiting to bump into a target receptor they can latch onto. Their abundance helps; in the ocean, there are about ten times as many phage as bacteria. Still, it might be a long wait, so evolution has fashioned some mechanisms to speed things up. One strategy is to target a receptor not on the cell body itself, but on a long, more easily-encountered cell appendage.

Some species of bacteria, including this *Escherichia coli* use "competence pili" assembled by a type IV secretion system to take up DNA from other cells. This "horizontal gene transfer" (in contrast to "vertical" heredity from mother to daughter) lets asexually-reproducing cells mix and match genes to potential advantage. It also blurs the line between species (see the *Phylogenetic Tree* in Appendix C for more about that). And in this case, it offers an advantage to these MS2 leviphage, which have evolved to recognize the pilus and inject their own RNA into the nucleic acid uptake channel.

Escherichia coli Collected by: Yi-Wei Chang DOI: 10.22002/D1.1590

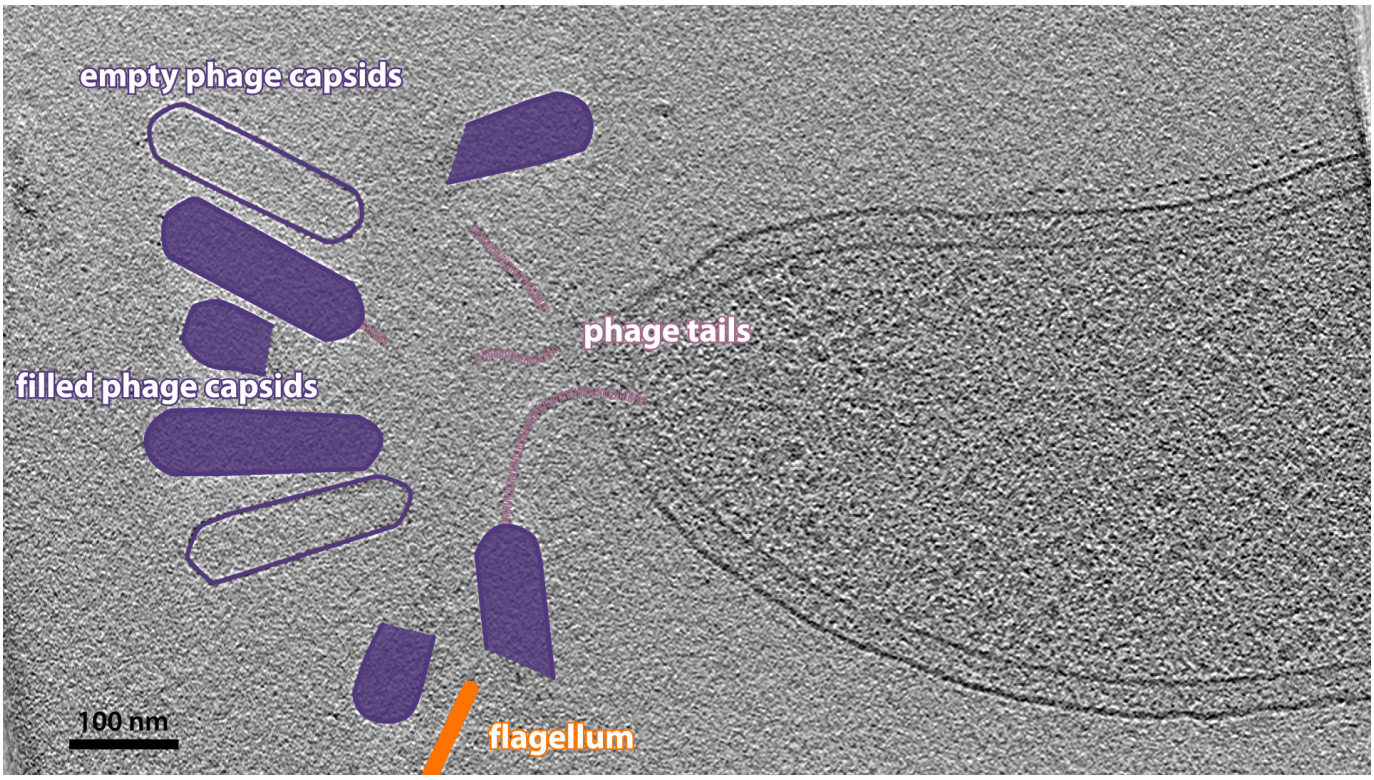

phage capsids
head fibers
flagellum
tail
100 nm


100 nm

6. Head Fibers

Other phage use bacterial appendages in an even more clever way. These φCb13 siphophage take advantage of the cell's motility to hitch a ride to the cell. They have a filament on their head that wraps into the helical groove of the *Caulobacter crescentus* flagellum. Remember that flagella can spin either clockwise or counter-clockwise. If this single polar flagellum spins clockwise, pushing the cell body, the attached phage would unscrew like a nut off the end of the flagellum. But if the flagellum spins counter-clockwise, the nut will instead spin down to the cell body, where its tail fibers will find their receptors and its tail will inject the genome (*Learn More* on the next page).

Caulobacter crescentus Collected by: Elizabeth Wright DOI: 10.22002/D1.1591

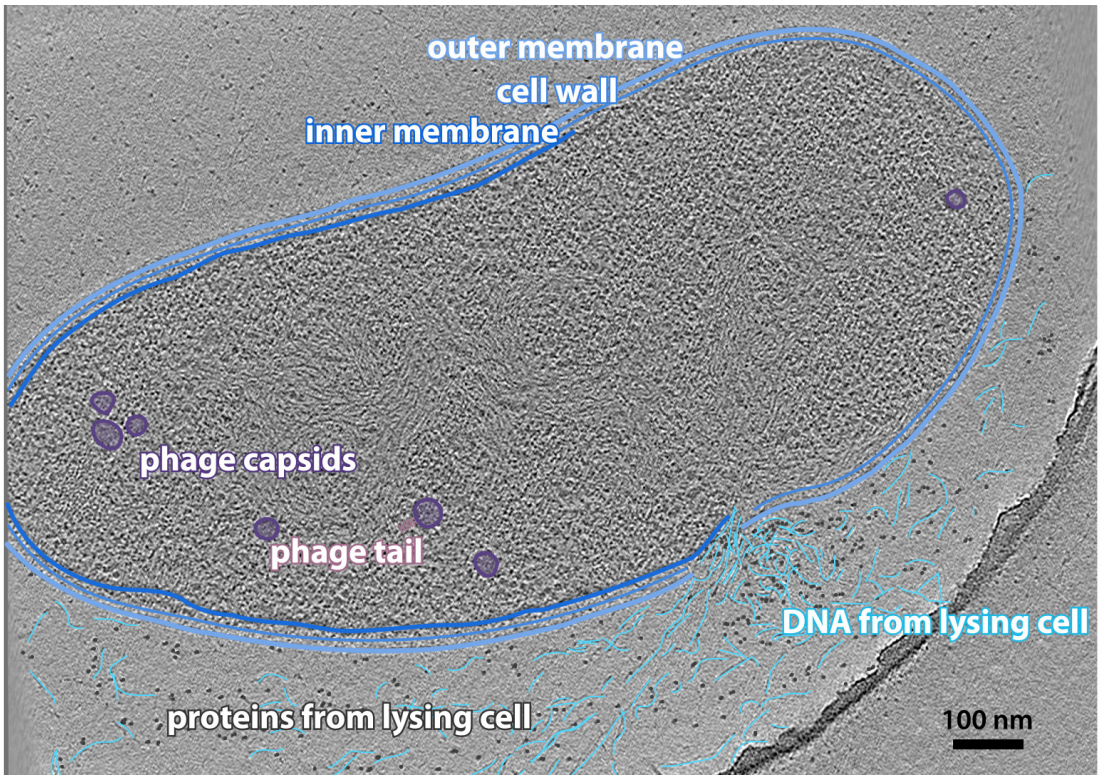

empty phage capsids
phage tails
filled phage capsids
100 nm
flagellum

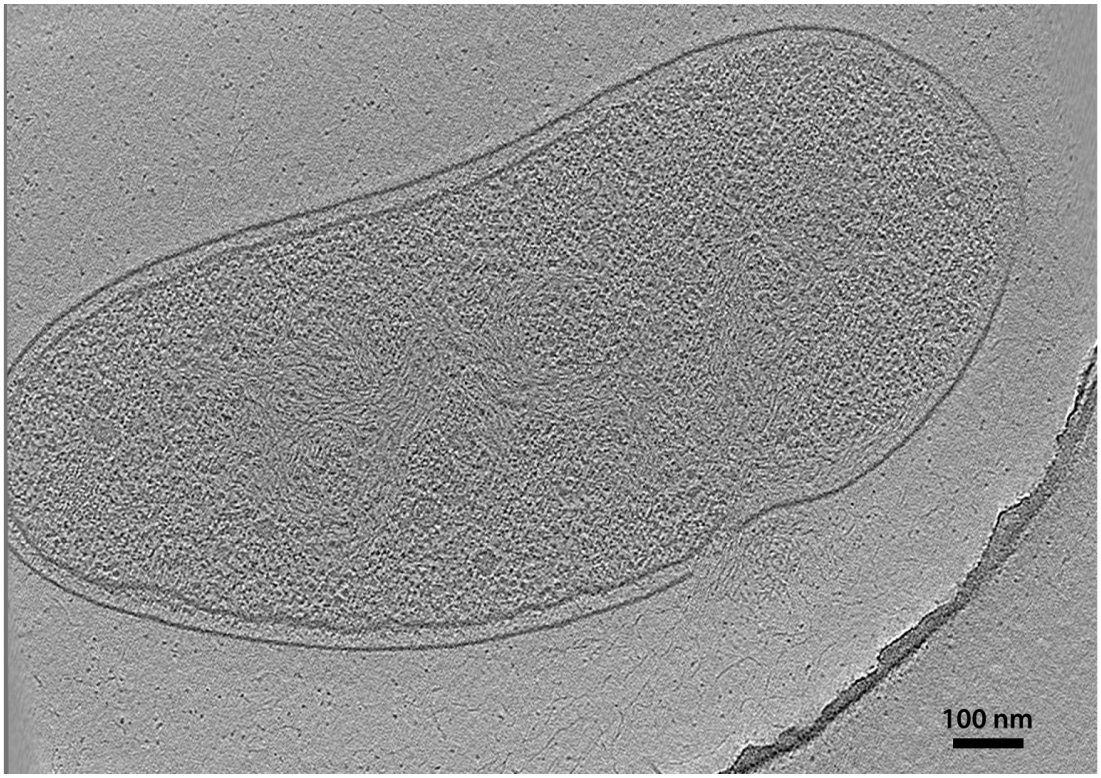
100 nm

Learn More: Infection

Here you see several φCb13 phage that have successfully reached their target receptors at the pole of a Caulobacter crescentus *and are in the process of infecting the cell (note the mix of filled capsids that have not yet released their genome and empty capsids that have). This particularly unlucky cell has already been infected by a different kind of phage, as you can see from the capsids assembling inside (more on that process on the next page).*

Caulobacter crescentus *Collected by: Elizabeth Wright* *DOI: 10.22002/D1.1596*


outer membrane
cell wall
inner membrane
phage capsids
phage tail
DNA from lysing cell
proteins from lysing cell
100 nm

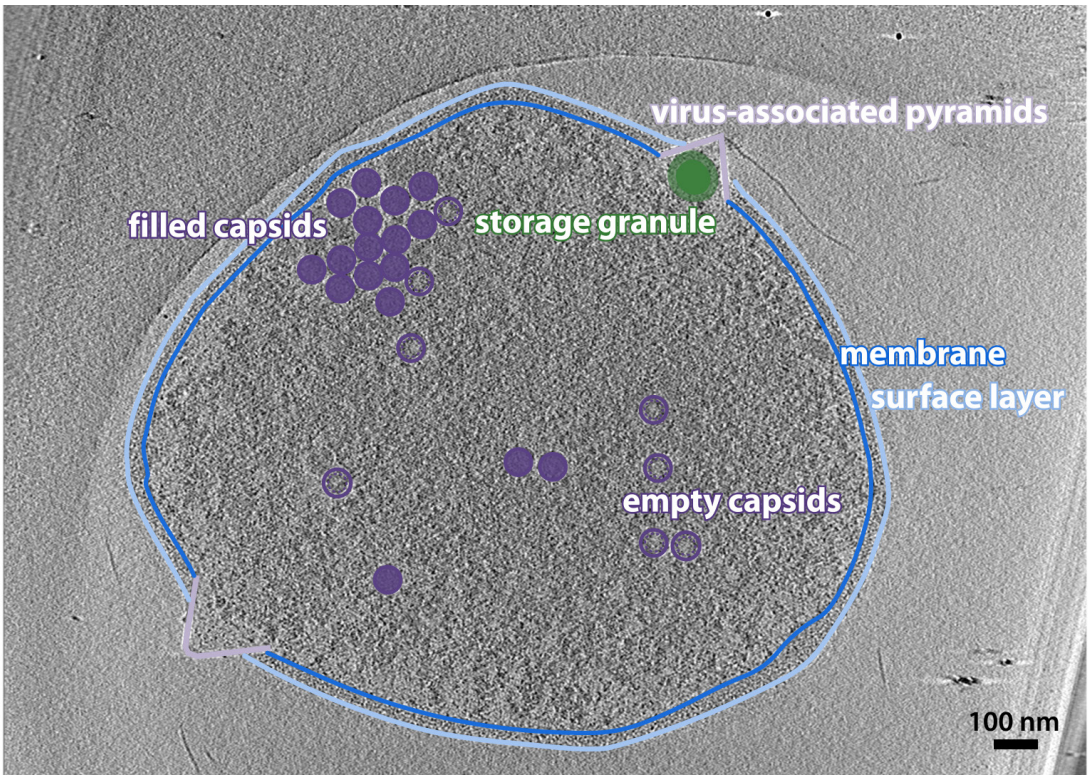

100 nm

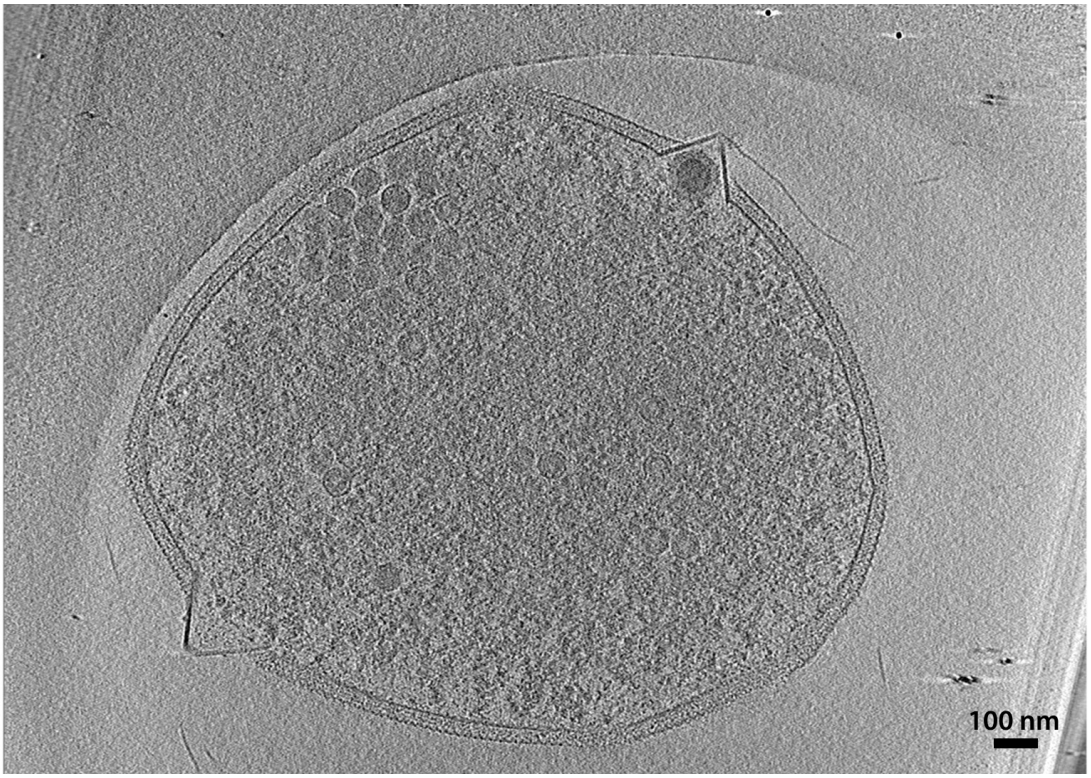
7. Replication

Once inside a cell, phage adopt one of two strategies. The first is to go through a straightforward round of replication, using their host's machinery. The second is to stick around a while. To do that, the phage inserts its genome into that of its host, creating an addition called a **prophage** that will be propagated indefinitely through cell replication. At some point in the future, usually in response to a stress that threatens the host, the prophage uses special DNA sequences at its ends akin to an ejection seat to pop its genes back out of the host genome, and carry out a normal viral replication and release cycle. Prophage are not simply passengers, though. Some confer a beneficial function on the host; a filamentous inophage carries the human-disease-causing "cholera toxin" in the *Vibrio cholerae* genome. Prophage also offer a source of genetic diversity to their host. Mutations sometimes alter the ejection sequences, rendering a prophage a permanent part of the genome, or genetic recombination may shuffle some or part of the prophage into a different part of the genome. Further evolution may then put these pieces to new use. This may be how bacteria got the contractile weapons you saw in the last chapter, repurposing genes encoding phage tail components.

Whether the phage replicates immediately or waits a while as a prophage first, once the cycle of replication and release starts, it is rapid. The virus hijacks the cell's protein-making machinery to churn out packaging proteins like capsid, and its replication machinery to churn out copies of the genome. Genomes are quickly (and very densely) packed into new heads, tails attached if necessary, and the mature phage released. Not all phage lyse their hosts; some filamentous phage (which are commonly beneficial) use secretion machinery to exit without harm. Most, though, use proteins that self-assemble into ports in the inner membrane, allowing another phage-encoded protein to access and chew up the cell wall, causing the cell to lyse. Here you can see a snapshot of this process in a *Vibrio cholerae* cell infected by a phage that is not its virulence-enhancing friend. The phage is replicating, and has just lysed the cell to make its escape.

Vibrio cholerae Collected by: Poorna Subramanian DOI: 10.22002/D1.1592

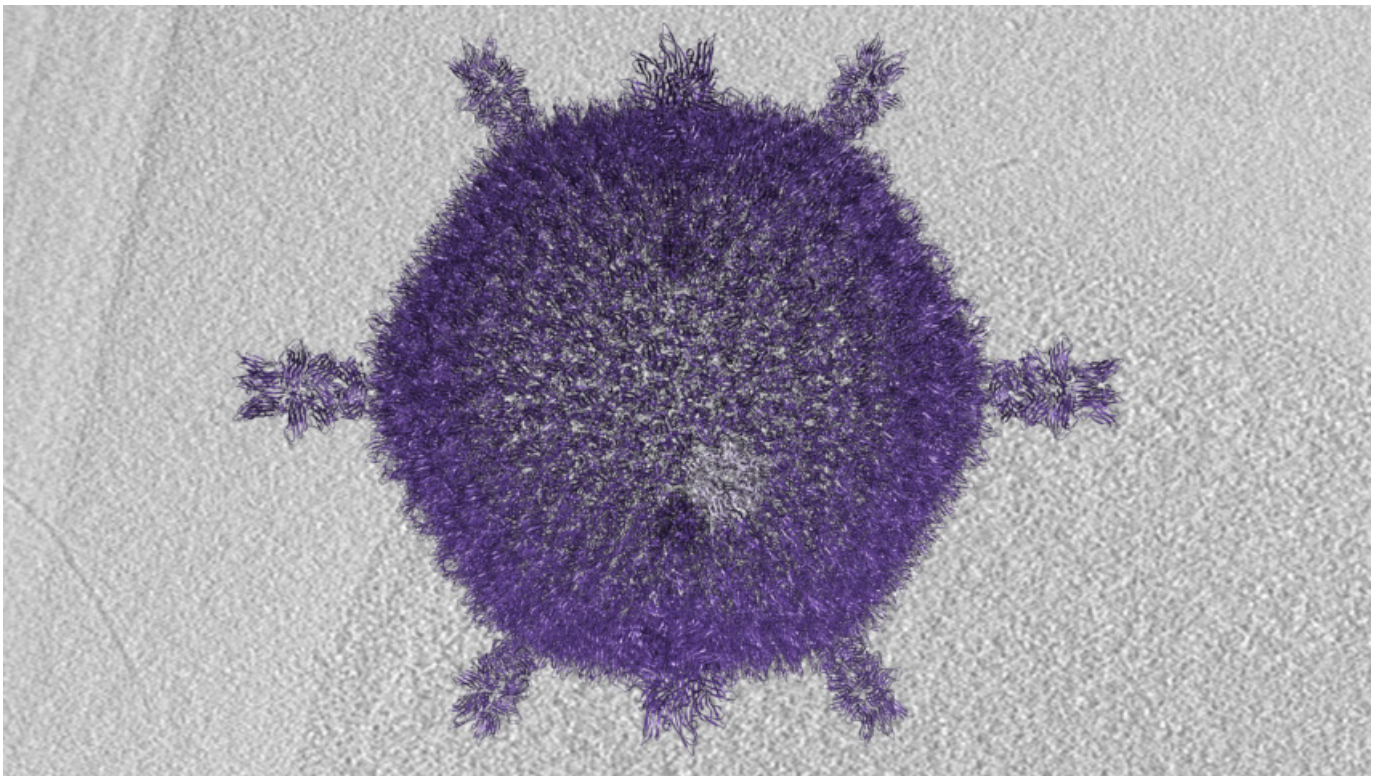

virus-associated pyramids
filled capsids
storage granule
membrane
surface layer
empty capsids
100 nm

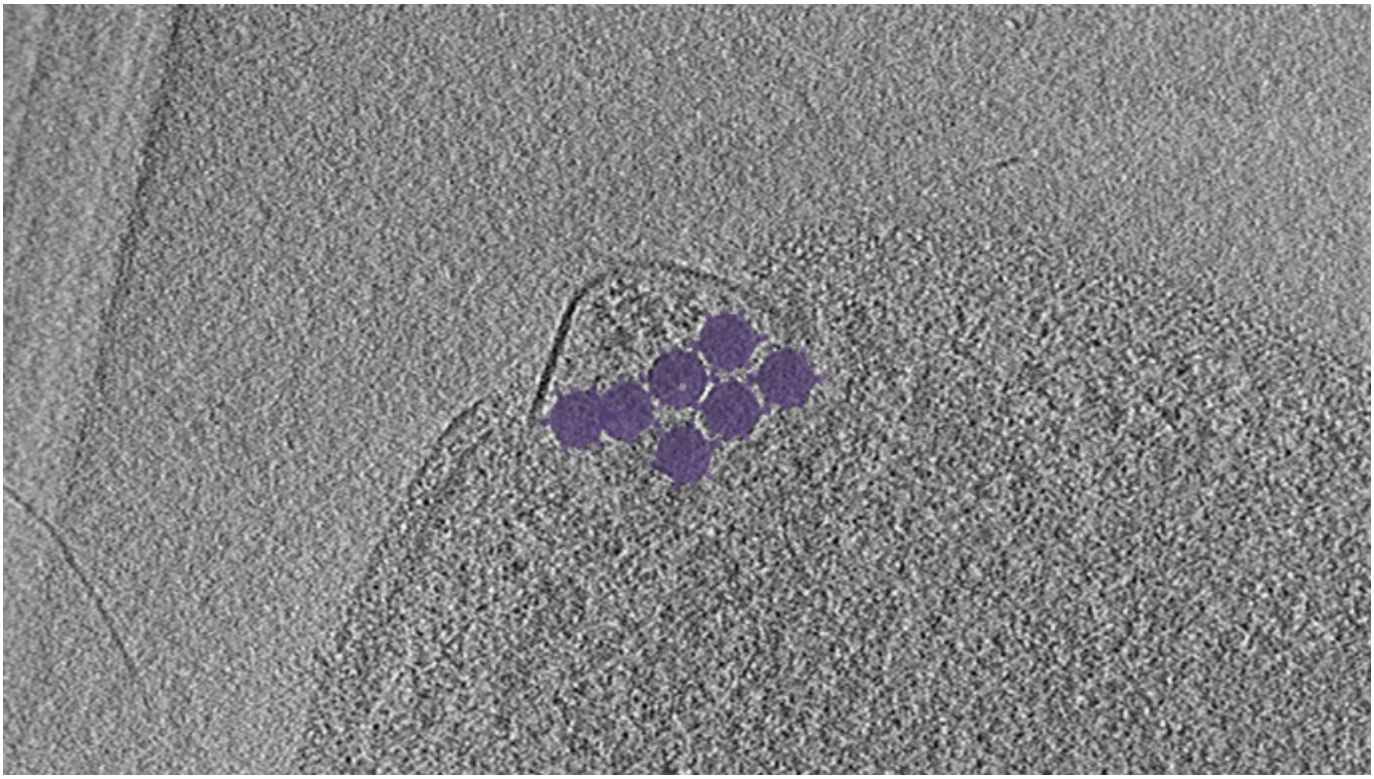

100 nm

8. Virus-Associated Pyramids

Some archaeal phage make an even more dramatic exit, through virus-associated pyramids, or **VAPs**. You can see these escape hatches in this *Sulfolobus solfataricus* cell infected with *Sulfolobus* Turreted Icosahedral Virus (STIV) (*Learn More* on the next page). The VAPs are made from a single phage-encoded protein that self-assembles into a seven-sided pyramid in the membrane. Note how the VAPs poke through and disrupt the cell's surface layer. Note also the many, many copies the phage has made. We sometimes see storage granules inside VAPs, likely simply pushed there by excluding forces from the densely-packed nucleoid and assembling viral capsids in the center of the cell.

Sulfolobus solfataricus Collected by: Lu Gan DOI: 10.22002/D1.1593

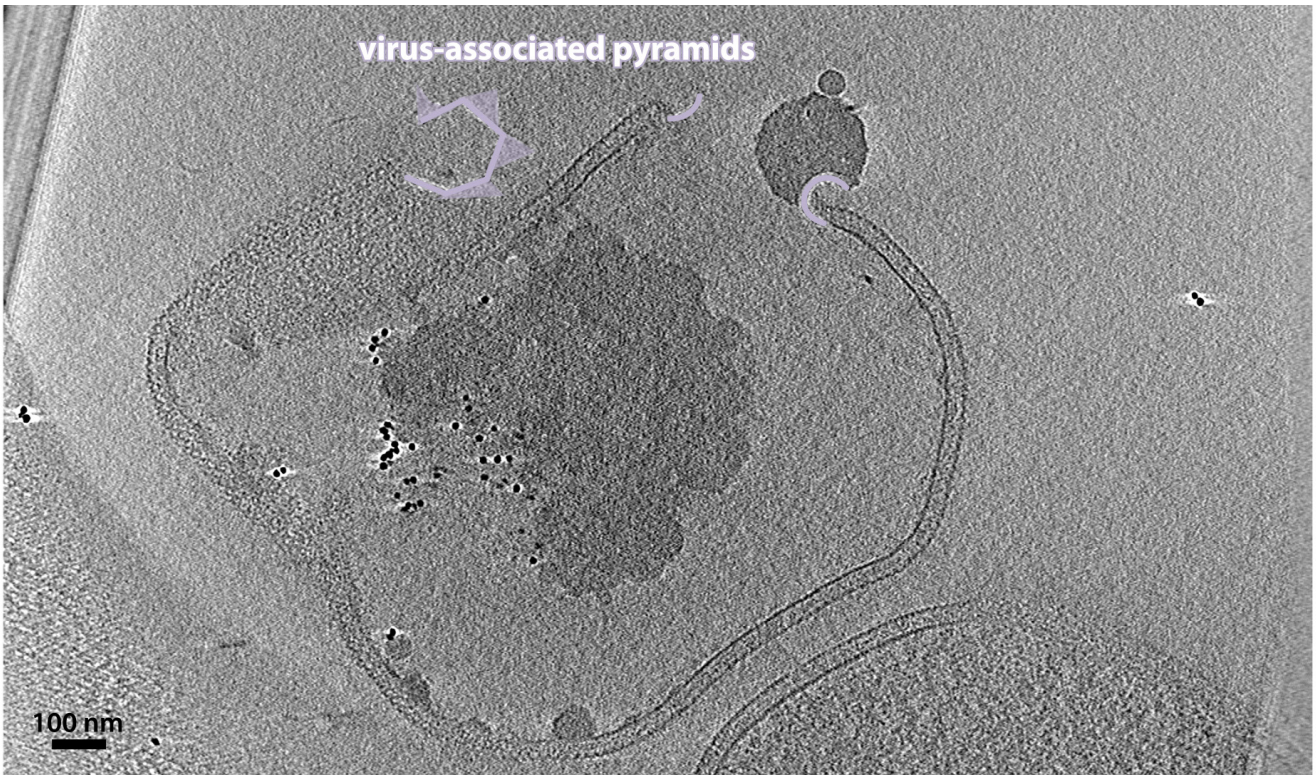


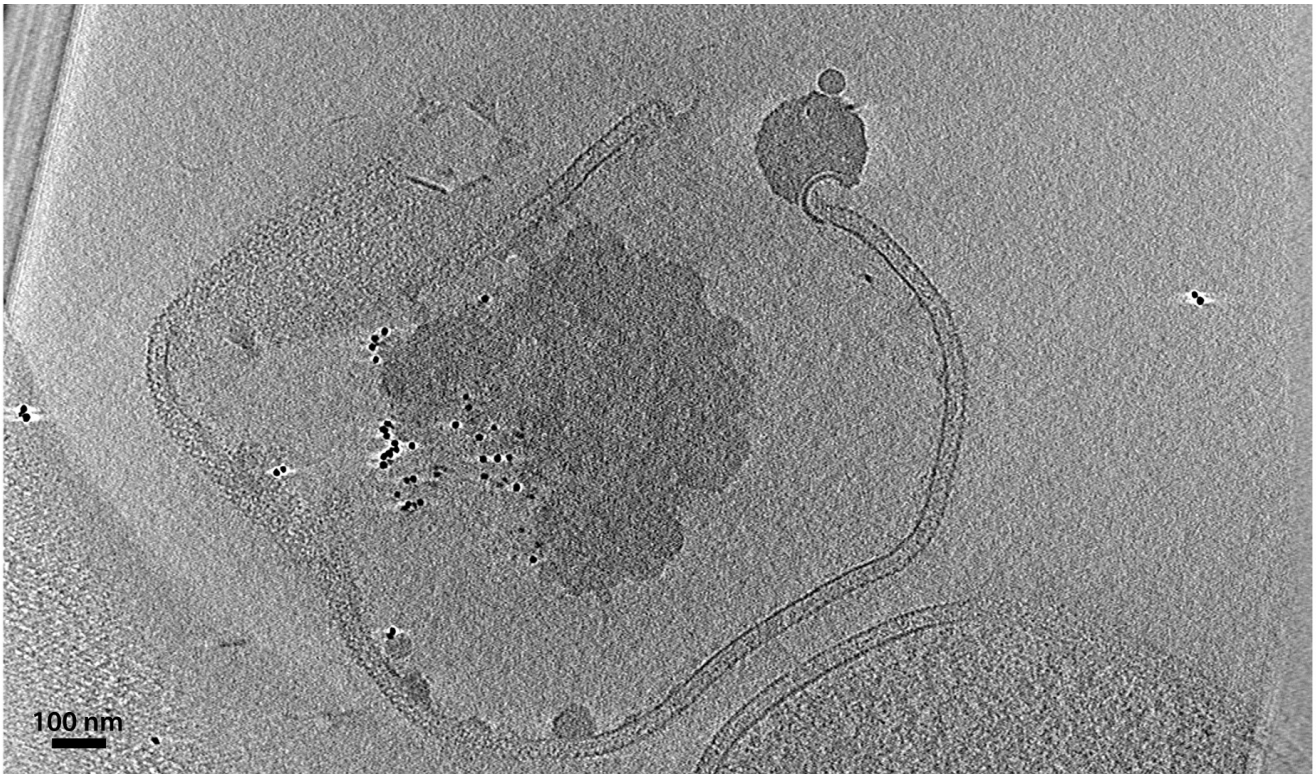


Learn More: Structure

As their name implies, STIV have icosahedral capsids with protruding turrets. The hexameric repeating unit in the shell, highlighted here in light purple, is formed by three copies of a double-lobed protein [92].

Source: Veesler et al. (2013) *Structure: PDB 3J31*

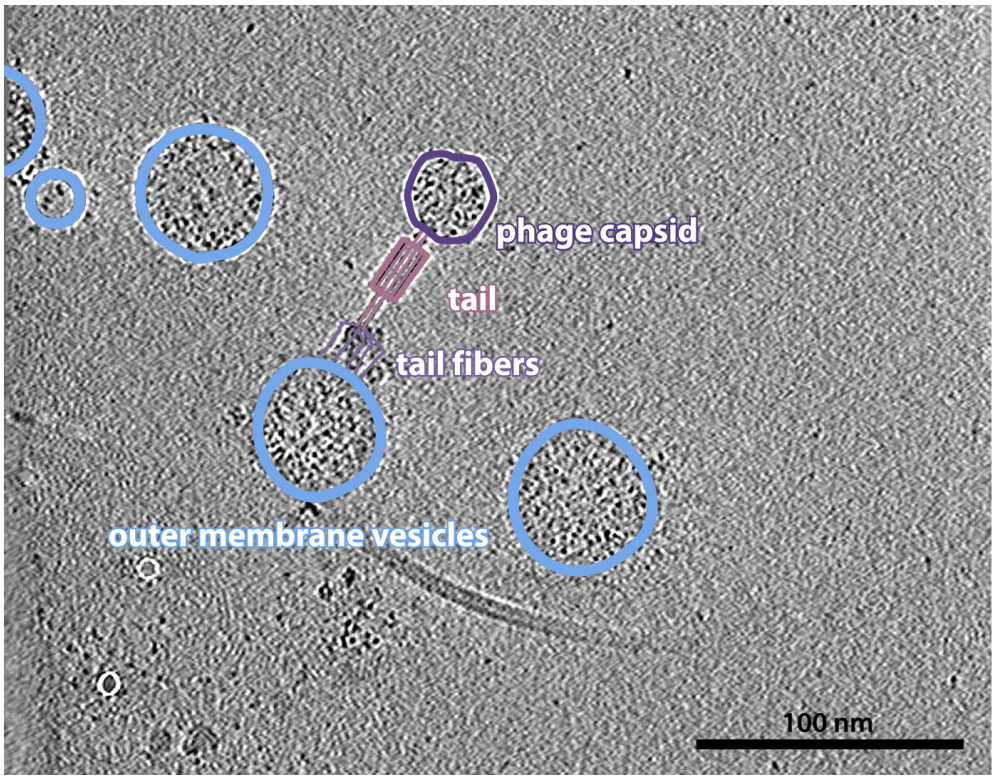

virus-associated pyramids
100 nm

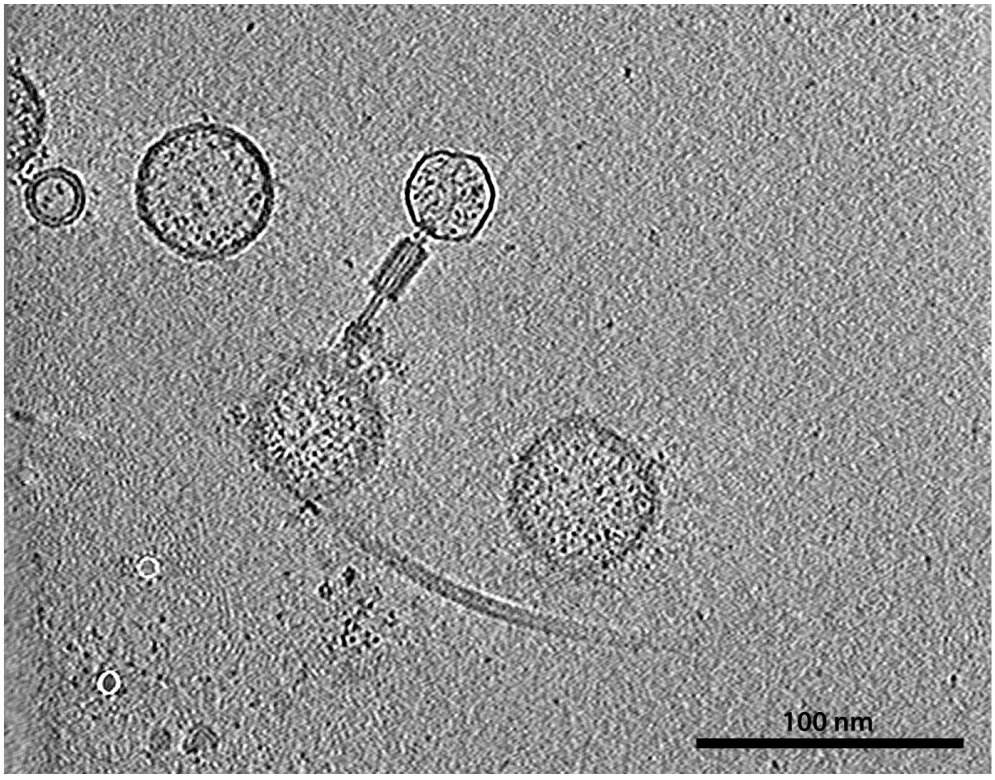

100 nm

9. Pyramid Release

When the STIV phage is ready to make its great escape, a still-unknown signal triggers the pyramids to open along their seams, peeling back to create 100 nm-wide holes for the phage, and all the other contents of the cell, to pour out, as you can see in this lysed *Sulfolobus solfataricus* cell infected with the same virus.

Sulfolobus solfataricus Collected by: Lu Gan DOI: 10.22002/D1.1594


phage capsid
tail
tail fibers
outer membrane vesicles
100 nm


100 nm

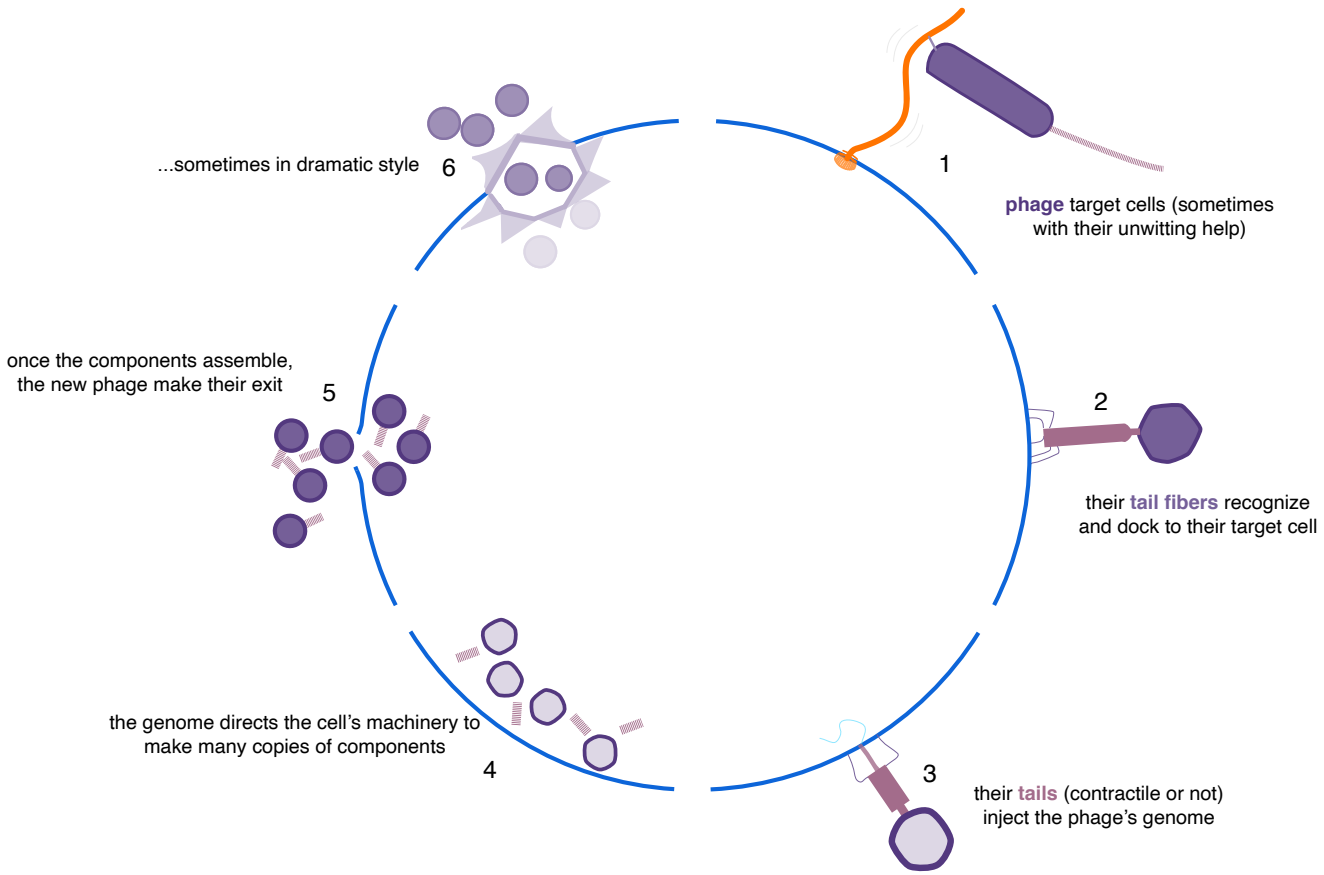
10. Cellular Defense

So what can your cell do to protect itself from these attackers? The most well-known defense is the CRISPR-Cas system now widely used to edit genomes across the tree of life. Its original function, though, is as an immune system similar to our own. Bacterial and archaeal cells "record" snippets of the genes of infecting phage in their own genome, enabling the cell to rapidly identify and chop up matching genes in future infections, much as our immune system stores the memory of antigen fragments for rapid neutralization in the future.

Defense can also be structural. Surface layers may protect cells from some phage. And then there is always distraction. One function of extracellular vesicles may be to provide decoys for phage to attack, as you can see with this myophage attacking a *Shewanella oneidensis* outer membrane vesicle.

The war between cells and viruses is an ancient one, and continually raging. Despite your cell's best efforts, it is very likely that it will end its life in a burst of phage. It is far from alone in this fate, though. In the ocean, it has been estimated that every day phage kill off half the bacterial population. But each lysed cell provides a feast for a starving neighbor, and DNA for more evolutionary tinkering, continuing to shape the weird, wonderful microbial world.

Shewanella oneidensis Collected by: Lauren Ann Metskas DOI: 10.22002/D1.1595


1
phage target cells (sometimes with their unwitting help)
2
their tail fibers recognize and dock to their target cell
3
their tails (contractile or not) inject the phage's genome
4
the genome directs the cell's machinery to make many copies of components
5
once the components assemble, the new phage make their exit
6
...sometimes in dramatic style

Summary

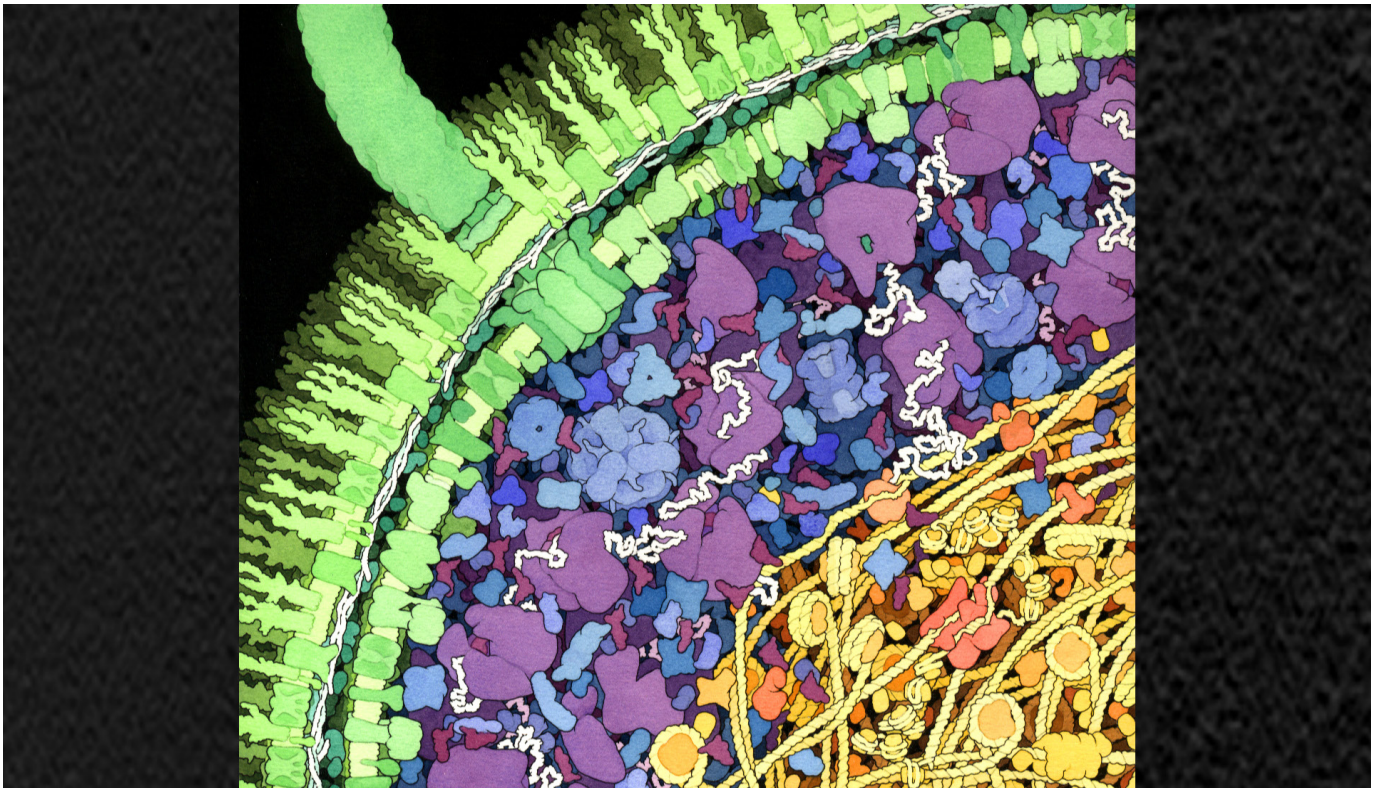
Concept Check Questions

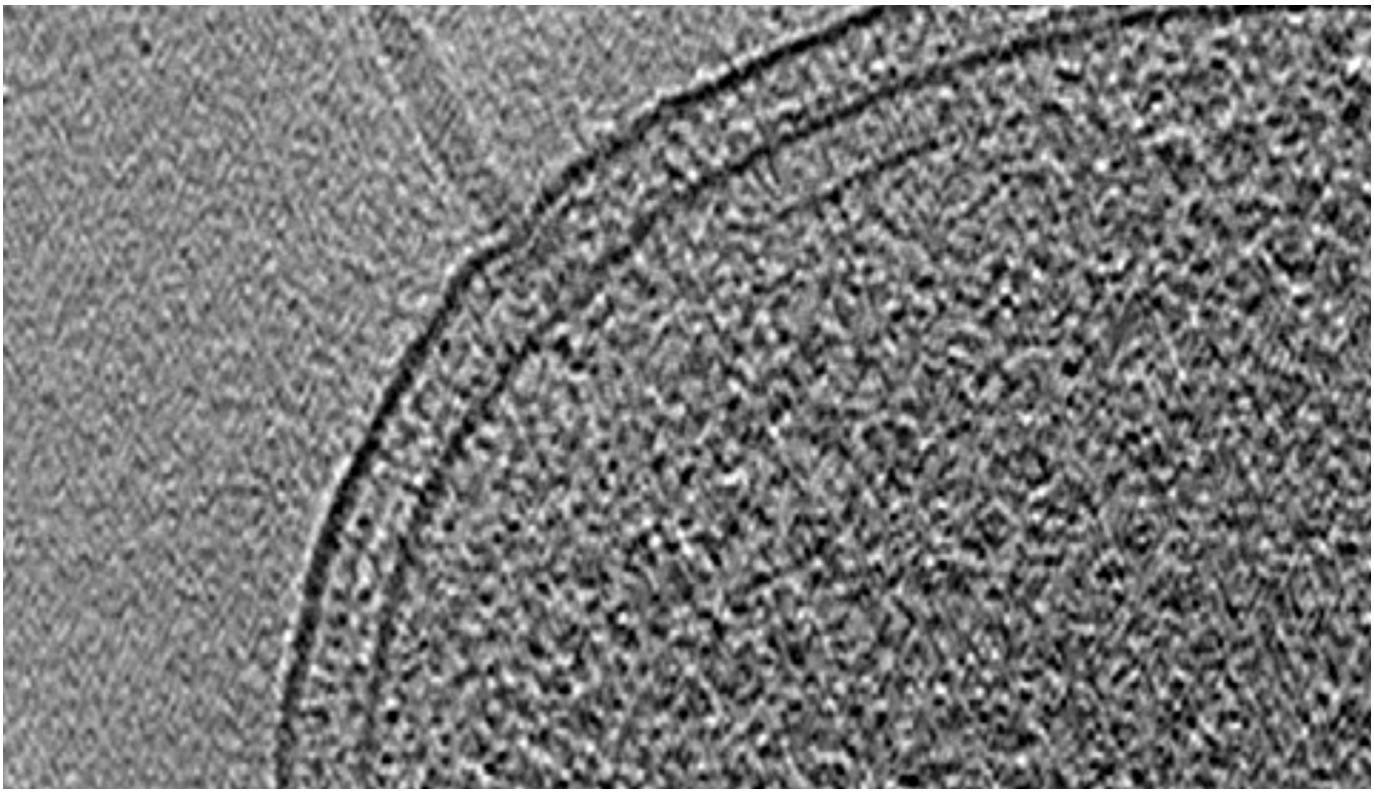
- Why are phage not considered a life form?
- Which macromolecule makes up the phage capsid?
- What structure do phage use to recognize their targets?
- Which bacterial structure is related to the phage tail?

Further Reading

- Keen (2015). *A century of phage research: Bacteriophages and the shaping of modern biology* [93].
- Prangishvili et al. (2017). *The enigmatic archaeal virosphere* [94].
- Rohwer et al. (2014). *Life in Our Phage World* [91].

Outlook





Keep Looking

To return to the quote from Richard Feynman that opened the book, "It is very easy to answer many of these fundamental biological questions; you just *look at the thing!*" Now that you have looked at many bacterial and archaeal cells, we hope you are beginning to appreciate the structure that underlies their complex lives.

It is important to keep in mind, though, that we have only highlighted a small fraction of the components of these cells. Look at any tomogram in the book and notice all the dark spots that are not labeled; each is a macromolecule, or larger complex, with a function for the cell. From illustrations of a cellular machine like the flagellar motor, it is easy to imagine it acting in isolation. In fact, though, its environment looks more like this beautiful illustration of an *Escherichia coli* cell by David Goodsell [95]. It is simply a part of the crowded life of the cell. And each of these parts, their functions and their interactions have been shaped, and will continue to be shaped, by evolution. Biology is very much a work in progress.

Our knowledge of biology is also a work in progress, and we are at an early stage. Many of the structures you saw in this book were characterized only in the past decade, and most are not yet fully characterized. Other structures have been observed inside cells, but we do not yet know what they are, or what function they may serve (e.g. [96]). You saw examples highlighted in white throughout the book.

Finally, the cells we have imaged represent only a fraction of the full diversity of bacteria and archaea. For every bacterial species that has been cultured in a lab, many more exist in nature, unseen and unknown. Archaea, which do not attract human attention with pathogenic functions, are even less studied. Some of the most tantalizing species, which promise to bring us closer to an understanding of how eukaryotes first evolved, have been identified from genetic fragments but have not yet made it into microscopes in the lab. Many questions remain unanswered, and much work remains to be done. We hope you will keep looking.

Escherichia coli Collected by: David Goodsell DOI: 10.22002/D1.1789

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Helicobacter pylori - 9.3 Type II and Type IV Secretion: Helicobacter pylori tubes
Hydrogenovibrio crunogenus - 4.6 Carboxysomes: Long carboxysomes
Hydrogenovibrio crunogenus - 4.8 Storage Granule Variety: Granule-containing carboxysomes
Hylemonella gracilis - 3.3 Length
Hylemonella gracilis - 6.3 Flagellar Assembly

Magnetospirillum magneticum - 7.6 Magnetotaxis
Methanoregula formicica - 3.2 Rod: Archaeal rods
Methanospirillum hungatei - 2.8 Sheath
Methanospirillum hungatei - 7.3 Chemosensory Array Conservation
Proteus mirabilis - 6.5 Peritrichous Flagella: Proteus mirabilis flagella
Pseudomonas aeruginosa - 6.3 Flagellar Assembly: Flagellar motor disassembly
Salmonella typhimurium - 7.2 Chemosensory Arrays
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 4.2 Nanowires
Shewanella oneidensis - 7.1 Chemotaxis
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Cone structure
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Organizing center
Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia
Vibrio cholerae - 10.4 Structural Variation
Vibrio cholerae - 10.7 Replication
Vibrio cholerae - 7.4 Chemoreceptor Variety
Vibrio cholerae - 7.5 Cytoplasmic Chemosensory Arrays: Vibrio cholerae chemosensory arrays
Vibrio cholerae - 9.7 Contractile Weapons

Chemosensory arrays (cytoplasmic)

Methanoregula formicica - 3.2 Rod: Archaeal rods
Methanoregula formicica - 7.5 Cytoplasmic Chemosensory Arrays
Vibrio cholerae - 7.5 Cytoplasmic Chemosensory Arrays: Vibrio cholerae chemosensory arrays

CTP synthase filaments

Caulobacter crescentus - 3.4 Vibrioid

Division plane

Agrobacterium tumefaciens - 5.8 Asymmetric Division
Caulobacter crescentus - 4.1 Stalk Bands
Caulobacter crescentus - 5.10 FtsZ
Caulobacter crescentus - 5.2 Fission
Caulobacter crescentus - 8.4 Differentiation
Cupriavidus necator - 3.2 Rod
Cupriavidus necator - 4.7 Bacterial Storage Granules
Cupriavidus necator - 5.9 Asynchronous Constriction
Halohasta litchfieldiae - 4.9 Archaeal Storage Granules: Halohasta litchfieldiae granules
Listeria monocytogenes - 2.2 Cell Wall
Porphyromonas gingivalis - 9.8 Type IX Secretion
Sphingopyxis alaskensis - 5.6 Diderm Cytokinesis: Sphingopyxis alaskensis division
Staphylococcus aureus - 5.4 Monoderm Septum
Sulfolobus acidocaldarius - 5.11 Archaeal Cytokinesis
Sulfolobus acidocaldarius - 5.12 Cdv
Thiomonas intermedia - 5.1 Copy Number

Division septum
Staphylococcus aureus - 5.4 Monoderm Septum
Tetrasphaera remsis - 5.5 Monoderm Cytokinesis
DNA
Haloarcula argentinensis - 2.9 DNA
Hyphomonas neptunium - 5.3 Budding
Vibrio cholerae - 10.7 Replication
Flagella (external, unsheathed)
Acetonema longum - 4.5 Microcompartments
Acetonema longum - 8.9 Diderm Sporulation
Agrobacterium tumefaciens - 9.1 Biofilm
Amoebophilus asiaticus - 9.7 Contractile Weapons: T6SS battery
Azospirillum brasilense - 7.4 Chemoreceptor Variety: Azospirillum brasilense aerotaxis
Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore engulfment
Campylobacter jejuni - 3.5 Helical
Campylobacter jejuni - 6.1 Flagellum
Campylobacter jejuni - 6.4 Flagella Patterns
Caulobacter crescentus - 10.6 Head Fibers
Caulobacter crescentus - 5.7 Outer Membrane Constriction
Caulobacter crescentus - 8.1 Stationary Phase
Caulobacter crescentus - 8.4 Differentiation
Escherichia coli - 9.10 Predator Entry: Bdellovibrio bacteriovorus entry
Helicobacter pylori - 6.4 Flagella Patterns: Lophotrichous bacteria
Hydrogenovibrio crunogenus - 2.3 Outer Membrane
Hylemonella gracilis - 3.3 Length
Hylemonella gracilis - 6.3 Flagellar Assembly
Listeria monocytogenes - 2.2 Cell Wall
Methylomicrobium alcaliphilum - 2.7 Surface Layer Variety: Methylomicrobium alcaliphilum
Proteus mirabilis - 6.5 Peritrichous Flagella: Proteus mirabilis flagella
Pseudomonas flexibilis - 6.5 Peritrichous Flagella
Salmonella typhimurium - 7.2 Chemosensory Arrays
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 10.10 Cellular Defense
Shewanella oneidensis - 4.2 Nanowires
Shewanella oneidensis - 7.1 Chemotaxis
Sporomusa acidovorans - 8.10 Diderm Spore Engulfment: Sporomusa acidovorans sporulation
Flagella (periplasmic)
Borrelia burgdorferi - 2.4 Vesicles: Pearled vesicles
Borrelia burgdorferi - 2.4 Vesicles: Tubular vesicles
Borrelia burgdorferi - 3.5 Helical: Borrelia burgdorferi shape
Borrelia burgdorferi - 6.7 Periplasmic Flagella

Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia

Flagella (sheathed)

Bdellovibrio bacteriovorus - 2.10 Nucleoid
Bdellovibrio bacteriovorus - 6.2 Flagellar Motor
Bdellovibrio bacteriovorus - 9.10 Predator Entry
Helicobacter hepaticus - 6.6 Sheathed Flagella
Vibrio cholerae - 7.4 Chemoreceptor Variety
Vibrio cholerae - 7.5 Cytoplasmic Chemosensory Arrays: Vibrio cholerae chemosensory arrays
Vibrio cholerae - 9.11 Predator Replication
Vibrio cholerae - 9.7 Contractile Weapons

Flagellar motor (assembling)

Hylemonella gracilis - 6.3 Flagellar Assembly

Flagellar motor (disassembled)

Caulobacter crescentus - 2.6 Surface Layer
Helicobacter pylori - 8.2 Genome Protection
Hylemonella gracilis - 3.3 Length
Pseudomonas aeruginosa - 6.3 Flagellar Assembly: Flagellar motor disassembly
Vibrio cholerae - 7.4 Chemoreceptor Variety
Vibrio cholerae - 7.5 Cytoplasmic Chemosensory Arrays: Vibrio cholerae chemosensory arrays
Vibrio cholerae - 9.9 Predation

Flagellar motors

Bdellovibrio bacteriovorus - 2.10 Nucleoid
Bdellovibrio bacteriovorus - 6.2 Flagellar Motor
Borrelia burgdorferi - 2.4 Vesicles: Pearled vesicles
Borrelia burgdorferi - 3.5 Helical: Borrelia burgdorferi shape
Borrelia burgdorferi - 6.7 Periplasmic Flagella
Campylobacter jejuni - 6.1 Flagellum
Campylobacter jejuni - 6.4 Flagella Patterns
Helicobacter hepaticus - 6.6 Sheathed Flagella
Helicobacter pylori - 6.4 Flagella Patterns: Lophotrichous bacteria
Hylemonella gracilis - 3.3 Length
Hylemonella gracilis - 6.3 Flagellar Assembly
Proteus mirabilis - 6.5 Peritrichous Flagella: Proteus mirabilis flagella
Pseudomonas aeruginosa - 6.3 Flagellar Assembly: Flagellar motor disassembly
Pseudomonas flexibilis - 6.5 Peritrichous Flagella
Shewanella oneidensis - 7.1 Chemotaxis
Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia
Vibrio cholerae - 7.4 Chemoreceptor Variety
Vibrio cholerae - 7.5 Cytoplasmic Chemosensory Arrays: Vibrio cholerae chemosensory arrays
Vibrio cholerae - 9.7 Contractile Weapons

FtsZ filaments
Caulobacter crescentus - 5.10 FtsZ
Cupriavidus necator - 5.9 Asynchronous Constriction
Gas vesicles
Halobacterium salinarum - 3.7 Square: Gas vesicles
Haloquadratum walsbyi - 3.7 Square
Gingipains
Porphyromonas gingivalis - 9.8 Type IX Secretion
Holdfast
Prosthecobacter vanneervenii - 8.4 Differentiation: Holdfast
Intracytoplasmic membrane
Methylomicrobium alcaliphilum - 2.7 Surface Layer Variety: Methylomicrobium alcaliphilum
Methyloprofundus sedimenti - 4.3 Intracytoplasmic Membrane: ICM variety
Rhodopseudomonas palustris - 4.3 Intracytoplasmic Membrane
Invasion portal
Bdellovibrio bacteriovorus - 9.10 Predator Entry
MACs
Pseudoalteromonas luteoviolacea - 9.7 Contractile Weapons: MACs
Magnetosomes
Magnetospirillum magneticum - 7.6 Magnetotaxis
MamK filaments
Magnetospirillum magneticum - 7.6 Magnetotaxis
Membrane (inner)
Acetonema longum - 4.5 Microcompartments
Acetonema longum - 8.10 Diderm Spore Engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Complete engulfment
Acetonema longum - 8.11 Diderm Germination
Acetonema longum - 8.9 Diderm Sporulation
Agrobacterium tumefaciens - 4.8 Storage Granule Variety
Agrobacterium tumefaciens - 5.8 Asymmetric Division
Agrobacterium tumefaciens - 9.1 Biofilm
Amoebophilus asiaticus - 9.7 Contractile Weapons: T6SS battery
Azospirillum brasilense - 7.4 Chemoreceptor Variety: Azospirillum brasilense aerotaxis
Bacteroidetes - 10.2 Contractile Tails
Bdellovibrio bacteriovorus - 2.10 Nucleoid
Bdellovibrio bacteriovorus - 6.2 Flagellar Motor
Bdellovibrio bacteriovorus - 9.10 Predator Entry
Bdellovibrio bacteriovorus - 9.10 Predator Entry: Bdellovibrio bacteriovorus entry
Bdellovibrio bacteriovorus - 9.11 Predator Replication
Bdellovibrio bacteriovorus - 9.9 Predation
Borrelia burgdorferi - 2.4 Vesicles: Pearled vesicles

Borrelia burgdorferi - 2.4 Vesicles: Tubular vesicles
Borrelia burgdorferi - 3.5 Helical: Borrelia burgdorferi shape
Borrelia burgdorferi - 6.7 Periplasmic Flagella
Brucella abortus - 3.2 Rod: Rod variety
Campylobacter jejuni - 3.5 Helical
Campylobacter jejuni - 6.4 Flagella Patterns
Caulobacter crescentus - 10.6 Head Fibers
Caulobacter crescentus - 10.6 Head Fibers: φCb13 infection
Caulobacter crescentus - 2.6 Surface Layer
Caulobacter crescentus - 3.4 Vibrioid
Caulobacter crescentus - 4.1 Stalk Bands
Caulobacter crescentus - 5.10 FtsZ
Caulobacter crescentus - 5.2 Fission
Caulobacter crescentus - 5.7 Outer Membrane Constriction
Caulobacter crescentus - 5.7 Outer Membrane Constriction: Caulobacter division
Caulobacter crescentus - 8.1 Stationary Phase
Caulobacter crescentus - 8.4 Differentiation
Cupriavidus necator - 3.2 Rod
Cupriavidus necator - 4.7 Bacterial Storage Granules
Cupriavidus necator - 5.9 Asynchronous Constriction
Cupriavidus necator - 5.9 Asynchronous Constriction: Asymmetric constriction
Delftia acidovorans - 2.6 Surface Layer: Nanopods
Escherichia coli - 10.5 Targeting
Escherichia coli - 9.5 Type V Secretion
Flavobacterium johnsoniae - 6.11 Type IX Secretion System
Gluconacetobacter hansenii - 9.2 Biofilm Matrix
Halothiobacillus neapolitanus - 4.6 Carboxysomes: Carboxysome variety
Halothiobacillus neapolitanus - 4.8 Storage Granule Variety: Carboxysome-granule connections
Helicobacter hepaticus - 6.6 Sheathed Flagella
Helicobacter pylori - 6.4 Flagella Patterns: Lophotrichous bacteria
Helicobacter pylori - 8.2 Genome Protection
Helicobacter pylori - 9.3 Type II and Type IV Secretion: Helicobacter pylori tubes
Hydrogenovibrio crunogenus - 2.3 Outer Membrane
Hydrogenovibrio crunogenus - 4.6 Carboxysomes: Long carboxysomes
Hydrogenovibrio crunogenus - 4.8 Storage Granule Variety: Granule-containing carboxysomes
Hylemonella gracilis - 3.3 Length
Hylemonella gracilis - 6.3 Flagellar Assembly
Hyphomonas neptunium - 5.3 Budding
Hyphomonas neptunium - 5.3 Budding: Hyphomonas lifecycle
Idiomarina loihiensis - 5.6 Diderm Cytokinesis
Legionella pneumophila - 9.3 Type II and Type IV Secretion

Lysobacter antibioticus - 4.7 Bacterial Storage Granules: Storage granule growth
Magnetospirillum magneticum - 7.6 Magnetotaxis
Methylomicrobium alcaliphilum - 2.7 Surface Layer Variety: Methylomicrobium alcaliphilum
Methyloprofundus sedimenti - 4.3 Intracytoplasmic Membrane: ICM variety
Mycobacterium marinum - 2.5 Classification Exceptions
Myxococcus xanthus - 2.4 Vesicles
Myxococcus xanthus - 2.4 Vesicles: Cytoplasmic vesicles
Myxococcus xanthus - 6.10 Type IV Pilus
Myxococcus xanthus - 9.6 Type VI Secretion
Porphyromonas gingivalis - 9.8 Type IX Secretion
Prosthecobacter debontii - 2.4 Vesicles: Cytoplasmic vesicle variety
Prosthecobacter vanneervenii - 3.6 Prosthecate
Prosthecobacter vanneervenii - 8.4 Differentiation: Holdfast
Proteus mirabilis - 6.5 Peritrichous Flagella: Proteus mirabilis flagella
Pseudomonas aeruginosa - 6.3 Flagellar Assembly: Flagellar motor disassembly
Pseudomonas aeruginosa - 9.4 Type III Secretion
Pseudomonas flexibilis - 6.5 Peritrichous Flagella
Rhodopseudomonas palustris - 4.3 Intracytoplasmic Membrane
Salmonella typhimurium - 7.2 Chemosensory Arrays
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 10.3 Noncontractile Tails
Shewanella oneidensis - 4.2 Nanowires
Shewanella oneidensis - 7.1 Chemotaxis
Simkania negevensis - 3.1 Coccoid
Sphingopyxis alaskensis - 5.6 Diderm Cytokinesis: Sphingopyxis alaskensis division
Sporomusa acidovorans - 8.10 Diderm Spore Engulfment: Sporomusa acidovorans sporulation
Thiomonas intermedia - 5.1 Copy Number
Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia
Verrucomicrobium spinosum - 3.6 Prosthecate
Vibrio cholerae - 10.4 Structural Variation
Vibrio cholerae - 10.7 Replication
Vibrio cholerae - 7.4 Chemoreceptor Variety
Vibrio cholerae - 7.5 Cytoplasmic Chemosensory Arrays: Vibrio cholerae chemosensory arrays
Vibrio cholerae - 9.7 Contractile Weapons

Membrane (monoderm)

Bacillus subtilis - 8.5 Monoderm Sporulation
Bacillus subtilis - 8.5 Monoderm Sporulation: Sporulation septum formation
Bacillus subtilis - 8.6 Monoderm Spore Engulfment
Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore engulfment
Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore coating

Clostridium thermocellum - 2.7 Surface Layer Variety: Clostridium thermocellum
Clostridium thermocellum - 4.4 Enzyme Filaments
Haloarcula argentinensis - 2.9 DNA
Halobacterium salinarum - 3.7 Square: Gas vesicles
Halobacterium salinarum - 6.9 Archaella Patterns: Lophotrichous Halobacteria
Haloferax gibbonsii - 4.9 Archaeal Storage Granules: Haloferax gibbonsii granules
Halohasta litchfieldiae - 4.9 Archaeal Storage Granules: Halohasta litchfieldiae granules
Halomicrobium mukohataei - 2.4 Vesicles: Archaeal vesicles
Haloquadratum walsbyi - 3.7 Square
Halorubrum litoreum - 4.9 Archaeal Storage Granules
Listeria monocytogenes - 2.2 Cell Wall
Methanoregula formicica - 2.7 Surface Layer Variety: Methanoregula formicica
Methanoregula formicica - 3.2 Rod: Archaeal rods
Methanoregula formicica - 6.8 Archaellum
Methanoregula formicica - 7.5 Cytoplasmic Chemosensory Arrays
Methanospirillum hungatei - 2.8 Sheath
Methanospirillum hungatei - 7.3 Chemosensory Array Conservation
Mycoplasma genitalium - 2.1 Membrane
Mycoplasma pneumoniae - 6.12 Terminal Organelle
Nitrosopumilus maritimus - 2.7 Surface Layer Variety: Nitrosopumilus maritimus
Staphylococcus aureus - 5.4 Monoderm Septum
Sulfolobus acidocaldarius - 5.11 Archaeal Cytokinesis
Sulfolobus acidocaldarius - 5.12 Cdv
Sulfolobus solfataricus - 10.8 Virus-Associated Pyramids
Sulfolobus solfataricus - 10.9 Pyramid Release
Sulfolobus solfataricus - 2.7 Surface Layer Variety
Tetrasphaera remsis - 5.5 Monoderm Cytokinesis
Thermococcus kodakaraensis - 6.9 Archaella Patterns
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Cone structure
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Organizing center
Thermococcus kodakaraensis - 8.3 Archaeal Stationary Phase

Membrane (outer)

Acetonema longum - 4.5 Microcompartments
Acetonema longum - 8.10 Diderm Spore Engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Complete engulfment
Acetonema longum - 8.11 Diderm Germination
Acetonema longum - 8.9 Diderm Sporulation
Agrobacterium tumefaciens - 4.8 Storage Granule Variety
Agrobacterium tumefaciens - 5.8 Asymmetric Division
Agrobacterium tumefaciens - 9.1 Biofilm
Amoebophilus asiaticus - 9.7 Contractile Weapons: T6SS battery
Azospirillum brasilense - 7.4 Chemoreceptor Variety: Azospirillum brasilense aerotaxis
Bacteroidetes - 10.2 Contractile Tails

Bdellovibrio bacteriovorus - 2.10 Nucleoid
Bdellovibrio bacteriovorus - 6.2 Flagellar Motor
Bdellovibrio bacteriovorus - 9.10 Predator Entry
Bdellovibrio bacteriovorus - 9.10 Predator Entry: Bdellovibrio bacteriovorus entry
Bdellovibrio bacteriovorus - 9.11 Predator Replication
Bdellovibrio bacteriovorus - 9.9 Predation
Borrelia burgdorferi - 2.4 Vesicles: Pearled vesicles
Borrelia burgdorferi - 2.4 Vesicles: Tubular vesicles
Borrelia burgdorferi - 3.5 Helical: Borrelia burgdorferi shape
Borrelia burgdorferi - 6.7 Periplasmic Flagella
Brucella abortus - 3.2 Rod: Rod variety
Campylobacter jejuni - 3.5 Helical
Campylobacter jejuni - 6.1 Flagellum
Campylobacter jejuni - 6.4 Flagella Patterns
Caulobacter crescentus - 10.6 Head Fibers
Caulobacter crescentus - 10.6 Head Fibers: φCb13 infection
Caulobacter crescentus - 2.6 Surface Layer
Caulobacter crescentus - 3.4 Vibrioid
Caulobacter crescentus - 4.1 Stalk Bands
Caulobacter crescentus - 5.10 FtsZ
Caulobacter crescentus - 5.2 Fission
Caulobacter crescentus - 5.7 Outer Membrane Constriction
Caulobacter crescentus - 5.7 Outer Membrane Constriction: Caulobacter division
Caulobacter crescentus - 8.1 Stationary Phase
Caulobacter crescentus - 8.4 Differentiation
Cupriavidus necator - 3.2 Rod
Cupriavidus necator - 4.7 Bacterial Storage Granules
Cupriavidus necator - 5.9 Asynchronous Constriction
Cupriavidus necator - 5.9 Asynchronous Constriction: Asymmetric constriction
Delftia acidovorans - 2.6 Surface Layer: Nanopods
Escherichia coli - 10.5 Targeting
Escherichia coli - 9.5 Type V Secretion
Flavobacterium johnsoniae - 6.11 Type IX Secretion System
Gluconacetobacter hansenii - 9.2 Biofilm Matrix
Halothiobacillus neapolitanus - 4.6 Carboxysomes: Carboxysome variety
Halothiobacillus neapolitanus - 4.8 Storage Granule Variety: Carboxysome-granule connections
Helicobacter hepaticus - 6.6 Sheathed Flagella
Helicobacter pylori - 6.4 Flagella Patterns: Lophotrichous bacteria
Helicobacter pylori - 8.2 Genome Protection
Helicobacter pylori - 9.3 Type II and Type IV Secretion: Helicobacter pylori tubes
Hydrogenovibrio crunogenus - 2.3 Outer Membrane
Hydrogenovibrio crunogenus - 4.6 Carboxysomes: Long carboxysomes

Hydrogenovibrio crunogenus - 4.8 Storage Granule Variety: Granule-containing carboxysomes
Hylemonella gracilis - 3.3 Length
Hylemonella gracilis - 6.3 Flagellar Assembly
Hyphomonas neptunium - 5.3 Budding
Hyphomonas neptunium - 5.3 Budding: Hyphomonas lifecycle
Idiomarina loihiensis - 5.6 Diderm Cytokinesis
Legionella pneumophila - 9.3 Type II and Type IV Secretion
Lysobacter antibioticus - 4.7 Bacterial Storage Granules: Storage granule growth
Magnetospirillum magneticum - 7.6 Magnetotaxis
Methylomicrobium alcaliphilum - 2.7 Surface Layer Variety: Methylomicrobium alcaliphilum
Methyloprofundus sedimenti - 4.3 Intracytoplasmic Membrane: ICM variety
Mycobacterium marinum - 2.5 Classification Exceptions
Myxococcus xanthus - 2.4 Vesicles
Myxococcus xanthus - 2.4 Vesicles: Cytoplasmic vesicles
Myxococcus xanthus - 6.10 Type IV Pilus
Myxococcus xanthus - 9.6 Type VI Secretion
Porphyromonas gingivalis - 9.8 Type IX Secretion
Prosthecobacter debontii - 2.4 Vesicles: Cytoplasmic vesicle variety
Prosthecobacter vanneervenii - 3.6 Prosthecate: Bacterial microtubules
Prosthecobacter vanneervenii - 8.4 Differentiation: Holdfast
Proteus mirabilis - 6.5 Peritrichous Flagella: Proteus mirabilis flagella
Pseudomonas aeruginosa - 6.3 Flagellar Assembly: Flagellar motor disassembly
Pseudomonas aeruginosa - 9.4 Type III Secretion
Pseudomonas flexibilis - 6.5 Peritrichous Flagella
Rhodopseudomonas palustris - 4.3 Intracytoplasmic Membrane
Salmonella typhimurium - 7.2 Chemosensory Arrays
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 10.3 Noncontractile Tails
Shewanella oneidensis - 4.2 Nanowires
Shewanella oneidensis - 7.1 Chemotaxis
Simkania negevensis - 3.1 Coccoid
Sphingopyxis alaskensis - 5.6 Diderm Cytokinesis: Sphingopyxis alaskensis division
Sporomusa acidovorans - 8.10 Diderm Spore Engulfment: Sporomusa acidovorans sporulation
Thiomonas intermedia - 5.1 Copy Number
Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia
Verrucomicrobium spinosum - 3.6 Prosthecate
Vibrio cholerae - 10.4 Structural Variation
Vibrio cholerae - 10.7 Replication
Vibrio cholerae - 7.4 Chemoreceptor Variety

Vibrio cholerae - 7.5 Cytoplasmic Chemosensory Arrays: Vibrio cholerae chemosensory arrays
Vibrio cholerae - 9.7 Contractile Weapons

Metal deposits
Shewanella oneidensis - 4.2 Nanowires

Microtubules
Prosthecobacter debontii - 2.4 Vesicles: Cytoplasmic vesicle variety
Prosthecobacter vanneervenii - 3.6 Prosthecate: Bacterial microtubules
Prosthecobacter vanneervenii - 8.4 Differentiation: Holdfast

Nanopods
Delftia acidovorans - 2.6 Surface Layer: Nanopods

Nanowires
Shewanella oneidensis - 4.2 Nanowires

Nucleoid
Bdellovibrio bacteriovorus - 2.10 Nucleoid
Bdellovibrio bacteriovorus - 6.2 Flagellar Motor
Bdellovibrio bacteriovorus - 9.10 Predator Entry
Bdellovibrio bacteriovorus - 9.10 Predator Entry: Bdellovibrio bacteriovorus entry
Bdellovibrio bacteriovorus - 9.11 Predator Replication
Bdellovibrio bacteriovorus - 9.9 Predation
Caulobacter crescentus - 8.1 Stationary Phase
Hylemonella gracilis - 3.3 Length
Hylemonella gracilis - 6.3 Flagellar Assembly
Vibrio cholerae - 10.7 Replication

Pdu microcompartments
Acetonema longum - 4.5 Microcompartments
Acetonema longum - 8.9 Diderm Sporulation

Phage capsids
Bacteroidetes - 10.2 Contractile Tails
Caulobacter crescentus - 10.6 Head Fibers
Caulobacter crescentus - 10.6 Head Fibers: φCb13 infection
Escherichia coli - 10.5 Targeting
Pseudomonas aeruginosa - 9.4 Type III Secretion
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 10.10 Cellular Defense
Shewanella oneidensis - 10.3 Noncontractile Tails
Sulfolobus solfataricus - 10.8 Virus-Associated Pyramids
Vibrio cholerae - 10.4 Structural Variation
Vibrio cholerae - 10.7 Replication

Phage head fibers
Caulobacter crescentus - 10.6 Head Fibers

Phage tail fibers

Bacteroidetes - 10.2 Contractile Tails
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 10.10 Cellular Defense
Shewanella oneidensis - 10.3 Noncontractile Tails
Vibrio cholerae - 10.4 Structural Variation

Phage tails

Bacteroidetes - 10.2 Contractile Tails
Caulobacter crescentus - 10.6 Head Fibers
Caulobacter crescentus - 10.6 Head Fibers: φCb13 infection
Pseudomonas aeruginosa - 9.4 Type III Secretion
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 10.10 Cellular Defense
Shewanella oneidensis - 10.3 Noncontractile Tails
Vibrio cholerae - 10.7 Replication

Pili

Amoebophilus asiaticus - 9.7 Contractile Weapons: T6SS battery
Bacteroidetes - 10.2 Contractile Tails
Caulobacter crescentus - 10.6 Head Fibers: φCb13 infection
Caulobacter crescentus - 2.6 Surface Layer
Caulobacter crescentus - 8.1 Stationary Phase
Clostridium thermocellum - 2.7 Surface Layer Variety: Clostridium thermocellum
Cupriavidus necator - 5.9 Asynchronous Constriction
Escherichia coli - 10.5 Targeting
Escherichia coli - 9.10 Predator Entry
Escherichia coli - 9.10 Predator Entry: Bdellovibrio bacteriovorus entry
Halothiobacillus neapolitanus - 4.6 Carboxysomes: Carboxysome variety
Hydrogenovibrio crunogenus - 2.3 Outer Membrane
Methanoregula formicica - 6.8 Archaellum
Methanospirillum hungatei - 2.8 Sheath
Methanospirillum hungatei - 7.3 Chemosensory Array Conservation
Prosthecobacter debontii - 2.4 Vesicles: Cytoplasmic vesicle variety
Prosthecobacter vanneervenii - 8.4 Differentiation: Holdfast
Pseudomonas flexibilis - 6.5 Peritrichous Flagella
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 7.1 Chemotaxis: Chemoreceptor structure
Sulfolobus solfataricus - 10.8 Virus-Associated Pyramids
Sulfolobus solfataricus - 10.9 Pyramid Release
Sulfolobus solfataricus - 2.7 Surface Layer Variety
Thiomonas intermedia - 4.6 Carboxysomes
Thiomonas intermedia - 5.1 Copy Number
Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia
Verrucomicrobium spinosum - 3.6 Prosthecate

Vibrio cholerae - 10.7 Replication
Vibrio cholerae - 9.7 Contractile Weapons

Pili (Type IV)

Bdellovibrio bacteriovorus - 2.10 Nucleoid
Bdellovibrio bacteriovorus - 6.2 Flagellar Motor
Bdellovibrio bacteriovorus - 9.9 Predation
Myxococcus xanthus - 2.4 Vesicles
Myxococcus xanthus - 6.10 Type IV Pilus
Myxococcus xanthus - 9.6 Type VI Secretion

PopZ

Caulobacter crescentus - 4.1 Stalk Bands
Caulobacter crescentus - 5.2 Fission
Caulobacter crescentus - 5.7 Outer Membrane Constriction

RecA/RadA filaments

Helicobacter pylori - 8.2 Genome Protection
Thermococcus kodakaraensis - 8.3 Archaeal Stationary Phase

Ribosomes

Acetonema longum - 4.5 Microcompartments
Acetonema longum - 8.11 Diderm Germination
Acetonema longum - 8.9 Diderm Sporulation
Agrobacterium tumefaciens - 4.8 Storage Granule Variety
Agrobacterium tumefaciens - 5.8 Asymmetric Division
Agrobacterium tumefaciens - 9.1 Biofilm
Amoebophilus asiaticus - 9.7 Contractile Weapons: T6SS battery
Azospirillum brasilense - 7.4 Chemoreceptor Variety: Azospirillum brasilense aerotaxis
Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore engulfment
Bacillus subtilis - 8.7 Monoderm Spores
Bacteroidetes - 10.2 Contractile Tails
Bdellovibrio bacteriovorus - 2.10 Nucleoid
Bdellovibrio bacteriovorus - 6.2 Flagellar Motor
Bdellovibrio bacteriovorus - 9.10 Predator Entry
Bdellovibrio bacteriovorus - 9.10 Predator Entry: Bdellovibrio bacteriovorus entry
Bdellovibrio bacteriovorus - 9.11 Predator Replication
Bdellovibrio bacteriovorus - 9.9 Predation
Borrelia burgdorferi - 2.4 Vesicles: Pearled vesicles
Borrelia burgdorferi - 2.4 Vesicles: Tubular vesicles
Borrelia burgdorferi - 3.5 Helical: Borrelia burgdorferi shape
Borrelia burgdorferi - 6.7 Periplasmic Flagella
Campylobacter jejuni - 3.5 Helical
Campylobacter jejuni - 6.4 Flagella Patterns
Caulobacter crescentus - 10.6 Head Fibers
Caulobacter crescentus - 10.6 Head Fibers: φCb13 infection
Caulobacter crescentus - 2.6 Surface Layer

Caulobacter crescentus - 3.4 Vibrioid
Caulobacter crescentus - 4.1 Stalk Bands
Caulobacter crescentus - 5.10 FtsZ
Caulobacter crescentus - 5.2 Fission
Caulobacter crescentus - 5.7 Outer Membrane Constriction
Caulobacter crescentus - 5.7 Outer Membrane Constriction: Caulobacter division
Caulobacter crescentus - 8.1 Stationary Phase
Caulobacter crescentus - 8.4 Differentiation
Clostridium thermocellum - 2.7 Surface Layer Variety: Clostridium thermocellum
Clostridium thermocellum - 4.4 Enzyme Filaments
Cupriavidus necator - 3.2 Rod
Cupriavidus necator - 4.7 Bacterial Storage Granules
Cupriavidus necator - 5.9 Asynchronous Constriction
Escherichia coli - 10.5 Targeting
Escherichia coli - 9.5 Type V Secretion
Flavobacterium johnsoniae - 6.11 Type IX Secretion System
Gluconacetobacter hansenii - 9.2 Biofilm Matrix
Halobacterium salinarum - 3.7 Square: Gas vesicles
Halobacterium salinarum - 6.9 Archaella Patterns: Lophotrichous Halobacteria
Haloferax gibbonsii - 4.9 Archaeal Storage Granules: Haloferax gibbonsii granules
Halohasta litchfieldiae - 4.9 Archaeal Storage Granules: Halohasta litchfieldiae granules
Halomicrobium mukohataei - 2.4 Vesicles: Archaeal vesicles
Haloquadratum walsbyi - 3.7 Square
Halorubrum litoreum - 4.9 Archaeal Storage Granules
Halothiobacillus neapolitanus - 4.6 Carboxysomes: Carboxysome variety
Halothiobacillus neapolitanus - 4.8 Storage Granule Variety: Carboxysome-granule connections
Helicobacter hepaticus - 6.6 Sheathed Flagella
Helicobacter pylori - 6.4 Flagella Patterns: Lophotrichous bacteria
Helicobacter pylori - 8.2 Genome Protection
Helicobacter pylori - 9.3 Type II and Type IV Secretion: Helicobacter pylori tubes
Hydrogenovibrio crunogenus - 2.3 Outer Membrane
Hydrogenovibrio crunogenus - 4.6 Carboxysomes: Long carboxysomes
Hydrogenovibrio crunogenus - 4.8 Storage Granule Variety: Granule-containing carboxysomes
Hylemonella gracilis - 3.3 Length
Hylemonella gracilis - 6.3 Flagellar Assembly
Hyphomonas neptunium - 5.3 Budding
Hyphomonas neptunium - 5.3 Budding: Hyphomonas lifecycle
Idiomarina loihiensis - 5.6 Diderm Cytokinesis
Legionella pneumophila - 9.3 Type II and Type IV Secretion
Listeria monocytogenes - 2.2 Cell Wall
Lysobacter antibioticus - 4.7 Bacterial Storage Granules: Storage granule growth

Magnetospirillum magneticum - 7.6 Magnetotaxis
Methanoregula formicica - 2.7 Surface Layer Variety: Methanoregula formicica
Methanoregula formicica - 3.2 Rod: Archaeal rods
Methanospirillum hungatei - 2.8 Sheath
Methanospirillum hungatei - 7.3 Chemosensory Array Conservation
Methyloprofundus sedimenti - 4.3 Intracytoplasmic Membrane: ICM variety
Mycobacterium marinum - 2.5 Classification Exceptions
Mycoplasma genitalium - 2.1 Membrane
Mycoplasma pneumoniae - 6.12 Terminal Organelle
Myxococcus xanthus - 2.4 Vesicles: Cytoplasmic vesicles
Myxococcus xanthus - 6.10 Type IV Pilus
Myxococcus xanthus - 9.6 Type VI Secretion
Nitrosopumilus maritimus - 2.7 Surface Layer Variety: Nitrosopumilus maritimus
Porphyromonas gingivalis - 9.8 Type IX Secretion
Prosthecobacter debontii - 2.4 Vesicles: Cytoplasmic vesicle variety
Prosthecobacter vanneervenii - 3.6 Prosthecate: Bacterial microtubules
Prosthecobacter vanneervenii - 8.4 Differentiation: Holdfast
Proteus mirabilis - 6.5 Peritrichous Flagella: Proteus mirabilis flagella
Pseudomonas aeruginosa - 6.3 Flagellar Assembly: Flagellar motor disassembly
Pseudomonas aeruginosa - 9.4 Type III Secretion
Pseudomonas flexibilis - 6.5 Peritrichous Flagella
Salmonella typhimurium - 7.2 Chemosensory Arrays
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 10.3 Noncontractile Tails
Shewanella oneidensis - 4.2 Nanowires
Shewanella oneidensis - 7.1 Chemotaxis
Simkania negevensis - 3.1 Coccoid
Sphingopyxis alaskensis - 5.6 Diderm Cytokinesis: Sphingopyxis alaskensis division
Sporomusa acidovorans - 8.10 Diderm Spore Engulfment: Sporomusa acidovorans sporulation
Staphylococcus aureus - 5.4 Monoderm Septum
Sulfolobus acidocaldarius - 5.11 Archaeal Cytokinesis
Sulfolobus acidocaldarius - 5.12 Cdv
Tetrasphaera remsis - 5.5 Monoderm Cytokinesis
Thermococcus kodakaraensis - 6.9 Archaella Patterns
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Cone structure
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Organizing center
Thermococcus kodakaraensis - 8.3 Archaeal Stationary Phase
Thiomonas intermedia - 4.6 Carboxysomes
Thiomonas intermedia - 5.1 Copy Number
Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia
Vibrio cholerae - 10.4 Structural Variation
Vibrio cholerae - 10.7 Replication

Vibrio cholerae - 7.4 Chemoreceptor Variety
Vibrio cholerae - 7.5 Cytoplasmic Chemosensory Arrays: Vibrio cholerae chemosensory arrays
Vibrio cholerae - 9.7 Contractile Weapons

Sheath

Methanospirillum hungatei - 2.8 Sheath
Methanospirillum hungatei - 7.3 Chemosensory Array Conservation

Spore coat

Acetonema longum - 8.10 Diderm Spore Engulfment: Mature spore
Acetonema longum - 8.11 Diderm Germination
Bacillus subtilis - 8.7 Monoderm Spores
Bacillus subtilis - 8.8 Monoderm Germination

Spore cortex

Acetonema longum - 8.10 Diderm Spore Engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Complete engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Mature spore
Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore coating
Bacillus subtilis - 8.7 Monoderm Spores
Bacillus subtilis - 8.8 Monoderm Germination

Spore membrane (inner)

Acetonema longum - 8.10 Diderm Spore Engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Complete engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Mature spore
Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore coating
Bacillus subtilis - 8.7 Monoderm Spores
Bacillus subtilis - 8.8 Monoderm Germination

Spore membrane (outer)

Acetonema longum - 8.10 Diderm Spore Engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Complete engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Mature spore
Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore coating
Bacillus subtilis - 8.7 Monoderm Spores
Bacillus subtilis - 8.8 Monoderm Germination

Sporulation plane

Bacillus subtilis - 8.5 Monoderm Sporulation: Sporulation septum formation

Sporulation septum

Acetonema longum - 8.9 Diderm Sporulation
Bacillus subtilis - 8.5 Monoderm Sporulation
Bacillus subtilis - 8.6 Monoderm Spore Engulfment
Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore engulfment

Stalk bands

Caulobacter crescentus - 4.1 Stalk Bands

Storage granules

Acetonema longum - 8.10 Diderm Spore Engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Complete engulfment
Acetonema longum - 8.10 Diderm Spore Engulfment: Mature spore
Acetonema longum - 8.11 Diderm Germination
Acetonema longum - 8.9 Diderm Sporulation
Agrobacterium tumefaciens - 4.8 Storage Granule Variety
Agrobacterium tumefaciens - 5.8 Asymmetric Division
Agrobacterium tumefaciens - 9.1 Biofilm
Azospirillum brasilense - 7.4 Chemoreceptor Variety: Azospirillum brasilense aerotaxis
Bacteroidetes - 10.2 Contractile Tails
Bdellovibrio bacteriovorus - 6.2 Flagellar Motor
Bdellovibrio bacteriovorus - 9.10 Predator Entry
Bdellovibrio bacteriovorus - 9.10 Predator Entry: Bdellovibrio bacteriovorus entry
Bdellovibrio bacteriovorus - 9.11 Predator Replication
Bdellovibrio bacteriovorus - 9.9 Predation
Brucella abortus - 3.2 Rod: Rod variety
Campylobacter jejuni - 3.5 Helical
Campylobacter jejuni - 6.4 Flagella Patterns
Caulobacter crescentus - 2.6 Surface Layer
Caulobacter crescentus - 3.4 Vibrioid
Caulobacter crescentus - 4.1 Stalk Bands
Caulobacter crescentus - 5.10 FtsZ
Caulobacter crescentus - 5.2 Fission
Caulobacter crescentus - 5.7 Outer Membrane Constriction
Caulobacter crescentus - 5.7 Outer Membrane Constriction: Caulobacter division
Caulobacter crescentus - 8.1 Stationary Phase
Caulobacter crescentus - 8.4 Differentiation
Cupriavidus necator - 3.2 Rod
Cupriavidus necator - 4.7 Bacterial Storage Granules
Cupriavidus necator - 5.9 Asynchronous Constriction
Cupriavidus necator - 5.9 Asynchronous Constriction: Asymmetric constriction
Escherichia coli - 9.5 Type V Secretion
Flavobacterium johnsoniae - 6.11 Type IX Secretion System
Gluconacetobacter hansenii - 9.2 Biofilm Matrix
Haloarcula argentinensis - 2.9 DNA
Haloferax gibbonsii - 4.9 Archaeal Storage Granules: Haloferax gibbonsii granules
Halohasta litchfieldiae - 4.9 Archaeal Storage Granules: Halohasta litchfieldiae granules
Haloquadratum walsbyi - 3.7 Square
Halorubrum litoreum - 4.9 Archaeal Storage Granules
Halothiobacillus neapolitanus - 4.6 Carboxysomes: Carboxysome variety
Halothiobacillus neapolitanus - 4.8 Storage Granule Variety: Carboxysome-granule connections

Helicobacter hepaticus - 6.6 Sheathed Flagella
Helicobacter pylori - 6.4 Flagella Patterns: Lophotrichous bacteria
Helicobacter pylori - 8.2 Genome Protection
Hydrogenovibrio crunogenus - 2.3 Outer Membrane
Hydrogenovibrio crunogenus - 4.6 Carboxysomes: Long carboxysomes
Hydrogenovibrio crunogenus - 4.8 Storage Granule Variety: Granule-containing carboxysomes
Legionella pneumophila - 9.3 Type II and Type IV Secretion
Lysobacter antibioticus - 4.7 Bacterial Storage Granules: Storage granule growth
Magnetospirillum magneticum - 7.6 Magnetotaxis
Methanospirillum hungatei - 2.8 Sheath
Methanospirillum hungatei - 7.3 Chemosensory Array Conservation
Methylomicrobium alcaliphilum - 2.7 Surface Layer Variety: Methylomicrobium alcaliphilum
Methyloprofundus sedimenti - 4.3 Intracytoplasmic Membrane: ICM variety
Mycobacterium marinum - 2.5 Classification Exceptions
Myxococcus xanthus - 2.4 Vesicles: Cytoplasmic vesicles
Myxococcus xanthus - 6.10 Type IV Pilus
Myxococcus xanthus - 9.6 Type VI Secretion
Prosthecobacter debontii - 2.4 Vesicles: Cytoplasmic vesicle variety
Prosthecobacter vanneervenii - 3.6 Prosthecate: Bacterial microtubules
Prosthecobacter vanneervenii - 8.4 Differentiation: Holdfast
Pseudomonas aeruginosa - 6.3 Flagellar Assembly: Flagellar motor disassembly
Pseudomonas aeruginosa - 9.4 Type III Secretion
Pseudomonas flexibilis - 6.5 Peritrichous Flagella
Rhodopseudomonas palustris - 4.3 Intracytoplasmic Membrane
Sphingopyxis alaskensis - 5.6 Diderm Cytokinesis: Sphingopyxis alaskensis division
Sulfolobus acidocaldarius - 5.11 Archaeal Cytokinesis
Sulfolobus acidocaldarius - 5.12 Cdv
Sulfolobus solfataricus - 10.8 Virus-Associated Pyramids
Sulfolobus solfataricus - 2.7 Surface Layer Variety
Tetrasphaera remsis - 5.5 Monoderm Cytokinesis
Thiomonas intermedia - 4.6 Carboxysomes
Thiomonas intermedia - 5.1 Copy Number
Verrucomicrobium spinosum - 3.6 Prosthecate
Vibrio cholerae - 10.4 Structural Variation
Vibrio cholerae - 9.7 Contractile Weapons

Surface layer

Caulobacter crescentus - 10.6 Head Fibers
Caulobacter crescentus - 10.6 Head Fibers: φCb13 infection
Caulobacter crescentus - 2.6 Surface Layer
Caulobacter crescentus - 3.4 Vibrioid
Caulobacter crescentus - 4.1 Stalk Bands

Caulobacter crescentus - 5.10 FtsZ
Caulobacter crescentus - 5.2 Fission
Caulobacter crescentus - 5.7 Outer Membrane Constriction
Caulobacter crescentus - 5.7 Outer Membrane Constriction: Caulobacter division
Caulobacter crescentus - 8.4 Differentiation
Clostridium thermocellum - 2.7 Surface Layer Variety: Clostridium thermocellum
Clostridium thermocellum - 4.4 Enzyme Filaments
Delftia acidovorans - 2.6 Surface Layer: Nanopods
Haloarcula argentinensis - 2.9 DNA
Halobacterium salinarum - 3.7 Square: Gas vesicles
Halobacterium salinarum - 6.9 Archaella Patterns: Lophotrichous Halobacteria
Haloferax gibbonsii - 4.9 Archaeal Storage Granules: Haloferax gibbonsii granules
Halohasta litchfieldiae - 4.9 Archaeal Storage Granules: Halohasta litchfieldiae granules
Halomicrobium mukohataei - 2.4 Vesicles: Archaeal vesicles
Haloquadratum walsbyi - 3.7 Square
Halorubrum litoreum - 4.9 Archaeal Storage Granules
Methanoregula formicica - 2.7 Surface Layer Variety: Methanoregula formicica
Methanoregula formicica - 3.2 Rod: Archaeal rods
Methanoregula formicica - 6.8 Archaellum
Methanoregula formicica - 7.5 Cytoplasmic Chemosensory Arrays
Methanospirillum hungatei - 2.8 Sheath
Methanospirillum hungatei - 7.3 Chemosensory Array Conservation
Methylomicrobium alcaliphilum - 2.7 Surface Layer Variety: Methylomicrobium alcaliphilum
Methyloprofundus sedimenti - 4.3 Intracytoplasmic Membrane: ICM variety
Nitrosopumilus maritimus - 2.7 Surface Layer Variety: Nitrosopumilus maritimus
Sulfolobus acidocaldarius - 5.11 Archaeal Cytokinesis
Sulfolobus acidocaldarius - 5.12 Cdv
Sulfolobus solfataricus - 10.8 Virus-Associated Pyramids
Sulfolobus solfataricus - 10.9 Pyramid Release
Sulfolobus solfataricus - 2.7 Surface Layer Variety
Thermococcus kodakaraensis - 6.9 Archaella Patterns
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Cone structure
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Organizing center
Thermococcus kodakaraensis - 8.3 Archaeal Stationary Phase
Thiomonas intermedia - 4.6 Carboxysomes
Thiomonas intermedia - 5.1 Copy Number

Terminal organelles

Mycoplasma genitalium - 2.1 Membrane
Mycoplasma pneumoniae - 6.12 Terminal Organelle

Type II secretion systems

Legionella pneumophila - 9.3 Type II and Type IV Secretion
Vibrio cholerae - 10.4 Structural Variation

Type III secretion systems
- Pseudomonas aeruginosa - 9.4 Type III Secretion

Type IV secretion systems
- Helicobacter pylori - 9.3 Type II and Type IV Secretion: Helicobacter pylori tubes
- Legionella pneumophila - 9.3 Type II and Type IV Secretion

Type V secretion systems
- Escherichia coli - 9.5 Type V Secretion

Type VI secretion systems
- Agrobacterium tumefaciens - 9.1 Biofilm
- Azospirillum brasilense - 9.7 Contractile Weapons: T6SS battery
- Myxococcus xanthus - 9.6 Type VI Secretion: Primed T6SS structure
- Proteus mirabilis - 6.5 Peritrichous Flagella: Proteus mirabilis flagella
- Vibrio cholerae - 9.7 Contractile Weapons: Fired T6SS structure

Type IX secretion systems
- Porphyromonas gingivalis - 9.8 Type IX Secretion

Unidentified structures
- Acetonema longum - 4.5 Microcompartments
- Acetonema longum - 8.9 Diderm Sporulation
- Agrobacterium tumefaciens - 9.1 Biofilm
- Amoebophilus asiaticus - 9.7 Contractile Weapons: T6SS battery
- Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore engulfment
- Bacillus subtilis - 8.6 Monoderm Spore Engulfment: Spore coating
- Bdellovibrio bacteriovorus - 2.10 Nucleoid
- Bdellovibrio bacteriovorus - 6.2 Flagellar Motor
- Borrelia burgdorferi - 3.5 Helical: Borrelia burgdorferi shape
- Campylobacter jejuni - 3.5 Helical
- Campylobacter jejuni - 6.4 Flagella Patterns
- Caulobacter crescentus - 8.4 Differentiation
- Cupriavidus necator - 5.9 Asynchronous Constriction
- Flavobacterium johnsoniae - 6.11 Type IX Secretion System
- Haloarcula argentinensis - 2.9 DNA
- Halohasta litchfieldiae - 4.9 Archaeal Storage Granules: Halohasta litchfieldiae granules
- Haloquadratum walsbyi - 3.7 Square
- Halorubrum litoreum - 4.9 Archaeal Storage Granules
- Halothiobacillus neapolitanus - 4.8 Storage Granule Variety: Carboxysome-granule connections
- Helicobacter pylori - 6.4 Flagella Patterns: Lophotrichous bacteria
- Helicobacter pylori - 9.3 Type II and Type IV Secretion: Helicobacter pylori tubes
- Hydrogenovibrio crunogenus - 4.6 Carboxysomes: Long carboxysomes
- Hydrogenovibrio crunogenus - 4.8 Storage Granule Variety: Granule-containing carboxysomes
- Listeria monocytogenes - 2.2 Cell Wall
- Methanoregula formicica - 2.7 Surface Layer Variety: Methanoregula formicica

Methanoregula formicica - 3.2 Rod: Archaeal rods
Methanoregula formicica - 6.8 Archaellum
Methanoregula formicica - 7.5 Cytoplasmic Chemosensory Arrays
Methylomicrobium alcaliphilum - 2.7 Surface Layer Variety: Methylomicrobium alcaliphilum
Mycobacterium marinum - 2.5 Classification Exceptions
Myxococcus xanthus - 2.4 Vesicles
Myxococcus xanthus - 9.6 Type VI Secretion
Nitrosopumilus maritimus - 2.7 Surface Layer Variety: Nitrosopumilus maritimus
Prosthecobacter debontii - 2.4 Vesicles: Cytoplasmic vesicle variety
Pseudoalteromonas luteoviolacea - 9.7 Contractile Weapons: MACs
Sporomusa acidovorans - 8.10 Diderm Spore Engulfment: Sporomusa acidovorans sporulation
Sulfolobus acidocaldarius - 5.12 Cdv
Thermococcus kodakaraensis - 6.9 Archaella Patterns
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Cone structure
Thermococcus kodakaraensis - 6.9 Archaella Patterns: Organizing center
Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia
Vibrio cholerae - 10.4 Structural Variation
Vibrio cholerae - 10.7 Replication
Vibrio cholerae - 9.7 Contractile Weapons

Unipolar polysaccharide

Agrobacterium tumefaciens - 9.1 Biofilm

Vesicles (cytoplasmic)

Acetonema longum - 8.9 Diderm Sporulation
Agrobacterium tumefaciens - 4.8 Storage Granule Variety
Amoebophilus asiaticus - 9.7 Contractile Weapons: T6SS battery
Azospirillum brasilense - 7.4 Chemoreceptor Variety: Azospirillum brasilense aerotaxis
Bdellovibrio bacteriovorus - 9.10 Predator Entry
Bdellovibrio bacteriovorus - 9.10 Predator Entry: Bdellovibrio bacteriovorus entry
Bdellovibrio bacteriovorus - 9.11 Predator Replication
Brucella abortus - 3.2 Rod: Rod variety
Caulobacter crescentus - 10.6 Head Fibers: φCb13 infection
Caulobacter crescentus - 3.4 Vibrioid: CTP synthase
Caulobacter crescentus - 5.2 Fission
Caulobacter crescentus - 5.7 Outer Membrane Constriction: Caulobacter division
Caulobacter crescentus - 8.1 Stationary Phase
Escherichia coli - 9.5 Type V Secretion
Gluconacetobacter hansenii - 9.2 Biofilm Matrix
Halobacterium salinarum - 6.9 Archaella Patterns: Lophotrichous Halobacteria
Halomicrobium mukohataei - 2.4 Vesicles: Archaeal vesicles
Helicobacter pylori - 6.4 Flagella Patterns: Lophotrichous bacteria
Helicobacter pylori - 8.2 Genome Protection

Legionella pneumophila - 9.3 Type II and Type IV Secretion
Listeria monocytogenes - 2.2 Cell Wall
Methanospirillum hungatei - 2.8 Sheath
Methanospirillum hungatei - 7.3 Chemosensory Array Conservation
Myxococcus xanthus - 2.4 Vesicles: Cytoplasmic vesicles
Prosthecobacter debontii - 2.4 Vesicles: Cytoplasmic vesicle variety
Prosthecobacter vanneervenii - 3.6 Prosthecate
Proteus mirabilis - 6.5 Peritrichous Flagella: Proteus mirabilis flagella
Pseudomonas aeruginosa - 6.3 Flagellar Assembly: Flagellar motor disassembly
Pseudomonas flexibilis - 6.5 Peritrichous Flagella
Shewanella oneidensis - 10.1 Phage
Shewanella oneidensis - 10.3 Noncontractile Tails
Shewanella oneidensis - 4.2 Nanowires
Simkania negevensis - 3.1 Coccoid
Sporomusa acidovorans - 8.10 Diderm Spore Engulfment: Sporomusa acidovorans sporulation
Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia
Vibrio cholerae - 9.7 Contractile Weapons

Vesicles (extracellular)

Acetonema longum - 4.5 Microcompartments
Acetonema longum - 8.11 Diderm Germination
Agrobacterium tumefaciens - 9.1 Biofilm
Bdellovibrio bacteriovorus - 9.11 Predator Replication
Borrelia burgdorferi - 2.4 Vesicles: Pearled vesicles
Borrelia burgdorferi - 2.4 Vesicles: Tubular vesicles
Cupriavidus necator - 3.2 Rod
Haloferax gibbonsii - 4.9 Archaeal Storage Granules: Haloferax gibbonsii granules
Halomicrobium mukohataei - 2.4 Vesicles: Archaeal vesicles
Haloquadratum walsbyi - 3.7 Square
Helicobacter pylori - 8.2 Genome Protection
Hylemonella gracilis - 3.3 Length
Hyphomonas neptunium - 5.3 Budding: Hyphomonas lifecycle
Idiomarina loihiensis - 5.6 Diderm Cytokinesis
Myxococcus xanthus - 2.4 Vesicles
Myxococcus xanthus - 2.4 Vesicles: Cytoplasmic vesicles
Prosthecobacter debontii - 2.4 Vesicles: Cytoplasmic vesicle variety
Pseudomonas aeruginosa - 9.4 Type III Secretion
Pseudomonas flexibilis - 6.5 Peritrichous Flagella
Shewanella oneidensis - 10.10 Cellular Defense
Shewanella oneidensis - 7.1 Chemotaxis
Sulfolobus solfataricus - 2.7 Surface Layer Variety
Thermococcus kodakaraensis - 8.3 Archaeal Stationary Phase
Treponema primitia - 6.7 Periplasmic Flagella: Treponema primitia

Vibrio cholerae - 10.4 Structural Variation

Vesicles (periplasmic)

Agrobacterium tumefaciens - 5.8 Asymmetric Division

Hydrogenovibrio crunogenus - 4.6 Carboxysomes: Long carboxysomes

Prosthecobacter vanneervenii - 3.6 Prosthecate

Appendix B

Scientist Profiles

Morgan Beeby

Morgan Beeby is a group leader at Imperial College London. In London. He earned his PhD with Todd O. Yeates at UCLA where he developed interests in evolution, bioinformatics, and structural biology. Electron cryotomography was the clear next step, prompting him to join Grant's lab as a postdoc from 2008 to 2012. In 2013 he established his own lab using cryoET as the backbone of an interdisciplinary research program to understand the evolution of molecular machines, with a focus on bacterial flagella.

Ariane Briegel

Ariane Briegel is Professor of Ultrastructural Biology at the Leiden University (The Netherlands) and co-director of the Dutch Center for Electron Nanoscopy (NeCEN). She earned her PhD in the laboratory of Prof. Wolfgang Baumeister at the Max Planck Institute in Martinsried Germany. Here, she learned the power of Electron Tomography to study microbes in three dimensions and at macromolecular resolution. She continued to explore the wonders of bacterial structure and function in the laboratory of Grant Jensen as a postdoc and research scientist. She has 20 years of experience using cryo-electron microscopy to study bacterial and archaeal ultrastructure. The Briegel laboratory focuses on investigating how microbes sense and respond to their environment using cryoEM methods.

Yi-Wei Chang

Yi-Wei Chang is a structural biology enthusiast and principal investigator at the University of Pennsylvania. Yi-Wei received his Ph.D. degree from National Tsing-Hua University and Academia Sinica in Taiwan, working with Chwan-Deng Hsiao in using X-ray crystallography to study atomic structural details of purified proteins. After being astonished by Grant Jensen's visiting seminar at Academia Sinica highlighting the power of cryo-electron tomography in resolving macromolecular structures directly in cellular contexts, Yi-Wei set this as his long term research direction and was lucky enough to join the Jensen laboratory at Caltech in 2011 as a postdoctoral scholar. After years of working with the amazing group to master cryo-electron tomography methods and visualize fascinating macromolecular structures in situ, Yi-Wei launched his own laboratory at the University of Pennsylvania Perelman School of Medicine in 2019 to continue exploring the magnificent in-cell structural biology and to nurture the next generation of outstanding scientists.

Songye Chen

Songye Chen is the managing co-director of the Caltech Cryo-EM Facility. She earned her PhD in physics at Caltech, for investigations on ultrafast electron crystallography with Dr. Ahmed Zewail. She joined the lab of Dr. Grant Jensen as a postdoc after that and began her career as an electron microscopist working in cryoEM. She has been enjoying developing new methods, working with biologists and facilitating their cutting-edge research with state-of-the-art cryoEM technology.

Georges Chreifi

Georges Chreifi is a postdoctoral scholar at Caltech. He earned his Ph.D. in the laboratory of Tom Poulos at UC Irvine studying structure-function relationships in heme proteins, with a focus in X-ray crystallography and enzymology. He joined Grant Jensen's lab in 2017 to do his postdoctoral research in cryo-EM. He is currently working on developing rapid tilt-series methods in cryo-ET and elucidating the architecture of the bacterial type IX secretion system.

Prabha Dias

Prabha Dias was an EM scientist and lab manager in Grant Jensen's lab at Caltech from 2002 to 2007. Full bio to follow….

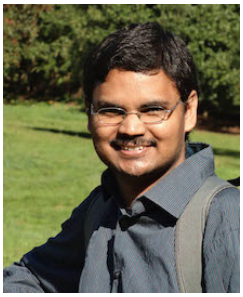
Megan Dobro

Dr. Megan Dobro is an Associate Professor of Biology at Hampshire College in Amherst, Massachusetts and Co-Founder and CEO of SafeTiva Labs. Dr. Dobro graduated with her PhD in Biology from the California Institute of Technology where she studied structural biology of viruses and bacteria using electron cryotomography in Dr. Grant Jensen's laboratory.

Lu Gan

Lu Gan is a principal investigator in the Centre for BioImaging Sciences and Department of Biological Sciences at the National University of Singapore. He did his undergraduate training at Caltech, where he studied antibody trafficking in Professor Pamela Bjorkman's lab. Lu then received PhD training in structural virology with Professor Jack Johnson at The Scripps Research Institute. In 2006, he joined Grant Jensen's lab as a postdoctoral scholar to study both bacterial cell-wall architecture and eukaryotic chromosome segregation. He moved to Singapore in 2011 to start his independent lab. Lu and his students continue to explore eukaryotic chromosomes and how they are regulated, all at molecular resolution.

Debnath Ghosal



Debnath Ghosal is a senior lecturer at University of Melbourne. Debnath received his PhD degree in structural biology from the MRC Laboratory of Molecular Biology (University of Cambridge, Darwin College), where he worked with Dr. Jan Lowe on bacterial cell division. Subsequently, he joined Dr. Grant Jensen's laboratory at Caltech for his postdoctoral training. At Caltech, he investigated the structure and function of bacterial secretion systems using electron cryotomography. Debnath established his own laboratory at University of Melbourne in 2020. His group is working on the structural and molecular biology of large bacterial protein complexes that are important for pathogenesis.

Gregory Henderson

Gregory Henderson was a graduate student in Grant Jensen's lab at Caltech from 2002 to 2007. Full bio to follow….

Cristina Iancu

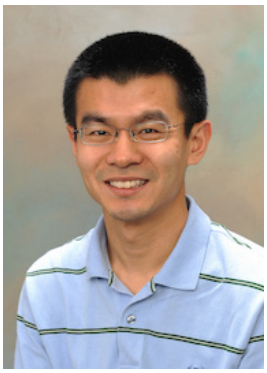
Cristina V. Iancu received her B.S. in Biophysics from the Faculty of Physics of the University of Bucharest, Romania, and her Ph.D. in Biochemistry from Iowa State University, Ames, IA, U.S. Her graduate work in the protein crystallography group of Dr. Richard B. Honzatko involved structural and functional studies of key enzymes in gluconeogenesis and de novo nucleotide synthesis. She joined the cryo-EM lab of Dr. Grant J. Jensen as his first postdoc, where she worked on cryo-ET method development projects and cryo-ET structure determination of protein complexes, isolated carboxysomes, and carboxysome-producing bacteria. Currently, she works on drug discovery targeting carbohydrate transporters with Dr. Jun-yong Choe.

Grant Jensen

Grant J. Jensen is Dean of the College of Physical and Mathematical Sciences at Brigham Young University. He earned his doctorate in Biophysics from Stanford, working on electron microscopy of RNA polymerase and other protein complexes with Dr. Roger Kornberg. He continued his work in protein electron microscopy as a Damon Runyon-Walter Winchell post-doctoral fellow under the supervision of Dr. Kenneth Downing at the Lawrence Berkeley National Lab. Here his interests expanded to include electron tomography of whole cells. Grant launched his own lab at Caltech in 2002, where his research has focused on three main areas: the ultrastructure of small cells, the structural biology of HIV, and the further development of cryoEM technology. Most of the cryotomograms featured in this atlas were collected by members of his lab while working with him at Caltech. In 2020, Grant moved to Brigham Young University.

Mohammed Kaplan

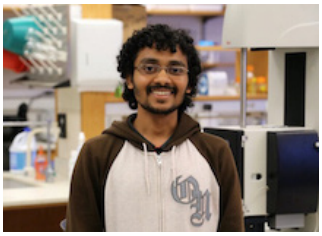
Mohammed Kaplan is currently a postdoc in the Jensen lab at Caltech. He did his PhD under the supervision of Prof. Marc Baldus at Utrecht University.



Zhuo Li

Zhuo Li is the director of the Electron Microscopy Core Facility at City of Hope Beckman Research Institute, in Duarte, California. He earned his PhD in biochemistry from the Institute of Biophysics, Chinese Academy of Sciences. He then did his postdoctoral research in the Jensen lab where he investigated the structure and function of bacterial cytoskeleton using cryogenic electron tomography.

Shrawan Mageswaran



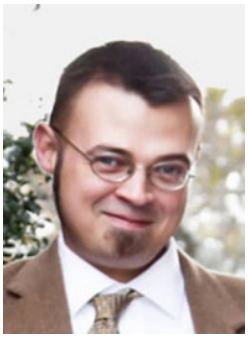
Shrawan Kumar Mageswaran is Cryo-ET Technical Director of the Beckman Cryo-EM Center at the University of Pennsylvania. He performed his graduate studies under the mentorship of Dr. Markus Babst at University of Utah. There he studied ESCRT (endosomal sorting complexes required for transport)-mediated biogenesis of multivesicular bodies (MVBs) using baker's yeast as a model system with implications in different cancers, neurodegenerative diseases and AIDS. He then underwent his postdoctoral training in cryo-electron tomography (cryo-ET), first with Dr. Grant Jensen at Caltech and subsequently with Dr. Yi-Wei Chang at University of Pennsylvania. During this period, he studied several membrane remodeling systems including the ESCRTs (in archaea, yeast and mammalian cells), plasma membrane and mitochondrial damage response (in mammalian cells), and rhoptry secretion system (in apicomplexan parasites). He used cryo-electron tomography as the principal method for these studies. He also developed/utilized other ancillary techniques for cryo-ET such as: (1) cryogenic correlative light and electron microscopy (to locate scarce/transient targets by cryo-ET and to provide molecular annotations for structures in tomograms using fluorescence) and, (2) cryogenic focused ion beam milling (to aid in artifact-free thinning of thicker samples). His interests in all of the above research topics continue to this day.

Alasdair McDowall

Alasdair McDowall is manager for the laboratory of Grant Jensen at Caltech. He is a career electron microscopist of 48 years and has managed, designed and directed microscopy centers and resources in the UK, USA, Germany and Australia. He is Emeritus Professor at the University of Queensland, Australia. He received his Doctorate from the University of Sorbonne, Paris. His thesis was enhanced by the unique discovery in 1981 when Dubochet and McDowall at the European Molecular Biology Laboratory (EMBL) reported the first vitrification of water at ambient pressures as seen in the electron microscope. This landmark result pioneered research that evolved into modern day molecular cryo-electron microscopy and the awarding of the 2017 Nobel Prize in Chemistry to Dubochet, Henderson and Frank. In 2019 he was appointed a Queen Elizabeth II honors recipient, and awarded Member of the Order of Australia. "For significant service to science, particularly in the field of electron microscopy".

Lauren Ann Metskas

Dr. Lauren Ann Metskas studied the conformational dynamics of disordered proteins during her PhD in Molecular Biophysics and Biochemistry at Yale University. After graduating in 2016, she transitioned to correlated cryo-fluorescence and cryo-electron microscopy methods development during her first postdoctoral position with the Briggs group at EMBL-Heidelberg and the MRC Laboratory of Molecular Biology. Lauren Ann joined the Jensen lab in 2018, whereupon she discovered a new interest in using tomography to study bacterial microcompartments and outer membrane vesicles. She is now an Assistant Professor in the Biological Sciences and Chemistry departments of Purdue University.



Gavin Murphy

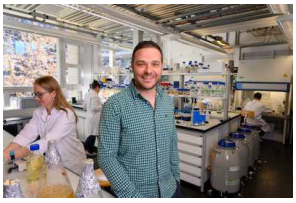
Following his graduation from the University of Dallas, Gavin Murphy spent two years studying X-ray crystallography as a Fulbright Scholar at the European Molecular Biology Laboratory in Hamburg, Germany. He then joined the lab of Grant Jensen at Caltech, applying cryo-electron tomography to solve the structures of bacterial macromolecular machinery. He received his doctorate in 2007, for work that included one of the first subtomogram averages ever published, revealing the ultrastructure of the flagellar motor. Wanting to make a more direct impact on human health, in his postdoctoral work with Sriram Subramanian at the National Institutes of Health he developed and applied methods for focused ion beam scanning electron microscopy (FIB-SEM) and correlative light and electron microscopy (CLEM) to visualize large volumes of eukaryotic cells and tissue. In 2012, Gavin became Director of the Electron Microscopy Center at Indiana University Bloomington. He died in 2017, and is fondly remembered and greatly missed by his colleagues.

William Nicolas

William J. Nicolas, a plant biologist and electron microscopist at heart, received his doctorate from the University of Bordeaux (France) working on the ultrastructure of the nanopores connecting plant cells to one-another called plasmodesmata. He joined Caltech as a postdoctoral scholar in the Jensen and Meyerowitz labs at the beginning of 2018 to work in the still vastly unexplored field of cryo-EM on the plant cell wall and cellulosic bacterial biofilms. He is currently an HHMI research specialist in the Gonen Lab at UCLA.

Catherine Oikonomou

Catherine M. Oikonomou is a research scientist and science writer at Caltech. She received her doctorate from the Rockefeller University, where she worked with Dr. Frederick Cross on cell cycle control in budding yeast. In 2012, she joined the lab of Dr. Grant Jensen at Caltech to explore microbial cell biology through cryo-electron tomography.



Martin Pilhofer

Martin Pilhofer is a Professor in the Institute of Molecular Biology & Biophysics at ETH Zürich in Switzerland. He earned his PhD in microbiology from the Technical University of Munich, working with Karl-Heinz Schleifer on bacterial phyla with unique cellular features. Recognizing the power of cryogenic electron tomography (cryo-ET) to characterize these structures, he joined Grant Jensen's lab at Caltech as a postdoc in 2008, where he used cryo-ET to reveal several fascinating bacterial structures, including cytoskeletal elements and contractile weaponry. In 2014 he established his own lab in Zürich, where he leads a team applying cryo-ET to understand these and other macromolecular machines mediating bacterial cell-cell interactions.

Sahand Pirbadian

Sahand Pirbadian received his doctorate in Physics in 2015 from University of Southern California, working with Moh El-Naggar on extracellular electron transfer in bacteria and bacterial outer membrane extensions. During his post-doctoral work, from 2015 to 2018, he collaborated with Grant Jensen's lab on cryo-electron microscopy of bacterial structures involved in extracellular electron transfer. Currently, he is a senior research engineer at Presidio Medical in South San Francisco, working on developing novel neuromodulation technologies.

Jian Shi

Jian Shi received his Bachelor's degree in Chemistry from Peking University, China and his Ph.D. in biophysics and molecular physiology from the School of Medicine at Vanderbilt University, USA. At Vanderbilt, he worked in Dr. Phoebe Stewart's lab to study small heat shock proteins with CryoEM and other biophysical methods. After his Ph.D., he joined Dr. Grant Jensen's lab at Caltech/HHMI as a CryoEM specialist to study ultra-structures in bacteria. After spending one year in the FEI Company, he has since been the manager of the Cryo-Electron Microscopy Facility at the National University of Singapore.

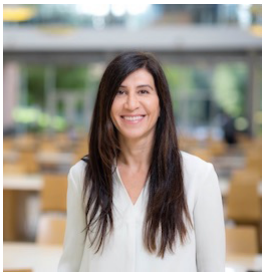
Poorna Subramanian

Poorna Subramanian is the Director of Enterprise Science and Innovation Partnerships at Thermo Fisher Scientific. She earned her PhD from Wayne State University, where she worked with Professor Tim Stemmler to understand molecular mechanisms of iron homeostasis in cells. In 2013, she joined Grant Jensen's lab at Caltech, where she used cryo-electron tomography to reveal the nanoscale machinery of bacterial cells, including nanowires, magnetosomes and flagellar motors. In 2018 she joined Thermo Fisher Scientific, where she helps bring cryo-electron microscopy technology to labs around the world.

Matthew Swulius

Matt Swulius received his Ph.D. from the University of Texas Health Science Center where he studied developmental changes in synaptic signaling complexes under Dr. Neal Waxham. He then did his postdoc at Caltech, with Dr. Grant Jensen, where he studied the microbial cytoskeleton using cryo-electron tomography. Now Matt has his own lab at the Penn State College of Medicine, where he uses his training in cryo-EM to study neuronal ultrastructure.

Elitza Tocheva



Dr. Elitza Tocheva graduated with her PhD in Microbiology and Immunology from the University of British Columbia in 2007, where she worked on characterizing the mechanism of bacterial enzymes involved in denitrification. She continued her structural biology studies as a postdoctoral scholar in the laboratory of Dr. Grant Jensen at Caltech where she applied cryo electron tomography and correlative microscopy approaches to study the ultrastructure of bacteria. She joined Université de Montréal as an Assistant Professor in June of 2015 and in January of 2019 was recruited to the Department of Microbiology and Immunology at UBC. Her lab combines microbiology, biochemistry and structural biology to study sporulation, membrane biogenesis, and host-pathogen interactions.

Steven Wang

Yuhang (Steven) Wang is a scientist at Quantum Simulation Technologies (QSimulate), Inc., in Cambridge, MA. He received his undergraduate degree in Biotechnology from Shandong University (China), an M.S. degree in Computational Chemistry from the University of Oklahoma, an M.S. degree in Applied Mathematics and a Ph.D. degree in Biophysics and Computational Biology from the University of Illinois, Urbana-Champaign. Before moving to his current position, Wang was a postdoctoral scholar in Prof. Grant Jensen's research group at the California Institute of Technology. He has worked on research projects in multiple areas of Molecular Biology, such as molecular immunology in crustaceans, Alzheimer's disease, human T-cell receptors, ion channel proteins, and bacterial mobility and sensing. Currently, he is working on developing new computational methods for pharmaceutical drug discoveries at QSimulate, Inc.

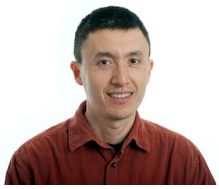
Elizabeth Wright

Elizabeth Wright is a Professor in the Department of Biochemistry at the University of Wisconsin, Madison. She is the director of the Cryo-EM Research Center (CEMRC) at UW-Madison and the Midwest Center for Cryo-Electron Tomography (MCCET), an NIH-funded National Center for Cryo-ET. She earned her PhD in Chemistry at Emory University in the laboratory of Prof. Vincent P. Conticello. It was during her PhD studies that she first began to explore macromolecular complexes and cellular systems with electron microscopy (EM) and cryo-EM. She joined Grant's lab at Caltech as a postdoc from 2003-2008. In Grant's lab she used cryo-ET to study HIV maturation, bacterial structure, and bacteria-bacteriophage interactions. In 2008, she established her cryo-EM lab where the group explores structure-function questions related to pathogenic viruses and bacteria, bacteriophage, blood cells and macromolecules, and neurons.

Qing Yao

Dr. Qing Yao received his Ph.D. in Biochemistry and Molecular Biology from Dr. Feng Shao's lab at National Institute of Biological Sciences, Beijing, where he worked on elucidating the molecular mechanisms of pathogen-host interactions from a structural biology perspective. He continued his structural biology studies as a postdoctoral scholar in the laboratory of Dr. Grant Jensen at Caltech. He studied bacterial cytokinesis using cryo-electron tomography at Caltech. He joined Amgen Thousand Oaks headquarters as a scientist in structural biology in November of 2020 and became a group leader and manager in March 2022.

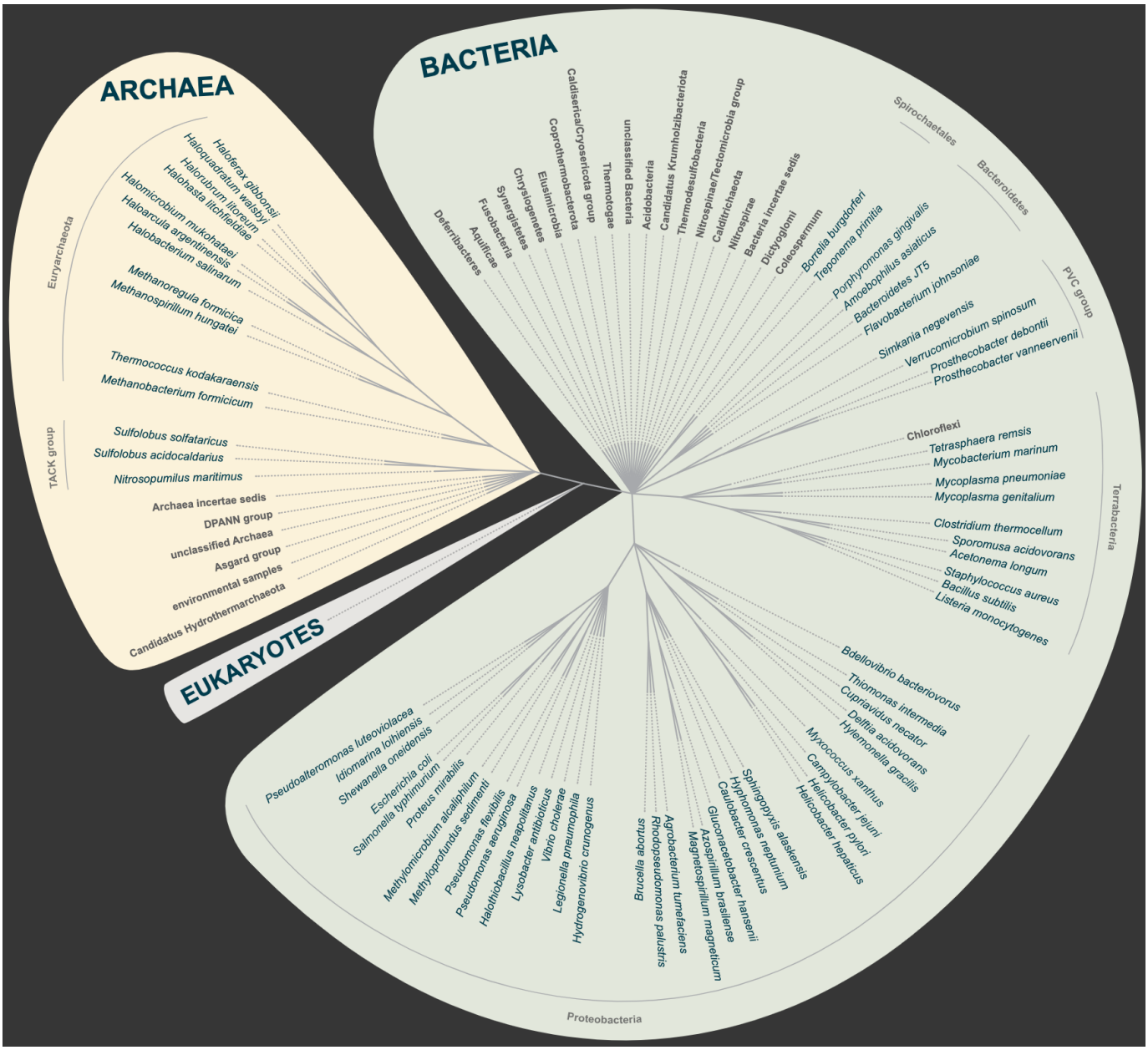
Zhiheng Yu



Zhiheng received his BS in physics from Peking University and received his MS and PhD degrees from Physics Department at Cornell University. At Cornell he worked with Professor John Silcox to characterize various nano-materials using high resolution transmission electron microscopy (TEM), high resolution scanning transmission electron microscopy (STEM), electron energy loss spectroscopy (EELS), electron nano-diffraction and computer simulation. After obtaining his PhD in 2004, he switched field from physics to biology and worked as a postdoc researcher in Professor Grant Jensen's lab at Caltech. There he learned and used cryogenic electron microscopy (cryoEM) to study the structure of various biological samples, including protein complexes, HIV virus and small bacterial cells with either single particle or cryo electron tomography techniques. In 2009 he joined FEI Company as a Research Scientist to work on the very early version of the now popular and powerful cryo electron microscope, the Titan Krios. In January 2011 he joined HHMI Janelia Research Campus to become the inaugural director of the CryoEM Shared Resource and has helped many HHMI users from various geographic locations.

Appendix C

Phylogenetic Tree


ARCHAEA
BACTERIA
EUKARYOTES
Euryarchaeota
TACK group
Haloferax gibbonsii
Haloquadratum walsbyi
Halorubrum litoreum
Halohasta litchfieldiae
Halomicrobium mukohataei
Haloarcula argentinensis
Halobacterium salinarum
Methanoregula formicica
Methanospirillum hungatei
Thermococcus kodakaraensis
Methanobacterium formicicum
Sulfolobus solfataricus
Sulfolobus acidocaldarius
Nitrosopumilus maritimus
Archaea incertae sedis
DPANN group
unclassified Archaea
Asgard group
environmental samples
Candidatus Hydrothermarchaeota
Deferribacteres
Aquificae
Fusobacteria
Synergistetes
Chrysiogenetes
Elusimicrobia
Coprothermobacterota
Caldisericia/Cryosericota group
Thermotogae
unclassified Bacteria
Acidobacteria
Candidatus Krumholzibacteriota
Thermodesulfobacteria
Nitrospinae/Tectomicrobia group
Calditrichaeota
Nitrospirae
Bacteria incertae sedis
Dictyoglomi
Coleospermum
Spirochaetales
Borrelia burgdorferi
Treponema primitia
Bacteroidetes
Porphyromonas gingivalis
Amoebophilus asiaticus
Bacteroidetes JT5
Flavobacterium johnsoniae
PVC group
Simkania negevensis
Verrucomicrobium spinosum
Prosthecobacter debontii
Prosthecobacter vanneervenii
Chloroflexi
Tetrasphaera remsis
Mycobacterium marinum
Mycoplasma pneumoniae
Mycoplasma genitalium
Terrabacteria
Clostridium thermocellum
Sporomusa acidovorans
Acetonema longum
Staphylococcus aureus
Bacillus subtilis
Listeria monocytogenes
Bdellovibrio bacteriovorus
Thiomonas intermedia
Cupriavidus necator
Delftia acidovorans
Hyiemonella gracilis
Myxococcus xanthus
Campylobacter jejuni
Helicobacter pylori
Helicobacter hepaticus
Sphingopyxis alaskensis
Hyphomonas neptunium
Caulobacter crescentus
Gluconacetobacter hansenii
Azospirillum brasilense
Magnetospirillum magneticum
Agrobacterium tumefaciens
Rhodopseudomonas palustris
Brucella abortus
Hydrogenovibrio crunogenus
Legionella pneumophila
Vibrio cholerae
Lysobacter antibioticus
Halothiobacillus neapolitanus
Pseudomonas aeruginosa
Pseudomonas flexibilis
Methyloprofundus sedimenti
Methylomicrobium alcaliphilum
Proteus mirabilis
Salmonella typhimurium
Escherichia coli
Shewanella oneidensis
Idiomarina lohiensis
Pseudoalteromonas luteoviolacea
Proteobacteria

A species is a unique group of organisms and is the first rung on the ladder of taxonomic classifications that stretches all the way up to the three domains of life: Bacteria, Archaea and Eukarya. How exactly a species is defined, though, is a surprisingly complicated question, especially for single-celled organisms. For most animals, species boundaries are defined by the infertility of offspring from matings across that boundary. This does not always work, though, and many species are defined by geographical, rather than reproductive, separation. For organisms that reproduce asexually, the boundaries are even more nebulous and often simply reflect a fairly arbitrary degree of difference, either morphological or genetic. Also, remember that bacteria and archaea frequently exchange genes, or larger stretches of DNA, through horizontal gene transfer (for a familiar example, think of the transfer of antibiotic resistance). This further blurs the lines between species. In an extreme view, perhaps we should think of environments less as collections of species than as pools of genes temporarily stored in a variety of containers. Still, despite its inexactness, taxonomic classification provides a useful way to trace biological traits so we can begin to answer questions like how the machines you see in this book may have evolved.

This phylogenetic tree shows the relatedness of the species in this book. The length of the branches separating two species from their last common ancestor is proportional to the amount of time that they have been evolving separately. Keep in mind that individual species of Bacteria and Archaea can be as evolutionarily divergent from one another as they are from us. The deeper we go into the past, toward the center of the tree, the less accurate the predictions of relatedness become. The branch point between Bacteria/Archaea and Eukaryotes is particularly hazy, and a topic of lively debate. We're still discovering new species, and even higher-order clades, and the computational tools we have to detect genetic relationships are improving, so this tree, too, will continue to evolve.

Appendix D

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