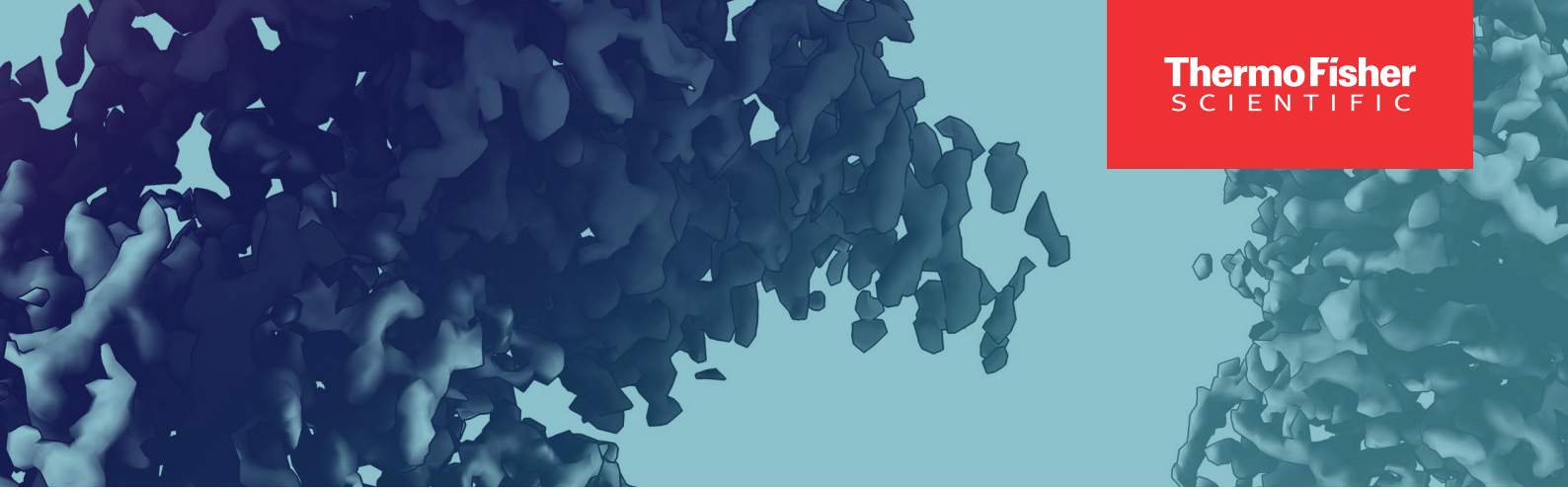


The evolution of 200 kV cryo-EM

Realizing the potential with the Glacios 2 Cryo-TEM

Cryo-electron microscopy (cryo-EM) has come a long way since the first viral structures were published by the Dubochet group in the early 80s. Increasingly seen as a vital, complementary technique to traditional structural biology methods, cryo-EM has gone through several "resolution revolutions" driven by improvements in both hardware and software. The results speak for themselves; there is an almost exponential increase in the number of cryo-EM structures submitted to the Protein Data Bank (PDB) year to year, many at resolutions <3 Å. Notably, these structures are often for membrane proteins and other systems that have been resistant to traditional crystallographic analysis.

The vast majority of these structures were determined with 300 kV cryo-transmission electron microscopes (cryo-TEMs) that were specifically designed for high-resolution data collection at cryogenic temperatures. In fact, it is safe to say that 300 kV has been treated as the *de facto* accelerating voltage for high-quality structural analysis. When they were introduced, 200 kV instruments such as the Thermo Scientific™ Talos Arctica™ and Thermo Scientific™ Glacios™ Cryo-TEMs were, therefore, largely seen as screening tools that could be used to optimize samples without spending time on the higher-resolution 300 kV instruments. However, advanced components and software are not reserved exclusively for 300 kV microscopes; they have allowed 200 kV cryo-TEMs to experience their own "resolution revolution," and research groups around the world are beginning to produce highly valuable results directly at 200 kV.

Glacios 2 Cryo-TEM: A revolution in productivity and resolution

	Glacios Cryo-TEM	AFIS upgrade	Selectris X Filter and Falcon 4i Detector upgrade	Glacios 2 Cryo-TEM
Energy filter	No-slit energy filter	No-slit energy filter	Selectris X Energy Filter	Selectris X Energy Filter
Electron detector	Falcon 3 Detector	Falcon 4 Detector	Falcon 4i Detector	Falcon 4i Detector
Images per hour	35	400	500	650
Apoferritin resolution	2.1 Å in 36 hours	1.9 Å in 9 hours	1.6 Å in 8 hours	1.6 Å in 7 hours

Attainable resolution and data collection time for apoferritin throughout the development of 200 kV cryo-TEM.

thermo scientific

The Thermo Scientific Glacios 2 Cryo-TEM.

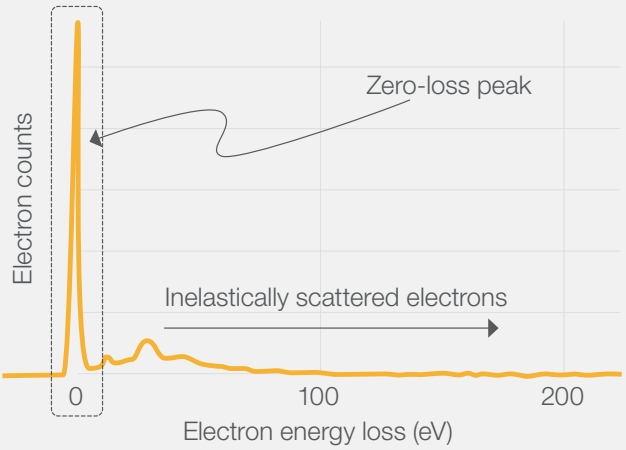
Glacios 2 Cryo-TEM

The Glacios 2 Cryo-TEM showcases many of the advances that have made high-quality analysis possible on 200 kV instruments. Hardware optimization has focused on improved specificity and sensitivity in electron detection. For instance, the Thermo Scientific™ Falcon™ 4i Direct Electron Detector enables high detective quantum efficiency (DQE) across the full spectral range, capturing both small/flexible proteins as well as larger structures using fewer images. When coupled with the novel Thermo Scientific™ Selectris™ or Selectris X Energy Filters, signal is limited to the zero-loss, elastically scattered electrons, producing high-contrast TEM data for higher throughput and higher resolution structures.

Energy filtering on 200 kV cryo-TEMs

Energy filtering is essential for cryo-EM acquisition conditions that generate a large amount of noise through interfering inelastic electron scattering. This can be the result of two main factors: sample thickness and accelerating voltage. (Sample thickness is especially important for cryo-tomography applications, as there is an inevitable increase in the travel path at larger tilt angles.) Notably, this means that energy filtering on a 200 kV instrument will have an inherently greater impact than on 300 kV tools, where there is less inelastic scattering.

Narrow, stable slit width is another key requirement for high-quality energy filtering. The narrower the width, the less of the unfiltered signal is sampled, and the less data is needed to produce high-quality results. With the Selectris X Energy Filter, extremely narrow slit widths are possible (<10 eV) with low slit drift (less than ±1.5 eV in 24 hours).



[See an example of the Glacios Cryo-TEM and Selectris Energy Filter in action as they are used to find the structure of the lysosomal tethering complex HOPS]

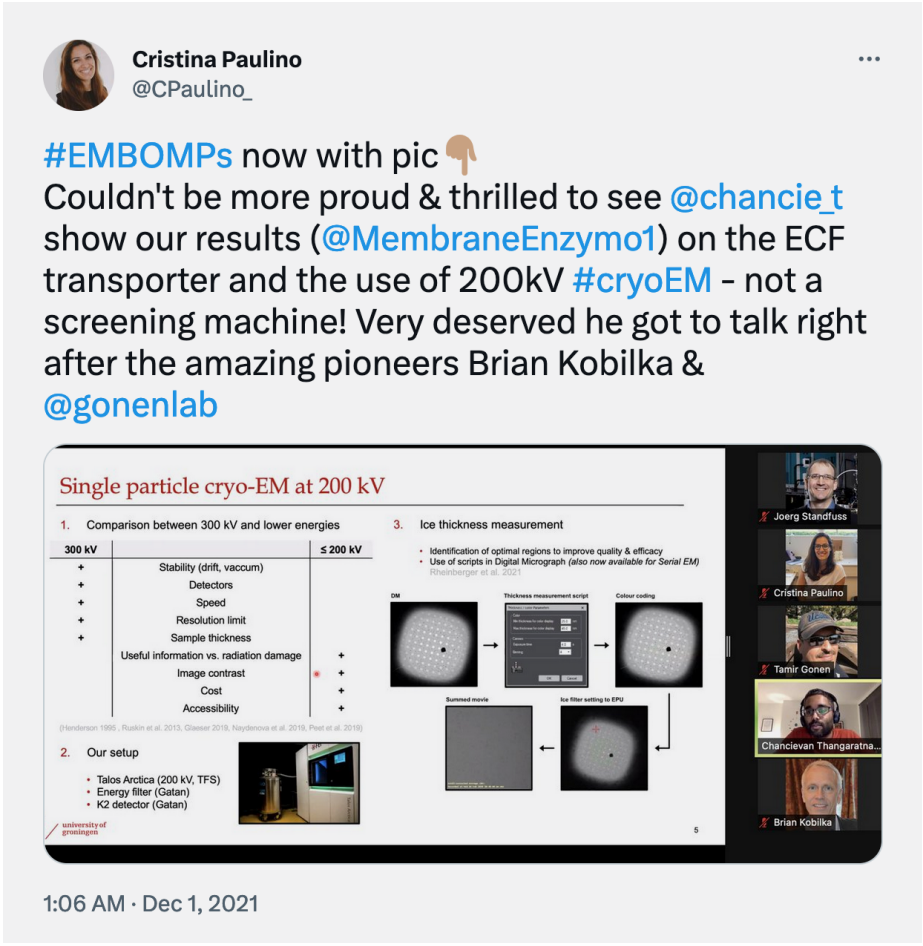
Glacios 2 Cryo-TEM results with and without the Selectris Energy Filter.

Sample	Falcon 4 Detector with no energy filtering		Falcon 4i Detector with 10 eV energy filtering by Selectris X Energy Filter	
	Number of particles	Resolution (Å)	Number of particles	Resolution (Å)
Apoferritin	250,000	1.9	200,000	1.6
T20S	102,200	2.3	102,200	2.1
GABAA	127,000	2.8	94,000	2.4
GLP1R*	632,000	3.2	219,000	2.6

*No-slit energy filter data collected with ligand; Selectris Energy Filter data is ligand-free.

Software improvements are centered on enhanced automation and data collection schemes. Thermo Scientific Smart EPU Software, for instance, enables both automated screening and data acquisition for single particle analysis through an approachable graphical user interface. Featuring unique AI algorithms trained on the decision-making process of cryo-EM specialists, Smart EPU Software allows for thousands of images to be collected reproducibly and with minimal user input.

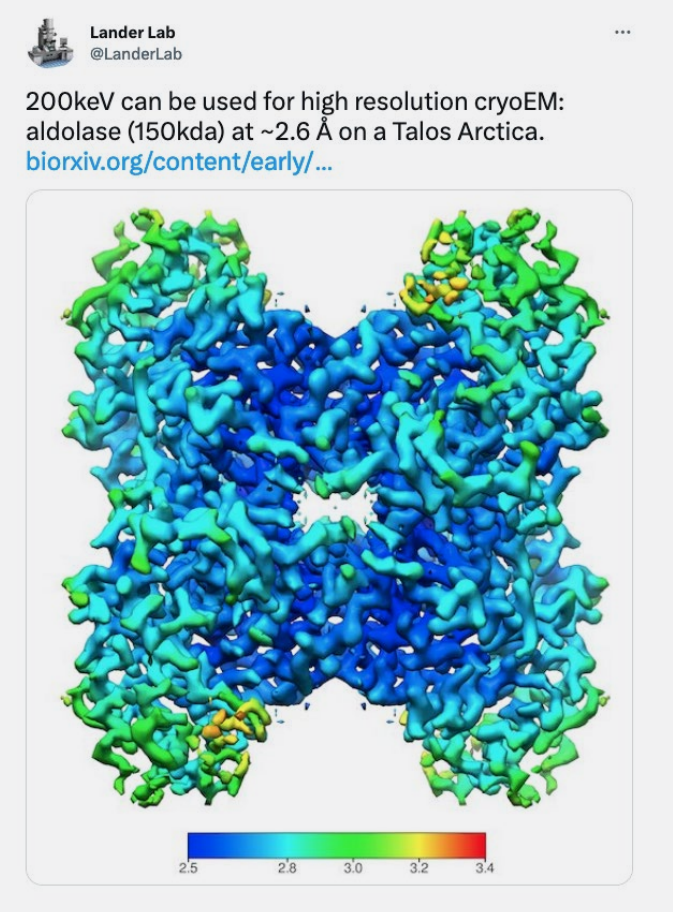
EPU Software also integrates aberration-free image shift (AFIS), a novel data collection scheme that uses image beam shift to avoid the aberrations typically caused by sample movement, producing more accurate, useable data with each scan. AFIS avoids sample drift by moving the image beam rather than stage; additional beam tilt and automated objective adjustments compensate for beam-shift aberrations such as coma and astigmatism.



Prof. Cristina Paulino at the University of Groningen highlighting her group's development of an optimized cryo-EM data-acquisition workflow by sample-thickness determination. Learn more

View their publication on cryo-EM studies of membrane proteins at 200 kV, including an ECF transporter. Learn more

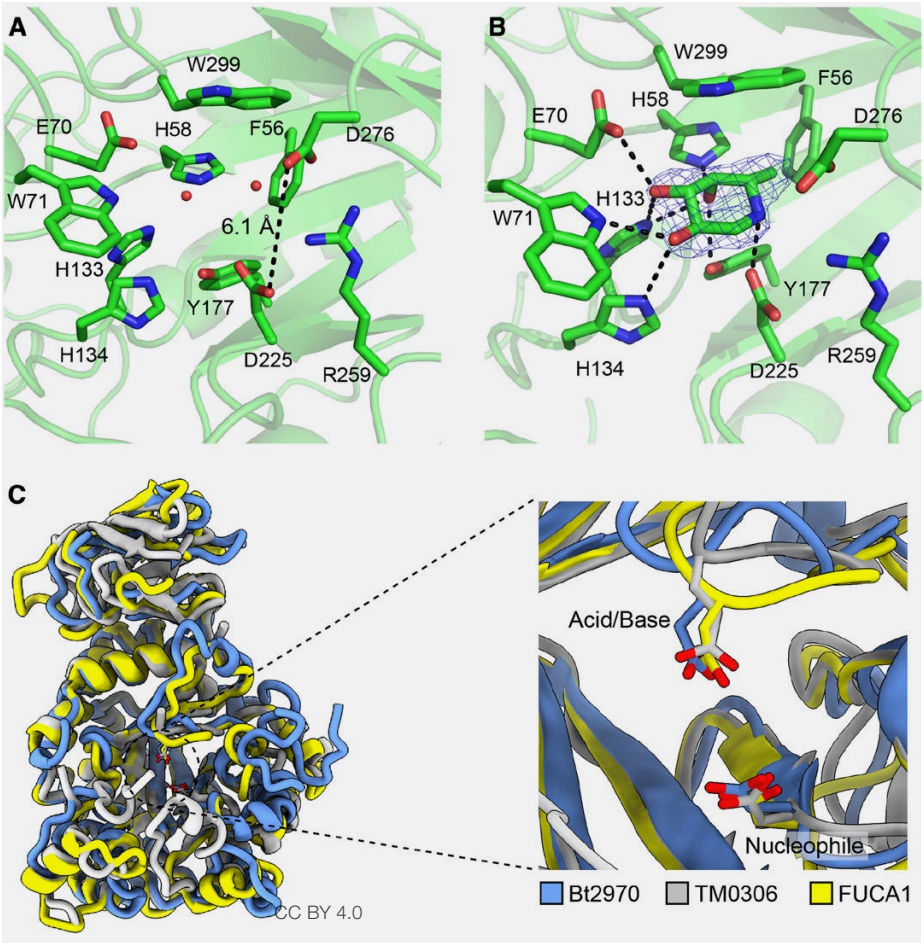
What follows are just a few examples of how 200 kV cryo-TEM is being utilized by researchers internationally to produce novel, actionable results. We hope their enthusiasm and excitement inspire you to see what question you can answer at 200 kV.



Prof. Gabe Lander's group at the Scripps Research Institute celebrates the publication of their ~1.75 Å resolution structure of apoferritin, determined with 200 kV cryo-TEM. Learn more

Cryo-EM of human fucosidase

The enzyme fucosidase plays a vital role in certain bacterial infections as well as fucosidosis, a neurodegenerative disorder impacting lysosomal storage. A complete understanding of fucosidase catalysis, along with any potential targeted drug design, has been hampered by the lack of 3D structures for the enzyme. Utilizing a 200 kV Glacios Cryo-TEM, researchers at the University of York were able to resolve the structure of human lysosomal α-L-fucosidase (FucA1) down to 2.49 Å resolution. Not only that, but they were also able to clearly visualize FucA1 in complex with an inhibitor (deoxyfuconojirimycin). At this level of detail, the precise architecture of the catalytic center could be determined as well as the location of various disease-related mutations. This serves as a promising step toward targeted treatment of fucosidase-related diseases and disorders. Learn more



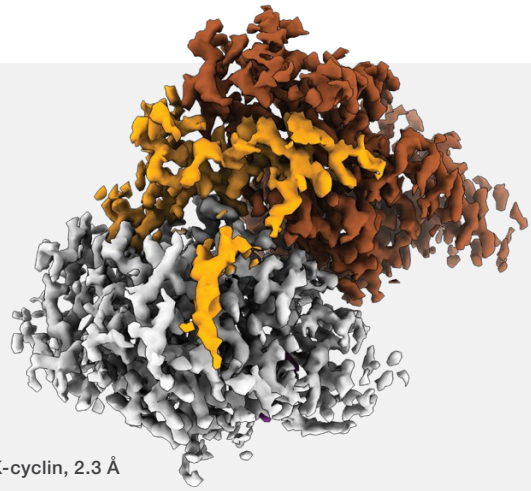
Close up of the FucA1 catalytic center without (A) and with (B) a bonded ligand. C) shows several bacterial fucosidases overlaid onto the structure of FucA1. Figure reproduced from the original article by Zachary Armstrong et al under CC BY 4.0.

Jamie Blaza, a contributor on the article, shares some of the beautiful 2D classes they obtained for FucA1.

Drug discovery at 200 kV

Structure-based drug design has been a longstanding goal of pharmaceutical research, as it enables the development of targeted treatments based on molecular insights. Efforts have largely been hampered by the speed at which actionable data can be collected and processed. Modern cryo-EM, however, has made significant strides toward structure-enabled therapeutics thanks to high-throughput, high-resolution data. For example, Prof. Basil Greber of the Institute of Cancer Research (London) has used the Glacios 2 Cryo-TEM to determine the structure of a CDK-activating kinase (CAK). These enzymes are involved in cell regulation and are actively being investigated as potential targets for cancer treatments.

Watch the on-demand webinar



CDK-cyclin, 2.3 Å

"Using the Glacios 2 Cryo-TEM, we developed a workflow that enables us to determine structures of small, asymmetric complexes at high resolution and with high throughput. Uncovering such structures provides us with detailed insight into inhibitor binding and suggests a mechanism for target selectivity in cancer therapeutics that we are currently testing."

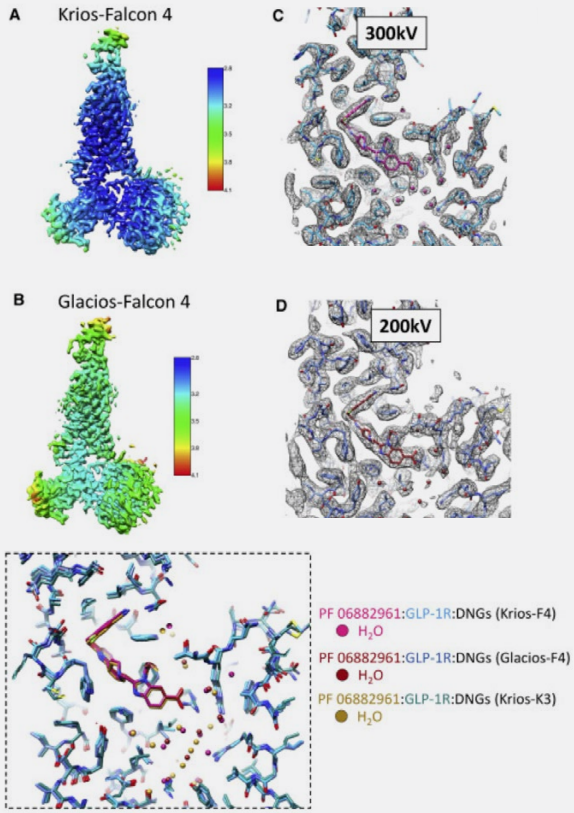
– Basil Greber, Institute of Cancer Research, London, UK

Cryo-EM structural analysis is not limited to academic investigation; the technique has already supported a number of industrial small molecule/biologics projects that are at the clinical stage or beyond.

GPCR structure determination

G protein-coupled receptors (GPCRs) compose a large class of membrane proteins that are promising therapeutic targets for a variety of diseases, but they are notoriously difficult to crystallize, hampering crystallographic characterization. Cryo-EM has made significant headway in the analysis of GPCRs in recent years, but this work has been limited to 300 kV instruments. However, researchers at Monash University have been able to successfully determine the structure of a GPCR to 3.2 Å resolution on a 200 kV Glacios Cryo-TEM, a platform whose general ease of use and increased throughput has the potential to significantly broaden structure-based drug design for GPCRs.

200 kV cryo-TEM is not limited to single particle analysis, or even cryo-EM. The following sections describe some of the ways that these instruments have been leveraged for the structural analysis of small molecules as well as in cryo-tomography.



Comparison of PF 06882961-GLP-1R-DNGs complexes imaged with 200 versus 300 kV cryo-EM

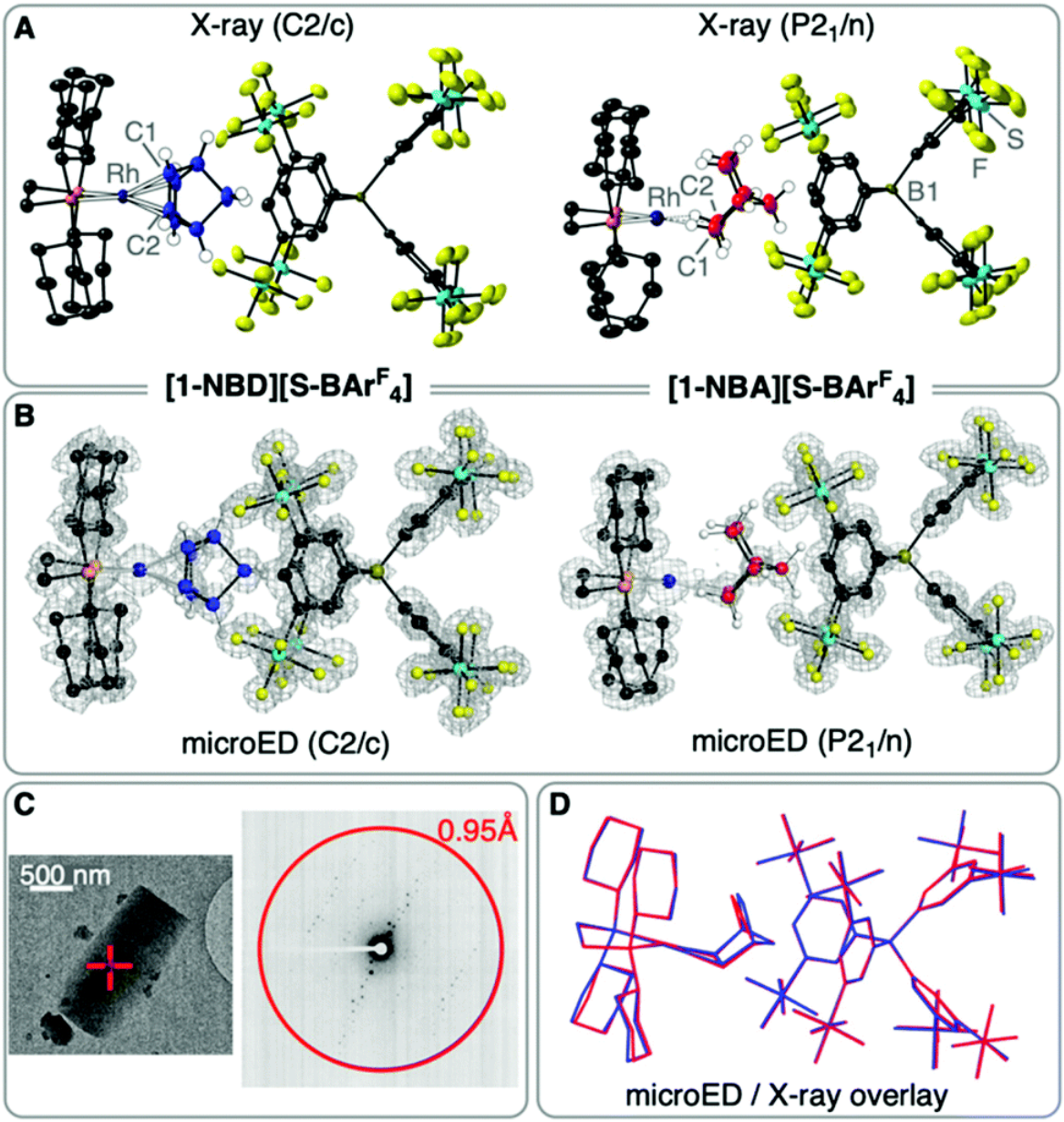
"Resolutions that can robustly support structure-assisted GPCR drug discovery can be achieved with a 200 kV cryo-EM, opening up broader application of cryo-EM within the pharmaceutical and biotechnology industries."

– Xin Zhang et al, "Evolving cryo-EM structural approaches for GPCR drug discovery"

MicroED of organometallics at 200 kV

Microcrystal electron diffraction is a burgeoning small molecule characterization technique propelled by the increased availability of high-resolution cryo-electron microscopy equipment. MicroED is capable of analyzing much smaller samples (i.e., nanocrystals) compared to X-ray diffraction, and, unlike other electron diffraction techniques, is designed to be compatible with sensitive organic samples such as protein crystals.

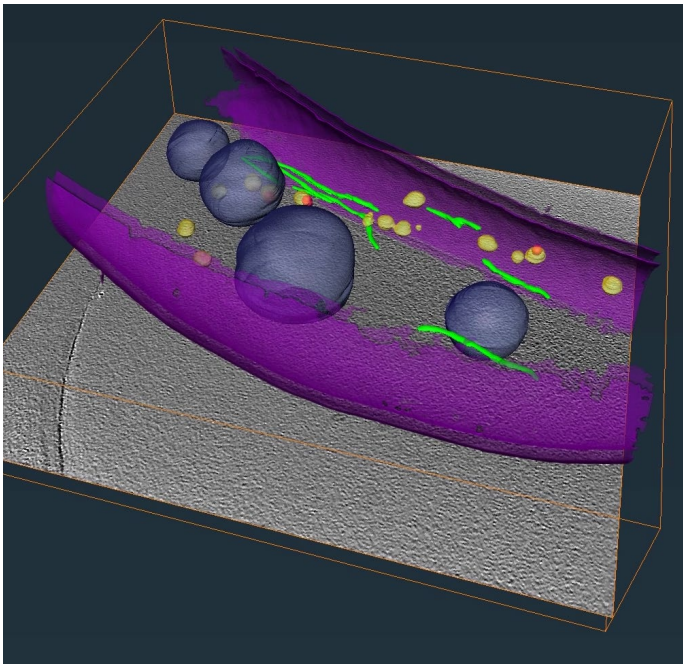
A recent study from the University of York showcases how 200 kV cryo-TEM can be used for the MicroED analysis of single-crystal organometallics. Specifically, single crystal to single crystal (SC-SC) transformations, performed on grid, can be used to generate and characterize otherwise highly reactive organometallic species. Here, nanocrystals composed of a norbornadiene complex were hydrogenated by the addition of H_2 gas into a σ-alkane (norbornane) complex. The resulting crystallographic structures were resolved down to 0.95 Å resolution. These results not only showcase the quality of MicroED data that can be generated at 200 kV, but more broadly demonstrate the potential of cryo-EM for on-grid single-crystal chemistry. Learn more



Comparison of MicroED data collected from nanocrystals (C) with traditional X-ray diffraction results obtained from larger crystals, which were much more difficult to obtain. Reproduced from the corresponding article by LR Doyle et al under CC BY 3.0.

Cryo-tomography on the Glacios 2 Cryo-TEM

Tomography is a reconstructive imaging technique that combines multiple 2D snapshots collected at different angles into a single 3D dataset. Cryo-tomography, specifically, is used on thinned cell samples to obtain high-resolution 3D information on organelles and proteins within their cellular context. Using the Glacios 2 Cryo-TEM, tomography data can be obtained from whole bacterial cells or lamellae prepared with Thermo Scientific™ Aquilos™ or Arctis™ Cryo-Focused Ion Beam (Cryo-FIB) instruments. In the Magnetospirillum example shown below, membranous compartments, filaments, and larger proteins can clearly be visualized within the 300 nm thick plunge-frozen bacterial cell.

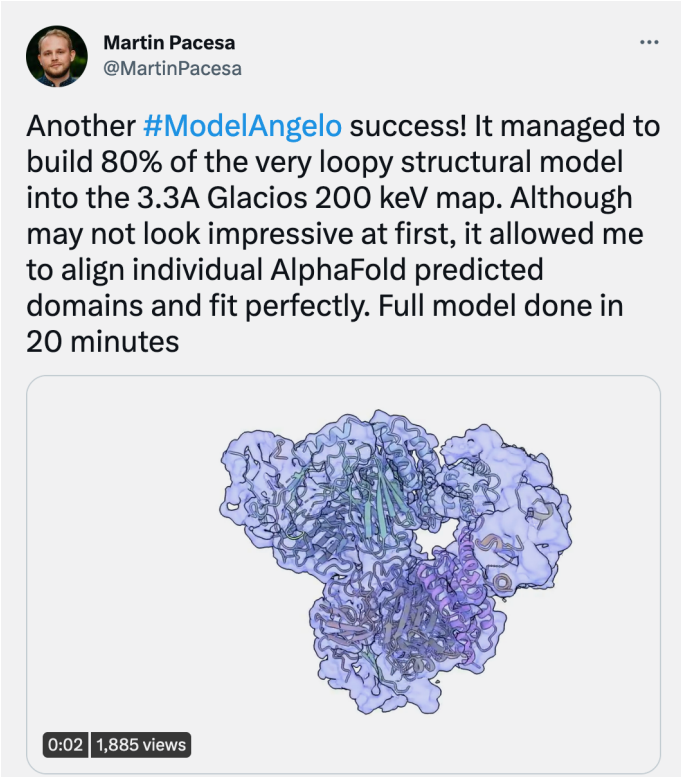


3D visualization of the cell membrane, liposomes, and filaments in a Magnetospirillum bacterium, generated using 200 kV Glacios 2 Cryo-TEM data. Image courtesy of Dirk Schüler, University of Bayreuth.

The future of 200 kV cryo-TEM

Cryo-EM software and hardware continue to improve at an astonishing pace, painting a bright future for the technique. Notably, cutting-edge data processing, along with AI-driven modeling of cryo-EM data, is showing incredible potential for taking us from sample to structure faster than ever before. For instance, researchers at the Martin Luther University of Halle-Wittenberg were able to utilize AI-assisted image analysis and data reconstruction to produce a number of *de novo* structures of highly heterogeneous metabolons.

Learn more



Martin Pacesa of EPFL shows the development of a cryo-EM reconstruction in under an hour using the novel Model Angelo modelling tool in combination with AlphaFold2. This map of a Legobody dimer (Wu et al. 2021, PNAS) was refined from 1,756 total micrographs to a 3.16 Å final map.

This article contains only a small portion of the results that have already been accomplished with the Glacios Cryo-TEM, showcasing how this instrument has evolved beyond its humble origins as screening tools for 300 kV data collection; 200 kV microscopes are now just as capable of producing actionable data for a variety of cryo-EM applications. For instance, the Glacios 2 Cryo-TEM can be found in a number of pharmaceutical labs, providing vital structural information that is guiding drug discovery for the next generation of therapeutics. We hope these exciting advancements have inspired you to see what you too can accomplish at 200 kV.

Learn more at **thermofisher.com/Glacios2**

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