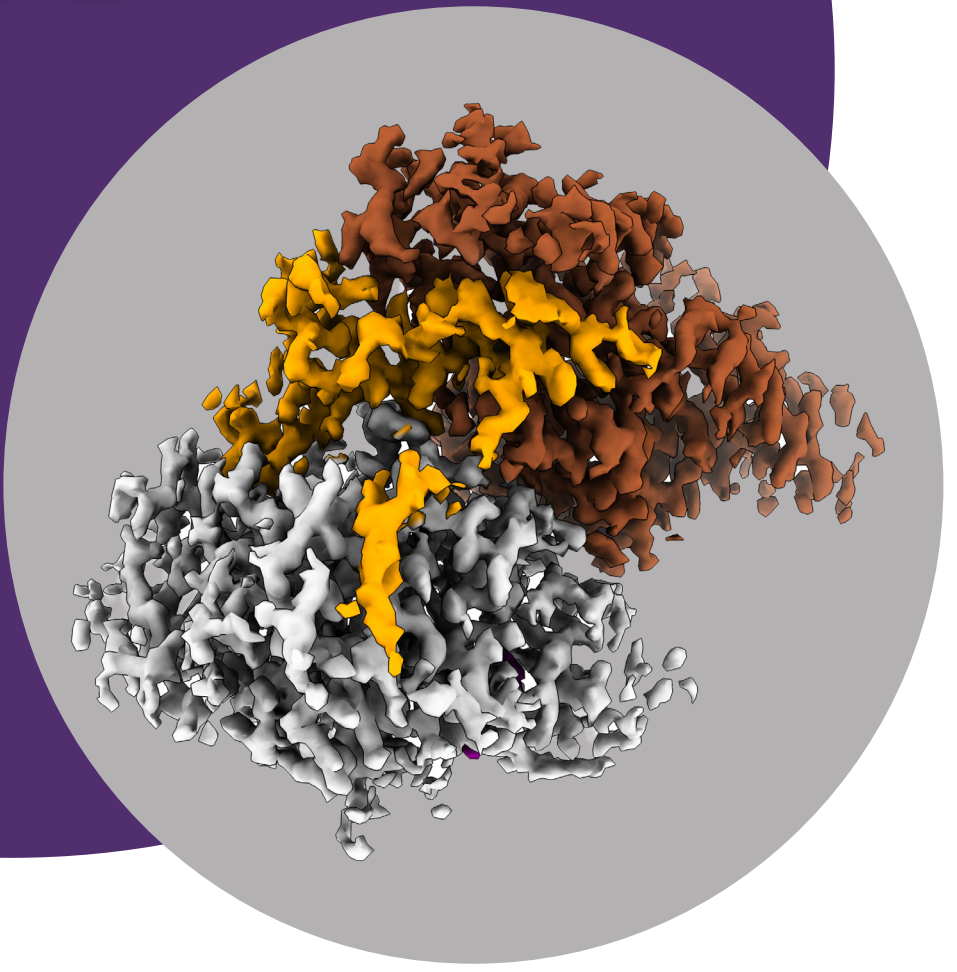
TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

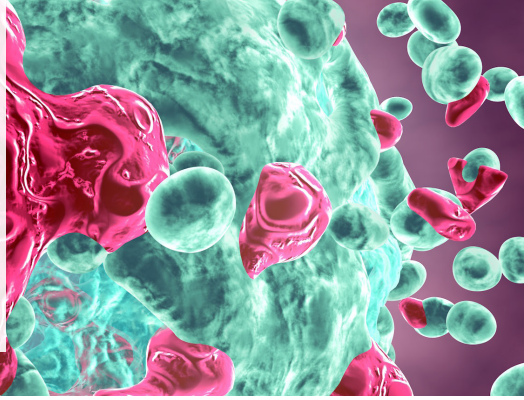
UNDERSTANDING THE COMPLEXITY OF CANCER WITH CRYO-EM



Sponsored By

ThermoFisher SCIENTIFIC

FINDING THE MOLECULAR DRIVERS OF CANCER



Cancer is a large group of diseases with a diverse array of etiologies and locations, governed by the behaviors and interactions of numerous distinct cell types, which are mediated by a vast number of protein-mediated signaling pathways. World Health Organization statistics show that nearly 10 million people worldwide died from cancer in 2020, with 20 million new cancer cases diagnosed in that year.[1]

While mutant gene products lead to altered pathway function leading to oncogenesis, the precise underlying mechanisms and potential protein-level therapeutic targets are largely unclear. This drives structural biologists to characterize how mutations affect protein structures and conformations and identify how these alterations impact signaling and function.[2]

Characterizing structures without crystallization

Characterizing protein structures has historically necessitated crystallization, a laborious process that often requires researchers to extensively alter the protein of interest. The advent of cryogenic electron microscopy (cryo-EM) changed that. In cryo-EM, samples are vitrified via flash-freezing and imaged using transmission electron microscopy. The resulting 2D images are then computationally reconstructed into a 3D structure.[3] Cryo-EM lets scientists observe biomolecules in their natural state, without the need for crystallization or other special preparations. This lets researchers capture complete and fully functional macromolecular complexes in different functional states.[3]

Cryo-EM has been particularly useful for researchers investigating membrane proteins, whose properties made them very challenging to crystalize. In 2019, John Rubinstein's team from the University of Toronto used cryo-EM to image a bacterial ATP synthase in three rotational states at a three Ångström resolution, allowing them to identify how subunit ε can inhibit ATP hydrolysis while allowing ATP synthesis.[4]

Unraveling cancer through structural biology

Determining how therapeutic agents interact with receptor structures is important for understanding drivers of anticancer drug efficacy and potential synergistic relationships. For example, both trastuzumab and pertuzumab reduce cellular growth by inhibiting human epidermal growth factor receptor 2 (HER2), and there has been a documented synergistic effect when they are used together. In 2019, Xin Huang's research group used cryo-EM to visualize the HER2-trastuzumab-pertuzumab complex, finding that the two antibodies can bind simultaneously to HER2, but that the binding of one does not affect the binding of the other. This indicates that the observed clinical synergism of the two agents arises from other mechanisms.[5]

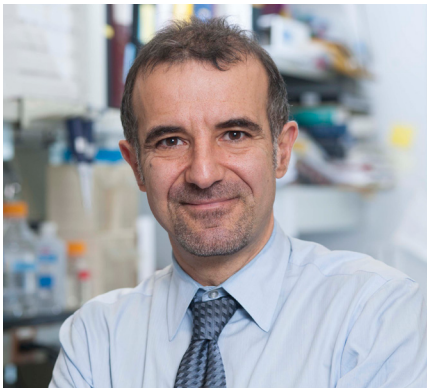
Rituximab is another anticancer therapeutic agent with a poorly understood mechanism of action. Researchers had identified that it bound CD20 to trigger cell death via complement activation, but precisely how this occurred was unclear. Furthermore, other CD20-targeting antibodies elicited different mechanisms. Alexis Rohou's research team recently used cryo-EM to characterize the structure of the CD20-rituximab complex. They found that CD20 forms a compact dimer with one rituximab antigen-binding fragment (Fab) bound to each monomer. These Fabs interact with each other to crosslink CD20 into well-ordered circular assemblies designed for complement recruitment.[6]

Finally, the malfunction or inactivation of DNA repair mechanisms such as the Fanconi anemia (FA) pathway is a major cancer promoter.[7] The FA pathway hinges on the monoubiquitination of a FANCD2–FANCI heterodimer by the FA core complex. However, how the FA core acts to maintain genome stability is unclear. To address this question, Lori Passmore's team used cryo-EM to determine the structure of an active, recombinant FA core complex. Their findings revealed two heterotrimers that act as a pivotal assembly scaffold for the entire complex, providing a foundation for more detailed study of this complex's E3 ubiquitin ligase activity and DNA interstrand crosslink repair.[8]

References:

1) "Cancer Today," Global Cancer Observatory: Cancer Today, International Agency for Research on Cancer, https://gco.iarc.fr/today. Accessed July 7, 2021.
2) D. Reid, C. Mattos, "Targeting cancer from a structural biology perspective," in *Unravelling Cancer Signaling Pathways: A Multidisciplinary Approach*, K. Bose, P. Chaudhari, eds., Singapore: Springer, 2019, pp. 295-320.
3) E. Nogales, S.H. Scheres, "Cryo-EM: A unique tool for the visualization of macromolecular complexity," *Mol Cell*, 58(4):677-89, 2015.
4) H. Guo et al., "Structure of a bacterial ATP synthase," *Elife*, 8:e43128, 2019.
5) Y. Hao et al., "Cryo-EM Structure of HER2-trastuzumab-pertuzumab complex," *PLoS One*, 14(5):e0216095, 2019.
6) L. Rougé et al., "Structure of CD20 in complex with the therapeutic monoclonal antibody rituximab," *Science*, 367(6483):1224-30, 2020.
7) A. Deans, S. West, "DNA interstrand crosslink repair and cancer," *Nat Rev Cancer*, 11:467-80, 2011.
8) S. Shakeel et al., "Structure of the Fanconi anaemia monoubiquitin ligase complex," *Nature*, 575:234-37, 2019.

FILLING IN THE GAPS: CHARACTERIZING PROTEIN MECHANISMS FOR CANCER RESEARCH



Nikola Pavletich, PhD
Memorial Sloan Kettering Cancer Center

Genomic integrity and cell growth regulation lie at the heart of cancer. Protein structures play a large role in mediating these mechanisms. Nikola Pavletich uses structural biology techniques to characterize said structures and identify how they behave during key cellular functions and mechanisms. The rise of cryogenic electron microscopy (cryo-EM) has made things much easier for Pavletich and his team at Memorial Sloan Kettering Cancer Center.

Q. Where does structural biology fit into cancer research?

Structural biology, in a nutshell, is about taking pictures of proteins. When a cancer pathway is uncovered, researchers in different areas employ different approaches to study it: cell biologists study mutated proteins in cell models and manipulate them using things like CRISPR, biochemists try to reconstitute the system using purified components to understand how A goes to B and B goes to C, and structural biologists try to map where the atoms are, where the active sites are, where the protein-protein interaction surfaces are, where the surfaces that promote and convey signaling are, and so forth. This provides both an atomic-level description of the system and a framework for discovering or improving pharmaceutical interventions.

Q. Why did you start using cryo-EM?

When I started my lab in 1993, the most powerful method for determining atomic structures at high resolution was X-ray crystallography. Electron microscopy existed, but the instrumentation at that time was not powerful enough to determine high resolution structures outside of a few specialized cases. To use X-ray crystallography, you need to make crystals, and this becomes exponentially harder the larger the protein gets. We had to figure out what parts of the protein inhibited crystallization and then genetically engineer them out. Sometimes, we would be forced to use simpler model organisms like zebrafish or yeast just because the protein analogs there were easier to crystallize.

The advent of cryo-EM changed all of that. We have been investigating mTOR (mammalian target of rapamycin) since 2008 because it is a cell cycle regulator and mediator of cell growth. It took us five years, but we managed to successfully crystalize part of the mTOR structure. After we acquired a cryo-EM system in 2013, it took us a further year-and-a-half to not just determine the structure of mTOR, but to crystalize the entire one million-Dalton mTOR Complex 1 (mTORC1) structure. We also got mTORC1 bound to its activator protein Rag, letting us characterize the "on" and "off" states of the complex for the first time.

Q. What can you do with cryo-EM but not with other imaging modalities?

The power of cryo-EM is that you can capture many different states. This lets scientists visualize the individual steps or stages of chemical reactions. For example, we took snapshots of the ubiquitination reaction as it was occurring, and we found all of these different states—pre-ubiquitination, bound but not ubiquitinated, mid-ubiquitination, and post-ubiquitination. We were able to computationally separate all of these different states, analyze them individually, and observe how the reaction progressed.

We used this capability to look at how RecA mediates homologous recombination. RecA is a bacterial homolog to RAD51 that is essential for DNA repair and maintenance. Through X-ray crystallography, we had characterized the RecA-substrate complex and the end-product, but we did not have anything in between. Cryo-EM let us classify the intermediates in this reaction, and through this, we were able to characterize the strand-exchange reaction mechanism central to homologous recombination.

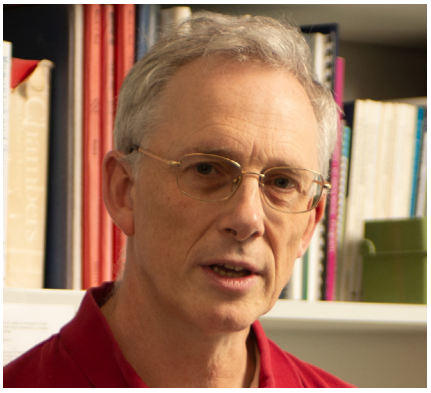
Q. Is cryo-EM becoming more accessible?

Absolutely. We've seen major investments, not only by individual institutions, but also by the National Institutes of Health. We are starting to see the fruits of this increased accessibility too. If you look at publications in my field, most of them these days use cryo-EM, and there are more studies being published in general. Of course, cryo-EM is a considerable investment, but the instrumentation is also improving while the costs remain stable. You can install a modern cryo-EM system for the same price as we paid for ours back in 2017, but these new instruments can reach two Ångström resolutions, whereas the 2017 model cannot.

To close with a little anecdote, back when the cryo-EM revolution was just taking hold, one of my former trainees told me that we were "losing our edge." We had become so good at tinkering with proteins to make them crystallizable, and now we did not need to do any of that any more. I said to him: "I'm so happy that we don't have to grow crystals any more, I'd rather lose that edge any day."

This interview has been edited for length and clarity.

THE IMPORTANCE OF MEMBRANE RECEPTOR STRUCTURES IN DRUG DISCOVERY



Chris Tate, PhD
Medical Research Council Laboratory of Molecular Biology, Cambridge, UK

Membrane proteins are fundamental to cellular function and survival, and their structures are integral to their function. Chris Tate has worked on membrane proteins for over three decades, the latter half focusing on G-protein coupled receptor (GPCR) structures. Defining GPCR structures has been historically challenging, but the advent of cryogenic electron microscopy (cryo-EM) has opened new doors for Tate and his team at the Medical Research Council (MRC) Laboratory of Molecular Biology (LMB) in Cambridge, UK.

Q. What drives the conformation that a GPCR adopts?

Not all receptors are the same, and that can be seen in something as trivial as basal activity in the absence of a ligand. For example, rhodopsin has virtually no basal activity—a spontaneous state transition occurs roughly once every 200 years—whereas something like a histamine receptor has quite a high level of basal activity, which is important for their function. What agonists do is change the receptor's energy landscape to promote transitions to the active state. There are a number of potential intermediate states between the basal and active states, and their properties depend on their thermodynamic stability. For example, the structure of a beta adrenergic receptor is virtually identical whether an agonist or an inverse agonist is bound to it. However, the adenosine receptor transitions from a basal state to an active-like intermediate—about 90% of the way to the fully active state—upon agonist binding.

Q. Why is it important to study GPCR conformation states?

When we think about drugs, we normally think about terminal states and ignore intermediate states. But that is changing because of biased agonism, where an agonist can activate different subsets of a receptor's signaling cascade. For example, one G protein-coupled receptor (GPCR) agonist could preferentially activate a Gs pathway, while another favors Gi subunit signaling. We do not really understand why this happens from a structural biology context, but this could be really important for drug development because side effects are often driven by signaling through one pathway rather than another.

Q. What led you to bring cryo-EM into your laboratory?

The major reason for doing cryo-EM is that obtaining crystals for X-ray crystallography is particularly difficult for membrane proteins, and particularly for complexes between a GPCR and a G protein. Before cryo-EM, there were only two published structures of GPCR-G protein complexes, and the first one won a Nobel Prize! X-ray crystallography is also unsuited to determine structures of membrane proteins in lipid nanodiscs, where the membrane protein is embedded in its own swimming pool of lipid contained by a proteinaceous ring.

Cryo-EM made what was an intractable problem of trying to obtain the structure of a GPCR-G protein complex much more tractable. We have been able to answer questions that would have been absolutely impossible to answer using X-ray crystallography. For example, one of our recently published structures was of a GPCR coupled to arrestin, which needs lipids for efficient coupling. What we did was purify the receptor, put it in a lipid nanodisc, and now you can couple arrestin. You cannot crystallize this thing, but with cryo-EM, it is tractable.

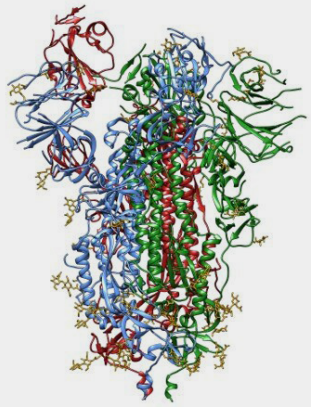
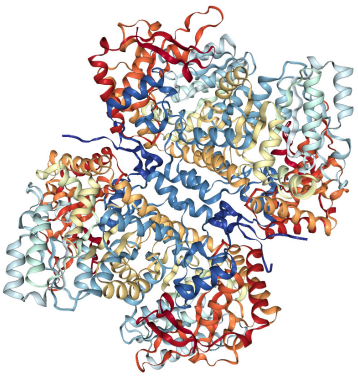
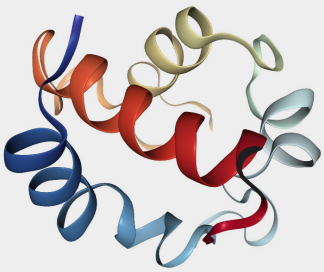
Another advantage of using cryo-EM is that engineering the GPCR to facilitate crystal formation becomes unnecessary. Very often flexible regions are removed, well-folded proteins are inserted into a loop, and themostabilizing mutations are added. Not engineering the receptor saves a huge amount of time, but more importantly, it means we do not let our assumptions impact the experiment. For example, our recent structure of a wild-type unengineered yeast GPCR dimer showed the N-terminus making crucial contributions to the dimer interface. If we had tried to engineer the receptor by removing flexible regions, perhaps we would have removed this region and never discovered the beautiful domain-swapped structure it forms.

Q. How do these insights affect drug discovery and development?

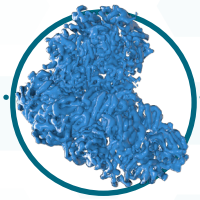
Structure-guided drug design is a well-validated and effective tool for making really good drugs. For example, there are cases where people have modeled small molecules bound to proteins, but when they obtained the structures, they found that the molecule was rotated 180 degrees in the binding pocket from what was expected, or moved by a few Ångstroms, or even bound in a completely different place. It really helps to have structures to understand how lead compounds actually bind and to design really good drugs in a rapid, efficient way. This is where cryo-EM and the structures stemming from cryo-EM are transforming drug discovery.

This interview has been edited for length and clarity.

Methods for Studying Protein Function

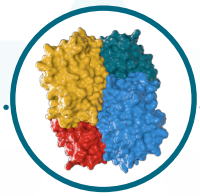
	 Cryo-EM	 X-ray crystallography	 NMR
Summary	Samples are rapidly frozen (vitrified), preserving the sample in its natural state. A transmission electron microscope (TEM) is used to capture two-dimensional projections of the specimen, which are then combined to make a 3D model.	Samples are crystalized 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

2012 Direct electron-detection cameras used to correct beam-induced motion by Nikolaus Grigorieff and colleagues, allowing much higher resolutions.[1]

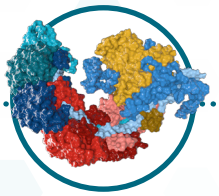


2014 David Barford's team characterizes the anaphase-promoting complex/cyclosome (APC/C), a key cell division regulator, at 7.4 Å resolution.[2]

2015 Single particle cryo-EM named Method of the Year by *Nature Methods*.

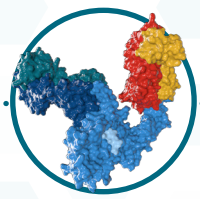


2016 Sriram Subramaniam and colleagues present 2.8 Å resolution structures of lactate dehydrogenase, which mediates energy generation in cancer cells.[3]

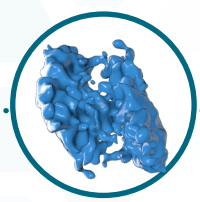


2017 Subramaniam's team uses cryo-EM to reveal the structural mechanisms underlying CRISPR-Cas system targeting of double-stranded DNA for degradation.[4]

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

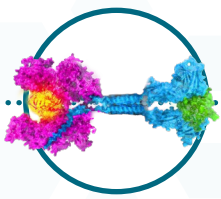


2019 Xin Huang's group presents the cryo-EM structure of HER2-trastuzumab-pertuzumab at 4.36 Å resolution, showing no cooperative interactions between the two anti-cancer antibodies.[5]



2020 Deborah Kelly and colleagues resolve structures for the tumor suppressor protein p53 in monomer and tetramer formats.[6]

2020 Atomic level resolution is achieved by two research groups, obtaining 1.22 and 1.25 Å resolution reconstructions of apoferritin.[7,8]



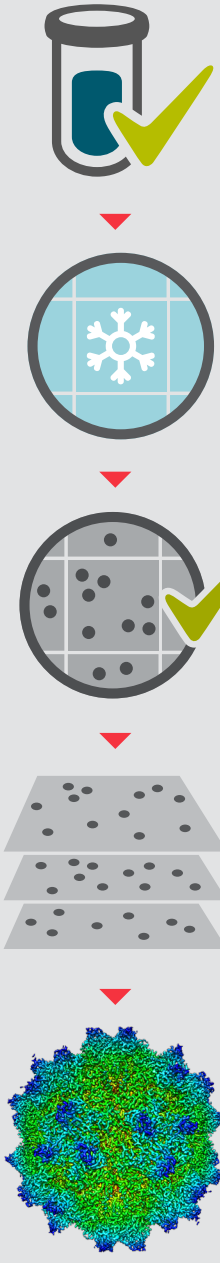
2022 The full length structure of a Janus kinase is solved by Chris Garcia, Caleb Glassman, and colleagues.[9]

References

1. E. Nogales, "The development of cryo-EM into a mainstream structural biology technique," *Nat Methods*, 13(1):24-27, 2016.
2. L. Chang et al., "Molecular architecture and mechanism of the anaphase-promoting complex," *Nature*, 513:388-93, 2014.
3. A. Merk et al., "Breaking cryo-EM resolution barriers to facilitate drug discovery," *Cell*, 165(7):1698-707, 2016.
4. T.W. Guo et al., "Cryo-EM structures reveal mechanism and inhibition of DNA targeting by a CRISPR-Cas surveillance complex," *Cell*, 171(2):414-26.e12, 2017.
5. Y. Hao et al., "Cryo-EM structure of HER2-trastuzumab-pertuzumab complex," *PLoS One*, 14(5):e0216095, 2019.
6. M.J. Solares et al., "Microchip-based structure determination of disease-relevant p53," *Anal Chem*, 92(23):15558-64, 2020.
7. T. Nakane et al., "Single-particle cryo-EM at atomic resolution," *Nature*, 587:152-56, 2020.
8. K.M. Yip et al., "Atomic-resolution protein structure determination by cryo-EM," *Nature*, 587(7832):157-61, 2020.
9. C.R. Glassman et al. "Structure of a Janus Kinase cytokine receptor complex reveals the basis for dimeric activation," *Science*, 376(6589):163-69, 2022.

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

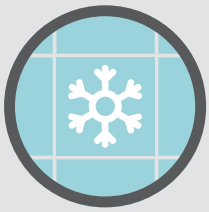
Once sufficient particle data is collected (ideally representing the sample from as many different orientations as possible) the data can be recombined into a 3D representation of the protein/protein complex. This uses 2D data from tens of thousands of particles and typically involves multiple data processing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

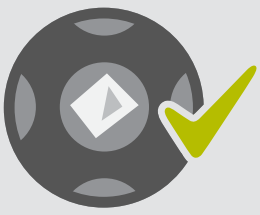
Sample preparation

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.



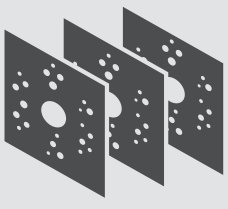
Vitrification

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).



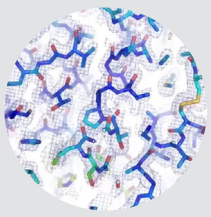
TEM low-dose screening

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.

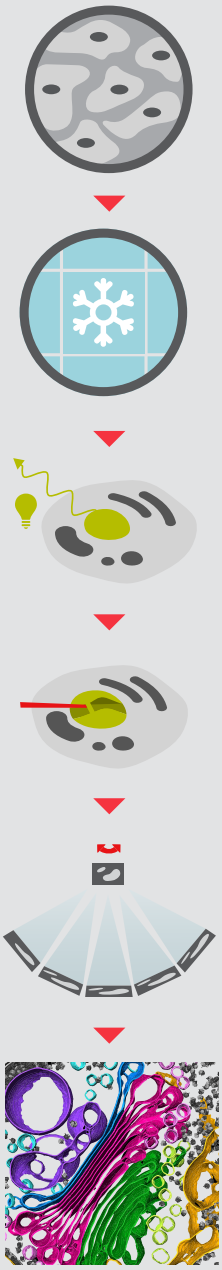


Reconstruction

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**

thermo scientific

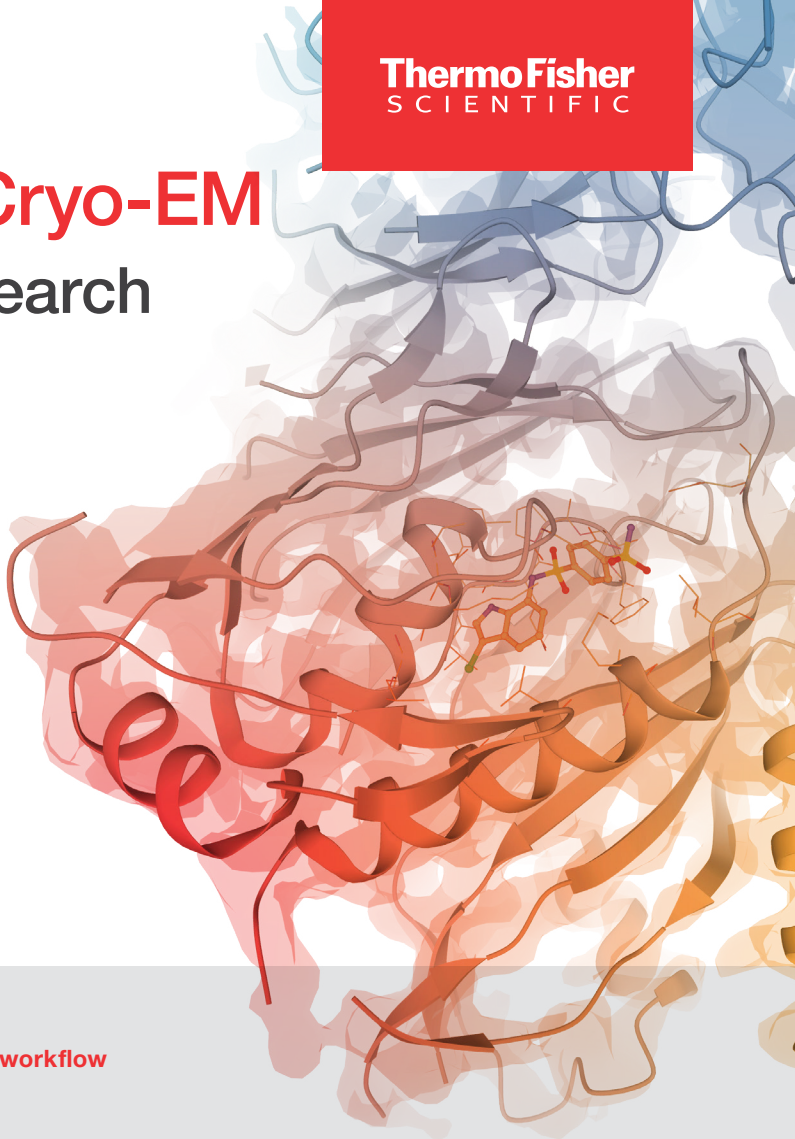
For research use only. Not for use in diagnostic procedures. For current certifications, visit thermofisher.com/certifications © 2022 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

ThermoFisher SCIENTIFIC

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**

thermo scientific

For research use only. Not for use in diagnostic procedures. For current certifications, visit thermofisher.com/certifications © 2022 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.