ThermoFisher SCIENTIFIC

Revolutionizing our understanding of biological systems

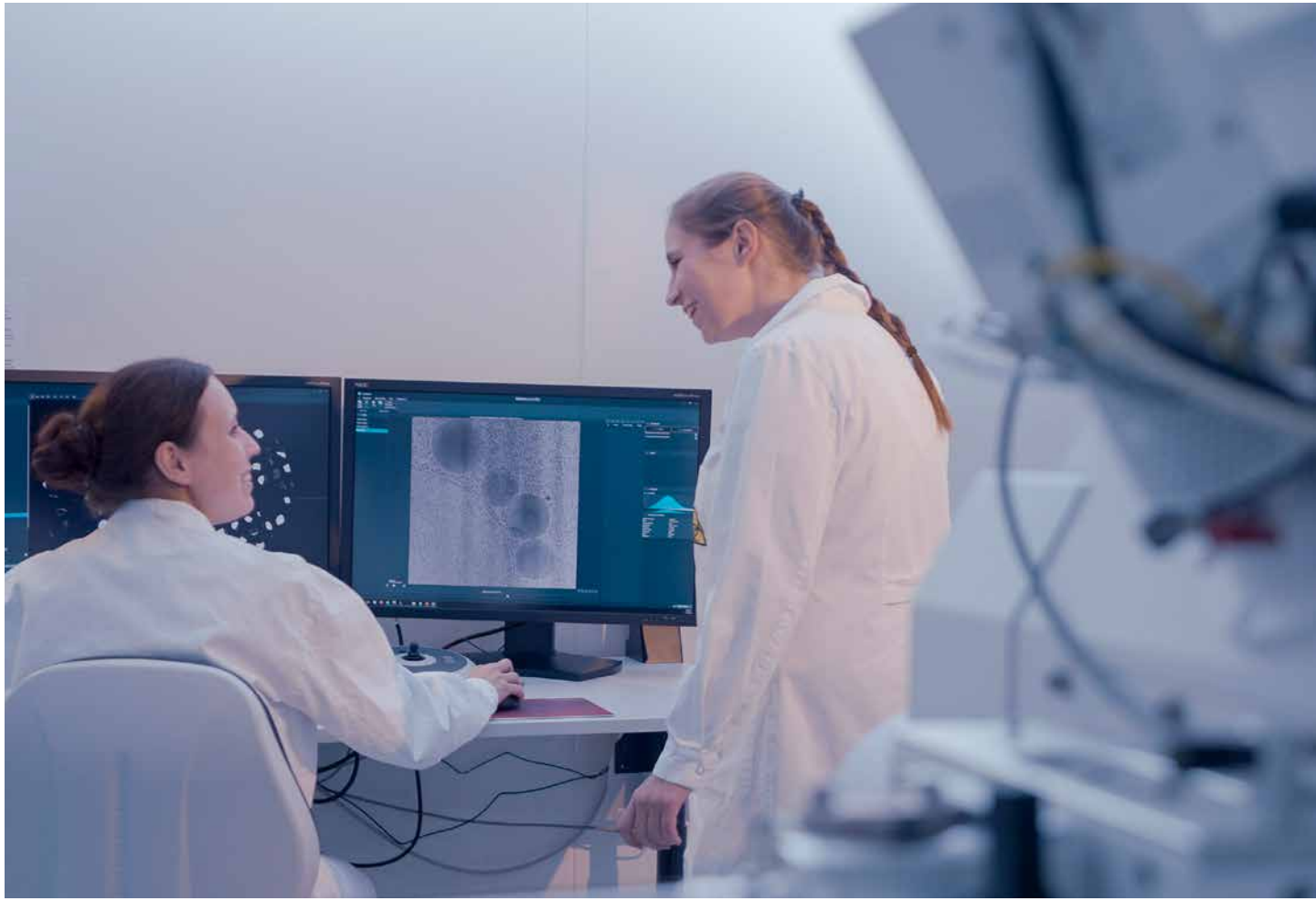
Find structure and function with cryo-electron tomography

thermo scientific

About Thermo Fisher Scientific

As the world leader in serving science, our innovative microscopy and application expertise helps customers find meaningful answers to the questions that accelerate breakthrough discoveries, increase productivity, and ultimately change the world.

Thermo Fisher Scientific's Materials & Structural Analysis Division develops our high-end electron microscopes, with development centers located in Eindhoven (NL), Brno (CZ) and Hillsboro, Oregon (US). At these sites, R&D engineers cover all disciplines necessary to develop an electron microscope, including physics, mechatronics, electronics and software. By continually expanding capabilities and driving innovation, Thermo Fisher Scientific is uniquely positioned to take cryo-electron microscopy (cryo-EM) technology to new heights, enabling you to explore biological complexity and make new discoveries for many years to come.



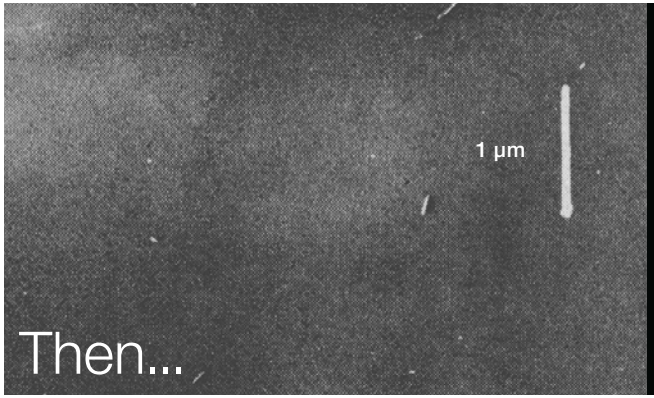
Introduction

In recent years, single-particle cryo-EM has emerged as a mainstream structural biology technique which can determine the 3D structure of proteins and protein complexes at atomic resolution. However, single particle cryo-EM is limited to highly purified and isolated proteins that are averaged to determine their 3D structure and lacks a connection to the cellular context. Here, cryo-electron-tomography fills the gap by visualizing proteins within their functional cellular environments.

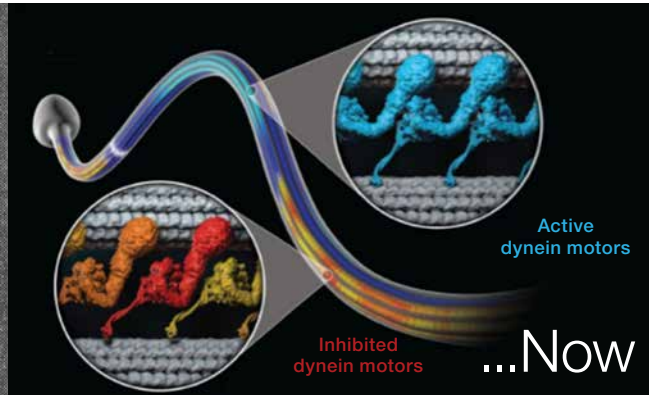
This allows for the observation of protein relationships and interactions with other cellular components and holds great promise for cell biology, where the ultimate goal is to understand every molecule in the cell – its structure, function, location, and interactions.

The 2017 Nobel Prize in Chemistry recognized the pioneering work of three scientists, Jacques Dubochet, Joachim Frank, and Richard Henderson, and their breakthrough developments in cryo-EM, for which they used Thermo Scientific™ instruments. It has taken decades of dedicated work to develop the technological advancements for the structural biology community – hardware, automation, software and detectors – that have made modern cryo-EM possible.

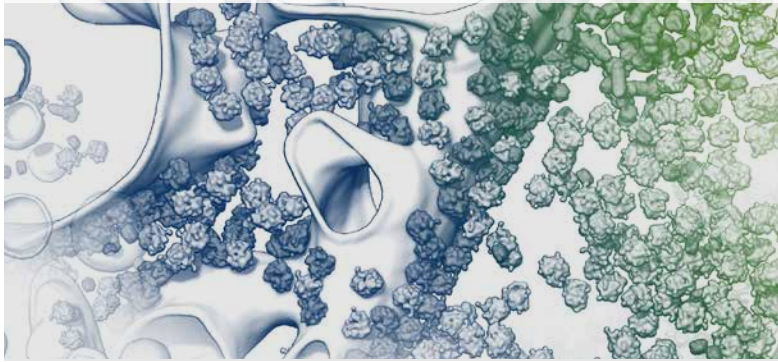
Video: Cryo-EM: Advancing research for more users than ever before



Electron micrograph of 30S dynein from cilia isolated from *Tetrahymena pyriformis*. Gibbons & Rowe, 1965.

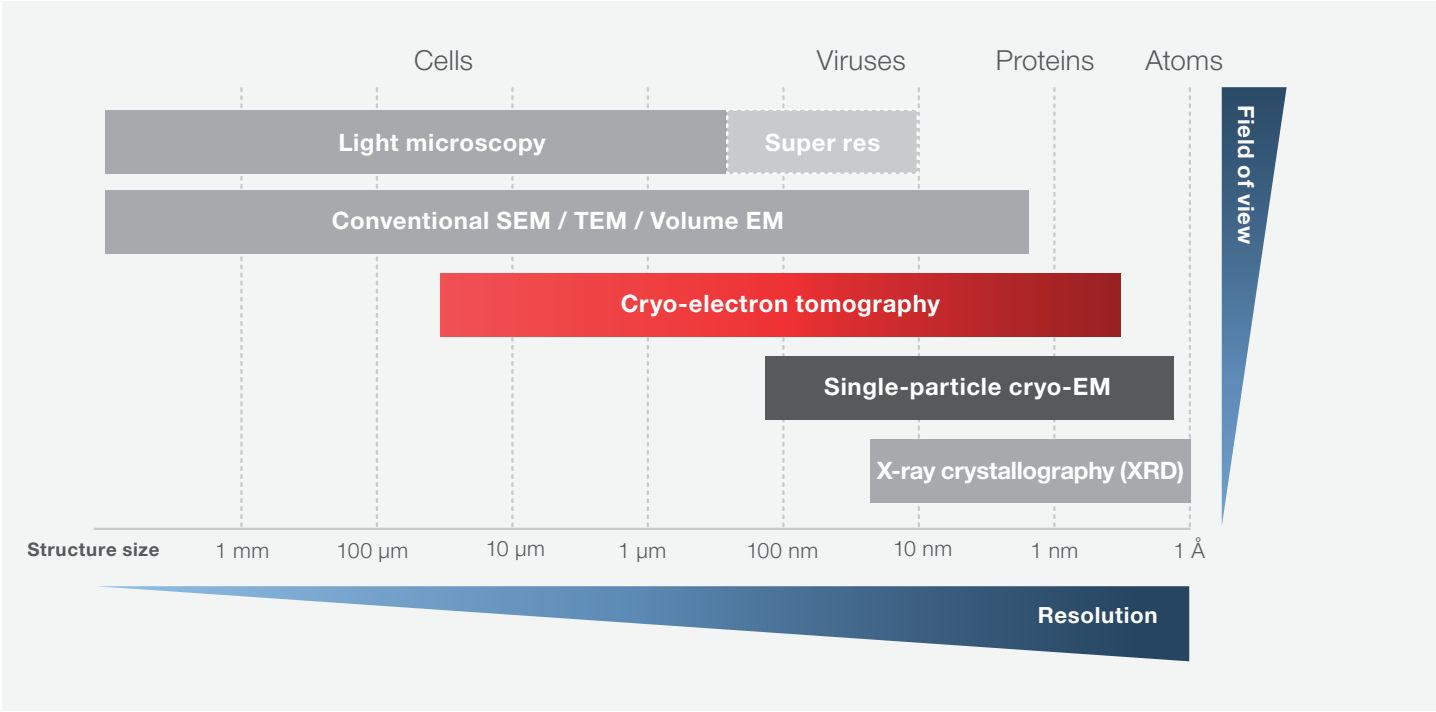


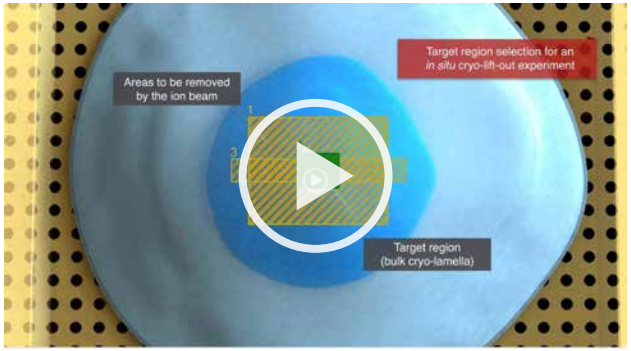
The structure and behavior of these flagella weren't revealed until the advent of cryo-electron microscopy.



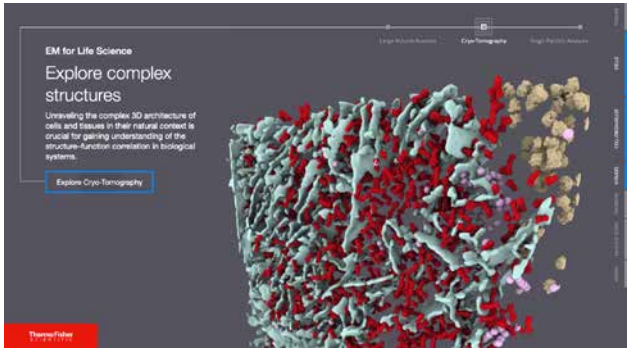
Cryo-electron tomography

Cryo-electron tomography (cryo-ET) combines the best possible structural preservation with nanometer-scale resolution. The method acquires 3D snapshots of the cellular interior and visualizes protein complexes within their crowded physiological environments. Such high-resolution 3D images of the cell interior provide new insights into cellular function and shed light on the arrangement and structure of native protein complexes. There is no longer just structural information; it is now linked to the spatial arrangement within a cell. The technique can bridge the gap between light microscopy and atomic-resolution techniques like single-particle cryo-electron microscopy.





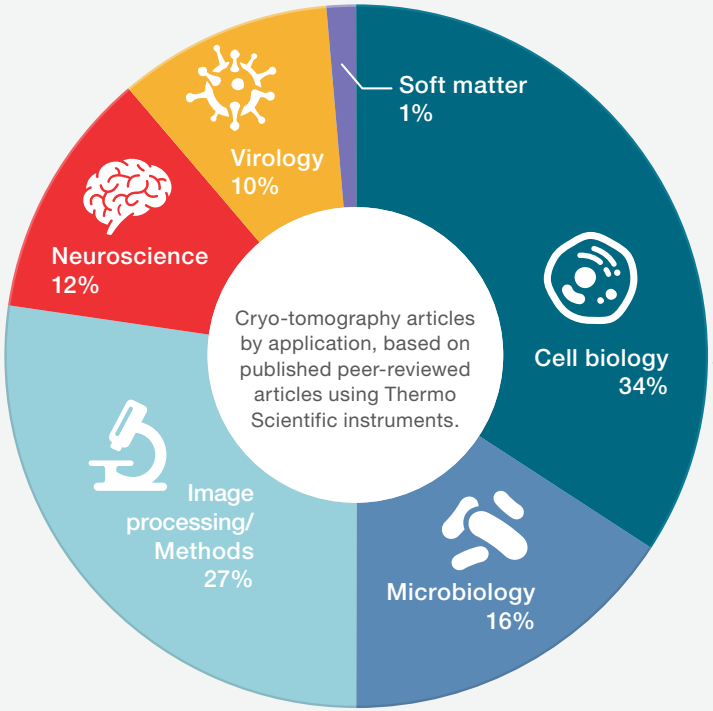
Video: How are cells prepared for cryo-ET?



Visit the virtual cryo-EM lab

In scientific literature

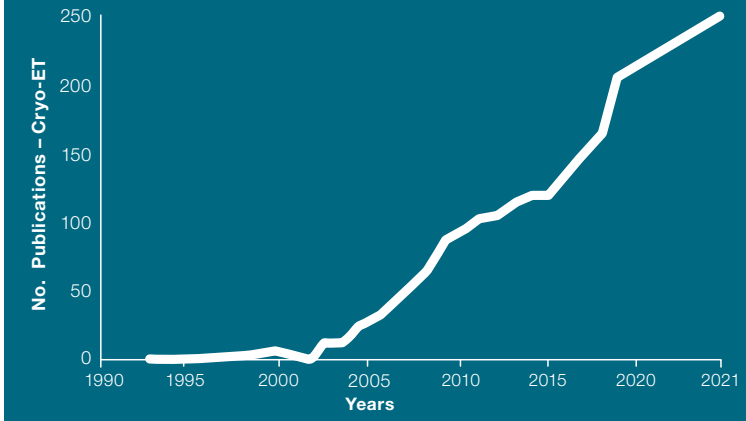
Cryo-ET has provided first insights into the cellular mechanisms underlying neurodegenerative diseases such as Huntington's, frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS).



Top 3 reasons cryo-ET is used to image cells

1. Maintains both molecular and structural integrity through the vitrification process
2. Enables the study of proteins at work, thus revealing their functional interactions
3. Provides label-free, fixation-free, 3D nanometer-resolution imaging of cells' inner workings

Since 2015, 32% of cryo-ET articles have appeared in *Cell*, *Science*, *Nature,* or *PNAS*, and 90% of these articles used Thermo Scientific instruments.



A method on the rise

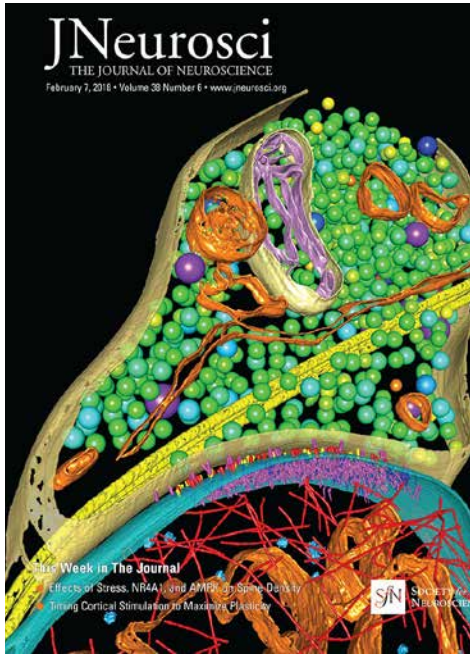
Review articles to feed the curious mind

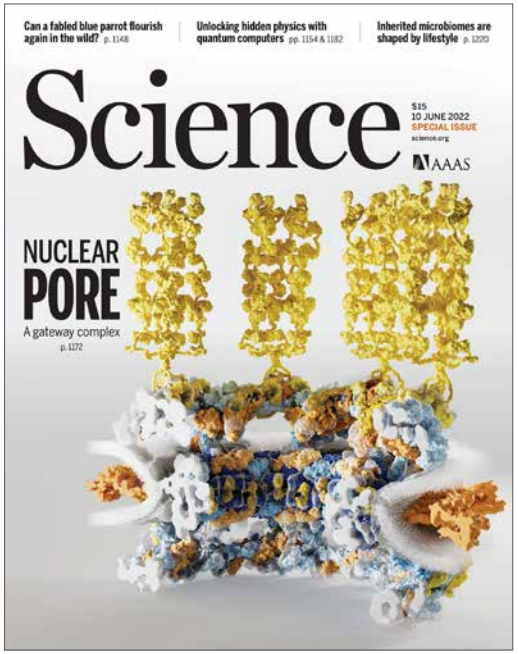
Cryo-electron tomography benefits cellular ultrastructure imaging because it can obtain nanometer-scale information about macromolecular complexes within frozen samples. Cryo-ET was selected by Nature Methods as a “Method to Watch” in 2016 and 2019. Since then, more and more cell biologists are adopting cryo-ET and methods continue to improve: increased automation for focused ion beam milling of cell and tissue samples, higher throughput and ease-of use for tomography data acquisition, as well as faster and more advanced imaging processing workflows.

Turk, M., Baumeister, W. 2020. The promise and the challenges of cryo-electron tomography. FEBS Letters doi: 10.1002/1873-3468.13948

Strack, R. 2020. Structures *in situ*. Nature Methods 17, 21. doi: 10.1038/s41592-019-0704-4

Bäuerlein FJB., Baumeister W. 2021. Towards Visual Proteomics at High Resolution. Journal of Molecular Biology. doi: 10.1016/j.jmb.2021.167187





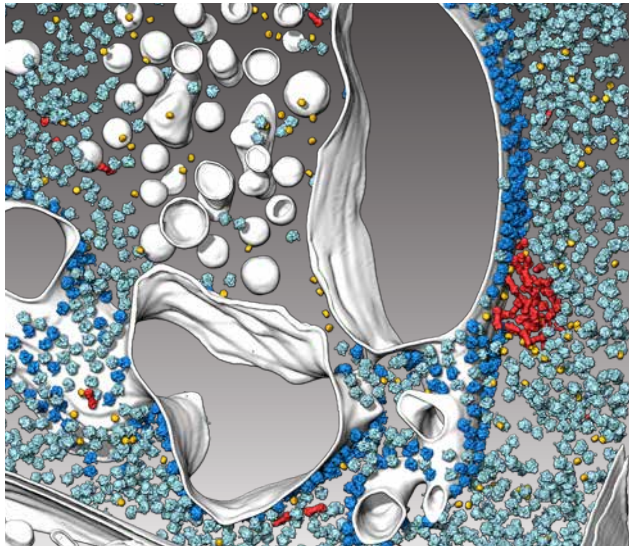
Cell biology

The study of cell morphology has been enhanced by electron microscopy, as it has one of the highest resolution limits of current morphological techniques. When combined with molecular detection methods, EM is the only technique with sufficient resolution to localize proteins to small membrane subdomains in the context of the cell. The astounding structural complexity of a cell arises from the action of a relatively small number of genes, raising the question of how this complexity is achieved. By examining how these parameters are controlled in a few specific cases, we can identify a handful of simple design principles that seem to underlie cellular architecture and assembly.

A detailed view of protein quality control in the cell

Cryo-electron tomography provides a close-up view of the endoplasmic-reticulum-associated degradation machinery at distinct microsites near the ER, giving rise to new questions: *How do these sites form? Are these sites examples of liquid-liquid phase separation? Do other sites within the cell exhibit this type of compartmentalization? How conserved are these sites across species?*

Albert, S., Wietrzynski, W., Lee, C.-W., et al. 2020. Direct visualization of degradation microcompartments at the ER membrane. *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.1905641117

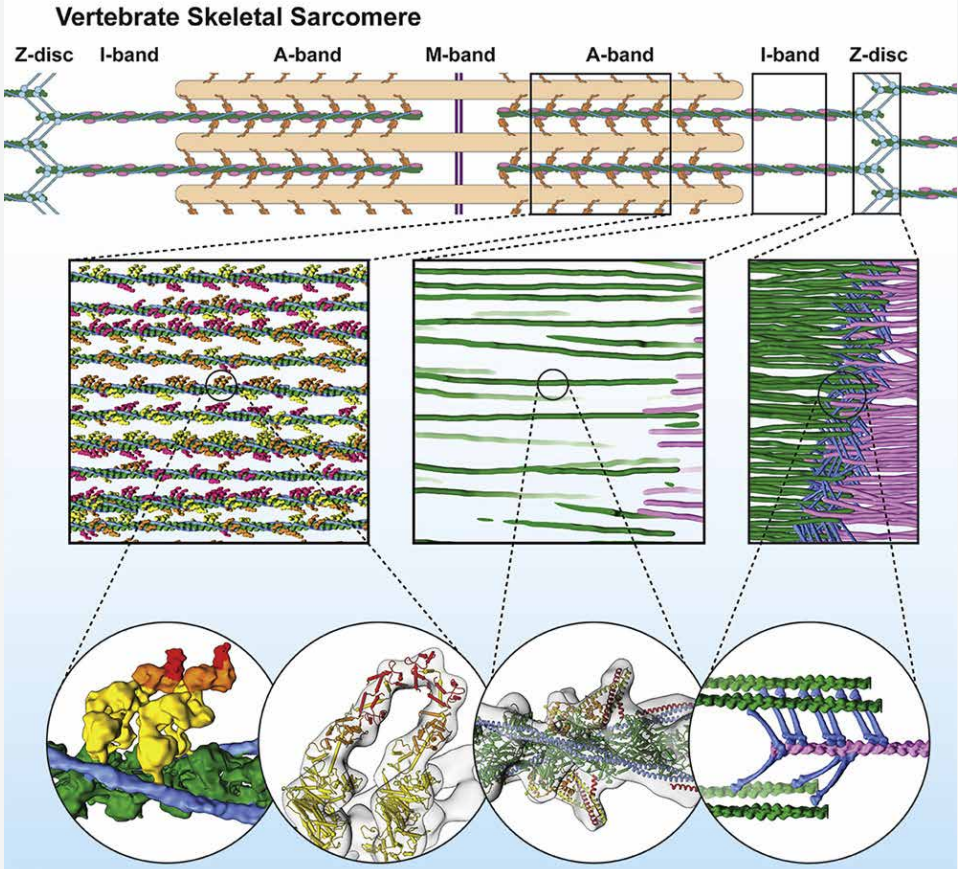


3D rendering of a degradation microcompartment within a cell. ER membrane (white), proteasomes (red), cytosolic ribosomes (light blue), membrane-bound ribosomes (dark blue), cdc48 (yellow). *Data courtesy of Dr. Benjamin Engel, formerly with Max Plank Institute for Biochemistry, currently at Biozentrum Basel. Data visualization with Thermo Scientific Amira™ Software.*

In situ cryo-electron tomogram of the native *Chlamydomonas* Golgi. *Image courtesy of Ben Engel from Bykov et al. Elife, 6, E32493, 2017.*

Molecular architecture of the sarcomere

Thin and thick actomyosin filaments are the key components of muscle. In skeletal muscle, the protein nebulin is essential for the length and strength of the thin filaments, with mutations of nebulin often leading to muscle diseases called nemaline myopathies. Wang et al. used cryo–electron tomography to identify nebulin integrated within the thin filament of native skeletal muscles. The authors determined a near-atomic *in situ* structure of nebulin and showed how it stabilizes thin filaments and functions as a "molecular ruler. " The structure of nebulin along thin filaments is key to understanding the pathogenicity of nemaline myopathies.



References:

Wang Z, Grange M, Pospich S et al. 2022. Science doi: 10.1126/science.abn1934

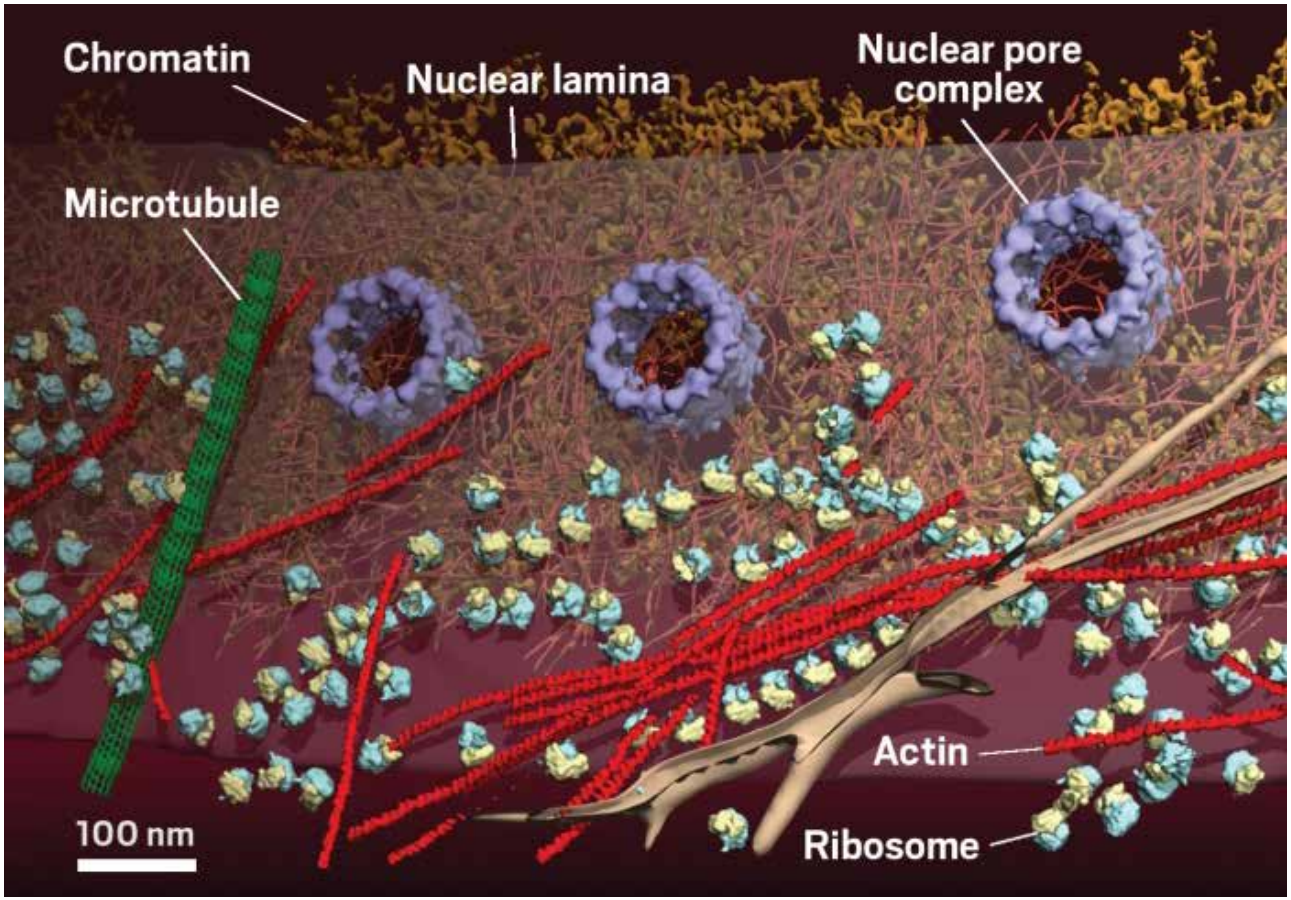
Wang Z, Grange M, Wagner T, et al. 2021. Cell doi: 10.1016/j.cell.2021.02.047

"Electron tomography cannot only be used for our muscle research, but of course, you can imagine for cellular processes like septations or segregation of chromosomes or all kinds of different cellular aspects that really happen inside the cell and that you cannot reconstitute easily."

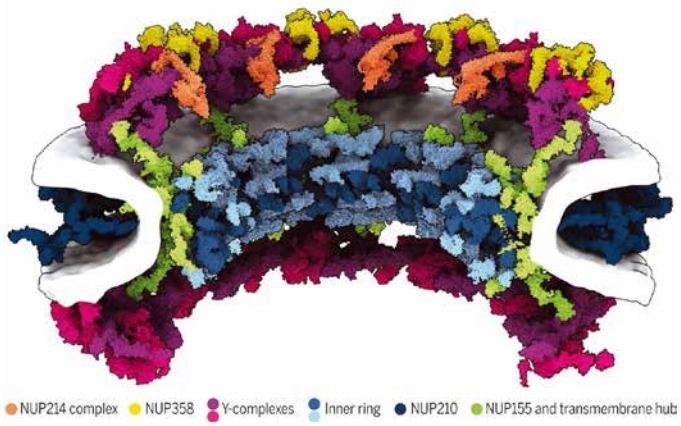
–Professor Stefan Raunser
Director of the Department of Structural Biochemistry
Max Planck Institute of Molecular Physiology
Dortmund, Germany

See inside the cell – the nuclear pore complex

Cryo-electron tomography provides the first view of a cell's nucleus in its natural, undisturbed environment. Cryo-ET reveals the molecular organization of various components of the HeLa cell in their natural environment. This technique shows that protein filaments make the nucleus the stiffest organelle around.



Data courtesy of Dr. J. Mahamid, formerly MPI Biochemistry, now EMBL Heidelberg. Mahamid, J., et al. 2016. Visualizing the molecular sociology at the HeLa cell nuclear periphery. Science, 351(6276), 969 LP – 972. doi: 10.1126/science.aad8857



Mosalaganti S., Obarska-Kosinska A., Siggel M. et al. 2022. AI-based structure prediction empowers integrative structural analysis of human nuclear pores. Science, doi: 10.1126/science.abm9506

Neuroscience

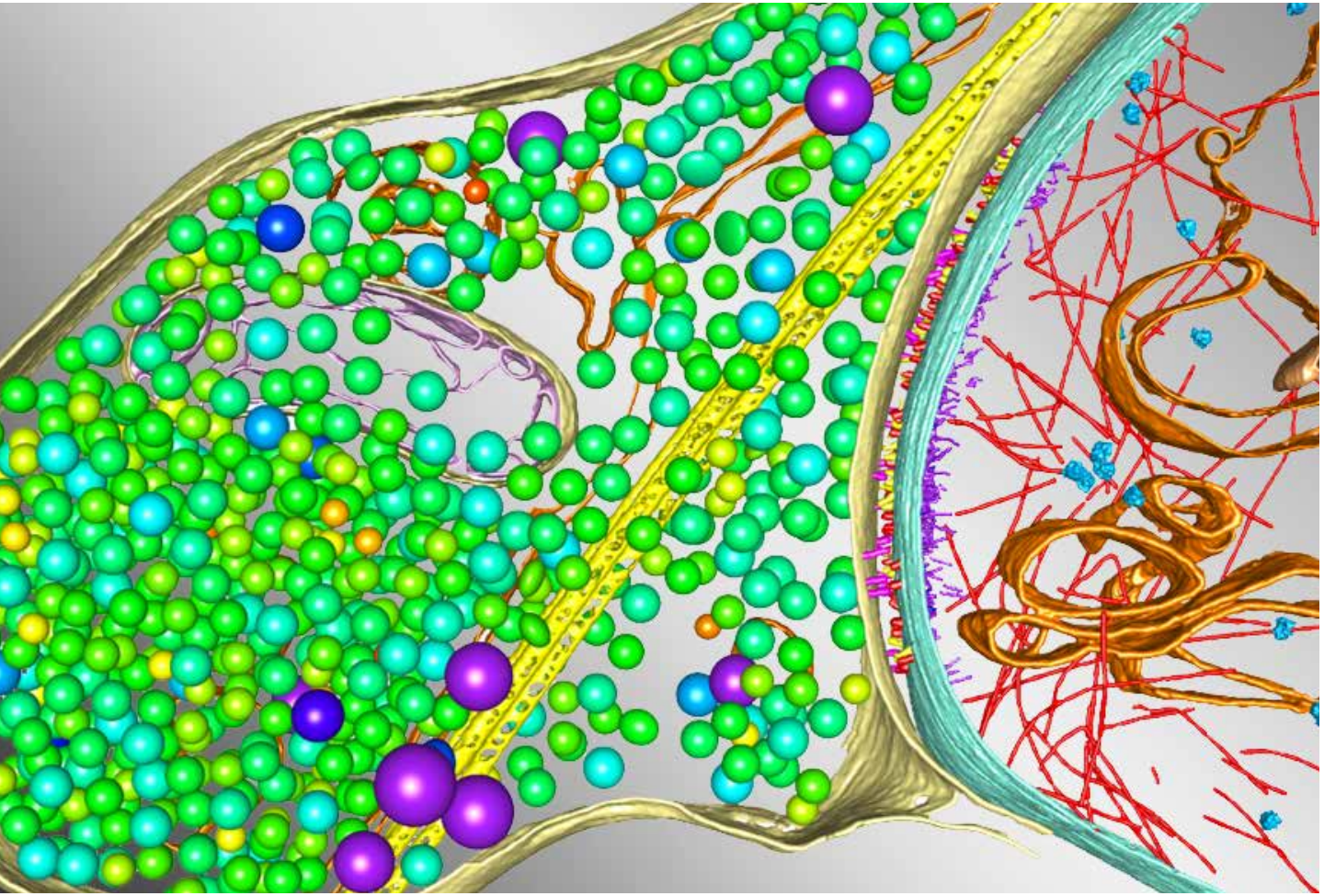


Image courtesy of G. Bi, USTC and H. Zhou, UCLA. Visualization by Thermo Scientific Amira™ Software. Tao CL, et al. JNeurosci, 2018.

How do the building blocks of a synapse work together? In order to understand synaptic mechanisms and their components it is necessary to visualize them. Understanding subcellular mechanisms and components is also essential for understanding larger life processes.

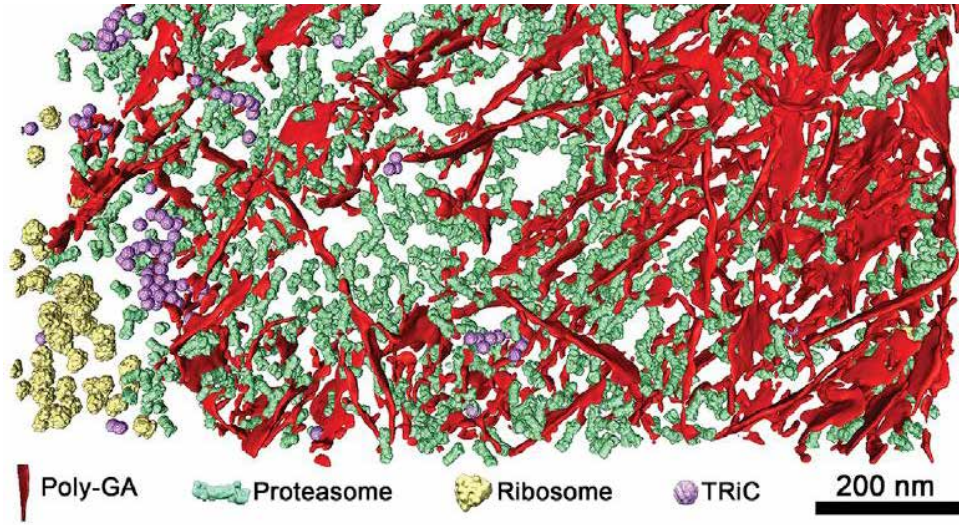
In situ analysis of ultrastructural organization underlying distinct synaptic functions

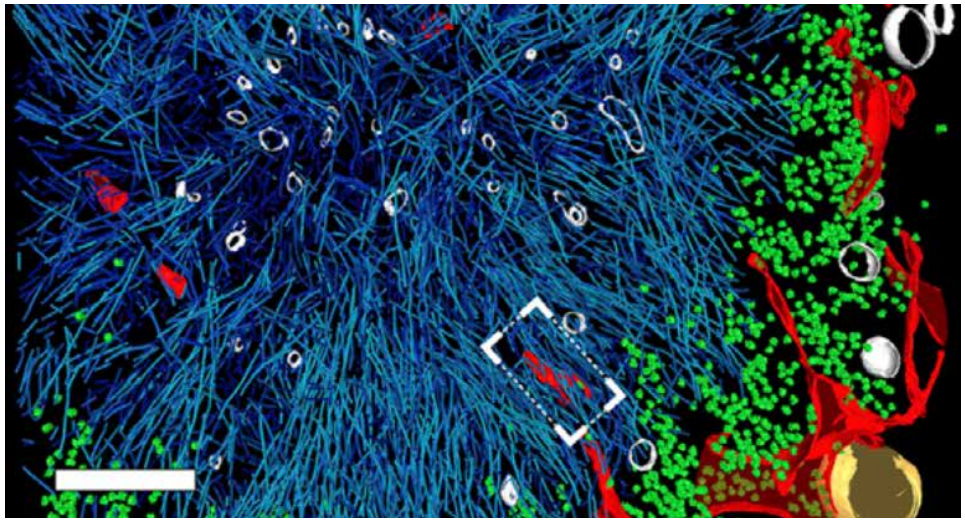
The image on the left shows a 3D view of an excitatory synapse between cultured hippocampal neurons revealed by cryo-ET. All essential structural synaptic elements are rendered to facilitate visualization. Post-synaptic structures like PSD filaments and glutamate receptors are clearly depicted as well as pre-synaptic structures such as vesicles or adhesion proteins. Cryo-electron tomography gives a unique insight into the structure of synapses and will lead to a better understanding of the interplay of its components.

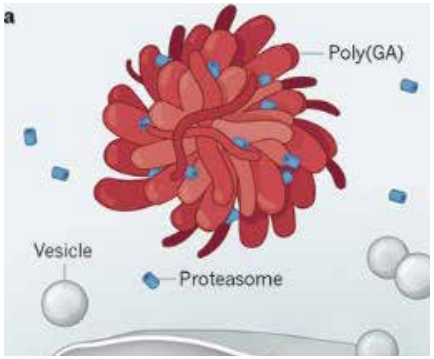
Liu YT, Tao CL, X. et al. 2020. Mesophasic organization of $GABA_A$ receptors in hippocampal inhibitory synapses. Nature Neuroscience. doi: 10.1038/s41593-020-00729-w

Protein aggregates and neurodegeneration

Protein aggregates involved in motor neuron disease (or ALS) have been captured stalling the molecular machines needed for normal protein degradation. Protein aggregates are a hallmark of neurodegeneration. High-resolution snapshots of the structure of one such aggregate offer an unprecedented view of how these proteins disrupt crucial cellular functions.



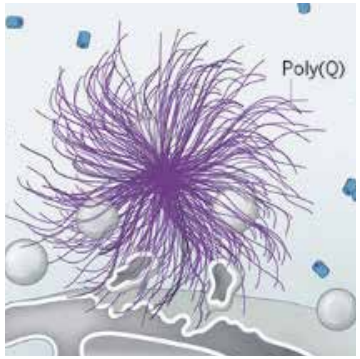




Are ALS dipeptide repeat ribbons entangling proteasomes?

In neurons, ALS/FTD poly-Gly-Ala peptides aggregate into a dense network of twisted ribbons. The ribbons sequester a large fraction of the cells' proteasomes. Many trapped proteasomes are frozen in a catalytic transition state.

Daisuke Ito, *ALZ Forum*, 02 Feb 2018

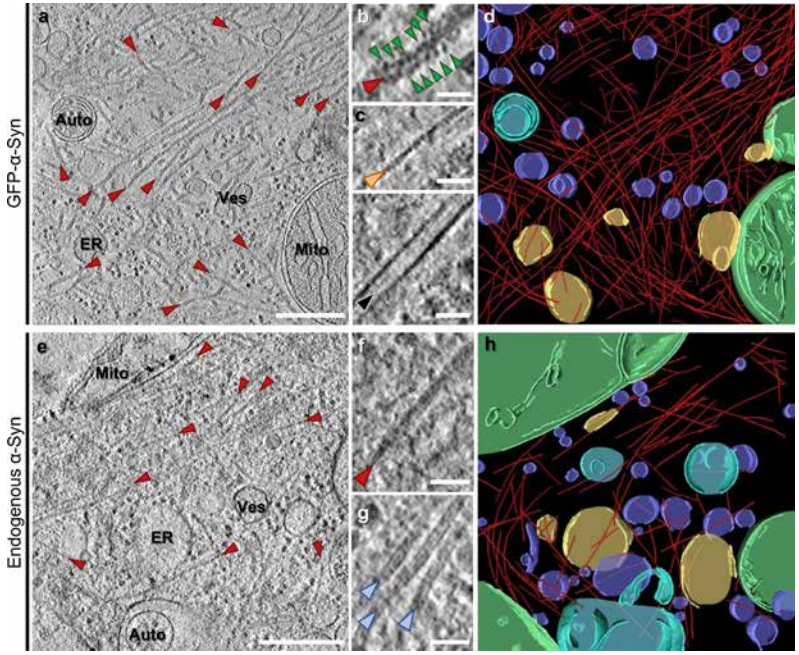


Weeds in the brain

A common feature of neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's is the accumulation of toxic protein deposits in the nerve cells of patients. Once these aggregates appear, they begin to proliferate like weeds. If and how these deposits damage nerve cells and lead to their demise remains largely unexplained. A detailed insight into the three-dimensional structure of the protein aggregates should help researchers to solve this puzzle.

MPI of Biochemistry, 07 Sept 2017

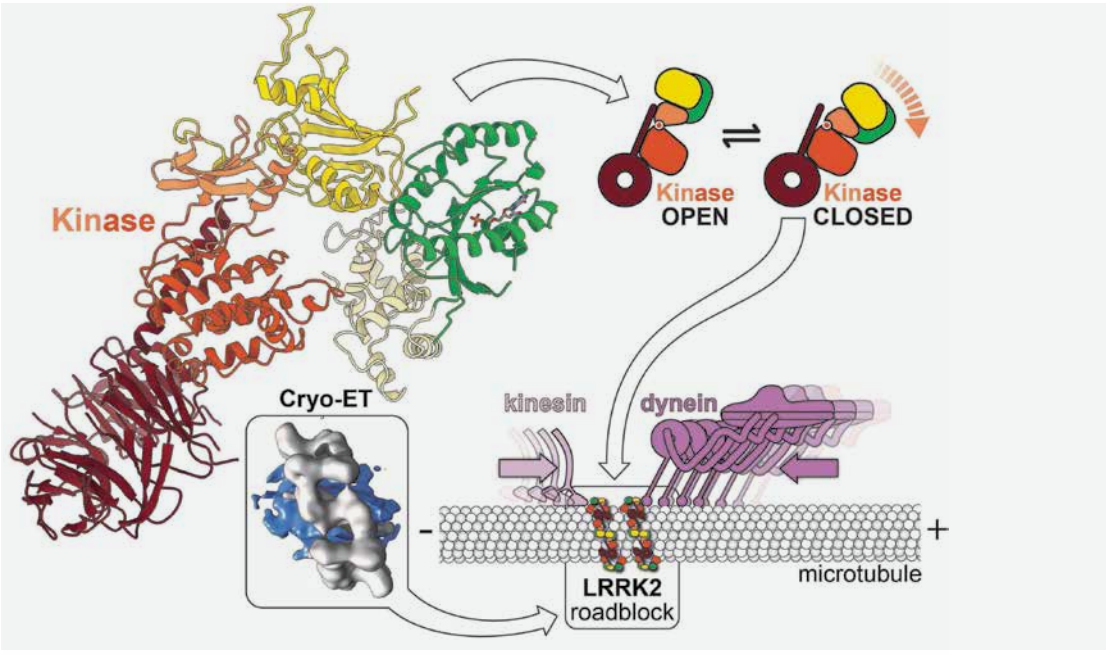
Protein aggregates and neurodegeneration



Microtubules in the axon

Cryo-electron tomography captures the ultrastructure of a growing axon in a lab-grown miniature brain (organoid). The axon (edged by dark blue) contains smaller structures such as microtubules (magenta) and actin filaments (orange) which provide structural support and carry all-important signals.

Trinkaus VA, Riera-Tur I, Martínez-Sánchez A, et al. 2021. *In situ* architecture of neuronal α-Synuclein inclusions. Nature Communications. doi: 1038/s41467-021-22108-0



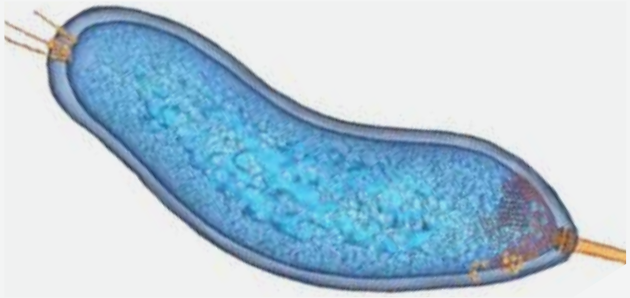
LRRK2 structure solved *in situ*

The top contributor to familial Parkinson's disease—the second most common neurodegenerative disease—is mutations in leucine-rich repeat kinase 2 (LRRK2), whose large and difficult structure has finally been solved, paving the way for targeted therapies. Lead author Dr. Watanbe says she is hopeful that "with this structure in hand, pharmaceutical companies can design a drug that can inhibit LRRK2 activity," which could provide a widely applicable cure for Parkinson's disease.

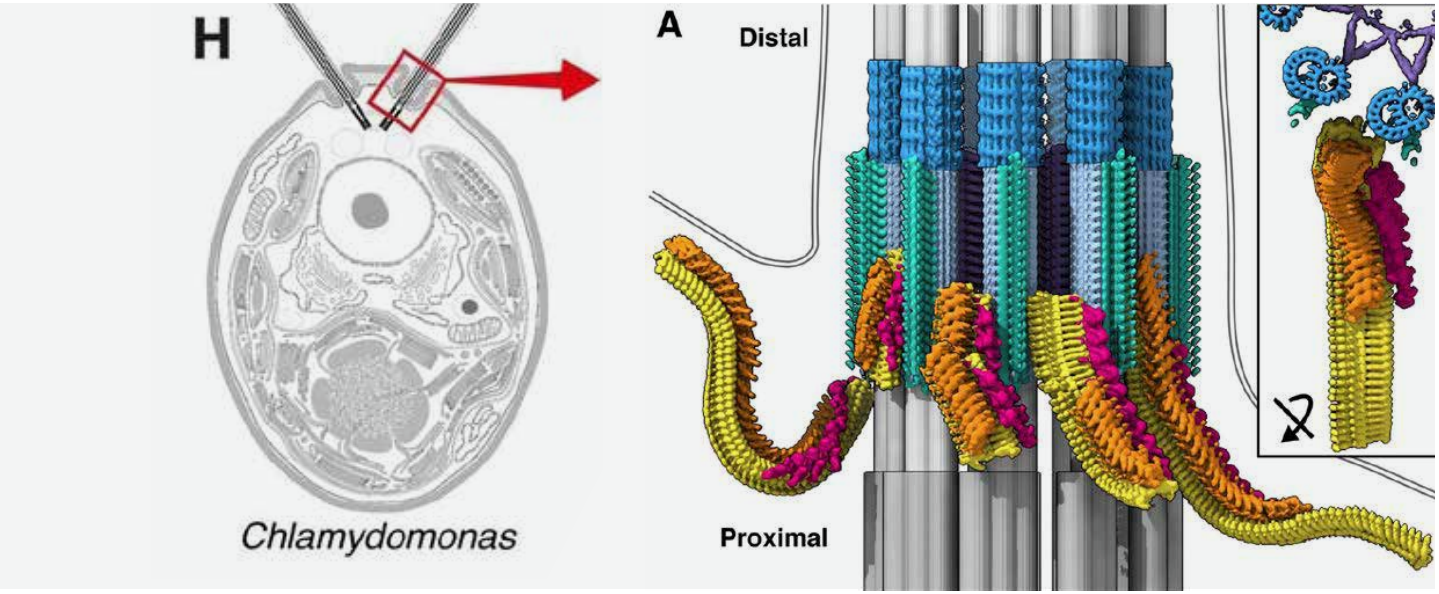
Watanabe R, Buschauer R, Böhning J, et al. 2020. The *In Situ* Structure of Parkinson's Disease-Linked LRRK2. Cell. doi: 10.1016/j.cell.2020.08.004

Microbiology

Bacteria and archaea can no longer be viewed as mainly undifferentiated sacs of jumbled enzymes. Cryo-ET has led to a broadened view of the intricate processes within prokaryotic cells. Finer details reveal organized assemblies of macromolecular machines that are optimized to travel through and interact with complex and dynamic environments. These fascinating observations raise a number of unanswered questions for uncharted areas of discovery.



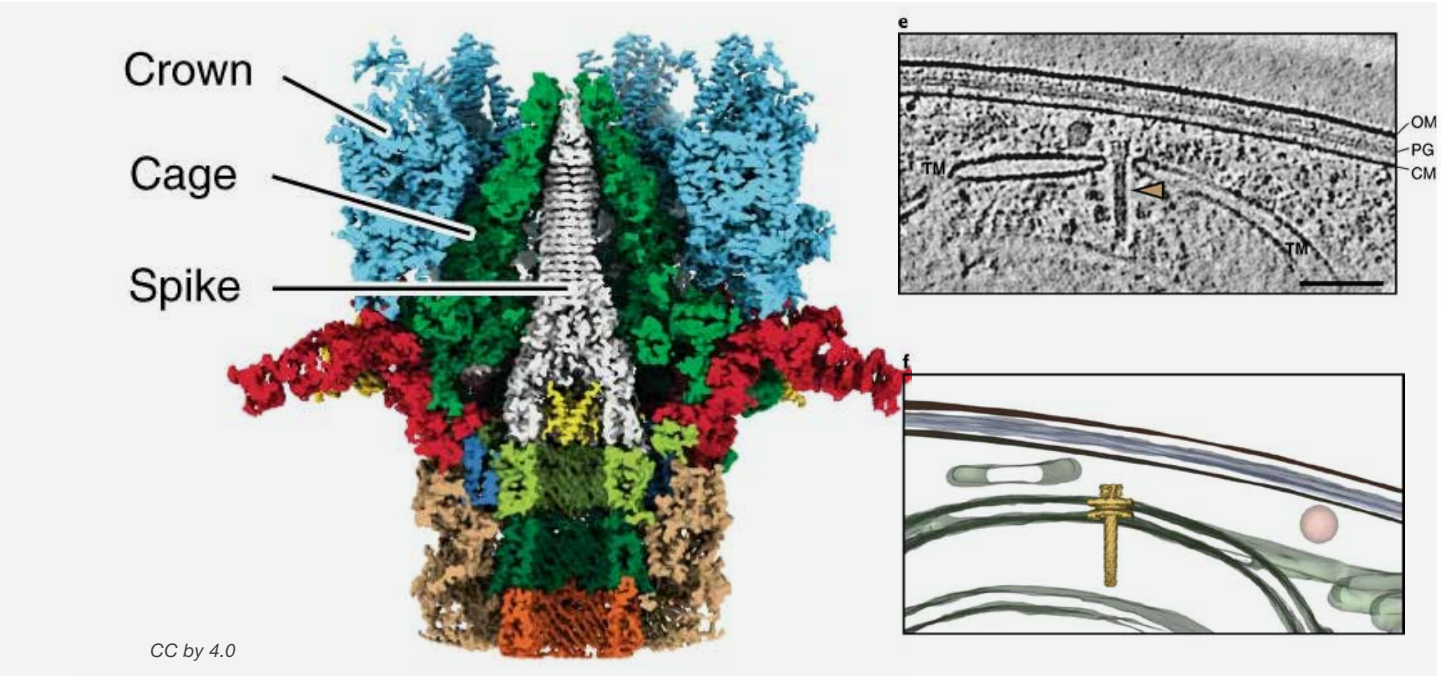
The Atlas of Bacterial & Archaeal Cell Structure -an open access digital textbook offers a tour of microbial cells guided by cutting-edge 3D electron microscopy. Courtesy Catherine M. Oikonomou & Grant J. Jensen California Institute of Technology
https://www.cellstructureatlas.org/

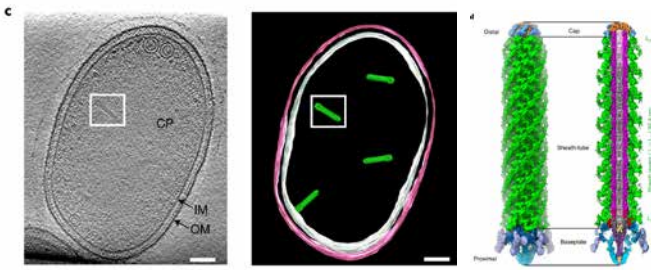


van den Hoek H, Klena N, Jordan MA, et al. 2021. *In situ* architecture of the ciliary base reveals the stepwise assembly of IFT trains. bioRxiv. doi: 10.1101/2021.10.17.464685

Bacterial nanomachines

Bacteria possess intricate macromolecular assemblies that enable vital functions like cell-cell interactions. For example, contractile injection systems, mega-dalton complexes that function like nanometer syringes, deliver effector proteins into target cells. Cryo-ET allows the study of these nanomachines *in situ*, leaving their complex architecture intact.





Weiss GL, Eisenstein F, Kieninger A-K, et al. 2022. Structure of a thylakoid-anchored contractile injection system in multicellular cyanobacteria. Nature Microbiology. doi: 10.1038/s41564-021-01055-y

Xu J, Ericson CF, Lien Y-W, et al. 2022. Identification and structure of an extracellular contractile injection system from the marine bacterium Algoriphagus machipongonensis. Nature Microbiology doi: 10.1038/s41564-022-01059-2

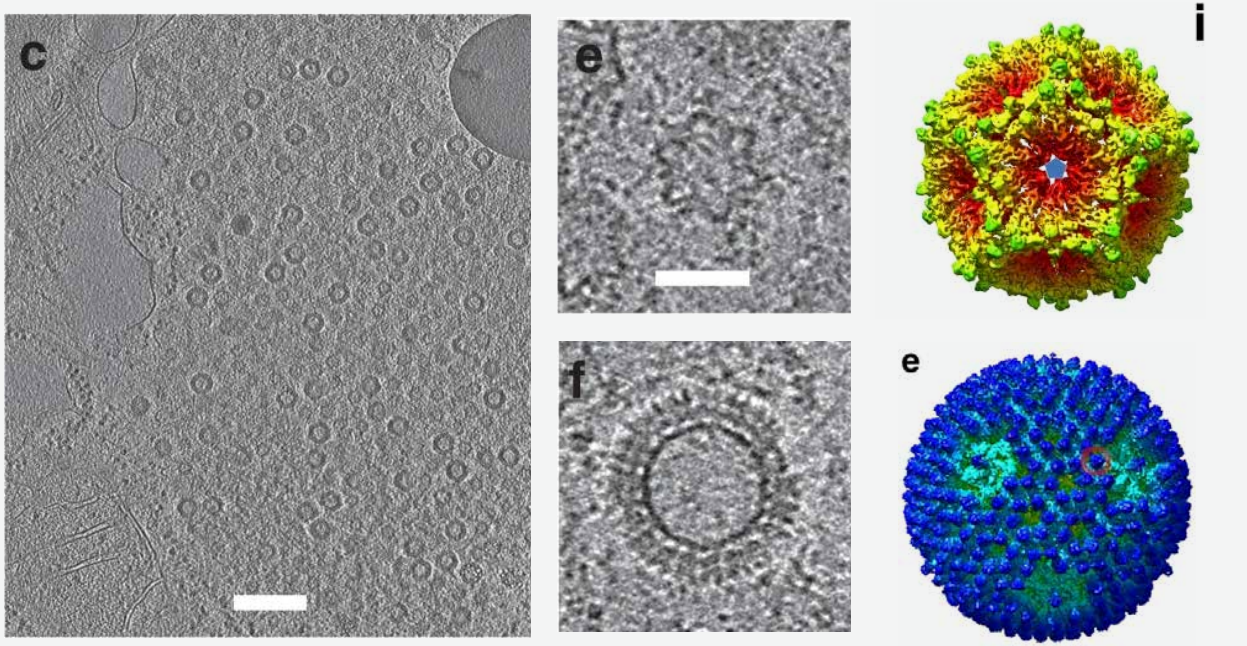
Video: Prof. Briegel and new discoveries in microbes with cryo-ET

"The thing I love the most about this work is that every cell is different. It's a glimpse into this unseen world and it's always fascinating."

–Ariane Briegel
Professor of Ultrastructural Biology,
Leiden University
Co-director of NeCEN

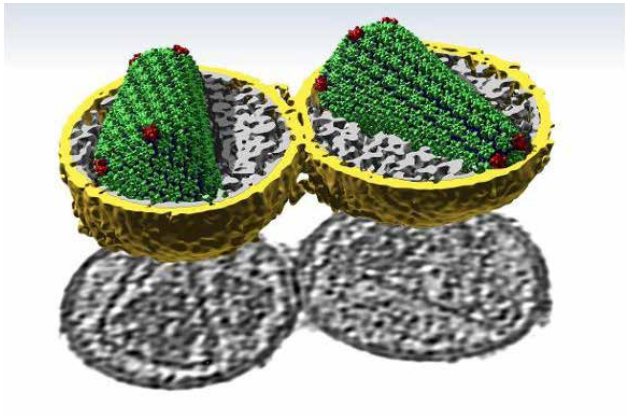
Virology

Viruses are infectious agents that come in a wide variety of shapes and sizes, with most having a diameter between ten and a few hundred nanometers. Cryo-EM has been used to study virus morphology for over 20 years, resolving the structures of viruses such as Ebola, HIV, and coronaviruses.

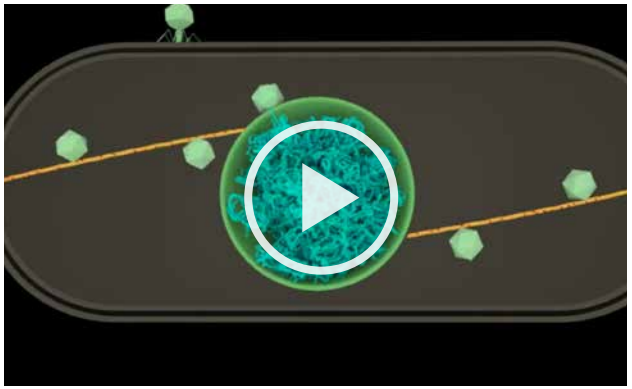


The near-atomic resolution information provided by single-particle analysis cryo-EM is critical for a better understanding of the molecular mechanisms behind antibody-antigen interactions. Cryo-ET provides structural information with broader cellular context, such as viral assembly within bacteria. Additionally, cryo-ET provides 3D analysis of asymmetric or pleomorphic viral particles such as HIV, herpesvirus, and influenza.

Sutton G, Sun D, Fu X, et al. 2020. Assembly intermediates of orthoreovirus captured in the cell. Nature Communications. doi: 10.1038/s41467-020-18243-9



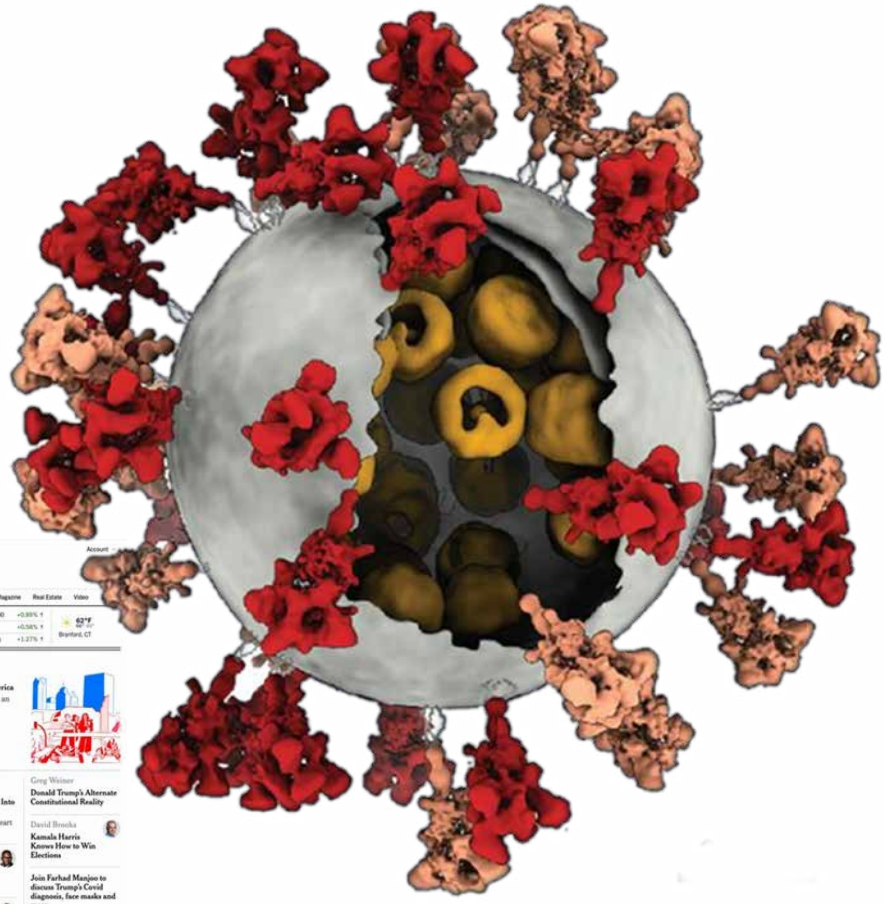
Insights into HIV at unprecedented resolution: The green and red CA protein structures form the conical protective envelope of the virus genome. *Image by Simone Mattei, EMBL Heidelberg.*



Video: Viral Capsid Trafficking along Treadmilling Tubulin Filaments in Bacteria. *Courtesy of the Villa Lab, Chaikeeratisak, Science, 2017.*

Cryo-tomography against SARS-CoV-2

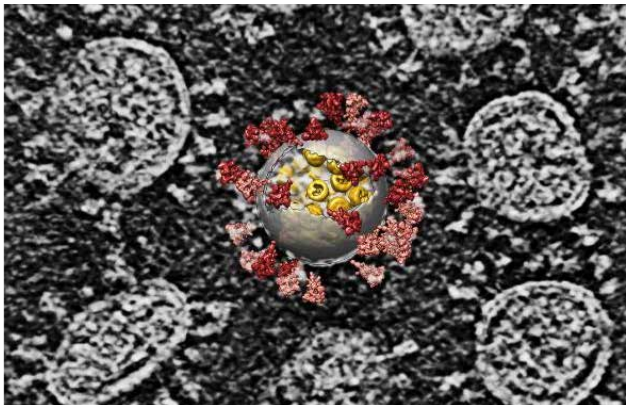
Global researchers have deposited over 1000 structures of SARS-CoV-2 biomolecules to the Worldwide Protein Data Bank (PDB) solved by cryo-EM or X-ray crystallography in 2020. Cryo-electron tomography (cryo-ET) and subtomogram averaging were used in studies to characterize the pleomorphic structure and the replication mechanism of SARS-CoV-2.



Molecular details of the intact virus

The molecular architecture of the authentic SARS-CoV-2 virus is unveiled by cryo-electron tomography and subtomogram averaging. Image courtesy of Professor Sai Li (Tsinghua University).

Yao, C. 2020. Molecular Architecture of the SARS-CoV-2 Virus, *Cell*, 183: 730- 739, doi: 10.1016/j.cell.2020.09.018



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The New York Times

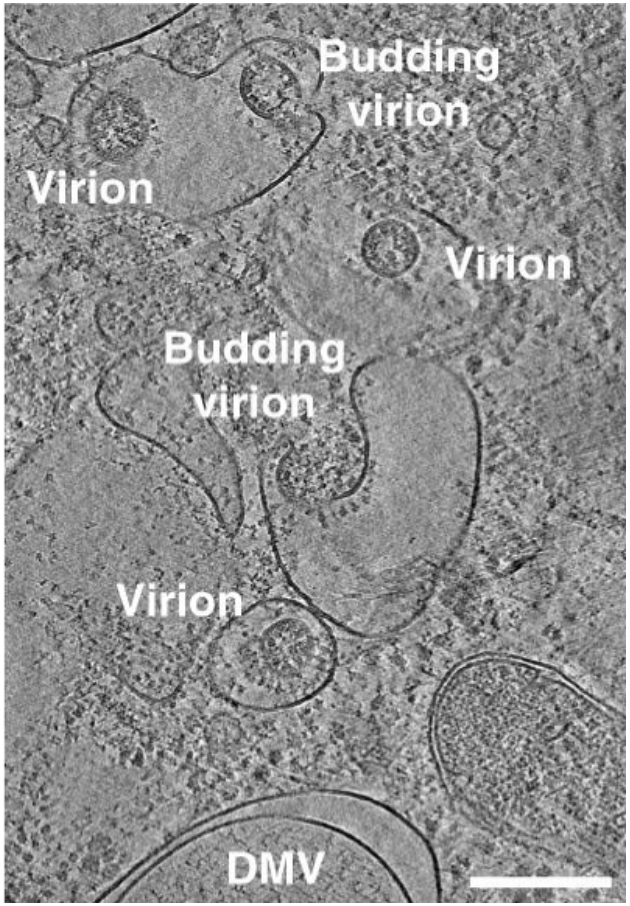
Trump's Taxes Show He Engineered a Sudden Windfall in 2016

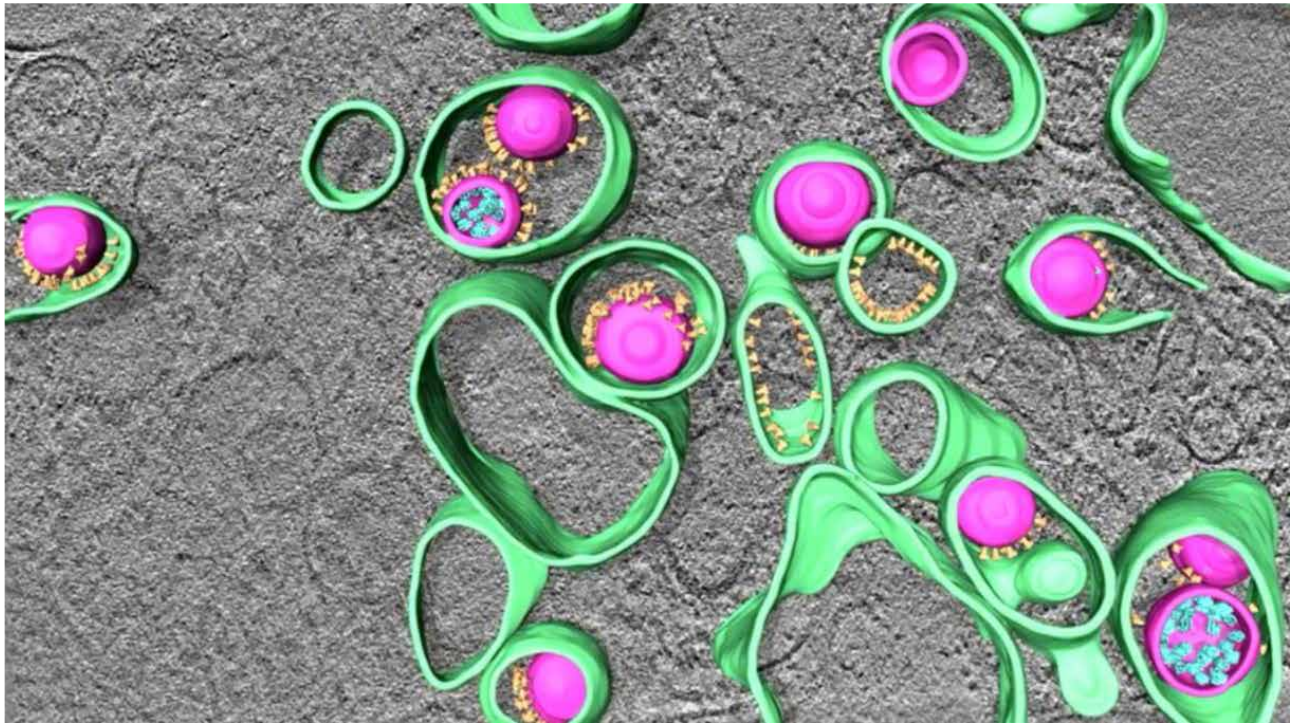
'Pandemic Fatigue' Complicates Efforts to Thwart Outbreaks

The Coronavirus Unveiled

Zimmer, Carl. "The Coronavirus Unveiled," New York Times 9 Oct 2020.

Cryo-tomography against SARS-CoV-2



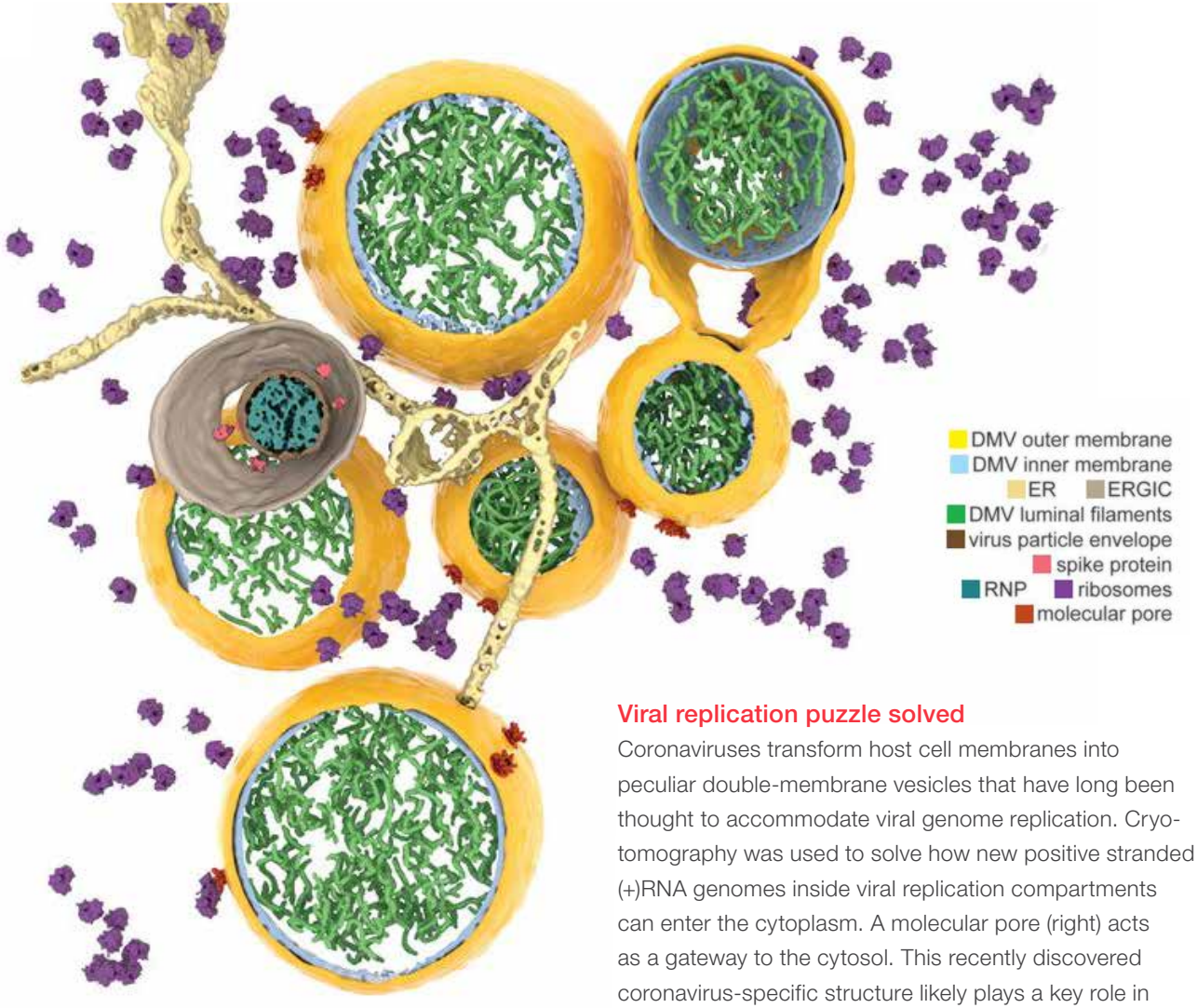


Virus induced changes in cellular membrane

Characterizing the replication cycle inside the host cell is key to understanding virulence and discovering targets restricting virus replication. Above, the SARS-CoV-2 virion is shown budding and assembling at the ERGIC membrane. The 3D volume rendering shows cellular and viral membranes in green and magenta respectively. Viral spike proteins are shown in yellow, and viral ribonucleoproteins in cyan.

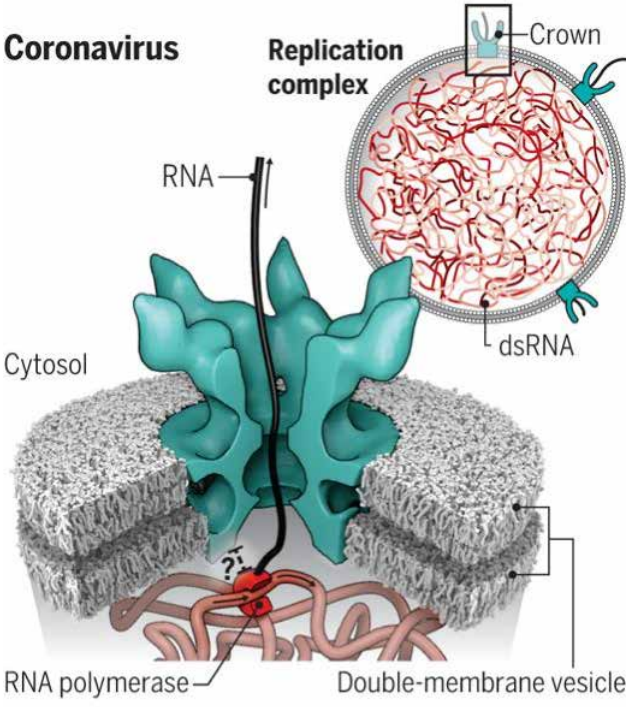
Klein, S. et al. 2020. *Nat Commun* 11, 5885, doi: 10.1038/s41467-020-19619-7

Cryo-tomography against SARS-CoV-2



Viral replication puzzle solved

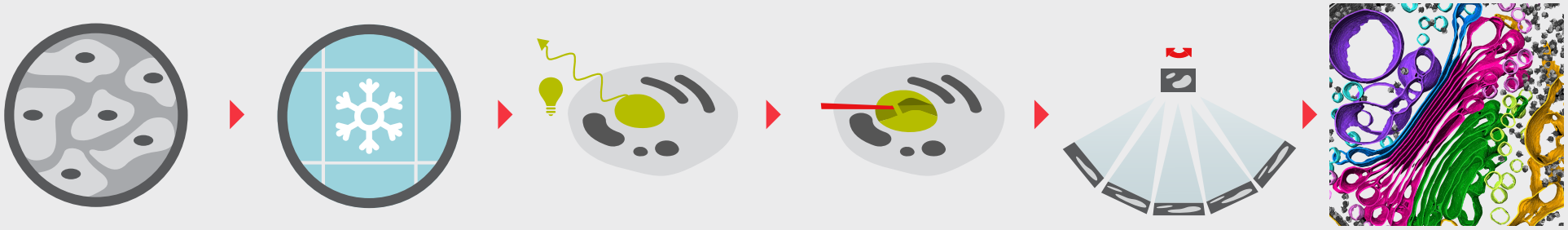
Coronaviruses transform host cell membranes into peculiar double-membrane vesicles that have long been thought to accommodate viral genome replication. Cryo-tomography was used to solve how new positive stranded (+)RNA genomes inside viral replication compartments can enter the cytoplasm. A molecular pore (right) acts as a gateway to the cytosol. This recently discovered coronavirus-specific structure likely plays a key role in coronavirus replication and thus constitutes a potential drug target.



Wolff, G. et al. 2020. A molecular pore spans the double membrane of the coronavirus replication organelle. *Science* 369, 1395, doi: 10.1126/science.abd3629

Cryo-electron tomography workflow

Cryo-ET provides label-free, fixation-free, nanometer-scale imaging of a cell's interior in 3D and visualizes protein complexes within their physiological environments. Using a correlative light and electron microscopy approach allows targeting of tagged proteins by fluorescence microscopy before subsequent cryo-EM higher-resolution imaging. Many cells are too thick for electrons, so the vitrified cells must be thinned with a cryo-focused ion beam microscope (cryo-FIB) prior to imaging in a transmission electron microscope.



Cell culture

Cells prepared by routine culture methods are grown on carbon-coated gold EM grids.

Sample preparation by vitrification

Cells are either vitrified through plunge-freezing (as in single particle analysis) or high pressure freezing (HPF). The water in the sample freezes rapidly and does not crystallize, thus avoiding the molecular-scale disruption (caused by ice-crystal formation) that would occur with a normal slow freezing process.

Localization by fluorescence

For cryo-correlative microscopy, the sample is transferred to a cryo-fluorescence light microscope (cryo-FLM), with which structures of interest are identified. A dedicated cryo-FLM stage keeps the sample in its vitrified state during cryo-fluorescence imaging.

Thinning by milling

A dedicated cryo-FIB prepares a thin, uniform lamella at the vitreous temperature (approximately -170°C).

Imaging by TEM

During cryo-ET, the sample is tilted in known increments about an axis. The individual projection images from the tomographic tilt series are then combined computationally in a procedure known as back-projection, which creates the 3D tomographic volume.

Reconstruction and visualization

The 3D tomogram featuring cellular structures can be segmented and colored in a variety of ways to enhance its display and presentation. Small subsets of data containing the structures of interest can be computationally extracted from the tomogram and subjected to image processing methods.

How do I get started with cryo-electron tomography?

EM-learning.com is a free learning platform that features over 70 hours of theoretical lectures and practical videos.

It was created by Thermo Fisher Scientific in collaboration with Professor Grant Jensen at Caltech and is intended for participants of all levels. Upon completion, you will have a fundamental base of knowledge in the field of cryo-electron microscopy.

Learn more at www.em-learning.com

Central cryo-electron microscopy core facilities are available worldwide.

To find the closest facility near you, visit this online directory of cryo-EM core facilities or service labs.

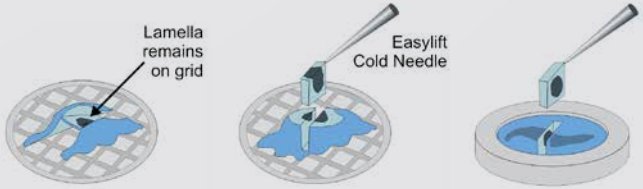
Video: Accelerate and Advance service packages for cryo-tomography

Accelerate your cryo-electron tomography research.

While this technology is transformative, implementing it can cause a lot of stress and introduce risk—especially if you are new to cryo-EM. You can rest assured that the Thermo Scientific Accelerate and Advance Service Package for Cryo-Electron Tomography will help you every step of the way to successfully implement this workflow in your lab. We will train your users and partner with you from the warranty period and beyond, delivering intensive support with onsite and remote support features. Learn more at thermofisher.com/cryo-tomo-service

What kind of samples can I image?

Samples for cryo-ET imaging can be prepared from cells and tissues. Cells can be grown on gold grids which are later used as carriers in the microscope (a). Target regions can be selected and 'lift out' tools (b) are currently available to automate the process on the new Thermo Scientific Aquilos™ 2 Cryo-FIB. High pressure frozen tissues can also be prepared in the Aquilos 2 Cryo-FIB through the cryo-lift out technique (b).

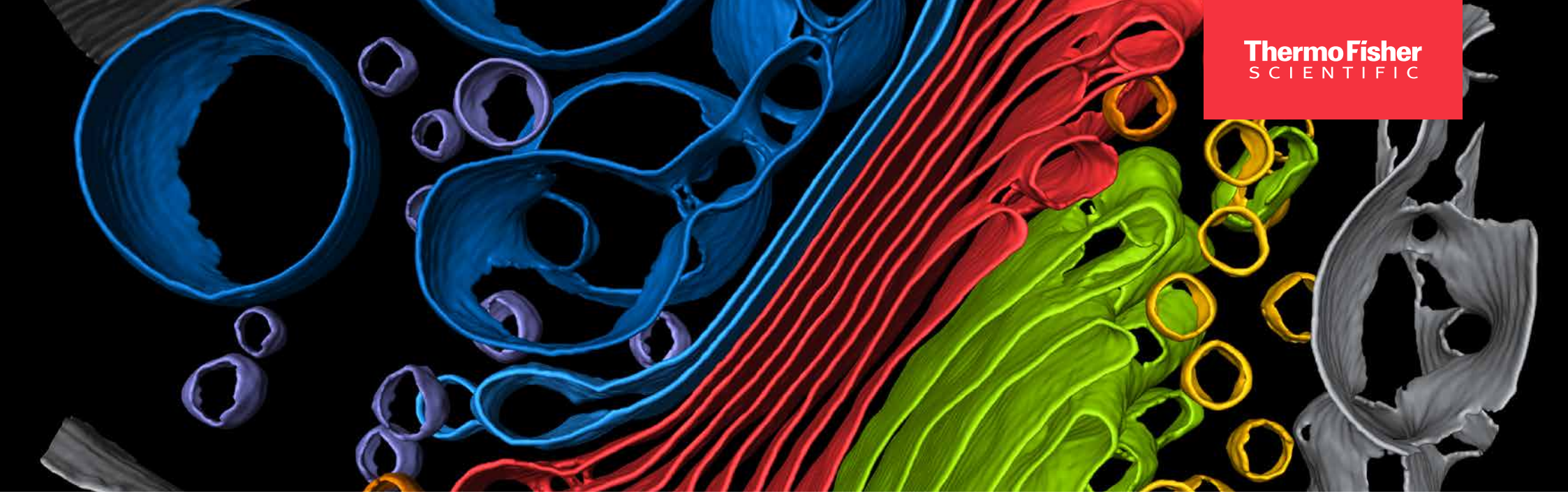


a On-the-grid Lamella

b On-the-grid Cryo-lift out

c Bulk HPF specimen Cryo-lift out

Learn more with **on-demand cryo-ET webinars**



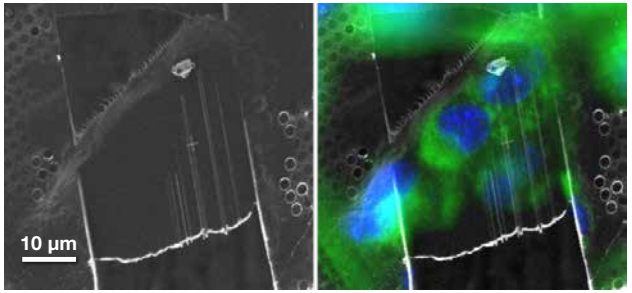
3D visualization of a *Chlamydomonas* Golgi apparatus obtained by cryo-electron tomography. Data courtesy of Dr. B. Engel, formerly with Max Plank Institute for Biochemistry, currently at Biozentrum Bazel.

Search less, discover more

iFLM Correlative System

Understanding the structural basis for cellular processes is essential for understanding how cells function. The Thermo Scientific iFLM™ Correlative System combines light and electron microscopy into one microscope, eliminating extra sample transfer steps and enabling users to create a streamlined cryo-correlative solution for cryo-tomography.

The iFLM Correlative System delivers the localization of fluorescent targets inside the chamber, allowing users to check the fluorescence signal inside milled lamellae and correlate two imaging modalities directly within one system. The iFLM Correlative System is available on the Aqulilos 2 Cryo-FIB, the Thermo Scientific Helios Hydra™ Plasma-FIB, and the Thermo Scientific Arctis™ Cryo-Plasma-FIB.



Cryo-lamella prepared with the Aquilos 2 Cryo-FIB (left). Overlay of SEM image with fluorescence image obtained with the iFLM Correlative System (right).

thermo scientific

Ask bold questions

Articles to feed the curious mind

Cell Biology

The interior of eukaryotic cells is mysterious. This review discusses some of the key ideas, strategies, auxiliary techniques, and innovations that an aspiring structural cell biologist will consider when asking bold questions.

Ng, C. T. & Gan, L. 2020. Investigating eukaryotic cells with cryo-ET. MBoC 31, 87–100. doi: 10.1091/mbc.E18-05-0329

Discovery of new endoplasmic reticulum subcompartment

Cryo-ET has sparked new interest in the endoplasmic reticulum (ER) via the discovery of ER subcompartments known as ribosome-associated vesicles (RSVs). Scientists now seek to answer how RSVs form and what role they play in cells.

Carter, SD, Hampton, CM, Langlois, R., et al. 2020. Ribosome-associated vesicles: A dynamic subcompartment of the endoplasmic reticulum in secretory cells. Science Advances, 6(14), eaay9572. doi: 10.1126/sciadv.aay9572

Four cryo-ET experts discuss recent advancements to encourage cell biologists to try cryo-ET, including the technology used for sample preparation and correlated light-electron microscopy as well as new cryo-EM access and training centers being set up through Common Fund grants from the National Institutes of Health.

Marx V., 2018. Calling all cell biologists to try cryo-ET. Nature Methods 15: 575–578. doi: 10.1038/s41592-018-0079-y

Doerr, A. 2017. Cryo-electron tomography. Nature Methods 14: 34. doi: 10.1038/nmeth.4115

Chakraborty, S., Jasnin, M., & Baumeister, W. 2020. Three-dimensional organization of the cytoskeleton: a cryo-electron tomography perspective. Protein Science, 2020. doi: 10.1002/pro.3858

Imaging and methods

In this special issue, Schur reviews the current status of methodology and provides an overview of *in vitro* and *in situ* applications of cryo-ET and subtomogram averaging. Nievergelt et al. provide a more in depth look at cryo-EM technology and correlative microscopy workflows for localization and complementary dynamic information.

Schur, F. K. 2019. Toward high-resolution *in situ* structural biology with cryo-electron tomography and subtomogram averaging. Current Opinion in Structural Biology, 58, 1-9. doi: 10.1016/j.sbi.2019.03.018

Nievergelt, A. P.; Viar, G. A. & Pigino, G. 2019. Towards a mechanistic understanding of cellular processes by cryoEM. Current Opinion in Structural Biology, 58, 149-158. doi: 10.1016/j.sbi.2019.06.008

Neuroscience

Iadanza, MG; Jackson, MP; Hewitt, EW; et al. 2018. A new era for understanding amyloid structures and disease. Nature Reviews Molecular Cell Biology, 19, 755-773. doi: 10.1038/s41580-018-0060-8

Zuber B, Lučić V. 2022. Neurons as a model system for cryo-electron tomography. Journal of Structural Biology: X. doi: 10.1016/j.yjsbx.2022.100067

Synapse ultrastructure

Brasch, J.; Goodman, KM; Noble, AJ et al. 2019. Visualization of clustered protocadherin neuronal self-recognition complexes. Nature, 569, 280-283. doi: 10.1038/s41586-019-1089-3

Synapse transmission

Correlative light and electron microscopy (CLEM) workflow was used to find transfected neurite varicosities for further investigation with cryo-ET.

Li, X, Radhakrishnan, A, Grushin, K, Kasula, R, et al. 2019. Symmetrical organization of proteins under docked synaptic vesicles. FEBS Letters, Wiley, 2019, 593, 144-153. doi: 10.1002/1873-3468.13316

Shahmoradian, SH, Lewis, AJ, Genoud, C. et al. 2019. Lewy pathology in Parkinson's disease consists of crowded organelles and lipid membranes. Nature Neuroscience, 22, 1099-1109. doi: 10.1038/s41593-019-0423-2

Microbiology

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

Wang, W., Briegel, A. 2020. Diversity of bacterial chemosensory arrays. Trends in Microbiology 28, 68-80. doi: 10.1016/j.tim.2019.08.002

Chaikeeratisak V., Nguyen K., Khanna K., et al. 2017. Assembly of a nucleus-like structure during viral replication in bacteria. Science 355: 194-197. doi: 10.1126/science.aal2130

Chaikeeratisak V., Khanna K., Nguyen KT., et al. 2019. Viral capsid trafficking along treadmilling tubulin filaments in bacteria. Cell, 177: 1771-1780. doi: 10.1016/j.cell.2019.05.032

Chang, YW, Shaffer, CL, Rettberg, LA, et al. 2018. In Vivo Structures of the Helicobacter pylori cag Type IV Secretion System. Cell Reports, 23(3), 673–681. doi: 10.1016/j.celrep.2018.03.085

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

Liedtke J, Depelteau JS, Briegel A. 2022. How advances in cryo-electron tomography have contributed to our current view of bacterial cell biology. Journal of Structural Biology: X. doi: 10.1016/j.yjsbx.2022.100065

Virology

Luque, D., Castón, J. R. 2020. Cryo-electron microscopy for the study of virus assembly. Nature Chemical Biology, 16, 231-239. doi: 10.1038/s41589-020-0477-1

Ebola

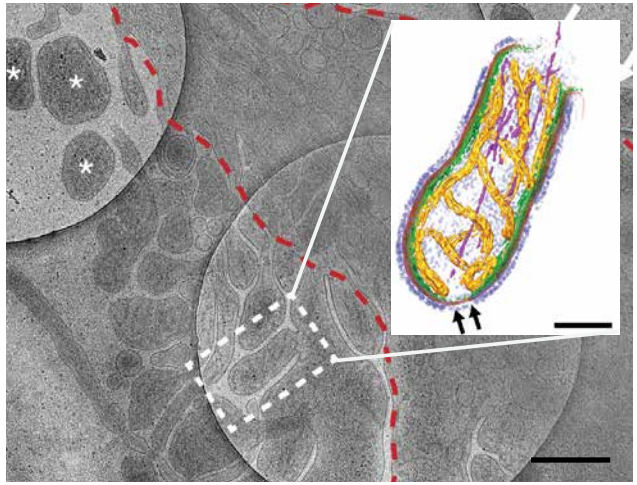
Wan, W. et al. 2017. Structure and assembly of the Ebola virus nucleocapsid. *Nature,* 551, 394–397 doi: 10.1038/nature24490

Quemin ERJ, Machala EA, Vollmer B, et al. 2020. Cellular Electron Cryo-Tomography to Study Virus-Host Interactions. Annual Review of Virology doi: 10.1146/annurev-virology-021920-115935

Li S. 2021. Cryo-electron tomography of enveloped viruses. Trends in Biochemical Sciences. doi: 10.1016/j.tibs.2021.08.005

Influenza

Gallagher J.R., Torian U., McCraw D.M., and Harris A.K. 2017. "Structural studies of influenza virus RNPs by electron microscopy indicate molecular contortions within NP supra-structures" *J. Structural Biology,* 197, 294–307. doi: 10.1016/j.jsb.2016.12.007



Cryo-ET of MeV-infected HeLa cell.

The site of measles virus (MeV) assembly is indicated by the dashed white box (above) where a 3D cryo-ET tomogram was collected (inset). Asterisks indicate released MeV particles and red dashed lines indicate the cell membrane. Inset is a false-colored 3D segmented view of the cryo-ET dataset. Scale bar is 100 nm (inset) and 500 nm.

Measles and Rubella

Ke, Z. et al. 2018. Promotion of virus assembly and organization by the measles virus matrix protein. *Nat. Commun.* 9, 1736. doi: 10.1038/s41467-018-04058-2

Mangala Prasad, V., Klose, T. & Rossmann, M. G.2017. Assembly, maturation and three-dimensional helical structure of the teratogenic rubella virus. *PLoS Pathog.* 13, e1006377. doi: 10.1371/journal.ppat.1006377

Herpes simplex virus 1 glycoprotein B

Ben-Mordehai, T. Z.; et al. 2016. Two distinct trimeric conformations of natively membrane-anchored full-length herpes simplex virus 1 glycoprotein B. PNAS, 113, 4176-418. doi: 10.1073/pnas.1523234113

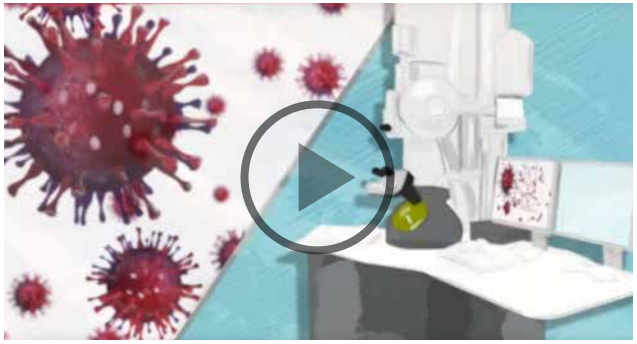
Meyer, N. L.; Hu, G.; Davulcu, O.; Xie, Q.; Noble, A. J.; Yoshioka, C.; Gingerich, D. S.; Trzynka, A.; David, L.; Stagg, S. M. & Chapman, M. S. 2019. Structure of the gene therapy vector, adeno-associated virus with its cell receptor, *eLife*, 8. doi: 10.7554/eLife.44707

Viral assembly in selected regions of virus-infected cells

The Elizabeth Wright lab at the University of Wisconsin—Madison describes its protocol for cryo-CLEM of virus-infected or transfected mammalian cells.

Hampton, C. M.; Strauss, J. D.; Ke, Z.; Dillard, R. S.; Hammonds, J. E.; Alonas, E.; Desai, T. M.; Marin, M.; Storms, R. E.; Leon, F. et al. 2017 Correlated fluorescence microscopy and cryo-electron tomography of virus-infected or transfected mammalian cells. *Nature protocols*, 12, 150. doi: 10.1038/nprot.2016.168

Fu, X.; Ning, J.; Zhong, Z.; Ambrose, Z.; Watkins, S. C. & Zhang, P. 2019 AutoCLEM: An Automated Workflow for Correlative Live-Cell Fluorescence Microscopy and Cryo-Electron Tomography. *Scientific Reports*, 9. doi: 10.1038/s41598-019-55766-8



Video: Thermo Fisher's COVID-19 Global Response

The Thermo Scientific cryo-ET workflow is an end-to-end workflow covering flash freezing of cells to final 3D visualization. Interior cellular regions are imaged at nanometer-scale resolution in a cryo-TEM from cryo-lamellae precisely prepared with cryo-FIB or cryo-PFIB microscopes.

Aquilos 2 Cryo-FIB

Dedicated cryo-FIB for cellular cryo-electron tomography sample preparation

Aquilos 2 Cryo-FIB produces cryo-lamellae for cryo-electron tomography; key steps can be automated through user-friendly milling recipes

Hydra Bio Plasma-FIB

Plasma-FIB for volume electron microscopy and cryo-electron tomography

Hydra Bio Plasma-FIB provides breakthrough capabilities for cryogenic and room temperature volume electron microscopy and versatile TEM lamella preparation for cryo-electron tomography

Arctis Cryo-Plasma-FIB

Automated cryo-plasma-FIB for throughput and connectivity in the cryo-ET workflow

Arctis Cryo-Plasma-FIB automates high-throughput TEM lamellae production and features Autoloader connectivity for the cryo-electron tomography workflow

Learn more at **thermofisher.com/cryo-tomography**