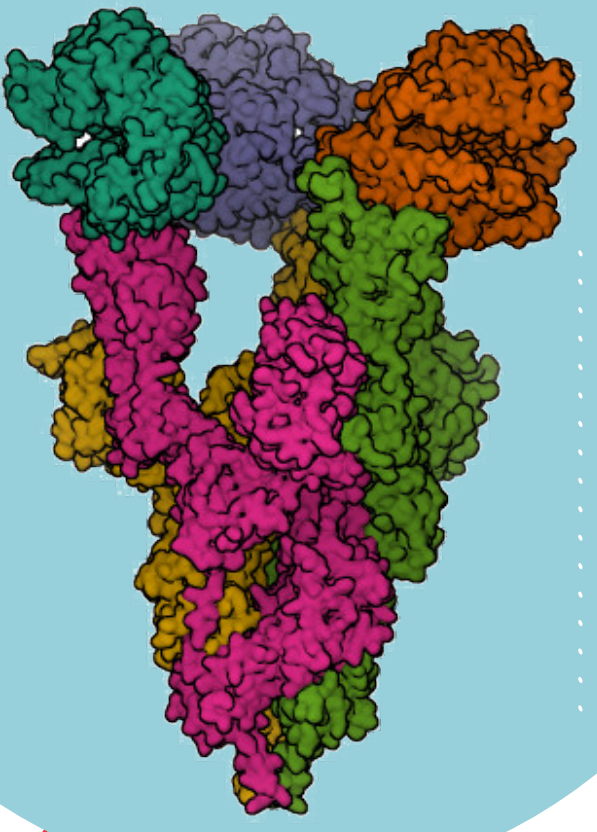
TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

THE RISE OF STRUCTURAL VIROLOGY



SOLVING STRUCTURAL PUZZLES IN VIROLOGY

Cryogenic electron microscopy (cryo-EM) is advancing virology in instances where purifying viral proteins is a challenge, protein homogeneity is difficult to achieve, and image averaging may be the only way to obtain structural information at high resolution. These structural insights are revolutionizing anti-viral drug discovery and vaccine design for newly emerging pathogens.[1,2]

The Burden of Viral Diseases

Viral diseases cause symptoms in humans such as spots, runny noses, swelling, and fever. In severe cases, viral infection may result in death. New viruses periodically emerge unpredictably and cause great personal and societal tragedy.[3,4] In the wake of recent outbreaks, such as HIV, COVID-19, Zika, and Ebola, structural virology studies have taken center stage. There is an urgent need to characterize viral biology and the host immune response to identify and develop therapeutics and vaccines.

Researchers can use cryo-EM to determine a structure quickly, even when the biochemistry involved in virus preparation is challenging.

Structural Virology Tools

A major challenge for virologists is to understand the molecular basis of viral life cycles and to use this knowledge to design new anti-viral strategies. The current understanding of viral life cycles comes from molecular biology combined with structural studies. Scientists use several techniques to determine protein structures.[3]

Nuclear magnetic resonance (NMR) provides unique information about protein dynamics and interactions, but this method restricts atomic structure determination to small complexes with molecular weights below 40–50 kDa.[3]

X-ray crystallography can achieve atomic details for smaller viruses, but visualizing larger multi-shelled viruses and complexes between viruses and antibodies or receptors is a challenge.[3]

Due to challenges that NMR and x-ray crystallography present for scientists analyzing virus structures, many have replaced these techniques with cryo-EM. In cryo-EM, researchers rapidly vitrify a cell, virus, molecular complex, or other structure to preserve samples in their natural state. An electron microscope shoots an electron beam at a sample, creating a two-dimensional (2D) projection of the sample on a digital detector. By creating hundreds of projections of the sample from many different angles and taking the average of these angles, scientists generate a 3D model of its structure.[2] Scientists using cryo-EM produce higher-resolution images compared to other structural techniques because of modern electron detectors and image-processing. The number of cryo-EM images uploaded to the Protein Data Bank has boomed in recent years, and the technique won its developers the 2017 Nobel Prize in Chemistry.[2]

The Future of Structural Virology

Early x-ray diffraction studies promoted a static view of viruses as robust protein containers, or capsids, protecting vulnerable genomes. With advanced structural biology tools such as cryo-EM, a more dynamic view emerged with the realization that the capsid must not only assemble appropriately but also disassemble, interact with cell surface receptors, be transported to appropriate cellular compartments, and, in some cases, participate in transcriptional activity.[2,3]

Scientists have to consider several important elements for applying structural information to vaccine design for newly emerging pathogens. This information should come from native samples at sufficiently high resolution to shed light on viral immunogenic surfaces. Researchers can use cryo-EM to determine a structure quickly, even when the biochemistry involved in virus preparation is challenging. Although these methods may not be generally applicable to every emerging pathogen, cryo-EM is now a routine tool to aid rapid vaccine design.

References:

1) R. Arya et al., "Structural insights into SARS-CoV-2 proteins," *J Mol Biol*, 433:166725-166734, 2021.
2) X. Bai et al., "How cryo-EM is revolutionizing structural biology," *Trends Biochem Sci*, 40:49-57, 2015.
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UNDERSTANDING OUR FOES WITH CRYO-EM

Erica Ollmann Saphire, PhD
President and Chief Executive Officer
Center for Infectious Disease and Vaccine Research
Professor, Immunology
La Jolla Institute for Immunology

Erica Ollmann Saphire fell in love with structural biology the first time she encountered an electron density map. Ever since, she has explored the inner workings of viruses and the hosts they infect. This work is made possible by cryogenic electron microscopy (cryo-EM), which allows her to visualize complex viral surface proteins and their binding partners in her own laboratory and as the leader of the Coronavirus Immunotherapy Consortium, a global research partnership dedicated to the discovery and optimization of life-saving antibody-based therapeutics against SARS-CoV-2.

What are the major questions that you tackle in your laboratory?

Viruses are fascinating and have beautiful molecular structures, which we use to understand how to defend human health. The viruses we study are very simple; they might have four or seven genes. We ask questions such as: how does something with so few working parts take over a cell, conducting it like an orchestra for its own needs? What structural assemblies do these viruses make? How do they evade the immune system? How can we devise defenses against them? We use cryo-EM to visualize what is happening between the immune system and the pathogen, and how the pathogen changes shape to change function.

Why did you adopt cryo-EM for your structural work?

The viral proteins that I work on are heterogeneous. They are covered in carbohydrates in order to evade the immune system, which makes the proteins exceptionally difficult to crystallize. They are also conformationally heterogeneous. Previously, we would work so hard to get crystals for one protein. For one project, we made 400 versions of an Ebola virus surface protein and we bound a dozen different monoclonal antibodies into each viral molecule. We screened hundreds of crystals and made so many point mutations to figure out how to stabilize this inherently soluble, heterogeneous molecule in order to grow a crystal.

With cryo-EM, we can finally see the complex array of heterogeneity that is important for biology—we can see different structural confirmations and capture them all at the same time. If an antibody binds with stoichiometric or substoichiometric occupancy, we can see that. We can capture the entire flexible, Y-shaped antibody as it exists in natural biology and understand how it interacts with the surface. That is important for understanding immunology and delivering therapeutics. We were never going to capture that with a crystal; we would have to cut it down into its most simple parts.

Also, with cryo-EM, we can see large things and we can look inside cells. That is so exciting because viruses are obligate intracellular parasites. The viruses that we work on have so few parts, and often they achieve their incredible array of functions by being different things at different times. Now we can look inside cells and see what the larger assemblies are and what the human factors are. We can understand the complexity that is biology.

How does cryo-EM help the efforts of the Coronavirus Immunotherapy Consortium?

We use cryo-EM to look at antibodies supplied by a group of international scientists. It lets us examine 400 different antibodies at scale to map their landscapes. There is no way one person can crystallize 400 molecules, but with electron microscopy, we can rapidly and broadly screen things. We can perform negative staining and get a sense of how the molecules bind, and then pursue the interesting ones at higher resolution with cryo-EM. If one wants to look at binding on the virus' surface, they can also use tomography. We can analyze entire biologically-relevant assemblies, which are too big and flexible to crystallize, more reliably and quickly than ever before. Through this work, we want to figure out what the different antibodies do and find ones that resist emerging SARS-CoV-2 variants.

It is profound to see the things that used to be out of reach, to visualize the molecular workings of our greatest foes and the immune system that keeps us alive. Having the tools to understand the incredible complexity that happens there is a remarkable gift.

This interview has been edited for length and clarity.

CRYO-EM: FROM ANTI-VIRAL DRUG DISCOVERY TO VACCINE DESIGN

Jason McLellan, PhD
Associate Professor, Molecular Biosciences
Robert A. Welch Chair in Chemistry
The University of Texas at Austin

For the past seven years, Jason McLellan has worked on coronaviruses, so his team at The University of Texas at Austin responded rapidly to the COVID-19 pandemic, conducting groundbreaking research on the conserved coronavirus spike protein using cryogenic electron microscopy (cryo-EM). They successfully redesigned the SARS-CoV-2 surface spike protein to enable faster and more stable production of vaccines worldwide. Their work highlights the important role of cryo-EM in virology research.

"It became clear that to remain relevant in structural biology and to be able to go after any target that we wanted for structure determination; we would have to learn cryo-EM."

How did you get interested in cryo-EM?

I was trained as an x-ray crystallographer when I went to graduate school at the Johns Hopkins University School of Medicine. In the early 2000s, x-ray crystallography was the only technique that provided high-resolution information, and cryo-EM only provided modest resolution. If you wanted to see side chains and get 2 Å resolution, x-ray crystallography was the way to go. In 2013 when I started my lab at Dartmouth College, new direct electron detectors came out with electron microscopy, and that started the resolution revolution. With the advanced detectors and new algorithms, cryo-EM was able to achieve similar resolutions as x-ray crystallography. It became clear that to remain relevant in structural biology and to be able to go after any target that we wanted for structure determination, we would have to learn cryo-EM. In no time, I was downloading data sets to build my cryo-EM lab.

How does cryo-EM aid viral studies?

Structural biology can help us understand proteins at a residue-by-residue level. For understanding viral replication mechanisms, it is important to analyze the enzymatic analysis or the specific binding interactions of viral proteins. With cryo-EM, we can see which amino acids are important for mediating receptor binding or recognition by a monoclonal antibody, allowing us to predict how variations in the viral proteins affect escape from the antibodies.

What are some challenges in structure-based vaccine design?

The hardest part is often the sample preparation and getting well-behaved proteins. Trying to purify an inactivated infectious virus or a recombinant protein is a difficult task. Data collection and data processing is not the hardest part as that continues to get easier as new algorithms develop. Taking the structure and determining which mutations and substitutions to make to develop an optimal vaccine antigen can be a rate-limiting step that requires a lot of protein engineering and testing of variants, including expression and purification, melting temperature, and antibody reactivity. Ultimately, you have to go into animal models, which also takes a long time. My advice to researchers is that it is good to have as much of the above done ahead of time. Then you might be in a good place to respond to an emerging pathogen.

Q. What role will cryo-EM play in anti-viral drug discovery and vaccine design?

Our cryo-EM studies revealed how SARS-CoV-2 spike proteins assemble and where the domains are located in the trimer. Using this information, my lab made stabilizing mutations that substantially boosted the expression of beta coronavirus spikes like those from the MERS coronavirus and the first SARS coronavirus.

I think we should fully expect similar situations in the future. We have had coronavirus outbreaks from 2012 to 2020. We are potentially on an eight-to-ten-year clock for coronavirus and novel influenza virus epidemics or pandemics. I think a lot of the advances in cryo-EM technology, such as methods development and improved algorithms for both x-ray crystallography and cryo-EM, will allow the scientific community to respond quickly in the future to help guide vaccine, antibody, and small molecule development. As developments in cryo-electron tomography increase, we can look at viruses at a higher resolution as they are assembling inside cells, budding, or infecting cells, and see how the viruses are interacting.

This interview has been edited for length and clarity.

To learn more, watch this video where Jason McLellan answers questions about cryo-EM, the spike protein structure, and vaccine trials with his collaborators at the National Institutes of Health. This video is courtesy of The University of Texas at Austin.

AN INSIDE VIEW OF HOST-PATHOGEN INTERACTIONS

Peijun Zhang, PhD
Director, Electron Bio-Imaging Centre (eBIC)
Diamond Light Source
Professor, Structural Biology
Nuffield Department of Medicine, University of Oxford

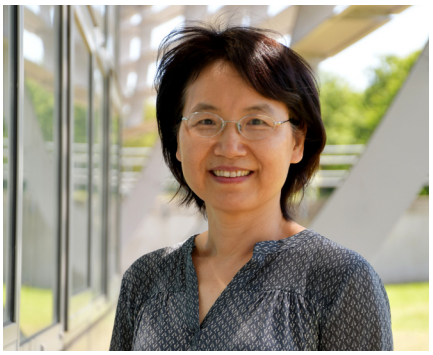


PHOTO COURTESY OF DIAMOND LIGHT SOURCE.

Peijun Zhang is a structural biology professor at the University of Oxford and is the director of the Electron Bio-Imaging Centre (eBIC) at Diamond Light Source, the United Kingdom's national synchrotron science facility. Over the past six years, she has built eBIC to be one of the largest cryogenic electron microscopy (cryo-EM) facilities in the world, offering scientists around-the-clock access to equipment, training, and expertise for cryo-EM and cryogenic electron tomography (cryo-ET).

What got you interested in structural biology and cryo-EM?

I took quite a journey, starting with my undergraduate degree in electrical engineering. When carrying out my master's degree in solid state physics, I got to use the most powerful microscope in China, a transmission electron microscope operating at 400 kilovolts. It was so powerful that I could see individual atoms. That sparked my interest. For my PhD, I decided to use cryo-EM to study biological problems. Now in my work, my engineering, material science, and physical science training help me develop better methods.

How has cryo-EM evolved since its early days as a niche technology?

I started my PhD thesis in 1992. Back then cryo-EM was mostly used to look at 2-D membrane protein crystals because it was very difficult to determine their structure with x-ray crystallography. We called the technology "blobology," as the majority of cryo-EM structures were at low resolution. For my thesis, I determined the structure of a calcium ATPase at 8 angstrom resolution; that was very exciting back then. There have been many developments over the years, especially the direct electron detector, better instrumentation, and improved software that have pushed cryo-EM to atomic or near-atomic resolution.

How do virologists utilize the eBIC facility?

Scientists use our center in many different ways because we offer different imaging modes. During the COVID-19 pandemic, eBIC made a huge impact in understanding SAR-CoV-2. Some groups performed single particle cryo-EM analysis to study in vitro interactions between the purified spike protein, antibodies, and the ACE2 cell receptor. They studied many spike variants from the different SARS-CoV-2 strains.

Scientists also use cryo-ET to study the infection process in situ. That can show where and how viral RNA is made and transported out, and how the virus assembles and buds into vesicles that are transported to the cell surface, allowing the virus to infect another cell. We studied this whole process by imaging natively inside of the cell during SARS-CoV-2 infection, utilizing a cryogenic focused ion-beam scanning electron microscope (cryo-FIB/SEM). This machine mills the cell into a very thin lamella layer—cells are 5-10 microns thick and an electron beam cannot penetrate them otherwise. After milling, the lamella is about 200 nanometers thick or less. We then collect a tilt image series and combine these different views to form a 3D structure of the virus infection process. The resulting knowledge helps us to better understand virus infection and find ways to treat or prevent infection.

How has cryo-EM provided unique insights into HIV-1 capsid assembly and host interactions?

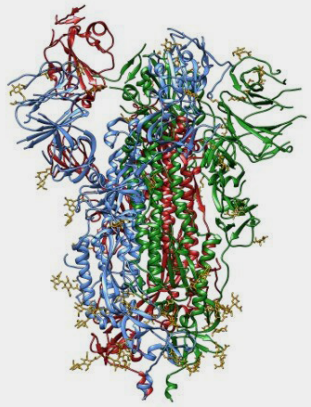
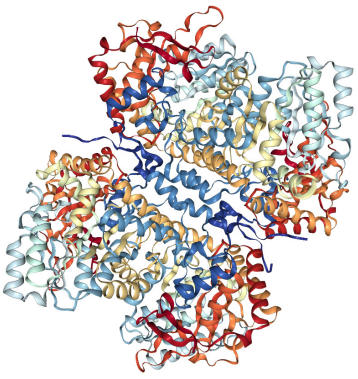
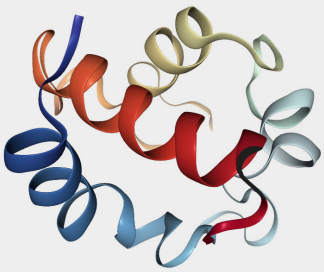
While the structures of the HIV capsid building blocks were determined by x-ray crystallography, this method cannot determine the whole assembled capsid structure. The capsid constitutes about 1,500 individual protein molecules with three million atoms. Not only can they not be crystallized, the capsids are very polymorphic in composition, size, and shape. Also, where host proteins bind to the capsid is mostly heterogeneous. Those factors make structural analysis by x-ray crystallography impossible, but cryo-EM and cryo-ET are powerful tools to study those assemblies.

We started with establishing the in vitro HIV-1 capsid assembly using purified recombinant protein and discerned the structure ultimately to three and a half angstrom resolution. That allowed us to tease out the details of the assembly interface and, with collaborators, we illustrated the atomic model of the entire capsid using molecular dynamic simulation. We could see how small molecules interact with the assembled capsids, which was not possible before. We are now using cryo-EM to study HIV-1 and host protein interaction. Many host factors, especially restriction factors that block HIV infection, do not bind the building blocks, they only bind the assembled capsid. They sense the pattern of the assembled capsid lattice, which requires cryo-EM to study.

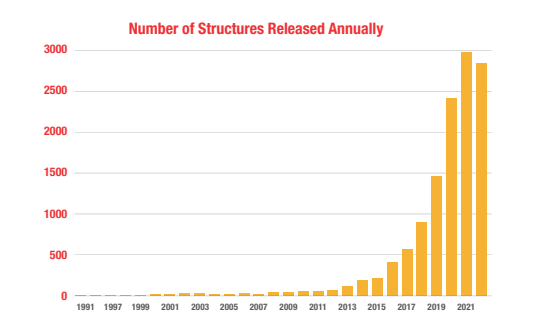
We have also studied the structure of immature capsid particles in complex with inhibitors that block viral maturation. Once we understand their mechanism for action we hope to come up with better drugs to block HIV-1 infection.

This interview has been edited for length and clarity.

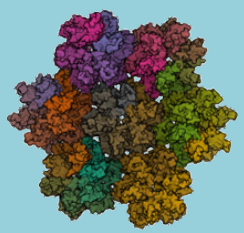
Methods for Studying Protein Function

	 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

The Cryo-EM Revolution



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.



HIV-1 capsid protein at 2.8 angstrom (2013)[1]

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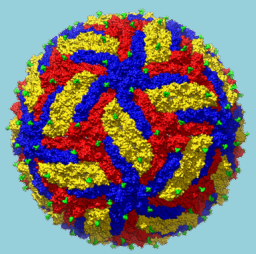

Zika virus at 3.1 angstrom (2018)[2]

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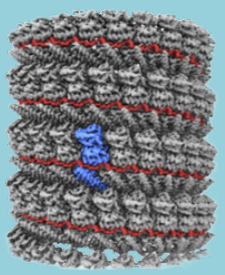
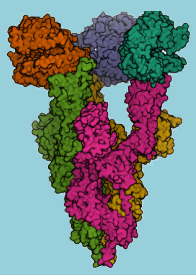


Ebola nucleoprotein nucleocapsid-like assembly at 5.8 angstrom (2018)[3]

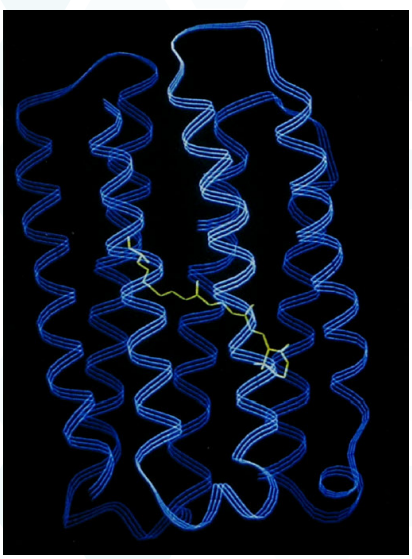
PDB 6C54



Triple ACE2-bound SARS-CoV-2 trimer spike at 3.64 angstrom (2020)[4]

PDB 7KMS

2017 Nobel Prize in Chemistry



The 2017 Nobel Prize in Chemistry was awarded to Jacques Dubochet, Joachim Frank, and Richard Henderson for the development of cryo-EM.

- Dubochet succeeded in vitrifying water, cooling it so rapidly that it solidifies in an amorphous form around a biological sample, allowing biomolecules to retain their natural shape even in a vacuum.
- Frank made the technology generally applicable by developing an image processing method that merged an electron microscope's fuzzy, two-dimensional images to reveal sharp, three-dimensional structures.
- Henderson generated a three-dimensional protein image (depicted on the left) of bacteriorhodopsin at atomic resolution.[5]

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1. G. Zhao et al., "Mature HIV-1 capsid structure by cryo-electron microscopy and all-atom molecular dynamics," *Nature*, 497:643-646, 2013.
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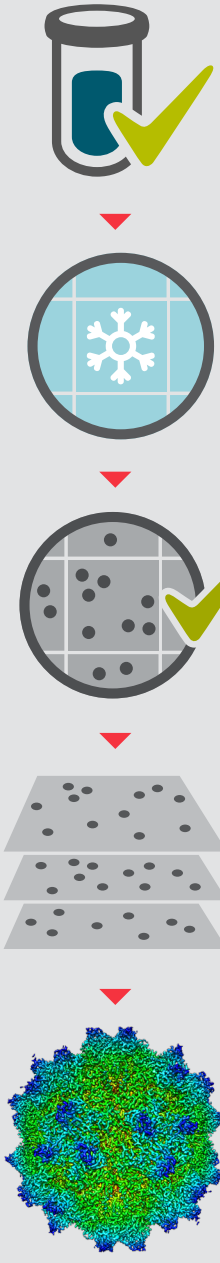
3. Z. Su et al., "Electron cryo-microscopy structure of Ebola virus nucleoprotein reveals a mechanism for nucleocapsid-like assembly," *Cell*, 172:966-978, 2018.
4. T. Zhou et al., "Cryo-EM structures of SARS-CoV-2 spike without and with ACE2 reveal a pH-dependent switch to mediate endosomal positioning of receptor-binding domains," *Cell Host Microbe*, 28:867-879, 2020.
5. R. Henderson et al., "Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy," *J Mol Biol*, 213(4):899-929, 1990.

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

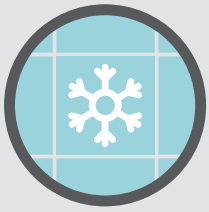
Once sufficient particle data is collected (ideally representing the sample from as many different orientations as possible) the data can be recombined into a 3D representation of the protein/protein complex. This uses 2D data from tens of thousands of particles and typically involves multiple data processing 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.

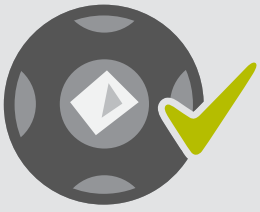
Sample preparation

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.



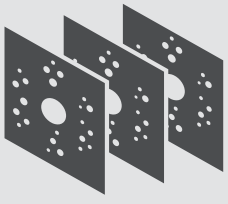
Vitrification

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).



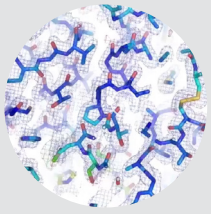
TEM low-dose screening

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.

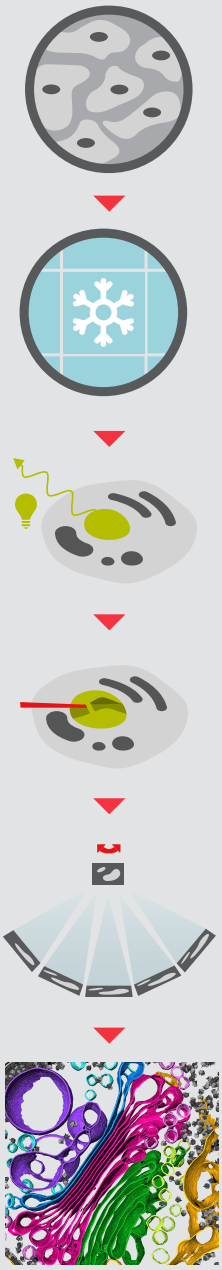


Reconstruction

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.

ThermoFisher SCIENTIFIC

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

Thermo Fisher Scientific offers 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

Thermo Scientific™ Tundra™ Cryo-TEM: accessible and 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

Thermo Scientific™ Glacios™ 2 Cryo-TEM: capable and versatile

- Maximized ease-of-use and excellent performance 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, <2.5 Å*
High throughput	Dataset in 30 minutes
Sample type	Proteins, crystals, cells
Applications	SPA, MicroED, tomography

Thermo Scientific™ Krios™ Cryo-TEM: powerful and productive

- Our highest productivity and image quality cryo-TEM with an integrated workflow solution
- Our 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, <1.5 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.

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

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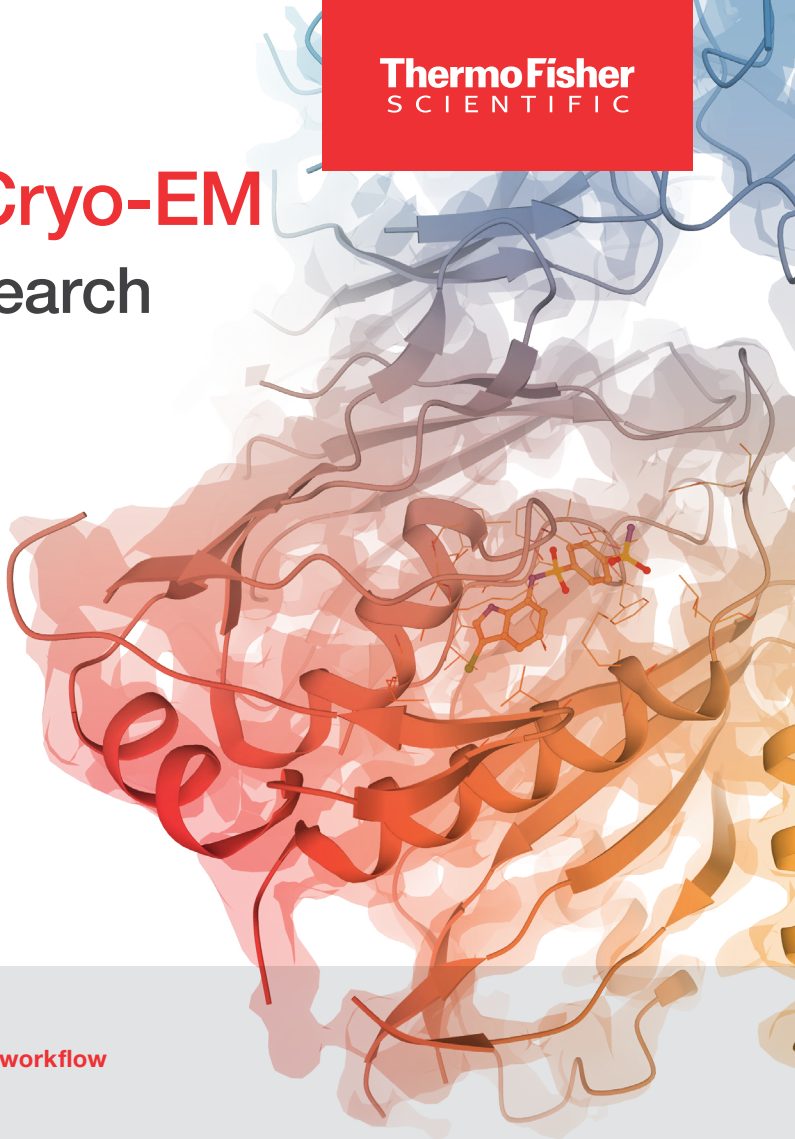
Getting Started in Cryo-EM

Revolutionize your research

Adopt cryo-EM quickly and seamlessly. As the market leader in cryo-EM, 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 Tundra and Glacios 2 Cryo-TEMs can provide entry-level 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
Our experts provide guidance to minimize environmental interference and maximize system performance.

Installation
Post installation, we provide training for high-quality sample preparation and data collection using validated workflows.

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 provides simplified single particle analysis, while the Glacios 2 Cryo-TEM offers improved efficiency, throughput, and ease of use for multiple applications.

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

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