ThermoFisher SCIENTIFIC

Getting Started with Cryo-EM

Capture the choreography of life

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Introduction

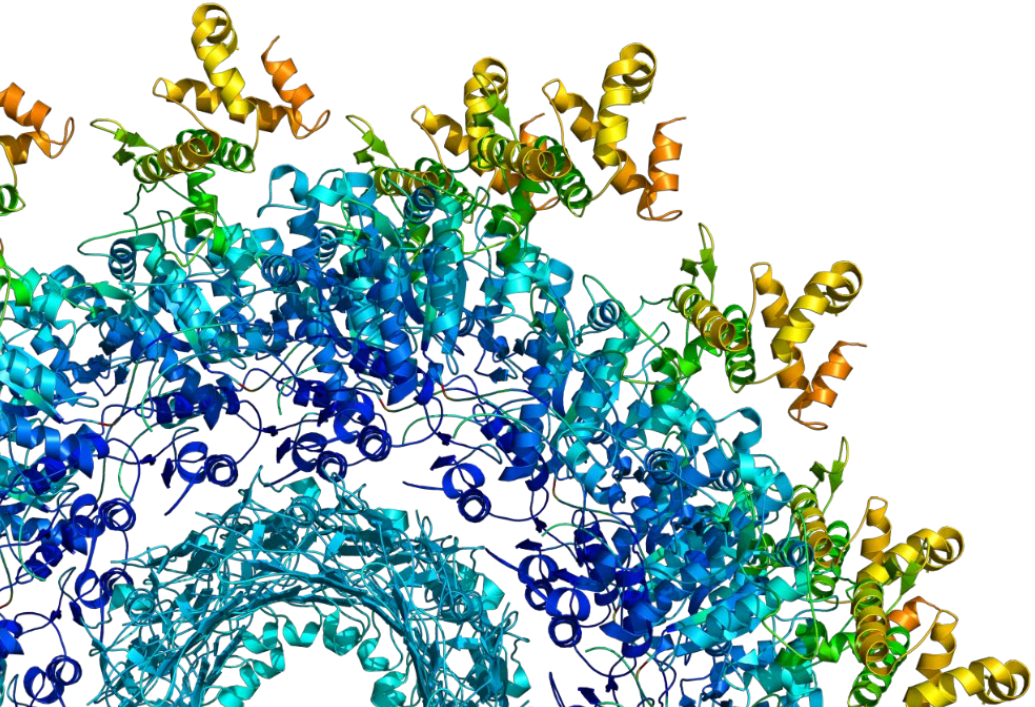
Cryo-electron microscopy (cryo-EM) can determine structures of challenging proteins and other macromolecules, without the need for crystals, while providing insight into how these proteins function in a way that other methods simply cannot. Although once considered a specialty tool used only by a small group of experts, cryo-electron microscopes are now increasingly easier to use, more affordable, and accessible to the wider scientific community.

Cryo-EM for studying protein function

What are the greatest challenges to obtaining deeper structural and functional insights for macromolecules?

Protein structure is often used to predict protein function. Commonly used techniques to identify structure include X-ray crystallography and nuclear magnetic resonance (NMR). However, scientists may have to infer function from indirect methods or to rely on sample manipulation to get a structure because proteins can have multiple conformations and form complexes, may be difficult to crystallize, or can be too large for NMR analysis.

When a target protein has multiple conformations, each conformation must be trapped and/or crystallized accordingly. If the protein is part of a complex, then a homogeneous version of the complex must be purified, which can be difficult. Membrane or post-translationally modified proteins typically have to be modified and cleaved into smaller fragments. Other challenges include low-abundance proteins that are difficult to purify in sufficient amounts or proteins that grow crystals that are very small, heterogeneous, or as part of slurries. Despite researchers knowing the structure of a protein at high resolution, it may still not be clear how this structure relates to function inside the cell.



A Nobel Prize worthy technique

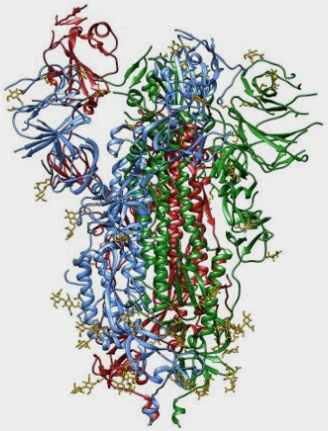
Interview with Dr. Richard Henderson, winner of the 2017 Nobel Prize in Chemistry, together with Dr. Jacques Dubochet and Dr. Joachim Frank, for "developing cryo-EM for the high-resolution structure determination of biomolecules in solution."

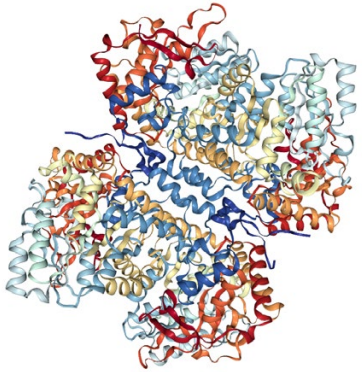
Courtesy of the Science Channel

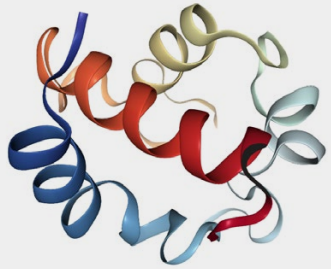
Learn how cryo-EM is powering biomedical research

Needing only tiny amounts of protein sample, researchers can use cryo-EM to get a larger picture of how membrane proteins function and contribute to disease, and access structures of macromolecular complexes for better drug design.

Cryo-EM for studying protein function





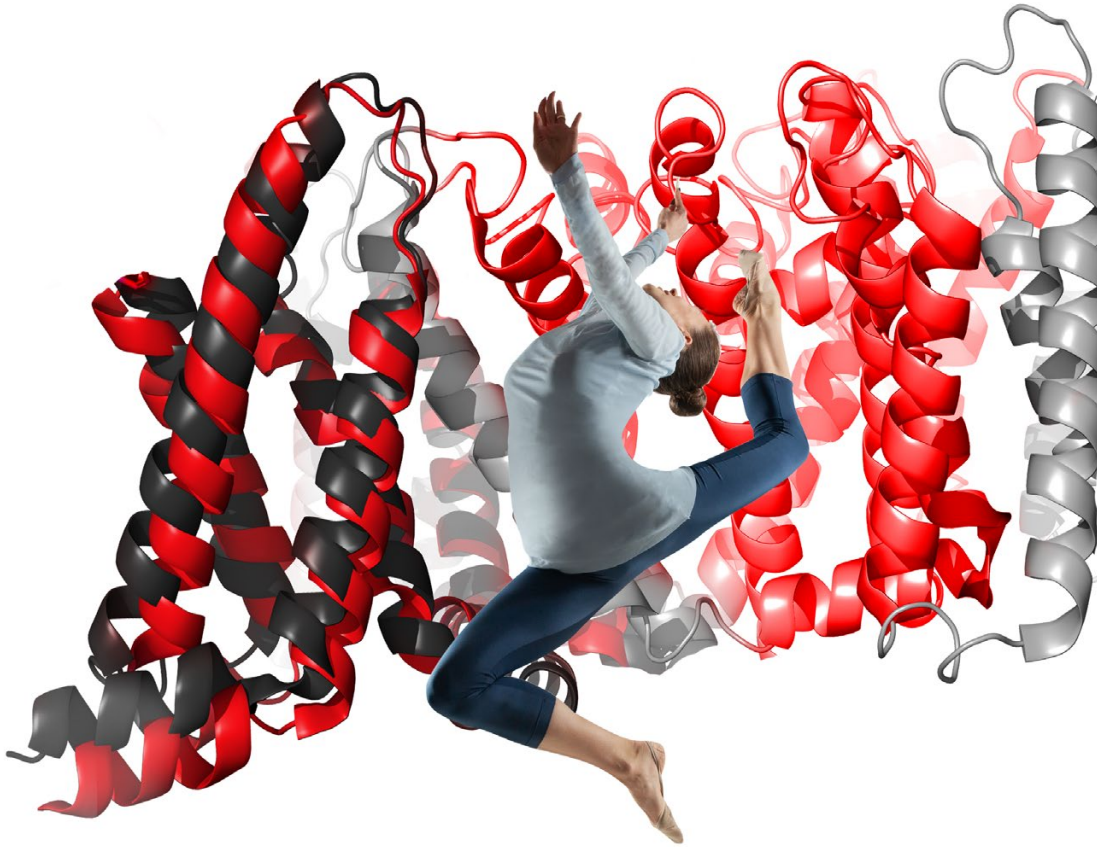


	Cryo-EM	X-ray crystallography	NMR
Summary	Samples are rapidly frozen (vitrified), preserving the sample in its natural state. A transmission electron microscope (TEM) is used to capture two-dimensional projections of the specimen, which are then combined to make a 3D model.	Samples are crystalized and an X-ray beam is used to create a diffraction pattern from which the position of each atom in the crystallized molecule is determined.	Samples are subjected to a large magnet inside an NMR spectrometer. A series of split-second radio-wave pulses are applied to the sample, which forces the nuclei to resonate at specific frequencies. A complete picture of the protein is developed by combining the measured resonance frequencies.
Sample types	• Membrane proteins • Large complex proteins • Ribosomes • Virions • Other macromolecules	• Crystallizable samples • Soluble proteins	• Proteins with MWs <40–50 kDa
Advantages	• Easier sample preparation • Only requires small sample size • Structures are obtained in native state	• Works well for broad molecular weight ranges • Easier model building	• Obtains 3D structures in solution
Current limitations	• Proteins with molecular weights >100 kDa are preferred, but the number of structures from proteins with smaller molecular weights have increased as technology rapidly improves	• Crystallization can be difficult and can take months to years to achieve • Solid structure is preferred • Results in static crystalline state • Diffraction can be difficult	• Needs high purity sample • Has a difficult computational simulation • Sample must be isotopically labeled
Sample amounts required	Nanograms to micrograms	Micrograms to milligrams	Micrograms to milligrams

Cryo-EM for studying protein function

How does cryo-EM provide a solution for protein scientists?

Cryo-EM enables researchers to analyze proteins in all their complex conformations, structures, and modified forms. We can look at multiple protein conformations in a single sample. Cryo-EM does not require crystal growth and needs only a small amount of sample. Cryo-EM can investigate heterogeneous complexes without construct optimization to remove post-translational modifications.



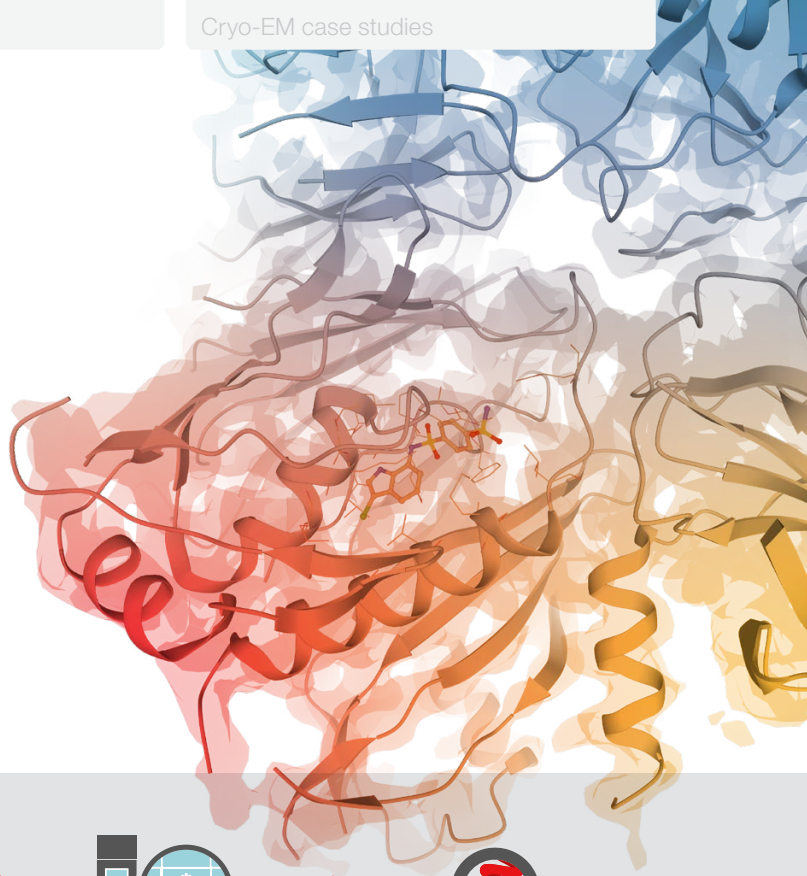
Why is cryo-EM beneficial for studying protein function?

- Maintains the sample in a near-native state with rapid sample vitrification
- Needs only a small amount of sample material
- Requires no protein crystallization
- Captures flexible conformations
- Needs no extensive construct optimization (e.g., no need to remove post-translational modifications)
- Can determine structures of heterogenous protein complexes

Learn why **cryo-EM is beneficial to structural biology**

Getting started in cryo-EM

Revolutionize your research



Cryo-electron microscopy (cryo-EM) techniques, such as single-particle analysis, can help you discover how proteins function – critical information for the development of cutting-edge infectious-disease treatments.

Adopt cryo-EM quickly and seamlessly. As a leader in cryo-EM innovation, we can help you and your team be successful at every stage of the adoption process, from financing to guidance on facility and data-processing requirements. Find out how the Thermo Scientific™ Tundra™ and Glacios™ Cryo-Transmission Electron Microscopes (Cryo-TEMs) offer cryo-EM solutions that fit your needs.

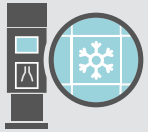
Products and support along every step of the cryo-EM workflow

Financing options
Competitive and flexible financing, leasing, lease-to-own options and more.

Site preparation services
Environmental engineering experts provide analysis and recommendations, minimizing environmental interference while maximizing system performance.

Installation
Following installation, our technicians train you to prepare samples and to gather data confidently and safely. Each workflow step is validated to ensure it is optimally tuned for high-quality data collection.

Sample preparation for vitrification
Maximize sample quality with a range of products – from protein expression to purification and clean up.



Sample vitrification
Preserve biological integrity and quickly produce high-quality samples with the Thermo Scientific Vitrobot™ Mark IV System.

Data collection
The Tundra Cryo-TEM is designed to bring single particle analysis to new users. The Glacios Cryo-TEM offers enhanced ease-of-use through automation, resulting in a more versatile microscope for higher-resolution data collection or for use in a shared facility.

Find out more at **thermofisher.com/CryoEMStartsHere**

Getting started in cryo-EM

How can I get started with cryo-EM?

In collaboration with the scientific community, we have developed a portfolio of solutions at different price points. We also provide programs to help with onboarding, training, and financing. Our offering includes improved ease of use and lower cost of ownership.

In addition, we offer comprehensive training and onboarding programs, dedicated customer success managers, and site preparation services to help ensure your success. You will find our instruments to be the easiest to use in the industry, thanks to automated alignments, performance monitoring, and seamless data connectivity.

Getting started in cryo-EM has become more affordable, through a range of leasing and financing options. The Tundra Cryo-TEM is a structure determination solution that is easy to use for new users. The Glacios Cryo-TEM is a capable and versatile fully integrated cryo-TEM solution. The Thermo Scientific Krios™ Cryo-TEM boasts ultimate performance and productivity in a compact design.

Getting started in cryo-EM is accessible. We have more than 450 systems placed globally in more than 150 labs. Our systems are used at workshops and schools that we sponsor worldwide. We even offer an online Cryo-EM University, which is accessible via our Scientific Workflows app on your mobile device.

"The major benefit of the Tundra [Cryo-TEM] is the very simple handling, specifically sample insertion, that will make it very simple for the newcomers to start their own cryo-EM experiments. It is not only an entry tool but can provide highly efficient sample screening that even established cryo-EM labs can use to collect high-quality data."

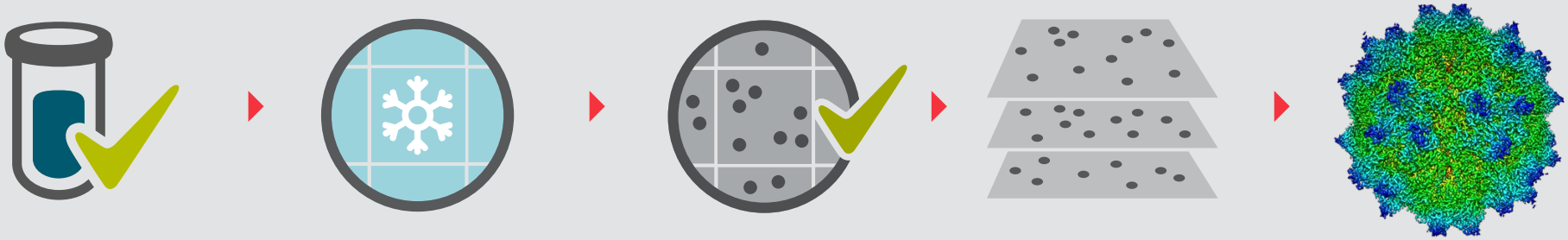
–Dr. Jiří Nováček
Head of Core Facility,
Central European Institute of Technology (CEITEC)
Connecticut, USA

"What excites me the most about the Tundra [Cryo-TEM] is the fact that you've got 100 KV affordable microscope that can deliver this type of resolution. The user interface is brilliant. The new technology is really going to help users enter the field, which I think is really important, and users can readily screen their samples and actually understand what they're looking at. Completely game changing."

–Dr. James Whisstock
Professor at Monash University, Australian Research Council
Laureate Fellow, and Scientific Head of EMBL Australia

Single particle analysis workflow

Single particle analysis is a revolutionary cryo-EM technique that has enabled the near-atomic structural determination of challenging proteins and protein complexes, without the need for crystallization. Samples can be studied directly in solution. High-quality data collection from cryo-EM has been facilitated by recent advances in sample preparation and data processing.



Sample preparation

High-quality cryo-EM starts with thorough sample preparation and screening. A variety of traditional sample preparation techniques can be used, including negative-stain screening and chromatography.

Vitrification

Once the aqueous sample is sufficiently purified, it must be rapidly frozen to suspend the specimens in a layer of amorphous (vitreous) ice (vitrification). By avoiding ice crystallization, the samples are preserved in a near-native state, essentially taking a snapshot of their structures in solution. Ice consistency as well as sample distribution and orientation are critical for data collection, and automated plunge freezing is the general method of choice for consistent sample vitrification.

Screening

Even the best vitrification system is not 100% consistent, and therefore the sample (frozen atop an EM grid) must be screened to find the optimal areas of ice for data collection. Ideally, the ice would uniformly cover the holes in the grid, and there is a large amount of specimen distributed evenly throughout the visible ice. Only a moderate-resolution TEM scan is required at this stage, as this is a largely qualitative scan.

Data aquisition

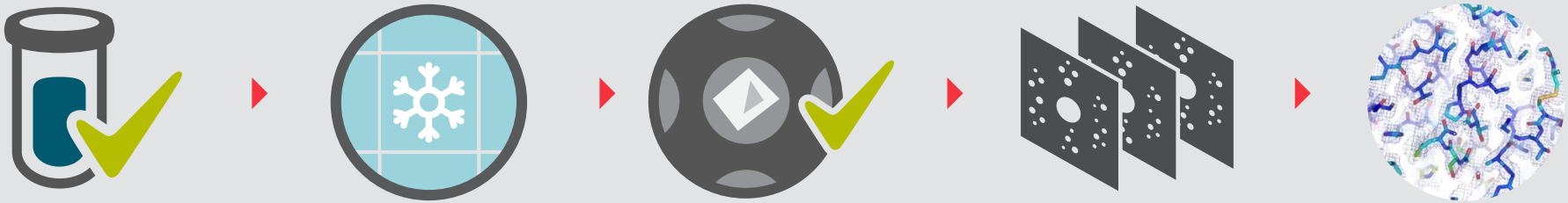
Data collection consists of high-resolution imaging with a TEM specifically designed for cryo-applications (also called cryo-TEMs). With advances in data collection software, individual particles can be automatically identified in the TEM image and grouped according to particle orientation. For every sample, robust, reliable automation simplifies and accelerates imaging and identification.

Structure visualization

Once sufficient particle data is collected (ideally representing the sample from as many different orientations as possible) the data can be recombined into a 3D representation of the protein/protein complex. This uses 2D data from tens of thousands of particles and typically involves multiple data processing steps, requiring high data storage capacity and computational power. A number of professionally developed and open-source data processing solutions exist to simplify and expedite this process.

MicroED workflow

Microcrystal electron diffraction (MicroED) enables fast, high-resolution, structural determination of small molecules and proteins. Atomic details can be extracted from individual nanocrystals (<200 nm in size), even in a heterogeneous mixture. Data is acquired on a cryo-TEM, using electrons as the incident beam.



Sample preparation

The creation of small crystals for MicroED depends on their sample type. Small molecule crystals, which are usually dry, may require mechanical grinding, or they may simply be crystallized spontaneously out of solution using evaporation. Protein crystals are typically kept in water to retain their hydrated native states.

Vitrification

Protein crystals are prepared by plunge freezing, a method that is similar to the one used for single particle analysis. After freezing, crystals that are too thick for MicroED are thinned using a cryo-focused ion beam (cryo-FIB).

TEM low-dose screening

Continuous rotation data is collected from a single crystal within the electron beam on a fast camera.

Data collection

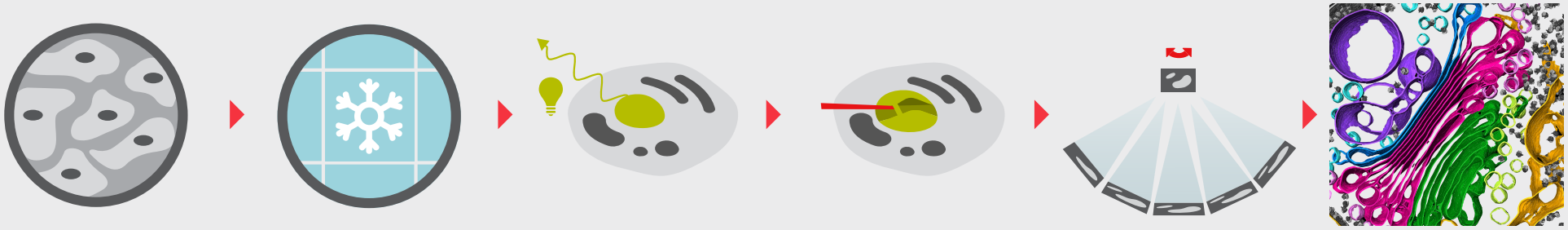
Individual images from the diffraction tilt series are combined computationally. Data collection is completed in only a few minutes, and 3D structures can be determined at atomic resolution.

Reconstruction

The electron diffraction data obtained by this method is fully compatible with the available X-ray crystallography software, which simplifies analysis.

Cryo-electron tomography workflow

Cryo-electron tomography (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 electron microscopy (EM) grids.

Sample preparation by vitrification
Cells are either vitrified through plunge-freezing (like SPA specimens) or High Pressure Freezing (HPF). The water in the sample freezes rapidly and does not crystallize, thus avoiding the molecular-scale disruption (by formed ice crystals) that would occur with a normal slow freezing process.

Localization by fluorescence
Using 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. From the tomogram small subsets of data containing the structures of interest can be computationally extracted and subjected to image processing methods.

Innovative, robust, and easy-to-use cryo-EM solutions

We offer integrated solutions and support from sample preparation to data analysis for multiple cryo-EM methods, including single particle analysis, MicroED and cryo-tomography. We provide long-term ongoing support, as well as flexible financing options to help you bring the right cryo-EM solution into your lab.

- Automated alignments and software
- Reduced need for user intervention
- Easily organize, view, and share data
- Analyze and visualize data remotely

Tundra Cryo-TEM: Accessible & Smart

- Easy, iterative loading and imaging for rapid sample-viability determination
- AI-guided automation with results displayed progressively
- Cost effective and space efficient

Intermediate-resolution SPA	100 kV, <3.5 Å*
Medium throughput	dataset in 24 hours
Sample type	proteins
Applications	SPA

Glacios Cryo-TEM: Capable & Versatile

- Maximized ease-of-use and excellent performance to offer a complete package for introducing cryo-TEM into your research
- Compact hardware footprint (minimizes installation requirements) at an affordable price

High-resolution SPA	200 kV, <3 Å*
High throughput	dataset in 30 minutes
Sample type	proteins, crystals
Applications	SPA, MicroED, Tomography

Krios Cryo-TEM: Powerful & Productive

- Ultimate productivity and image quality with an integrated workflow solution
- Highest level of automation from sample vitrification to data analysis
- Compact design fits in standard room without costly renovations

Ultra-high-resolution SPA	300 kV, <2 Å*
Highest throughput	dataset in minutes
Sample type	proteins, crystals, cells
Applications	SPA, MicroED, Tomography

* Based on best published performance, actual results will depend on non-microscope factors such as sample and user experience. Not a promise of biological resolution performance.

Portfolio instrument comparison matrix

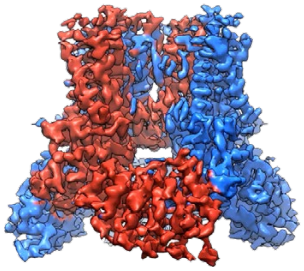
		Thermo Scientific Tundra Cryo-TEM (100 kV)	Thermo Scientific Glacios Cryo-TEM (200 kV)	Thermo Scientific Krios Cryo-TEM (300 kV)
Sample Type	Proteins (Single Particle Analysis)	✓✓✓	✓✓✓	✓✓✓
	Crystals (Micro-electron Diffraction)	×	✓✓✓	✓✓✓
	Cells (Tomography)	×	✓	✓✓✓
Performance	Highest resolution	2.6 Å*	1.6 Å**	1.22 Å
Throughput & Productivity	Time to get to 3.5 Å (apoferritin)***	24 hours	30 minutes	A few minutes
	Time to get to 2.5 Å (apoferritin)***	×	2 hours	10 minutes
Ease of Use	For new users	✓✓✓	✓	✓✓
	For pushing near-atomic resolution	×	✓✓	✓✓✓
Comparison		$	$$	$$$

* Data for 100 kV achieved on apoferritin, unpublished
** Data for 200 kV achieved on apoferritin, unpublished
*** Based on assumptions that will vary for microscope-independent factors including, but not limited to, user experience, sample type, quality, and concentration

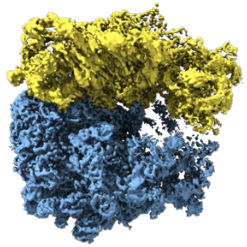
"In our lab at the University of Washington we are equipped with a couple of autoloader systems, Glacios and Krios Cryo-TEMs. The Glacios Cryo-TEM is primarily used as a microscope for optimizing cryo-EM grids to identify the perfect grids for high resolution data collection on the Krios Cryo-TEM. We are very happy with the performance of our Glacios Cryo-TEM and routinely obtain structures near 3Å resolution."

–Dr. Justin Kollman
University of Washington,
Seattle, Washington, USA

Unravelling complex protein structures with Tundra cryo-TEM

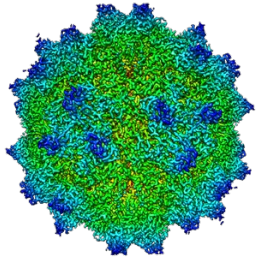


4.2Å
TRPV5

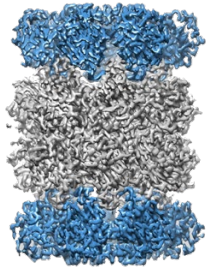


3.7Å
70S Ribosome

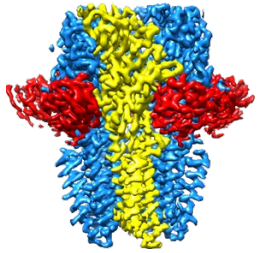
2.6Å
Apoferritin



3.0Å
AAV6



3.0Å
T20S Proteasome



3.4Å
$GABA_A$ Receptor

Molecular weight	~330 kDa	~2.5 MDa	~500 kDa	~3.7 MDa	~700 kDa	~220 kDa
Pixel size (Å)	0.75					
Exposure time(s)	1.3 ~ 1.8					
Flux (e*/pixel/s)	12.95 ~ 14.66					
Number of images	10,876	21,552	1,510	5,058	5,176	4,073
Number of particles	184,800 (C4)	~150,000 (C1)	165,082 (octahedral)	46,646 (Icosahedral)	183,486 (D7)	92,997 (C5)
Acquisition time (hrs)	**~30**	**~37**	**~6**	**~17**	**~56**	**~50**

TRPV5 sample courtesy of Vera Moiseenkova-Bell, Beckman Center for Cryo Electron Microscopy, University of Pennsylvania and Image courtesy of Abhay Kotecha, Thermo Fisher Scientific.

GABAA receptor sample courtesy of Radu Aricescu, Medical Research Council Laboratory of Molecular Biology, Cambridge and Image courtesy of Abhay Kotecha, Thermo Fisher Scientific.

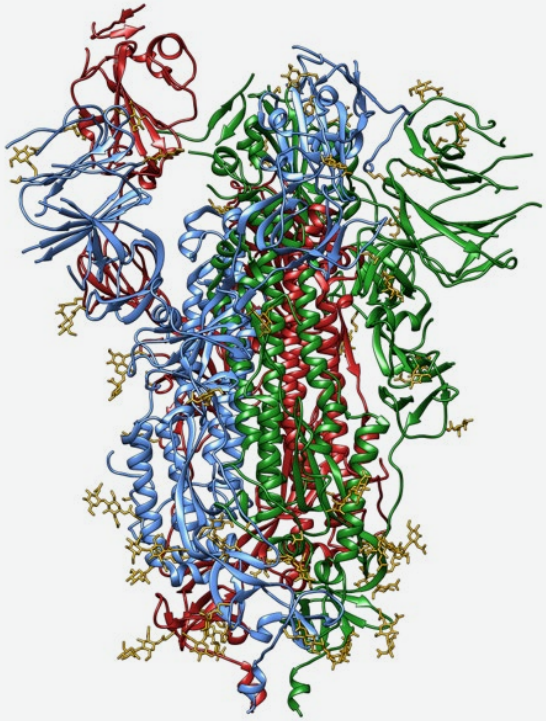
T20S proteosome sample courtesy of Juergen Plitzko, Max Planck Institute of Biochemistry, Martinsried, Germany.

70S ribosome sample courtesy Prof. Daniel Wilson, Hamburg University & Image courtesy of Jiri Novacek, CEITIC (Central European Institute of Technology).

Fast mode (AFIS) throughput: 250 ~380 images/hour

Standard mode throughput: 80~90 images/hour

Cryo-EM to study viruses: Structural details of SARS-CoV-2 variants



Cryo-EM structure of SARS-CoV-2 spike glycoprotein in the prefusion conformation, with the three subunits of the trimer in red, green, and blue, and glycosylation in yellow (3.5 Å resolution). Image created from PDB data (6VSB).

Research challenge:
Several fast-spreading variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have become the dominant circulating strains in the COVID-19 pandemic. Studying the structures of the full-length spike (S) trimers of the Alpha, Beta, Gamma, Kappa, and Delta variants will enable scientists to better understand their function and antigenic properties.

Biological sample:
Expressed, purified proteins

References:
Zhang, J., Chen, B et al. (2021) Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. *Science*.

Cryo-EM advantage:
Provides structural details on how SARS-CoV-2 has evolved to have enhanced viral fitness and increased immunity evasion.

Webinar:
Understanding SARS-CoV-2 variants by cryo-EM

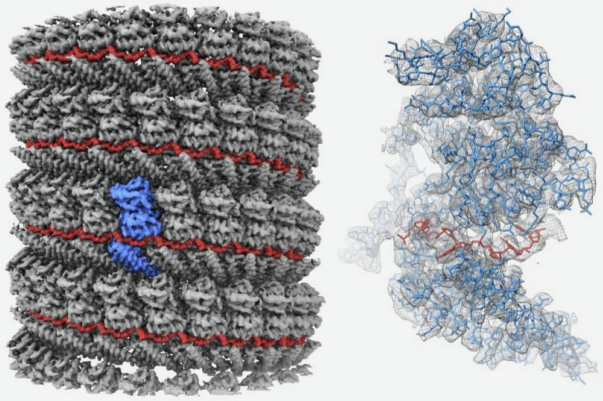
Dr. Bing Chen
Professor of Pediatrics, Harvard Medical School and Boston Children's Hospital

Watch webinar

Virology eBook
Cryo-EM technology is helping researchers overcome challenges related to virus purification and protein homogeneity and revolutionizing antiviral drug discovery and vaccine design. Learn more about how cryo-EM can help solve structural puzzles in virology.

Download eBook

Cryo-tomography of viruses: Visualization of enterovirus virion assembly



A B

3D rendering of the cryo-EM reconstruction. (A) The RNA and NP are colored in red and grey, respectively. A single NP molecule is highlighted in blue. (B) An atomic model of a single NP molecule with the RNA in the complex. The model is superposed with the cryo-EM map in a polygon mesh representation.
Courtesy of Yukihiko Sugita, OIST.

Research challenge:
Enteroviruses are non-enveloped positive-sense RNA viruses that cause diverse diseases in humans. Their rapid multiplication depends on remodeling of cytoplasmic membranes for viral genome replication. It is unknown how virions assemble around these newly synthesized genomes or how they are then loaded into autophagic membranes for release through secretory autophagy.

Biological sample:
Infected HeLa cells (poliovirus type 1)

References:
Dahmane, S et al. (2021) Membrane-assisted assembly and selective autophagy of enteroviruses, Cold Spring Harbor Laboratory. *bioRxiv.*

Cryo-EM advantage:
Visualize how virions assemble directly on replication membranes

Webinar:
Enterovirus membrane-assisted assembly and release revealed by cryo-electron tomography

Dr. Selma Dahmane
Umea University

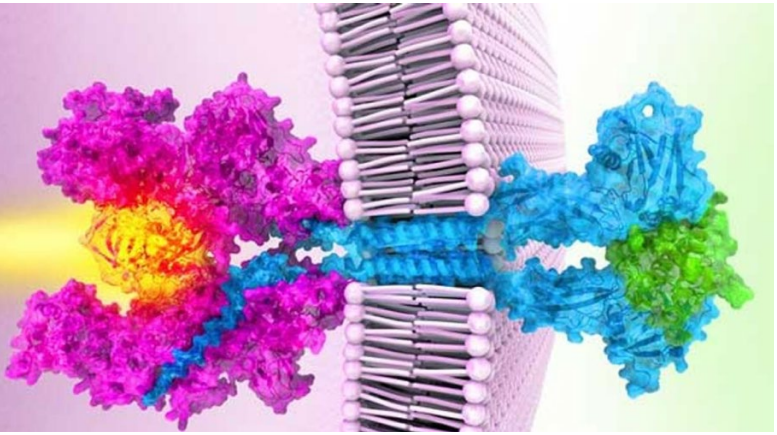
Dr. Kristian Wadel
Thermo Fisher Scientific

Watch webinar

Tomography eBook
Access the inner workings of cells through 3D sample reconstruction at unprecedented nanoscale resolution. Results from this technique are having profound effects on our understanding of cell biology, revealing native cellular architecture with molecular clarity. Explore a curated collection of publications highlighting the use of cryo-ET.

Download eBook

Cryo-EM to study proteins: Structural insights into p53 mutations for rational drug design



When a (green) cytokine binds to (teal) receptors, two parts of the (pink) Janus kinase protein come together, activating it to send signals inside In some cancers, mutations in the kinase lock it together, keeping it abnormally active.
Credit: Eric Smith/Chris Garcia/Howard Hughes Medical Institute.
Source: Technology Networks.

Full length structure of Janus kinase solved

Researchers at Stanford University used our cryo-EM solutions to solve the long-sought-after structure of Janus kinase, a large signaling protein involved in responses to infection, inflammation, the generation of immune cells, and, when dysregulated by mutation, the emergence of blood cancers.

Researchers captured the full structure of this important signaling molecule as well as the mechanism for how these kinases work. Because protein behavior can go awry in disease, these results could lead to new and better drugs against certain cancers.

Learn more

Research challenge:

How can the structural determination of mutations in protein factor p53, the most altered gene in human cancer, be paired with biochemical and cellular studies to help understand differences in function? This could lead to new interventions based on structural oncology approaches.

Biological sample:

Human cancer cells

References:

Solares, M., Kelly, D et al. (2020) Microchip-based structure determination of disease-relevant p53. *Anal Chem.*

Cryo-EM advantage:

Offers a fresh tool to evaluate native protein assemblies that can advance the development of medically-relevant macromolecules for therapeutic purposes.

Webinar:

Structural oncology—fighting cancer with cryo-EM

Dr. Deb Kelly
Professor of Biomedical Engineering, Pennsylvania State University

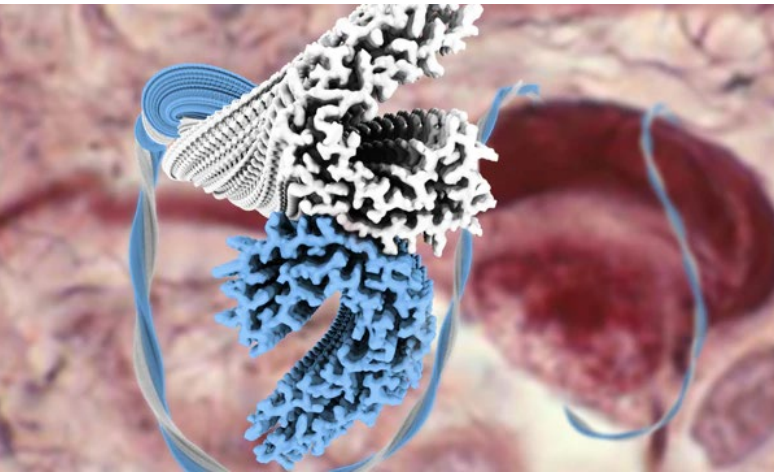
Watch webinar

Oncology eBook

Leverage structural insights to better understand the conditions for cancer-cell growth and identify new ways to treat cancer. Learn how cryo-EM is revolutionizing cancer research.

Download eBook

Cryo-EM of proteins: Visualizing misfolded tau protein in filaments for disease characterization



Using cryo-EM, researchers solved the structure of tau filament structures, rendered in blue and white in this photo. Knowing how tau proteins assemble into their filaments is expected to lead to more targeted treatment of Alzheimer's.
Source: Technology Networks

Research challenge:

Abnormal accumulation of misfolded tau protein filaments characterizes more than 20 neurodegenerative diseases, collectively called tauopathies, including: Alzheimer's disease, primary age-related tauopathy (PART), chronic traumatic encephalopathy (CTE), Pick's disease, and corticobasal degeneration (CBD). These diseases are distinguished by different tau folds. To understand the relevance of these folds, better model systems are required that can recapitulate this *in vitro*.

Biological sample:

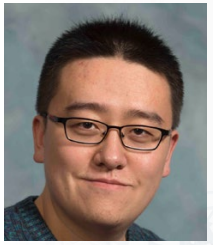
Brain tissue

References:

Lövestam, S, et al. (2022) Assembly of recombinant tau into filaments identical to those of Alzheimer's disease and chronic traumatic encephalopathy. *eLife.*

Cryo-EM advantage:

Enables a hierarchical classification of tauopathies on the basis of their filament folds, complementing clinical diagnosis and neuropathology while also allowing the identification of new tauopathies.



Webinar:

Tale of amyloid filaments in neurodegenerative diseases

Dr. Yang Shi
Dr. Sofia Lövestam
MRC Laboratory of Molecular Biology

Dr. Abhay Kotecha
Thermo Fisher Scientific

Watch webinar

Neurodegeneration eBook

Cryo-EM technology is used to uncover the atomic structures of numerous misfolded proteins and their aggregates, including tau filaments, α-synuclein fibrils, and amyloid-ß aggregates, as well as small-molecule drug candidates that bind to these proteins. Learn how cryo-EM can enable the structure-based classification of tauopathies.

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Research publications featuring data generated with cryo-EM

Wasmuth E, Sawyers C, et al. (2022) **Allosteric interactions prime androgen receptor dimerization and activation.** *Mol Cell.*

Glassman, C, Garcia C, et al. (2022) **Structure of a Janus kinase cytokine receptor complex reveals the basis for dimeric activation.** *Science*.

Milligan, JC, Ollmann Saphire, E et al. (2022) **Asymmetric and non-stoichiometric glycoprotein recognition by two distinct antibodies results in broad protection against ebolaviruses.** *Cell.*

Subramaniam S. (2020) **COVID-19 and cryo-EM.** *IUCrJ*.

Wandzik JM, Kouba T, Karuppasamy M, et al. (2020) **A structure-based model for the complete transcription cycle of influenza polymerase.** *Cell*.

Jessop M, Arragain B, Miras R, et al. (2020) **Structural insights into ATP hydrolysis by the MoxR ATPase RavA and the LdcI-RavA cage-like complex.** *Communications Biology*.

Ni T, Jiao F, Yu X, et al. (2020) **Structure and mechanism of bactericidal mammalian perforin-2, an ancient agent of innate immunity.** *Science Advances*.

Baek K, Krist DT, Prabu JR, et al. (2020) **NEDD8 nucleates a multivalent cullin–RING–UBE2D ubiquitin ligation assembly.** *Nature*.

Bhogaraju S, Bonn F, Mukherjee R, et al. (2019) **Inhibition of bacterial ubiquitin ligases by SidJ–calmodulin catalysed glutamylation.** *Nature*.

Learn more at **thermofisher.com/CryoEMStartsHere**

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