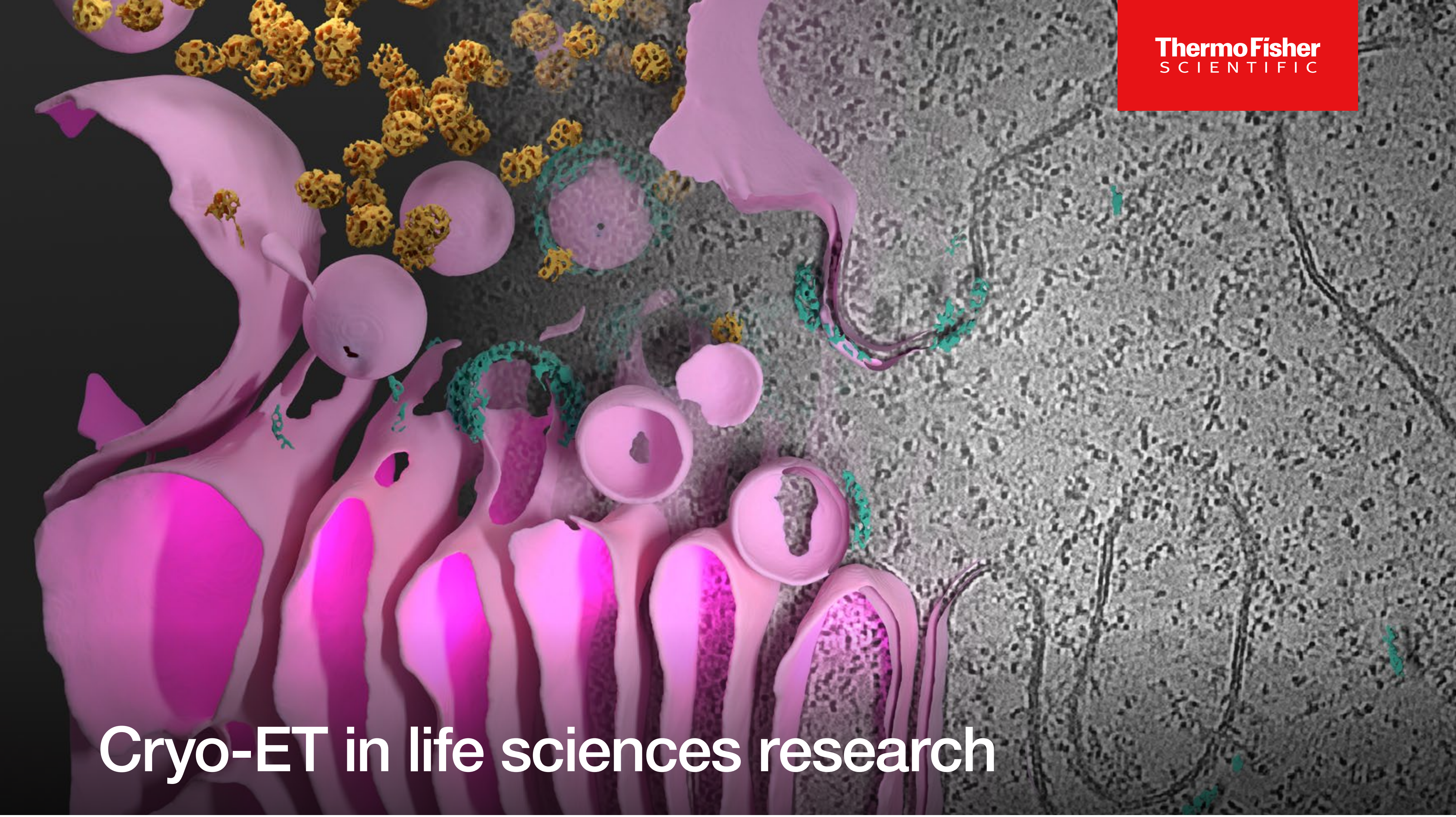


Cryo-ET in life sciences research

A curated reference guide to recent advances

See biology in context with cryo-ET

Cryo-electron tomography (cryo-ET) enables three-dimensional visualization of biological specimens in a near-native, vitrified state at nanometer resolution. By acquiring a series of tilted projection images in a transmission electron microscope and computationally reconstructing them into a tomogram, cryo-ET reveals the structural organization of macromolecular complexes, organelles, and cellular environments directly inside cells.

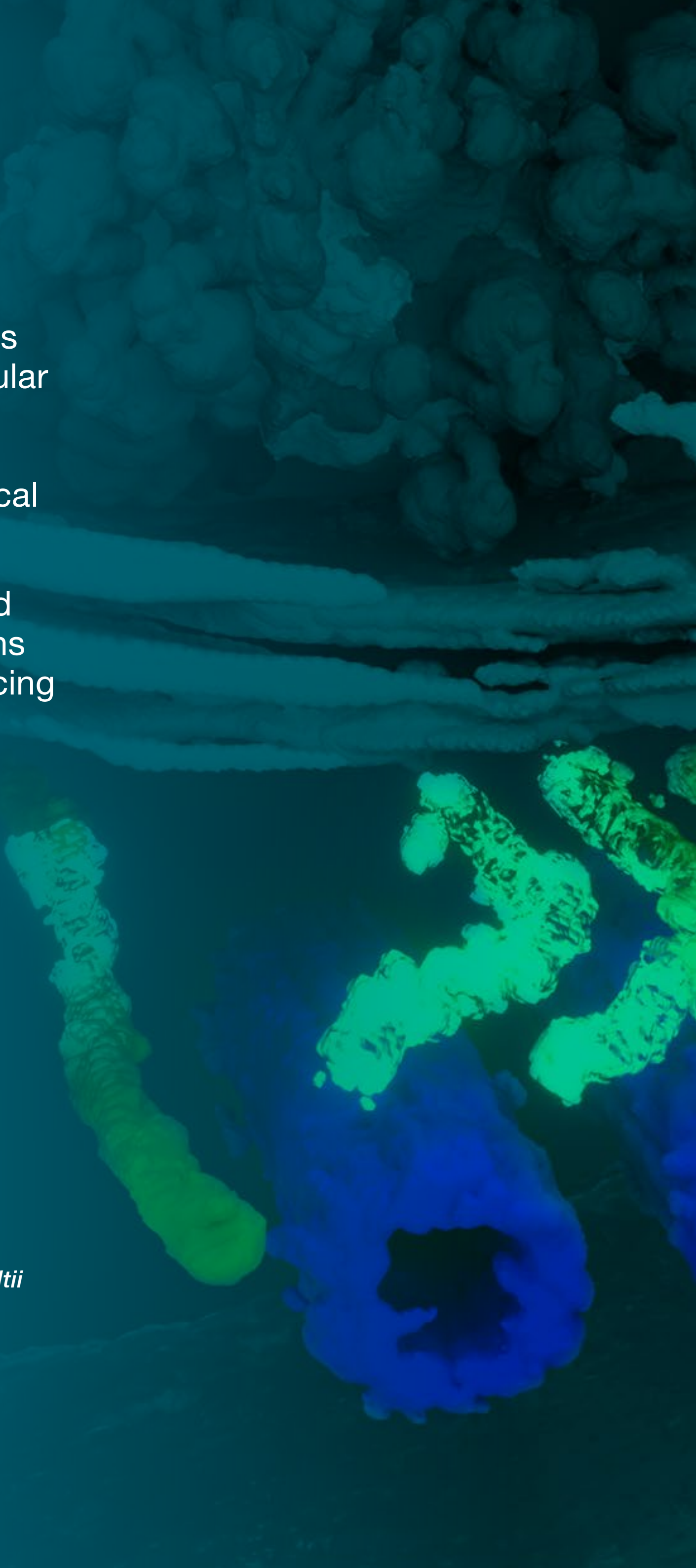
Unlike single particle cryo-electron microscopy, which determines structures of isolated macromolecules, cryo-ET preserves spatial context. This allows researchers to investigate molecular machinery *in situ* and understand how proteins assemble, interact, and function within the native cellular environment. Cryo-ET complements single particle cryo-EM by providing structural information that cannot be obtained from purified preparations alone.

Advances in cryogenic sample preparation, focused ion beam milling, automated tilt series acquisition, and computational analysis have significantly improved the accessibility and throughput of cryo-ET workflows.

These developments are helping a rapidly growing number of researchers study cellular architecture and molecular organization directly within intact biological systems, driving adoption across an expanding range of biological applications.

The following pages present a curated selection of peer-reviewed publications demonstrating how cryo-ET is advancing discovery across six life sciences research areas:

- **Fundamental cell biology**
- **Tissue and cancer biology**
- **Neuroscience**
- **Plant research**
- **Virology**
- **Microbiology**



Cryo-ET reconstruction of *Chlamydomonas reinhardtii* adapted from Kelley *et al* (2025). under CC BY 4.0.

The cryo-ET workflow

Biological samples are rapidly vitrified to preserve their native structure, after which regions of interest are identified and targeted for cryogenic focused ion beam milling to produce electron-transparent lamellae. Tilt series are then acquired using cryogenic transmission electron microscopy (TEM) and computationally reconstructed into 3D tomograms for visualization and quantitative analysis.

Preserve

Rapid vitrification prevents ice crystal formation, preserving native molecular organization.

Thermo Scientific™ Vitrobot™ System

Target

Cryo-fluorescence microscopy (integrated CLEM) localizes specific cells or regions before lamella preparation.

Thermo Scientific™ Hydra™ Bio Plasma-FIB for cells and grids in cryo-ET

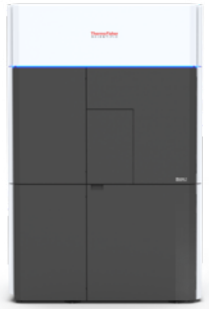
Thin

Cryo-focused ion beam milling thins vitrified specimens into electron-transparent lamellae.

Thermo Scientific™ Arctis™ Cryo-Plasma-FIB for cells, tissues and larger samples in cryo-ET and volume EM

Image

A cryo-TEM acquires a tilt series to generate three-dimensional cellular detail.

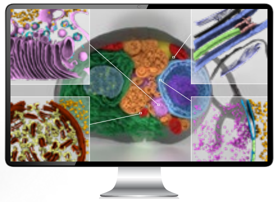


Thermo Scientific™ Glacios™ 3 Cryo-TEM

Thermo Scientific™ Krios™ 5 Cryo-TEM

Quantify

Computational reconstruction generates tomograms for segmentation and statistically robust analysis.



Thermo Scientific™ Amira™ Software

Cryo-ET software

- **Ar** Arctis Web UI
- **AS** Autoscript
- iFLM
- **Ma** Maps
- **AT** AutoTEM
- **AS** Autoscript
- iFLM
- **ASV** ASV
- **Ma** Maps
- **CT** CryoTomo Software Suite
- **CF** CryoFlow Software

Fundamental cell biology

Understanding the fundamentals of cell biology depends not only on what proteins do in isolation but also on how they are organized, assembled, and coordinated within the crowded environment of the living cell. Cryo-ET is uniquely positioned to address this, preserving the three-dimensional spatial context of macromolecular complexes, cytoskeletal networks, membranes, and organelles in their near-native state. Studies in this area provide important insights into how cellular machines function *in situ*, revealing fundamental cellular processes and molecular organization that are inaccessible to biochemical analyses or single particle cryo-EM.

Typical applications include:
Studies of cytoskeletal organization, membrane remodeling, intracellular transport, and organelle architecture.

Revealing how a major regulatory assembly is reorganized into a membraneless compartment *in situ*

Using cryo-ET to examine yeast, this study showed that inactive doubly capped 26S proteasomes assemble into approximately 75 MDa trimeric units during the transition from proliferation to quiescence. First, the assembly is seen in the nucleoplasm and near the nuclear envelope, then as bundled paracrystalline fibers within cytoplasmic proteosome storage granules. Cryo-ET structures at 9 Å revealed how cells store fully assembled but inactive proteasomes in membraneless compartments for rapid reuse.

Instruments used: *Thermo Scientific™ Krios™ Cryo-TEM, Vitrobot™ Mark IV System, Falcon 4i Detector, Selectris™ Imaging Filter, Aquilos™ Cryo-FIB, and Amira™ Software*

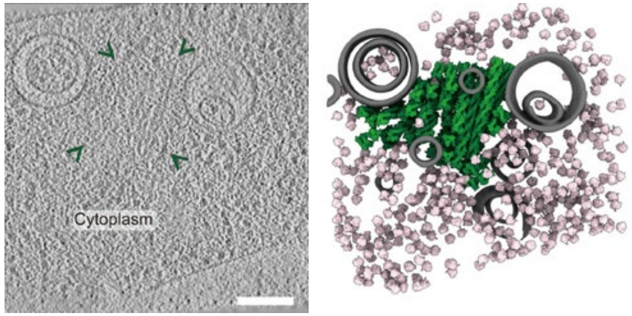
Read the study

Linking centriole architecture to disease by identifying the molecular basis of a key structural connection within centrioles

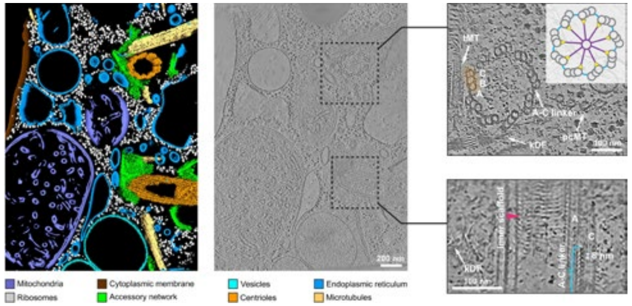
Using cryo-ET together with single particle cryo-EM, this study identified the proteins composing the centriolar A-C linker and showed that it is structurally variable along the centriole axis. Several of these components are linked to microcephaly, primordial dwarfism, and cancer predisposition, connecting centriole ultrastructure to human disease.

Instruments used: *Krios G4 Cryo-TEM and Vitrobot Mark IV System*

Read the study



Cryo-ET slice and segmentation of a stationary-phase yeast cell showing a proteasome storage granule composed of bundled fibers of proteasome trimers. Figure adapted from Tang *et al.* (2026) under CC BY 4.0.



Segmented surfaces and a cryo-tomogram slice of a FIB-milled *Tetrahymena* cell showing two centrioles analyzed to resolve the A-C linker architecture. Figure adapted from Cai *et al.* (2025) under CC BY 4.0.

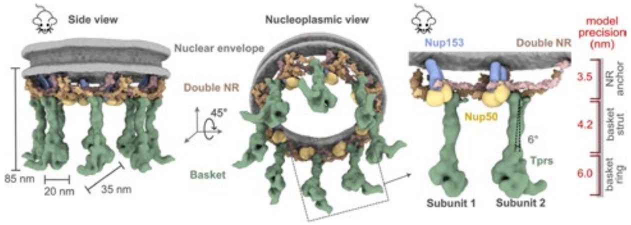
Fundamental cell biology

Defining the architecture of the nuclear basket and explaining variable stability across nuclear pores

Cryo-ET imaged 1,604 tilt series in three species to reveal that a stable nuclear basket forms only on nuclear pore complexes (NPCs) with a double nuclear ring, explaining long-standing observations of variable basket stability. The study resolved the basket's coiled-coil struts and captured a roughly 20 nm chromatin exclusion zone, providing a complete structural model of this mRNA surveillance platform.

Instruments used: *Krios G3 Cryo-TEM and Aquilos Cryo-FIB*

Read the study



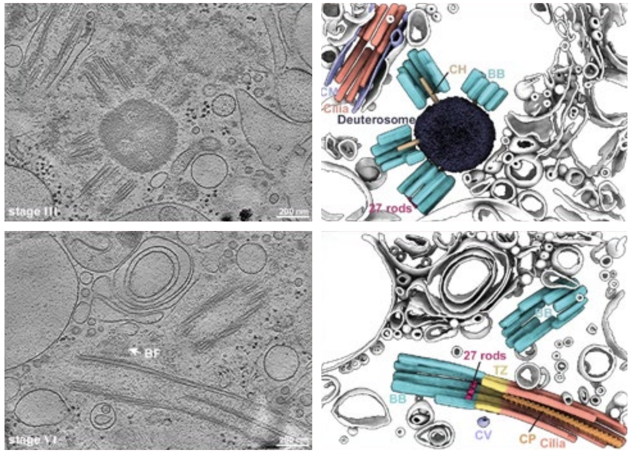
Cryo-ET-derived structural model of the mammalian nuclear basket showing its modular organization and overall topology relative to the nuclear ring. Figure taken from Singh *et al.* (2024) under CC BY 4.0.

Capturing mammalian cilia biogenesis *in situ* and linking distinct assembly stages to ciliopathy-relevant proteins

In situ cryo-ET of mouse ependymal cells captured basal body assembly across multiple stages and identified three distinct structural zones, as well as a boundary ring of 27 rods that had not been previously visualized in mammalian cells. The study also tracked progressive exchange of internal microtubule proteins during cilia maturation and identified CEP41, a protein mutated in Joubert syndrome, as a key transitional component.

Instruments used: *Krios Cryo-TEM, Vitrobot Mark IV System, Aquilos 2 Cryo-FIB, and Amira Software*

Read the study



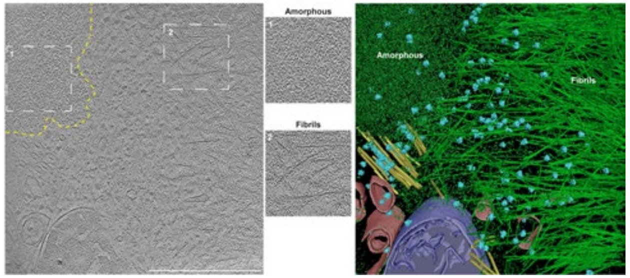
In situ cryo-ET snapshots showing successive stages of basal body maturation and early cilium formation in mouse ependymal cells. Figure reproduced from Ma *et al.* (2025) under CC BY 4.0.

Revealing an autophagy mechanism inaccessible to biochemical approaches and providing a structural explanation relevant to neurodegeneration

Cryo-ET in intact cells showed that polyQ aggregates exist as both a deformable amorphous outer region and a rigid fibrillar core, with autophagosomes selectively engaging only the amorphous phase. The study also directly visualized phagophores becoming trapped within fibrillar cores, providing a structural explanation for why autophagy stalls at mature protein inclusions.

Instruments used: *Krios Cryo-TEM, Vitrobot Mark IV System, and Amira Software*

Read the study



Cryo-ET slices and segmentations of intracellular polyQ aggregates showing the coexistence of amorphous and fibrillar regions and the interaction of autophagic membranes with aggregate structures. Scale bar is 500nm. Figure adapted from Zhao *et al.* (2024) under CC BY 4.0.

Tissue and cancer biology

Understanding how cells are organized within tissues and how this organization changes in disease is central to modern biomedical research. Cryo-ET enables investigation of cellular and molecular architecture within complex biological systems, including primary tissues and organoid models, at nanometer resolution.

These studies are revealing how macromolecular assemblies, organelles, and cell–cell interactions are organized within native tissue environments. Although cancer-specific cryo-ET is still emerging, advances in tissue imaging workflows are creating new opportunities to investigate tumor organization and the cancer microenvironment.

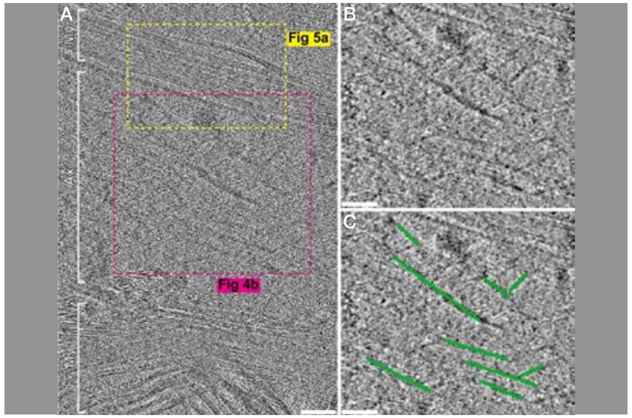
Typical applications include:
Studies of native tissue ultrastructure, extracellular matrix organization, targeted molecular biopsy across organ types, and structural analysis of disease-relevant tissue specimens.

Bringing human clinical tissue into reach and creating a path toward structural histopathology in disease

This study showed for the first time that unfixed, never previously frozen human post-mortem brain tissue can be vitrified and imaged by cryo-ET, preserving intact subcellular architecture without chemical fixation artifacts. Autophagic structures and tau-like fibrils were identified directly in Alzheimer's disease tissue, showing that patient-derived human pathology can be examined *in situ* at nanometer resolution.

Instruments used: *Krios G3i Cryo-TEM*

Read the study



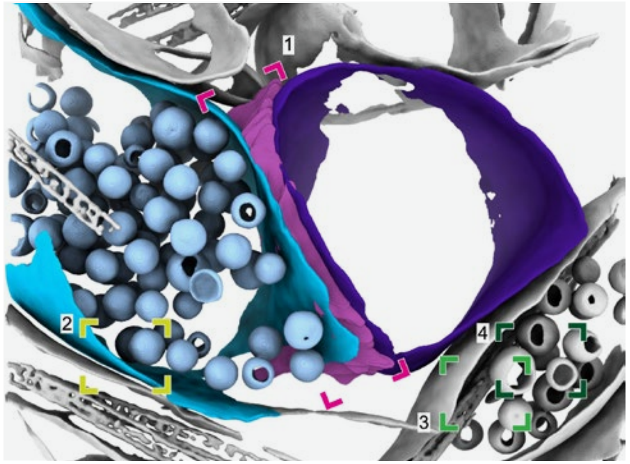
Above: Cross-section of a tomogram showing a myelinated (My) axon (Ax) containing twisting filaments consistent with tau fibrils. The pink box from (a) is shown with denoising in (b) to highlight the potential twisting tau fibrils that are traced in (c). Scale bars, 100 nm. Figure adapted from B. Creekmore *et al.* (2024) under CC BY 4.0.

Turning tissue imaging into a targeted molecular biopsy approach for nanoscale analysis of defined regions within complex tissue

Using cryo-correlative light and electron microscopy (cryo-CLEM) targeting in intact mouse hippocampal tissue, this study generated a molecular atlas of the CA1 region at nanometer resolution, capturing synaptic vesicle fusion events, postsynaptic densities, and astrocyte-neuron contacts within a specific sub-region. Because the workflow can be directed to any chosen location in any tissue type, it provides a practical route for studying defined pathological sites such as inflammatory lesions and tumor microenvironments.

Instruments used: *Thermo Scientific™ Krios™ G4 Cryo-TEM, Helios Hydra™ 5 CX PFIB, Arctis™ Cryo-PFIB, Vitrobot™ Mark IV System, Falcon 4i Detector, Selectris™ Imaging Filter, and Amira™ Software*

Read the study



Right: Cryo-ET segmentation of a CA1 stratum radiatum synapse in vitreous brain tissue showing synaptic vesicles, the synaptic cleft, and pre- and postsynaptic membranes. Figure adapted from Glynn *et al.* (2025) under CC BY 4.0.

Tissue and cancer biology

Providing the first native structural view of extracellular matrix organization in a fully hydrated state

This study visualized native extracellular matrix (ECM) in a fully hydrated state and resolved fibronectin, fibrillin microfibrils, and collagen VI in their genuine three-dimensional context alongside matrix-secreting cells. It also identified previously unseen filamentous bridges between matrix components, providing a structural baseline for understanding how ECM remodeling controls tumor stiffness, invasion routes, and immune exclusion in disease.

Instruments used: *Krios G3i Cryo-TEM, Aquilos 2 Cryo-FIB, and Amira Software*

Read the study

Making intact solid tissue specimens and organoids broadly accessible for molecular-level structural analysis

Using serialized on-grid lift-out sectioning, this study produced molecular-resolution views from the interior of intact solid mammalian tissues, including mouse liver, heart, and brain. The approach resolved ribosome organization, ER morphology, and vesicle trafficking directly in native tissue, and was also demonstrated across multiple organ types including human forebrain organoids, establishing that no tissue type is inaccessible by cryo-ET.

Instruments used: *Krios G4i Cryo-TEM, Falcon 4i Detector, Selectris X Imaging Filter, Aquilos 2 Cryo-FIB, and Amira Software*

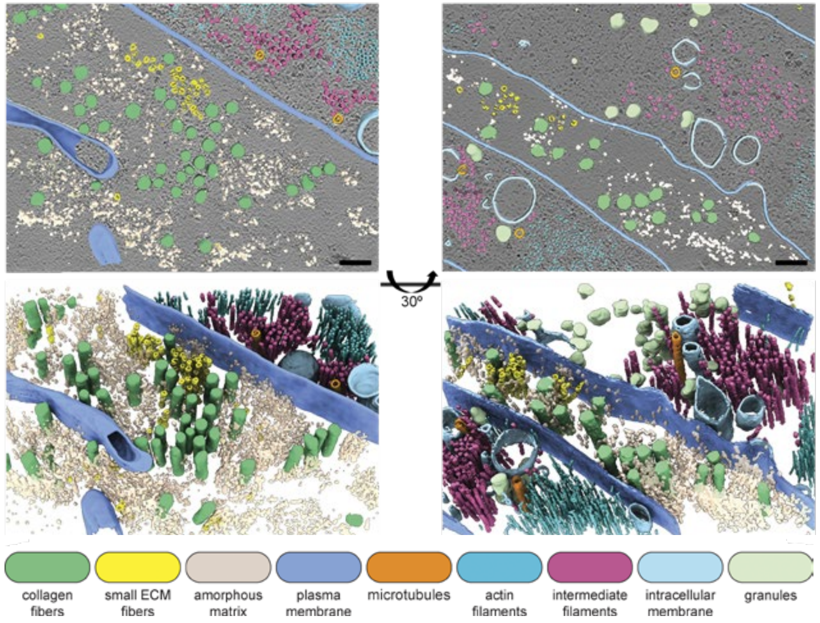
Read the study

Resolving the native organization of the cardiac thick filament *in situ* to provide a structural framework for muscle regulation and cardiomyopathy biology

Cryo-ET of relaxed muscle cardiac myofibrils resolved the molecular architecture of the thick filament *in situ*, defining how myosin, titin, and cardiac myosin-binding protein C (cMyBP-C) are arranged from the M band to the C zone. The study showed that myosin heads adopt region-specific organizations along the filament, clarified how titin chains run through the thick filament, and revealed how cMyBP-C links thick and thin filaments while stabilizing the relaxed OFF state of myosin.

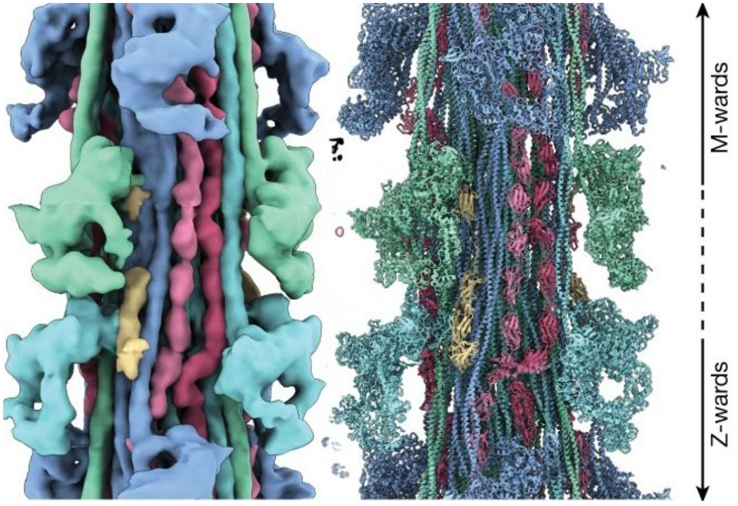
Instruments used: *Krios Cryo-TEM and Vitrobot Mark IV System*

Read the study



Left: Cryo-ET segmentation of cell-derived matrix showing ECM fibers and adjacent cellular structures in their native spatial context. Figure adapted from Zens *et al.* (2024) under CC BY 4.0.

Right: Cryo-ET structure and atomic model of the relaxed cardiac thick filament C zone, showing the *in situ* arrangement of myosin, titin, and cMyBP-C. Figure adapted from Tamborrini *et al.* (2023) under CC BY 4.0.



Neuroscience

The nervous system poses particular challenges for structural biology. Many of its most important features, including synaptic junctions, axonal projections, and disease-associated protein aggregates, exist within dense and complex tissue. Cryo-ET enables three-dimensional visualization of these structures in their native context, from the organization of vesicles and scaffolding proteins at synapses to the propagation of pathological filaments between neurons. Studies in this area are providing new insights into neuronal organization and function, as well as mechanisms underlying neurodegenerative disease.

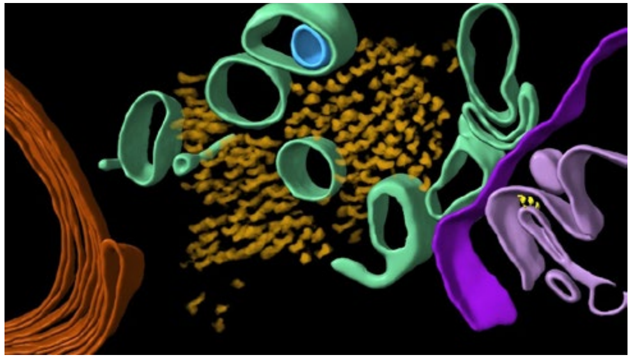
Typical applications include:
Studies of synaptic architecture, vesicle organization, cytoskeletal dynamics in axons, structural markers of neurodegeneration, and membrane-associated protein complexes.

Providing structural context for amyloid pathology directly in human brain tissue

Cryo-ET of post-mortem human Alzheimer's disease brain tissue showed that β-amyloid plaques contain branched fibrils, protofilaments, and extracellular vesicles in spatial arrangements not seen in tissue-extracted or *in vitro* preparations. Subtomogram averaging of tau filaments within intact tissue also showed that tau structure varies with subcellular location, suggesting templated propagation as the mechanism of tau spread in Alzheimer's disease.

Instruments used: *Krios G2 Cryo-TEM, X-FEG, Falcon 4i Detector, and Selectris Imaging Filter*

Read the study



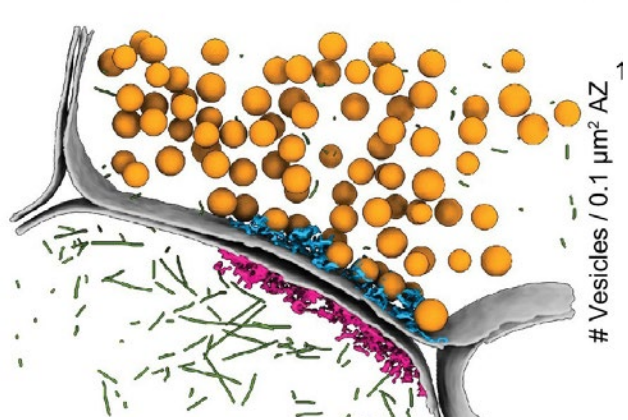
Representative tomographic slice and segmentation of extracellular tau pathology in post-mortem Alzheimer's disease brain tissue, showing ultrastructural features only visible in intact tissue. Figure adapted from Gilbert *et al.* (2024) under CC BY 4.0.

Showing that synaptic vesicle release sites are offset from aligned scaffold nanoclusters

Using cryo-ET to visualize synapses, this study resolved both membrane-proximal synaptic vesicles and aligned presynaptic and postsynaptic scaffold clusters. It showed that vesicle release sites are offset from these aligned scaffold complexes, refining current models of how nanoscale synaptic geometry shapes neurotransmission.

Instruments used: *Krios Cryo-TEM, Aquilos 2 Cryo-FIB, and Amira Software*

Read the study



Cryo-ET slice and segmentation of a FIB-milled synapse showing synaptic vesicles, aligned presynaptic and postsynaptic protein densities, and actin filaments. Figure adapted from Held *et al.* (2024) under CC BY 4.0.

Neuroscience

Enabling efficient, minimally invasive targeting of excitatory synapses in primary neurons

This study established a cryo-CLEM workflow for targeting synapses in primary neuronal cultures using minimally invasive labeling. The method improved targeting efficiency, enabling systematic *in situ* imaging of excitatory synapses for future cryo-ET studies of neurotransmission, plasticity, and synaptic disease.

Instruments used: *Krios G4 Cryo-TEM, Falcon 4i Detector, Selectris Imaging Filter, Aquilos 2 Cryo-FIB, and Amira Software*

[Read the study]

Linking electrophysiological behavior to molecular ultrastructure

This study combined voltage imaging and cryo-ET in the same neuron, allowing electrophysiological firing patterns to be related directly to molecular architecture. Neurons with different activity profiles showed measurable differences in cytoskeletal organization and synaptic vesicle arrangement, linking function and structural state at the single-cell level.

Instruments used: *Krios G4 Cryo-TEM, Falcon 4i Detector, Selectris X Imaging Filter, and Aquilos 2 Cryo-FIB*

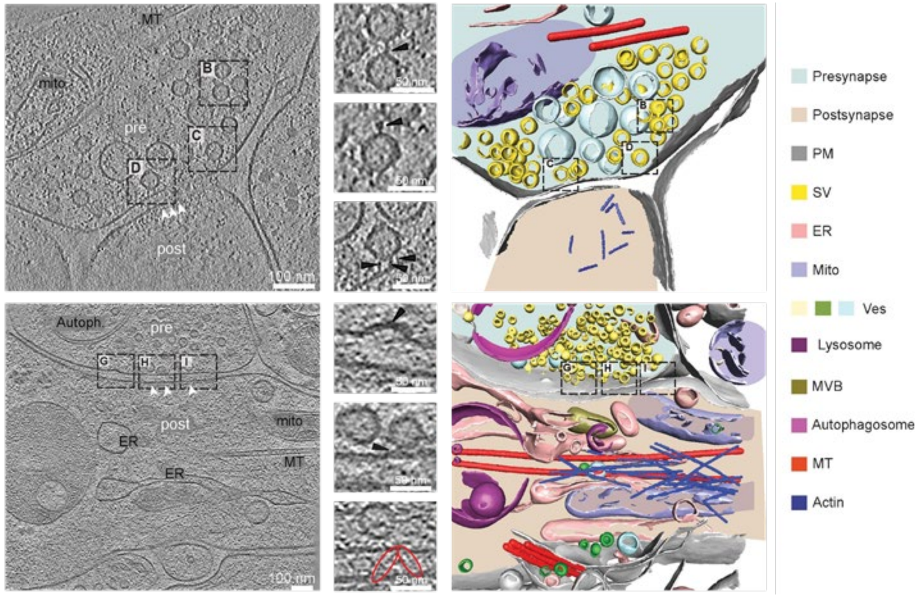
[Read the study]

Resolving endogenous glutamate receptors at a mammalian synapse in intact brain tissue

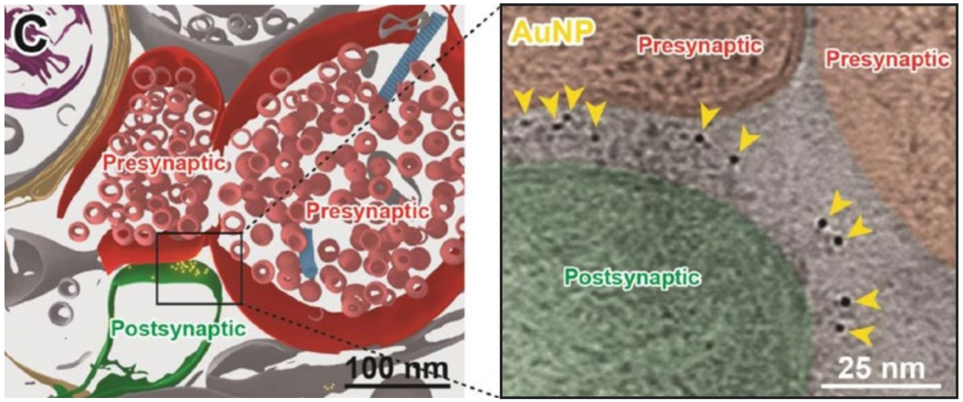
Using cryo-CLEM targeting of synapses in knock-in mice, this study resolved AMPA receptors, synaptic vesicles, presynaptic scaffolds, and postsynaptic densities simultaneously within native hippocampal tissue. It also identified globular densities consistent with prefusion SNARE-synaptotagmin complexes at the vesicle-membrane interface, providing structural evidence for inhibited prefusion machinery at resting calcium.

Instruments used: *Krios Cryo-TEM and Aquilos 2 Cryo-FIB*

[Read the study]



Cryo-ET slices and segmentations of labeled neuronal synapses showing synaptic vesicles, postsynaptic densities, vesicle tethering, and fusion intermediates. [Figure adapted from Do *et al.* (2026)] under [CC BY 4.0.]



Cryo-ET segmentation and tomogram slice of mouse hippocampal CA1 tissue labelled with anti-GluA2 Fab-AuNP, with gold particles visible between the pre- and postsynaptic membranes. [Figure adapted from Matsui *et al.* (2024)] under [CC BY 4.0.]

Plant research

Plant and algal cells carry out processes of global importance, including photosynthesis and carbon fixation, within organelles and membrane systems whose native three-dimensional architecture has been challenging to access using conventional approaches. Cryo-ET is enabling structural studies of cell walls, chloroplasts, and pyrenoids within intact cells, as well as the organization of complex membrane systems and the specialized structures that support function. These studies are helping to define plant cell organization and development in ways that are relevant to engineering photosynthetic efficiency.

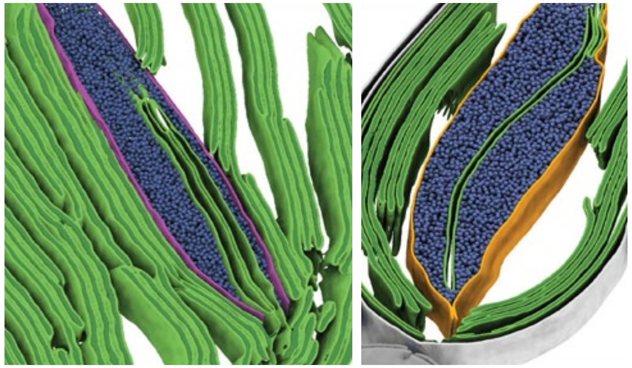
Typical applications include:
Studies of chloroplast structure, cell wall organization, membrane systems, and plant cell development.

Identifying a previously unknown organelle enclosure strategy that underpins efficient carbon fixation in diatoms

Cryo-ET of two diatom species showed that pyrenoids, which are responsible for a substantial fraction of global CO$_2$ fixation, are enclosed by a previously unknown protein shell named PyShell. The shell maintains pyrenoid shape and positions CO$_2$-generating thylakoids against the Rubisco matrix, while PyShell knockouts caused fragmentation of the Rubisco matrix and loss of CO$_2$-concentrating efficiency.

Instruments used: *Krios Cryo-TEM, Falcon 4 Detector, Selectris X Imaging Filter, Vitrobot Mark IV, Aquilos Cryo-FIB, and Amira Software*

[Read the study]



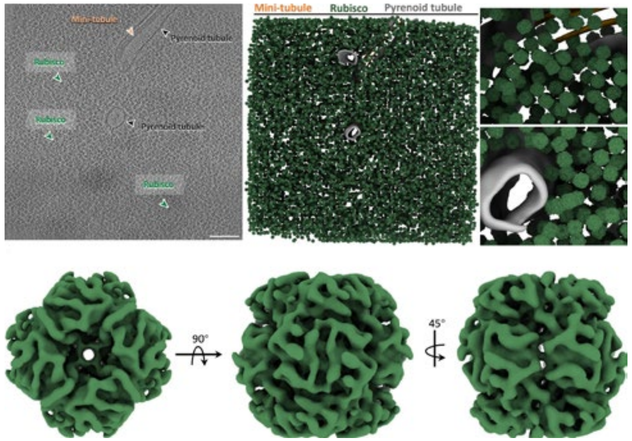
Tomographic slices and segmentations of pyrenoids from two diatom species, showing the PyShell enclosure surrounding the Rubisco matrix and associated thylakoid membranes. Figure adapted from Shimakawa *et al.* (2024) under CC BY 4.0.

Resolving the native conformation of Rubisco inside the living pyrenoid and revealing structural heterogeneity within the same cell

Cryo-ET of intact algal cells resolved the native in-cell structure of Rubisco at 8.1 Å resolution and showed that the enzyme adopts an active closed conformation inside the living pyrenoid. The study also identified multiple structurally distinct Rubisco populations consistent with different catalytic states within the same cell.

Instruments used: *Krios G4 Cryo-TEM, Falcon 4i Detector, Selectris X Imaging Filter, Aquilos Cryo-FIB, and Arctis Cryo-PFIB*

[Read the study]



Representative tomographic slice, segmentation, and subtomogram average of pyrenoid Rubisco in *C. reinhardtii*, showing its native in-cell organization relative to thylakoids and pyrenoid tubules. Figure adapted from Elad *et al.* (2024) under CC BY 4.0.

Plant research

Defining how photosystems are partitioned within native thylakoid membranes

In situ cryo-ET of intact spinach chloroplasts mapped thylakoid membrane components at single-molecule precision using AI-assisted segmentation, extending plant cryo-ET beyond earlier work in algae. The study showed strict lateral segregation of photosystems between appressed grana and stromal lamellae domains, resolving a decades-old debate about photosystem mixing at grana margins.

Instruments used: *Krios Cryo-TEM, Vitrobot Mark IV System, Aquilos Cryo-FIB, and Amira Software*

Read the study

Setting a benchmark for in-cell visual proteomics in plant and algal systems

This study generated 1,829 cryo-ET tomograms of *Chlamydomonas reinhardtii* and produced sub-nanometer resolution averages for seven major complexes, including ribosomes, Rubisco, nucleosomes, and ATP synthase, all inside living cells. Released as an open-access community resource, the dataset establishes large-scale in-cell visual proteomics as a practical tool for plant and algal biology.

Instruments used: *Thermo Scientific™ Krios™ G4 Cryo-TEM, Falcon 4i Detector, Selectris™ X Imaging Filter, Vitrobot™ Mark IV System, Arctis™ Cryo-PFIB, Hydra™ Bio Cryo-PFIB, and Amira™ Software*

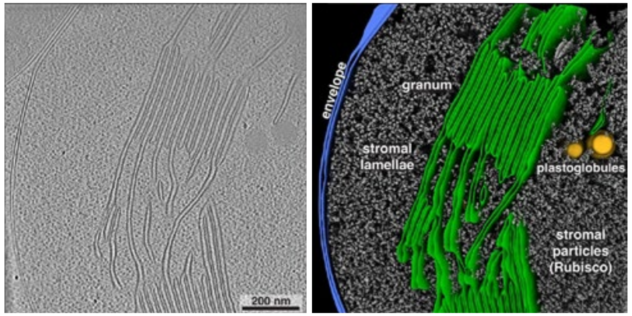
Read the study

Extending structural imaging to specialized plant cell walls and deep tissue targets that were previously inaccessible in a native hydrated state

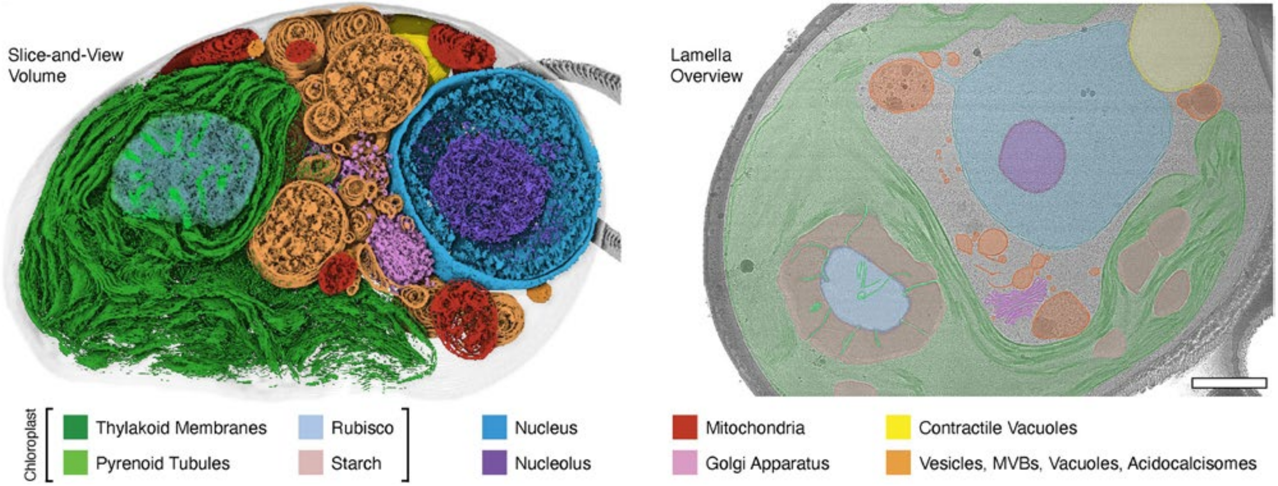
This study examined intact *Arabidopsis thaliana* roots and resolved Casparian strips, suberin lamellae, and xylem vessel walls in their native hydrated state at a depth of 50 microns, targets that were inaccessible by any previous plant EM approach. It also identified previously unseen membrane structures and extracellular vesicles at specialized cell wall interfaces, opening new questions about cell wall assembly and cell–cell communication.

Instruments used: Krios G4 Cryo-TEM, Falcon 4i Detector, Selectris X Imaging Filter, and Aquilos 2 Cryo-FIB

Read the study



Cryo-ET slice and segmentation of intact spinach chloroplast thylakoid membranes showing the organization of membrane architecture across grana and stromal lamellae. Figure adapted from Wietrzynski *et al.* (2025) under CC BY 4.0.



3D segmentation and tomographic slice of a vitreous *C. reinhardtii* cell from the large-scale cryo-ET dataset, illustrating the overall cellular architecture captured for community-scale visual proteomics. Figure adapted from Kelley *et al.* (2025) under CC BY 4.0.

Virology

Viruses replicate, assemble, and hijack host cells through processes that are spatially organized and structurally precise. Cryo-ET enables direct visualization of viruses within their native environment, providing three-dimensional insight into viral architecture, assembly, and host cell remodeling during infection. Because many stages of the viral life cycle occur within complex intracellular environments, cryo-ET is particularly valuable for understanding how viral and host components interact during infection.

Studies in this area support investigations into infection mechanisms and provide structural context for the development of antiviral therapies.

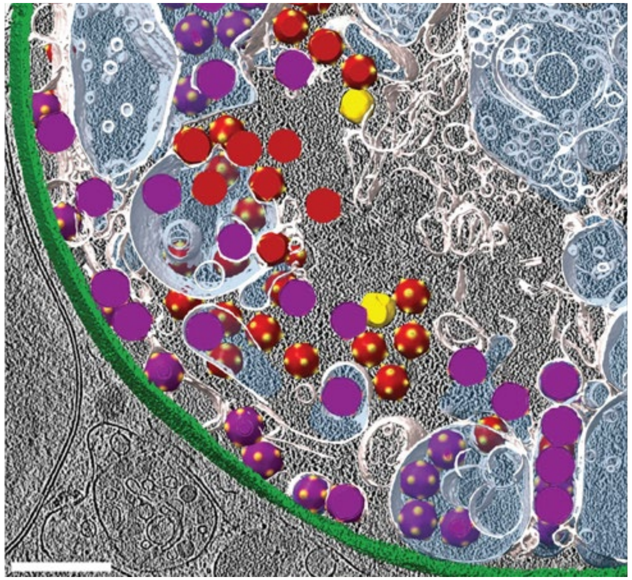
Typical applications include:
Studies of viral entry, capsid organization, genome packaging, replication, and virus–host interactions.

Defining the complete infection and assembly cycle of an algal virus *in situ*, linking structural virology to marine ecosystem function

Cryo-ET showed that EhV-201 infects the alga *Emiliania huxleyi* by binding through a fivefold vertex and fusing its inner membrane with the host plasma membrane. It also captured capsid assembly, genome packaging, outer membrane acquisition, and virion release *in situ*, providing a structural glimpse into the full replication cycle of a climate-relevant algal virus.

Instruments used: *Krios G2 Cryo-TEM, Vitrobot Mark IV System, and Helios Hydra 5 CX PFIB*

Read the study



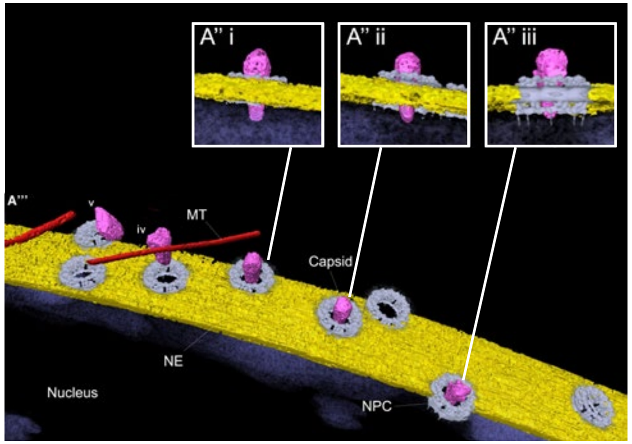
Cryo-ET reconstruction of an EhV-201-infected *Emiliania huxleyi* cell showing viral assembly intermediates, mature particles, intracellular vesicles, and host cellular architecture. Figure adapted from Homola *et al.* (2024) under CC BY 4.0.

Capturing HIV capsids translocating through nuclear pores *in situ*, revealing nuclear entry as a mechanically destructive process that could not be resolved by fluorescence microscopy

HIV-1 capsids were captured entering nuclear pores narrow-end first, physically cracking the NPC scaffold rings as the wider cone forces through—structural damage that was not detectable by fluorescence microscopy. These findings reframe HIV nuclear entry as a process that mechanically disrupts the pore and provides structural context relevant to how capsid-targeting antivirals such as lenacapavir may act.

Instruments used: *Krios G4 Cryo-TEM, E-CFEG, Falcon 4 Detector, Selectris X Imaging Filter, Aquilos 2 Cryo-FIB, and Amira Software*

Read the study



Segmented cryo-ET view of HIV-1 capsids at the nuclear envelope, showing capsids deeply engaged with the nuclear pore during translocation. Figure adapted from Kreysing *et al.* (2025) under CC BY 4.0.

Virology

Providing the statistical scale and mechanistic detail needed to define the structural rules of HIV nuclear import

Cryo-ET captured 1,489 native HIV-1 cores across four stages of nuclear import and showed that nuclear pores selectively filter capsids according to shape and size. Capsid elasticity and CPSF6 binding were identified as two distinct mechanistic requirements for successful import, complementing the pore-cracking study and helping define the full structural basis of HIV nuclear entry.

Instruments used: *Krios Cryo-TEM, Falcon 4i Detector, Selectris X Imaging Filter, Aquilos 2 Cryo-FIB, Arctis Cryo-PFIB, and Amira Software*

Read the study

Capturing the full replication cycle of flaviviruses and extending structural virology into infected tissue

Cryo-ET of flavivirus-infected human cells resolved replication organelles as two distinct bud states and captured virions assembling near replication sites, connected by a 500 kDa neck complex that links RNA replication to packaging. The same structural features were also confirmed in infected mouse brain tissue, the first cryo-ET analysis of viral replication within brain tissue.

Instruments used: Krios Cryo-TEM, Falcon 4i Detector, Selectris Imaging Filter, Vitrobot Mark IV System, Aquilos 2 Cryo-FIB, and Amira Software

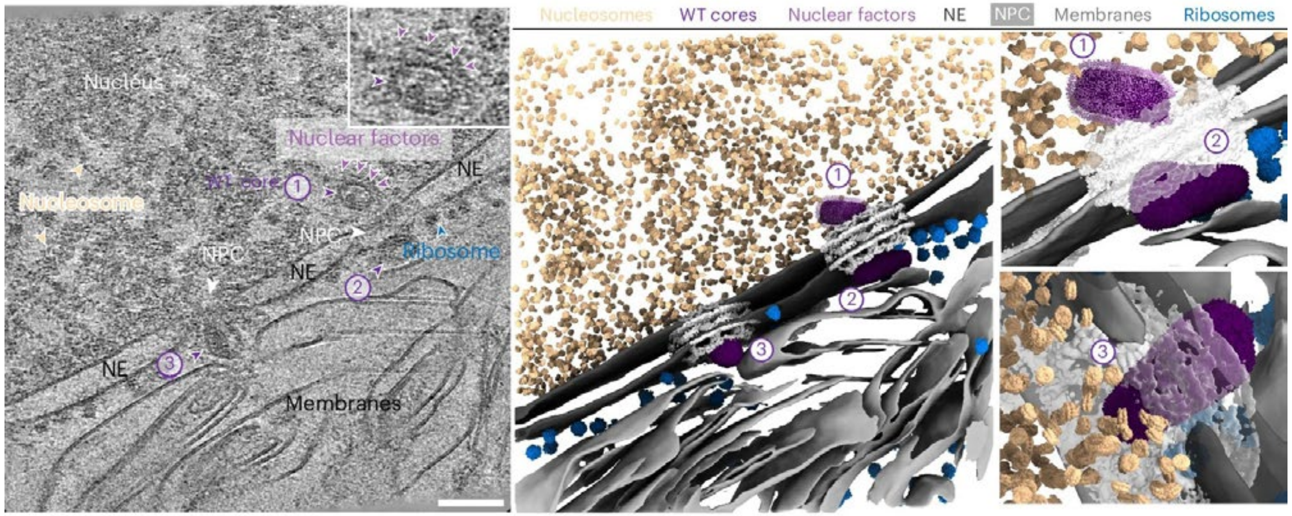
Read the study

Answering a central question to how influenza A virus packages its segmented genome inside infected cells

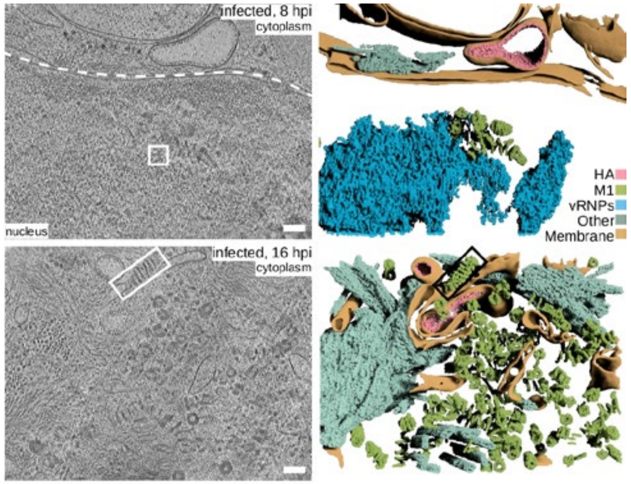
In situ cryo-ET of influenza A virus assembly showed that viral ribonucleoproteins cluster at HA-containing membranes through Rab11a-dependent platforms, while M1 matrix protein reorganizes filamentous structures to coordinate genome packaging. This study resolves how all eight influenza genome segments are selectively co-packaged during assembly with implications for understanding reassortment and pandemic potential.

Instruments used: *Krios Cryo-TEM and Aquilos 2 Cryo-FIB*

Read the study



Segmented cryo-ET volume of native HIV-1 cores at the nuclear pore, showing docked, traversing, and imported states during nuclear entry.
Figure adapted from Hou *et al.* (2025) under CC BY 4.0.



Representative cryo-ET slices and segmentations of influenza A virus-infected cells showing compact and loosely coiled M1 assemblies in different cellular compartments during infection.
Figure adapted from Wachsmuth-Melm *et al.* (2025) under CC BY 4.0.

Microbiology

Microorganisms exhibit diverse and highly organized cellular architectures that are closely linked to their function and behavior. Cryo-ET allows researchers to examine the organization of bacterial and archaeal cells in a near-native state, revealing macromolecular complexes, cell envelope organization, and intracellular interactions. This approach supports studies of cellular organization, motility, and interactions with the surrounding environment, revealing new mechanisms of pathogenesis, toxin delivery, and adaptation.

Typical applications include:
Studies of cell envelope and surface layer organization, secretion system architecture, motility systems, intracellular complexes, and environmental interactions.

Capturing bacterial vacuole escape *in situ* and revealing that a secretion system can rupture host membranes via mechanical force

Using cryo-ET, this study captured *Shigella flexneri* type III secretion systems puncturing the vacuolar membrane during the earliest stages of cytosolic escape. The data showed that membrane injury occurs at tight, high-tension bacteria-vacuole interfaces where long T3SS needles press against constricted membranes, establishing mechanoporation as a new model for pathogen-induced vacuole rupture.

Instruments used: *Krios Cryo-TEM, Falcon 4i Detector, Selectris X Imaging Filter, and Aquilos 2 Cryo-FIB*

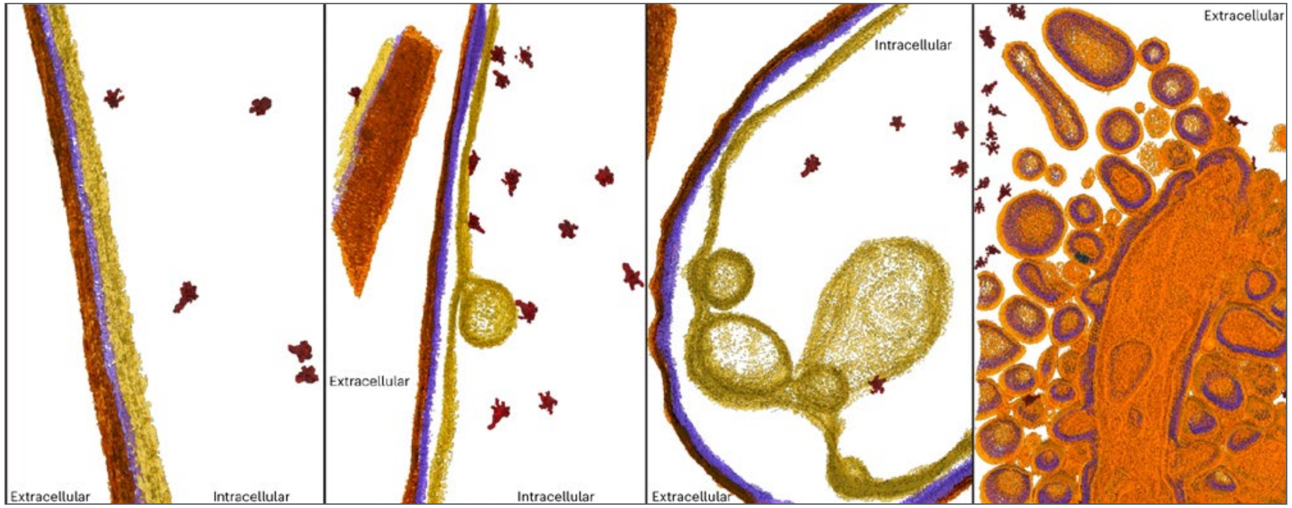
Read the study

Resolving rare toxin release *in situ* and revealing a secretion mechanism with direct relevance to pathogenesis and antimicrobial development

Cryo-ET of FIB-milled *Yersinia entomophaga* cells showed that pH-dependent T10SS drives lytic release of YenTc toxin from a minority cell subpopulation, with spanin-mediated membrane fusion triggering controlled cell death for concentrated toxin release. The study established T10SS as a secretion mechanism distinct from T1SS to T9SS systems and relevant to a broader set of bacterial pathogens.

Instruments used: *Krios Cryo-TEM, Vitrobot Mark IV, and Aquilos 2 Cryo-FIB*

Read the study



Cryo-ET slices and annotated densities of an Ara-RoeA cell showing step-by-step stages of YenTc secretion, from cytoplasm holotoxin assembly to cell envelope remodeling and extracellular toxin release. Figure adapted from Sitsel *et al.* (2024) under CC BY 4.0.

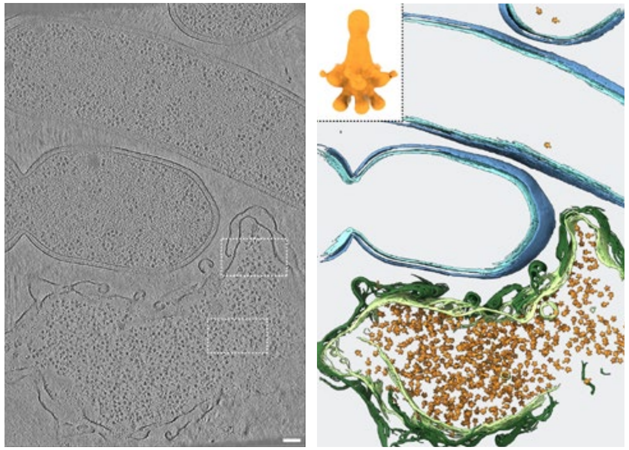
Microbiology

Resolving rare bacterial secretion states *in situ* and uncovering mechanisms that are inaccessible to bulk analysis methods

Cryo-ET revealed that YenTc toxin is produced exclusively in a primed subpopulation of *Yersinia entomophaga* cells, a form of population heterogeneity invisible to bulk biochemistry. Ghost cells were identified as assembly compartments before lytic release, with the study independently confirming that T10SS enables export of cytoplasmic toxin complexes too large for previously known secretion systems.

Instruments used: *Krios Cryo-TEM and Vitrobot Mark IV System*

Read the study



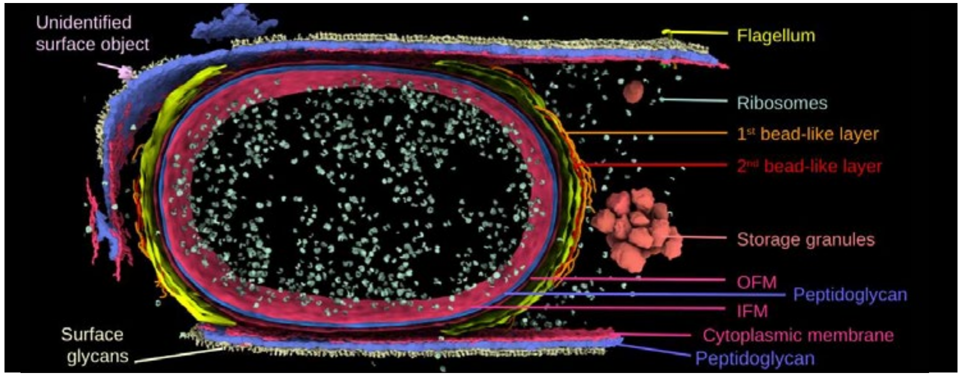
Cryo-ET tomogram and segmentation of cryo-FIB-milled *Yersinia entomophaga* cells showing YenTc-like densities enriched in a lysing subpopulation during toxin release. Figure adapted from Feldmüller *et al.* (2024) under CC BY 4.0.

Resolving bacterial spore ultrastructure *in situ* to reveal protective architecture underlying extreme resistance

Using cryo-ET of cryo-FIB-milled *Bacillus subtilis sporangia*, this study visualized early spore maturation directly inside intact cells. The work revealed a toroidal forespore chromosome organization, distinct membrane and peptidoglycan layers, and ordered coat-associated structures surrounding the developing spore. By mapping these macromolecular assemblies in their native cellular context, the study provides an ultrastructural foundation for understanding how bacterial spores build the protective architecture that supports long-term dormancy and resistance.

Instruments used: *Vitrobot Mark IV System and Titan Krios Cryo-TEM*

Read the study



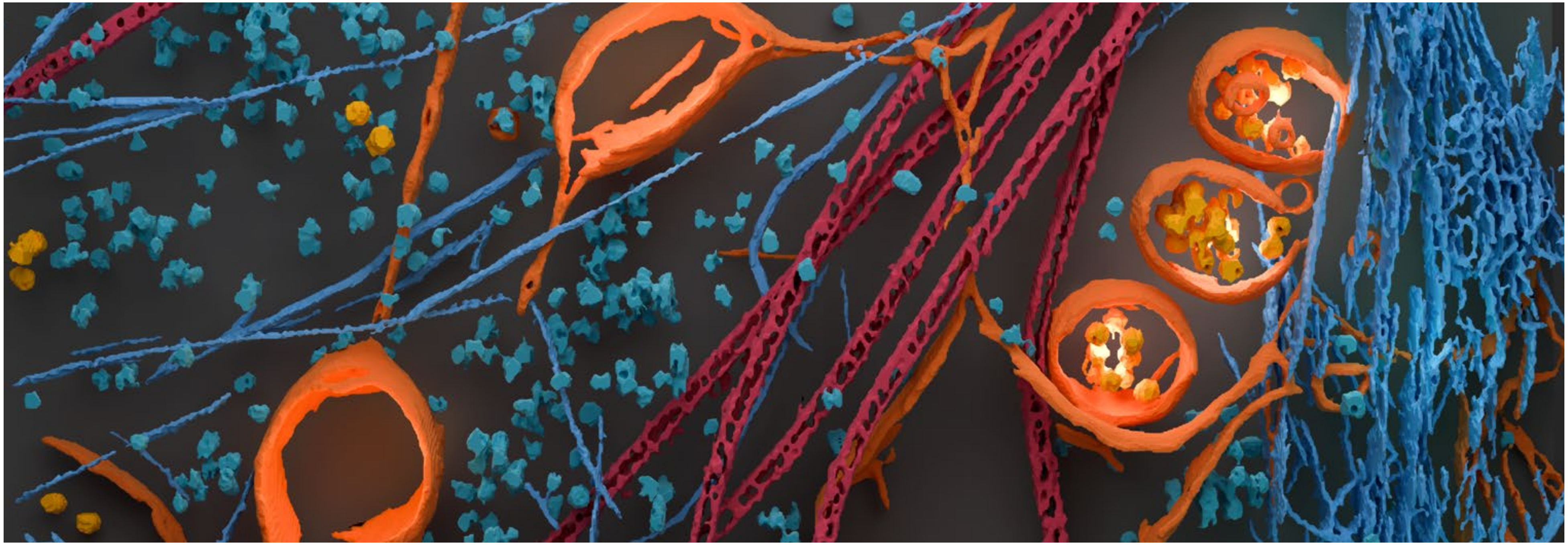
Cryo-ET segmentation of a stage-III ΔspoIVB *Bacillus subtilis sporangium* showing forespore and mother-cell ultrastructures, including envelope layers, peptidoglycan, membranes, ribosomes, storage granules, surface glycans, and a flagellum. Figure adapted from Bauda *et al.* (2024), Supplementary Figure 2a, under CC BY 4.0.

Defining the native organization of the unusual corynebacterial cell envelope, showing how *in situ* imaging can map complex surface architecture in bacteria with distinctive outer layers

Using cryo-ET and single particle cryo-EM, this study mapped the major layers of the *Corynebacterium glutamicum* cell envelope *in situ*, showing that its S-layer decorates the mycomembrane in a patchy rather than continuous manner and suggesting that it functions similarly to extended appendage-like pili or adhesions. These findings enable better understanding of the evolution and organization of mycomembrane-containing cell surfaces.

Instruments used: *Krios Cryo-TEM, Falcon 4i Detector, Selectris X Imaging Filter, Vitrobot Mark IV System, and Aquilos 2 Cryo-FIB*

Read the study



Cryo-ET of rhinovirus-infected COS-7 cells. Created with data from EMPIAR-12270. Segmented using Thermo Scientific™ Amira™ Software and visualized with Blender.

Summary

The publications in this reference guide illustrate the growing impact of cryo-ET across a broad range of life sciences research areas. From fundamental studies of cellular organization to investigations of disease, neuroscience, plant biology, and host–pathogen interactions, these examples demonstrate how cryo-ET enables visualization of biological structures directly within their native cellular environment.

This rapid progress is supported by advances in cryogenic workflows, automation, and high-performance instrumentation. Integrated approaches spanning vitrification, lamella preparation, tomography acquisition, and analysis are helping to make cryo-ET more accessible and scalable for life sciences research.

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