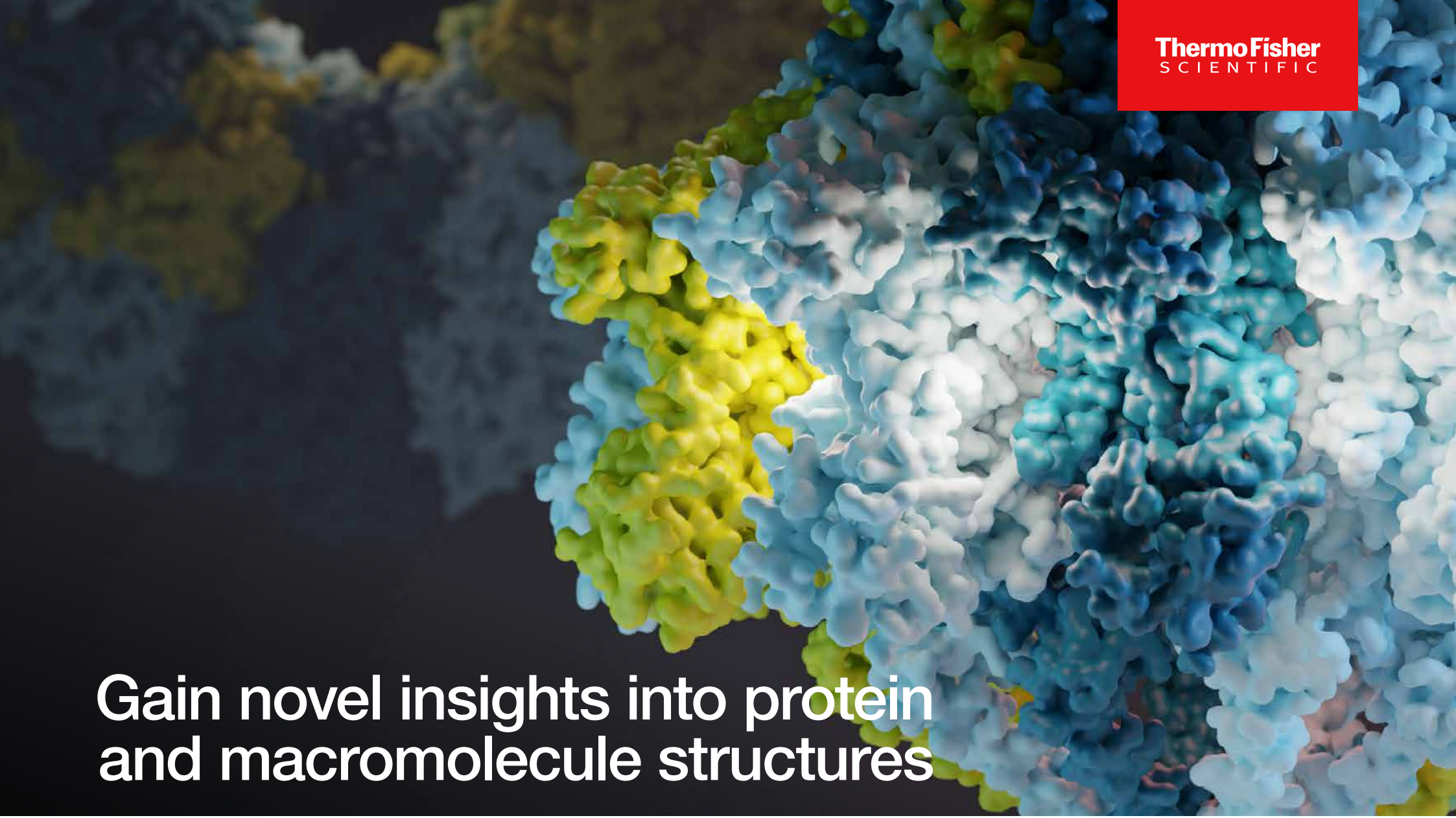

ThermoFisher SCIENTIFIC

Gain novel insights into protein and macromolecule structures

With single particle cryo-electron microscopy

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About Thermo Fisher Scientific

As the world leader in serving science, our innovative microscopy solutions and application expertise help scientists find meaningful answers that accelerate breakthrough discoveries, increase productivity, and ultimately change the world.

We develop high-end electron microscopes, with key sites located in Eindhoven (NL), Brno (CZ), and Hillsboro, Oregon (US). At these locations, R&D engineers and scientists are trained in all specialties needed to develop electron microscopes and workflows, including physics, mechatronics, electronics, software, and biochemistry. By continually expanding our capabilities and driving innovation, we are helping to advance electron microscopy for the life sciences, enabling unique biological insights, from fundamental research to drug discovery. Notably, recent advances in technology, automation, and artificial intelligence (AI), have made cryo-electron microscopes increasingly easier to use, more affordable, and accessible to the wider scientific community.

Virginia Commonwealth University's cryo-EM facility will offer a course in both cryo-EM and image processing. With the Tundra Cryo-TEM's simplified workflow, new users can be trained on the instrument in a fraction of the time needed for other cryo-EM solutions, making it an ideal training tool to help emerging scientists enhance their career opportunities. Read blog>

Learn how instruments like the Thermo Scientific Tundra Cryo-TEM broaden the accessibility of cryo-electron microscopy. **Watch demo**

Cover image:
CDK-activating protein, CAK-ICEC0942 complex.

Introduction

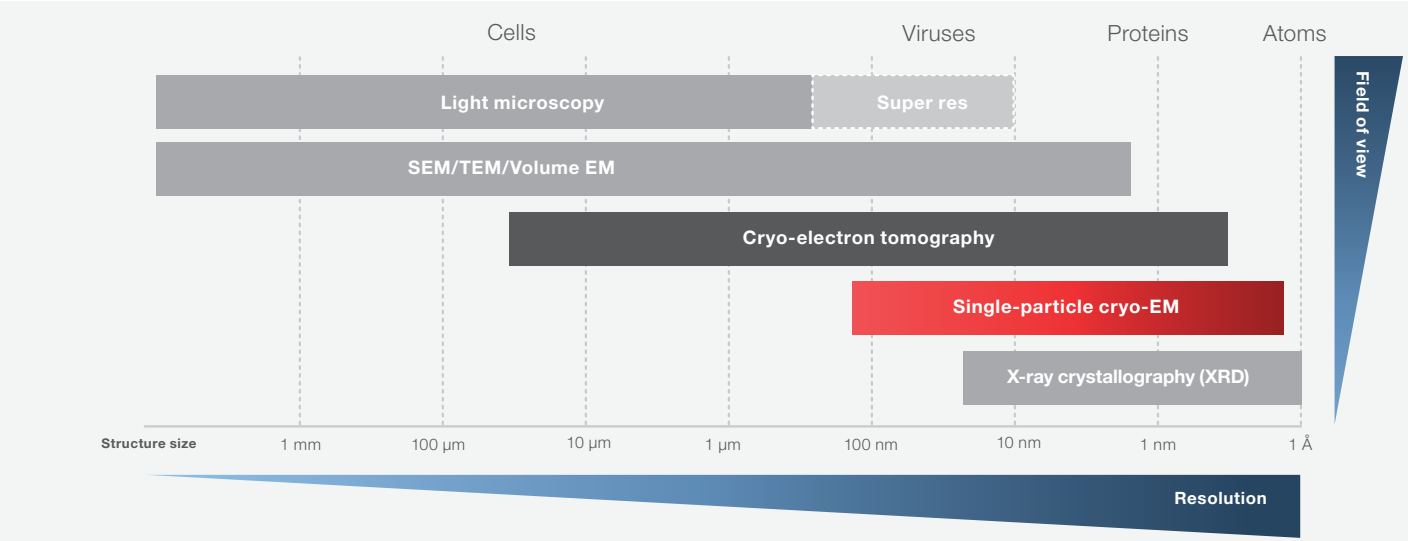
Investigating proteins and macromolecules with cryo-electron microscopy

Protein, RNA, and DNA structure is often used to predict function. X-ray crystallography (XRD) and nuclear magnetic resonance (NMR) spectroscopy are commonly used techniques for structural analysis, but each has its inherent difficulties and limitations.

Consequently, it may be necessary to derive function from indirect methods, or rely on sample manipulation to get a structure. Macromolecules can be particularly challenging for traditional structural analysis techniques, as they can have multiple conformations and form complexes, may be difficult to crystallize, or can be too large for NMR analysis.

If a protein of interest has multiple conformations, each must be trapped and/or crystallized accordingly. If the protein is part of a complex, a homogeneous version of the complex must be purified, which can be difficult. Membrane or post-translationally modified proteins typically have to be altered and cleaved into smaller fragments. Other challenges for traditional structural analysis 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. Additionally, even if the structure of a protein is known at high resolution, it may still be unclear how it relates to function inside the cell.

In recent years, single-particle analysis (at both cryogenic and ambient temperatures) has emerged as a mainstream structural biology technique for the 3D characterization of macromolecules, proteins, and protein complexes at atomic resolution. In fact, the 2017 Nobel Prize in Chemistry recognized the breakthrough developments in cryo-EM that make single particle analysis possible. Decades of dedicated work have refined the hardware, automation, and software needed to bring modern, accessible, and reliable single particle analysis to the structural biology community and beyond.



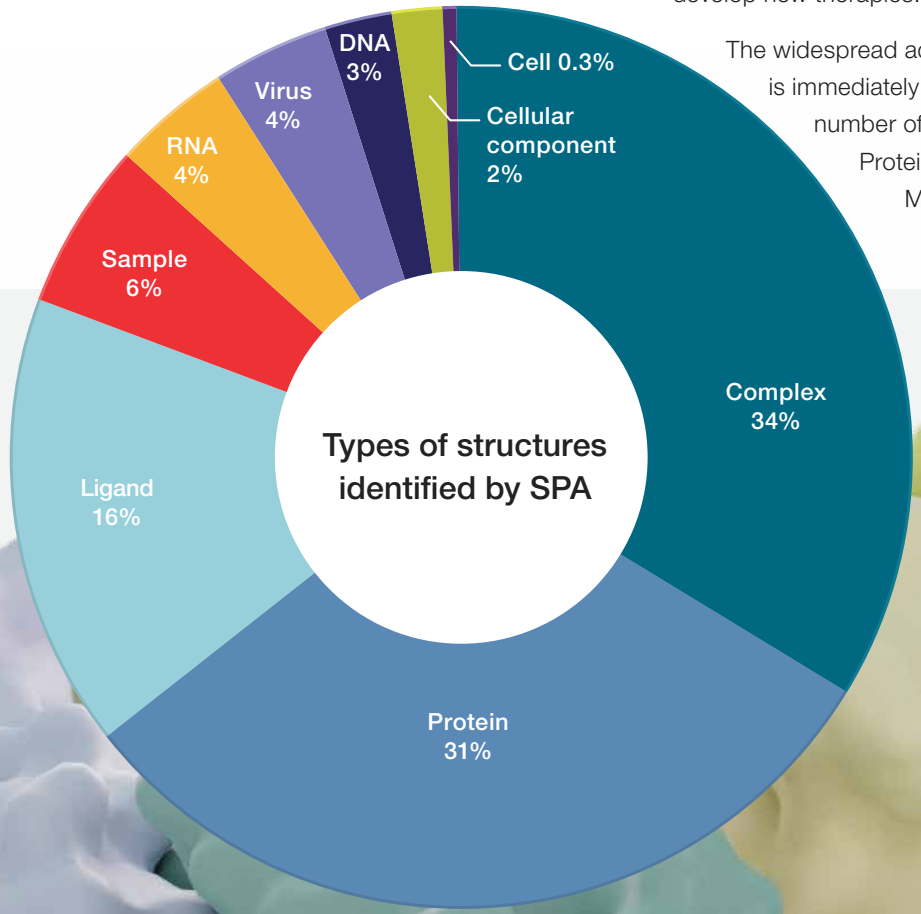
Dr. Joachim Frank, a professor of biochemistry, molecular biophysics, and biological sciences at Columbia University and a 2017 Nobel Prize winner, reflects on his research, how he uses peripheral vision to find unexpected opportunities, and his favorite TV shows.

Single particle analysis in scientific literature

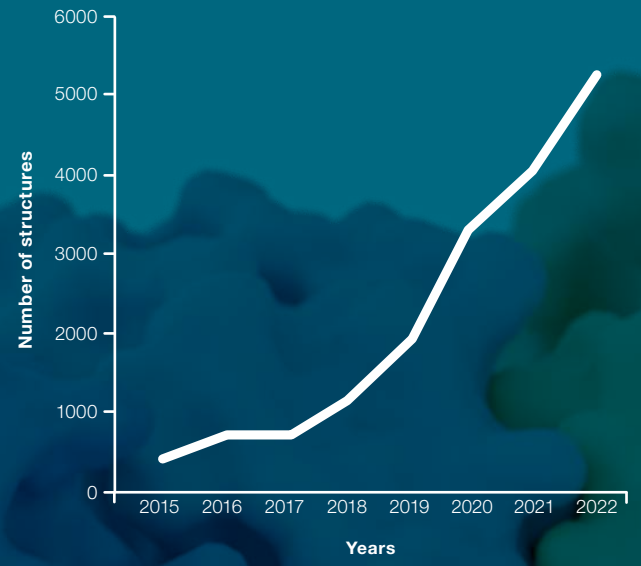
Advances in resolution, automation, and the artificial intelligence used in single particle analysis (SPA) are helping scientists gain greater insights into multiple diseases and their underlying mechanisms.

This includes a range of pressing and debilitating disorders such as Alzheimer's disease, SARS CoV-2, and respiratory syncytial virus (RSV), as well as the mechanical processes underlying the mitochondrial stress response, cancer signaling pathways, and more. As a result, cryo-EM has become an important tool for pharmaceutical and biotechnology organizations as they strive to discover and develop new therapies.

The widespread adoption of single particle analysis is immediately clear through the increasing number of cryo-EM structures found in the Protein Databank (PDB) and Electron Microscopy Databank (EMDB).



New structures identified by single particle analysis by year

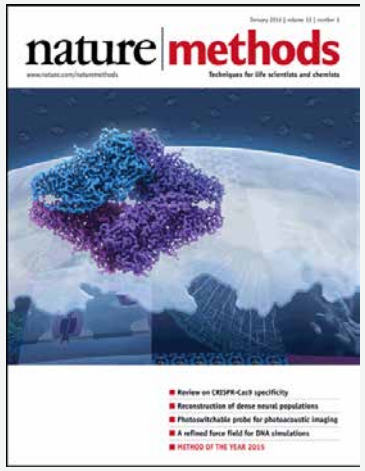


Literature reviews on single particle analysis

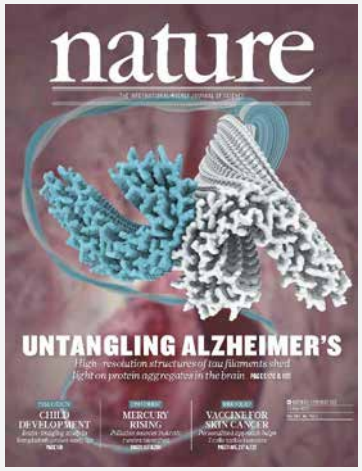
A key method for structural insights

Single particle cryo-EM has improved substantially over the years and is now routinely capable of atomic-resolution structural analysis of proteins. Since it was first cited as the **Method of the Year** in 2015, improvements in resolution, speed, and ease-of-use have only further revolutionized and democratized the technique.

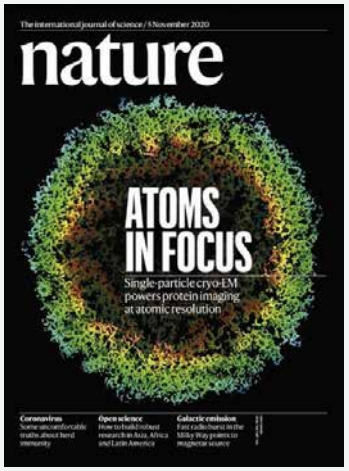
In 2017, **Fitzpatrick *et al.*** revealed the first high-resolution structures of two tau-filament types found in Alzheimer's disease, helping to pave the way for novel diagnostics and therapeutic compounds. In 2020, two groups reported high-resolution structures for apoferritin; **Nakane *et al.*** first reported the highest resolution structure at 1.22 Å while **Yip *et al.*** also achieved a comparable resolution of 1.24 Å. These results showed that the technique had become significantly more amenable to structure-based drug design. In 2021, the structural details of numerous complex, challenging proteins were identified and published, including the **mitochondrial ribosome** and a critical transcription co-activator (**PIC-Mediator**).



The End of 'blob-ology': Single-Particle Cryo-EM is Now Being Used to Solve Macromolecular Structures at High Resolution, *Nature Methods,* 13:1, 2015.



Cryo-EM structures of tau filaments from Alzheimer's disease, *Nature,* 547 p185–190, 2017.



Single-particle cryo-EM at atomic resolution, *Nature,* 587 p152–156, 2020.

Mechanism of membrane-tethered mitochondrial protein synthesis, *Science,* 371 p846–849, 2021.

Structures of +1 nucleosome–bound PIC-Mediator complex, *Science,* 378 p62-68, 2022.

Membrane proteins

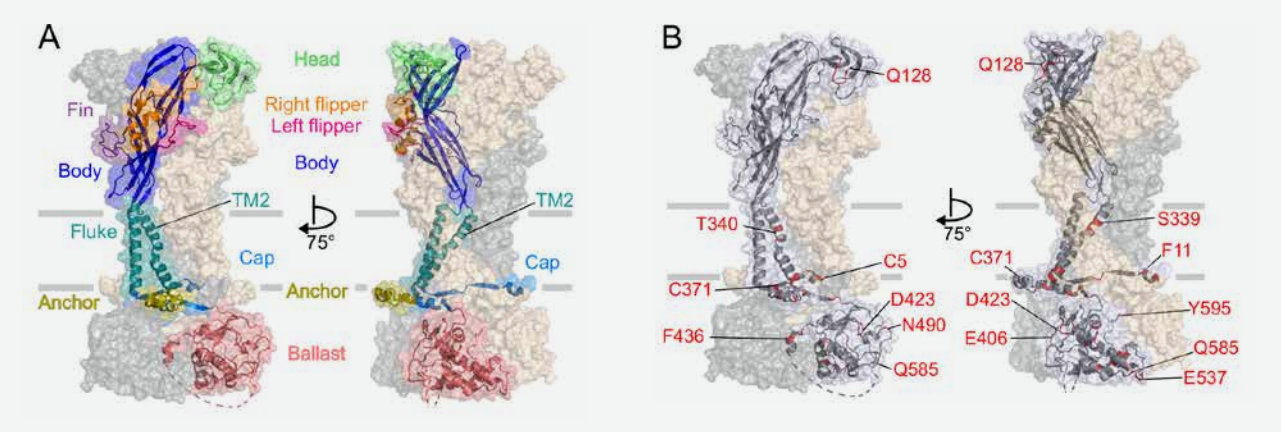
Membrane proteins play a critical role in cellular function by mediating signaling, transport, and recognition processes. Despite their importance, limited structural characterization has been possible due to their flexibility and instability outside the cell membrane.

In particular, G protein-coupled receptors (GPCRs) are a class of membrane proteins that are highly desirable drug targets, with approximately 40% of medical prescriptions targeting a member of this receptor family. Ion channel proteins are also an attractive drug target because of their roles in neuronal and cardiac diseases. Structural analysis, enabled by cryo-EM techniques, has led to breakthroughs in our understanding of membrane protein function.

Understanding the role of intracellular domains

Durner *et al.* show the conformational rearrangements of the challenging P2X7 receptor throughout various domains, with a focus on the intracellular "ballast" domain, providing strong evidence that this domain is unlikely to undergo major conformational changes upon ATP-induced gating.

Durner, A, *et al.* **Improved ANAP incorporation and VCF analysis reveal details of P2X7 current facilitation and a limited conformational interplay between ATP binding and the intracellular ballast domain.** *eLife* 12:e82479, 2023. doi.org/10.7554/eLife.82479

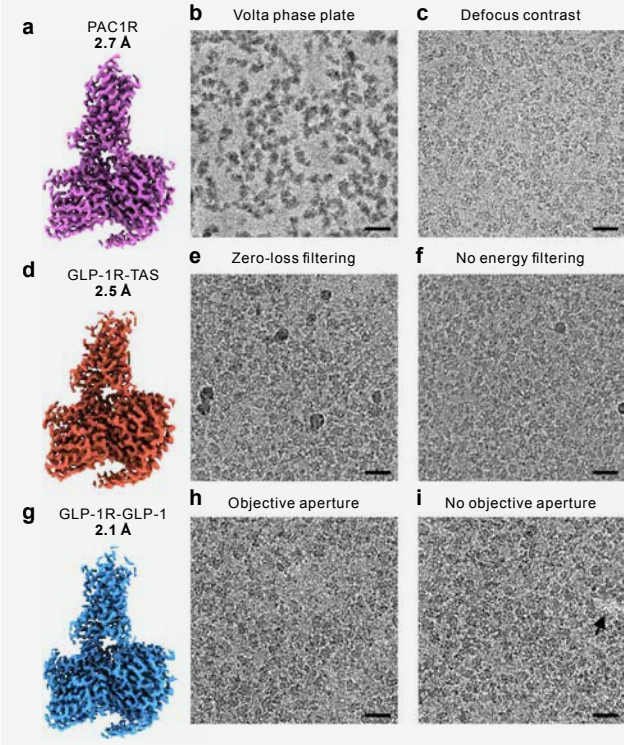


Open-state structure of P2X7 determined by Durner *et al.* with single particle analysis. (PDB ID: 6u9w) Sites of fluorescent L-3-(6-acetylnaphthalen-2-ylamino)–2-aminopropanoic acid (ANAP) substitutions are shown in B. Figure reproduced under CC BY 4.0.

Pushing the boundaries of GPCR structure determination

Danev *et al.* describe their optimization of cryo-EM parameters that enabled their lab to routinely determine the structures of small membrane proteins, such as GPCRs, at resolutions better than 2.5 Å.

Danev, R, *et al.* **Routine sub-2.5 Å cryo-EM structure determination of GPCRs.** *Nature Communications* 12:4333, 2021. doi.org/10.1038/s41467-021-24650-3



GPCR data, including 3D maps and micrographs, used in the optimization of experimental parameters by Danev *et al.* Figure reproduced under CC BY 4.0.

Cell signaling

Signaling proteins are implicated in multiple human diseases including cancer, hypertension, heart disease, diabetes, and neurodegeneration, making these pathways important targets for drug discovery and development. Two signaling proteins of interest are DELE1 and cereblon; recent structural studies with cryo-EM have provided vital insights into potential therapeutic strategies.

Obtaining insights into the mitochondrial stress response

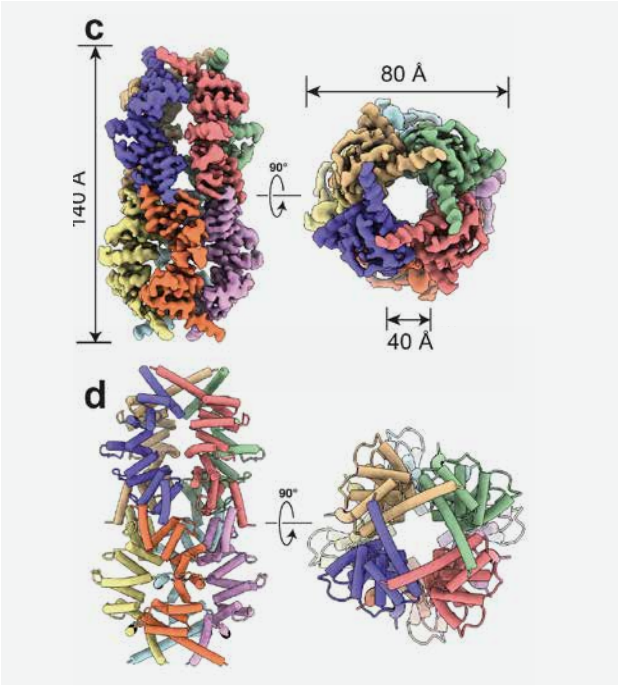
Yang *et al.* reveal the molecular description of DELE1, a key signaling factor that links mitochondrial dysfunction to the integrated stress response. DELE1 is associated with a range of human mitochondrial pathologies in cancers as well as heart and neurodegenerative diseases. Novel therapeutics that target DELE1 are being explored as potential interventions for these pathologies.

Yang, J, *et al.* **DELE1 oligomerization promotes integrated stress response activation.** *bioRxiv*, 2022. doi.org/10.1101/2022.10.01.510468

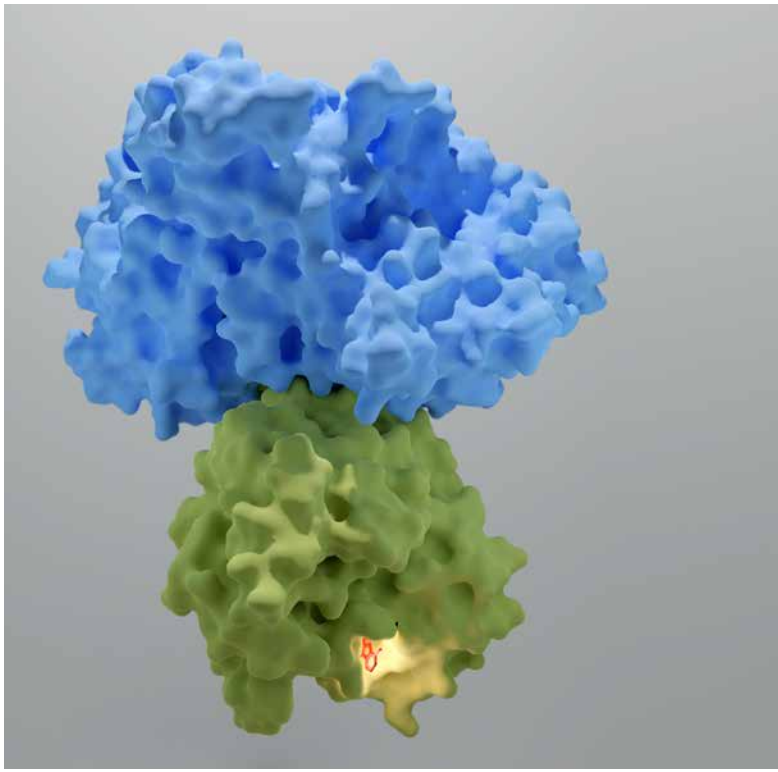
Deconvoluting protein degradation

Watson *et al.* investigate how a class of small molecules (CRBN E3 ligase modulatory drugs, or CELMoDs) alter the conformational landscape of cereblon (CRBN), the enzyme that recognizes and marks substrates for degradation. CELMoDs trigger a conformational rearrangement of CRBN from an entirely open and defunct form to an active, closed form, but only for a subset of CRBN proteins. Next-generation CELMoDs, which promote closure more efficiently, are improving future drug development.

Watson, ER, *et al.* **Molecular glue CELMoD compounds are regulators of cereblon conformation.** *Science* 378:6619, 2022. doi.org/10.1126/science.add7574



Orthogonal views of the D4-symmetric DELE1 oligomer determined with C) cryo-EM and D) atomic modeling. Figure reproduced under CC BY-ND 4.0.



Cereblon-DDB1 bound to Pomalidomide. PDB ID: 8D81.

In this webinar, Dr. Jie Yang discusses the role of DELE1 in the mitochondrial stress response. **Watch now**

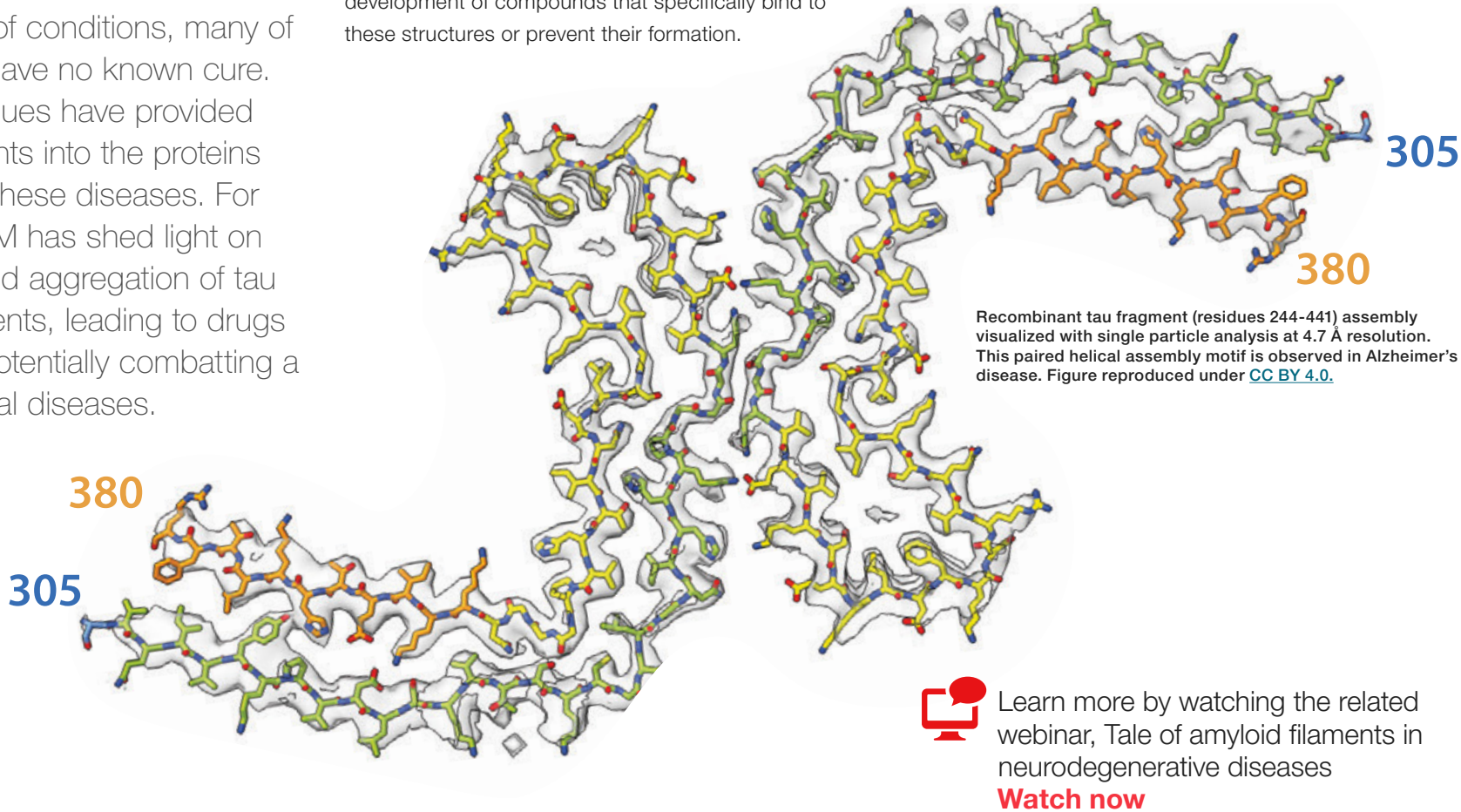
Neuroscience

Neurodegenerative diseases encompass a broad range of disorders involving progressive damage to cells and connections in the nervous system, impacting cognition, mobility, and more. These diseases are linked to a wide range of conditions, many of which currently have no known cure. Cryo-EM techniques have provided critical new insights into the proteins associated with these diseases. For instance, cryo-EM has shed light on the misfolding and aggregation of tau protein into filaments, leading to drugs that target tau, potentially combatting a variety of neuronal diseases.

Revealing distinct tau folds to characterize different diseases

Lövestam *et al.* report 76 cryo-EM structures of recombinant tau filaments assembled *in vitro*. Understanding the assembly of tau structures into disease-relevant filaments will facilitate studies that determine their roles in different diseases, as well as the development of compounds that specifically bind to these structures or prevent their formation.

Lövestam, S, *et al.* **Assembly of recombinant tau into filaments identical to those of Alzheimer's disease and chronic traumatic encephalopathy.** *eLife* 11:e76494, 2022. doi.org/10.7554/eLife.76494



Recombinant tau fragment (residues 244-441) assembly visualized with single particle analysis at 4.7 Å resolution. This paired helical assembly motif is observed in Alzheimer's disease. Figure reproduced under CC BY 4.0.

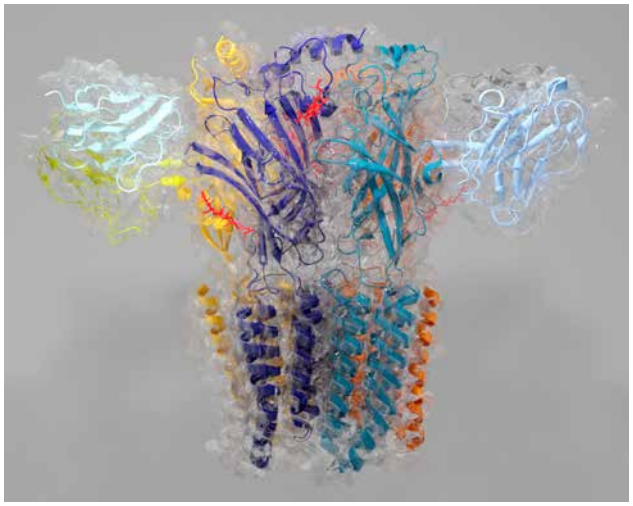
Learn more by watching the related webinar, Tale of amyloid filaments in neurodegenerative diseases
Watch now

Alzheimer's disease associated TMEM106B filaments. PDB ID: 7QVC.

Solving the structure of a novel protein linked to neurodegeneration

Chang *et al.* showed that a previously unsolved amyloid fibril is composed of TMEM106B, a transmembrane lysosomal/endosomal protein. These fibrils are prevalent in several human neurodegenerative diseases, including those characterized by abnormal aggregation of TDP-43, tau, or α-synuclein protein. TMEM106B fibrils are common to a broad range of debilitating human disorders, indicating a shared fibrillization pathway that may initiate or accelerate neurodegeneration.

Chang, A, *et al.* **Homotypic fibrillization of TMEM106B across diverse neurodegenerative diseases.** *Cell* 185:8, 2022. doi.org/10.1016/j.cell.2022.02.026

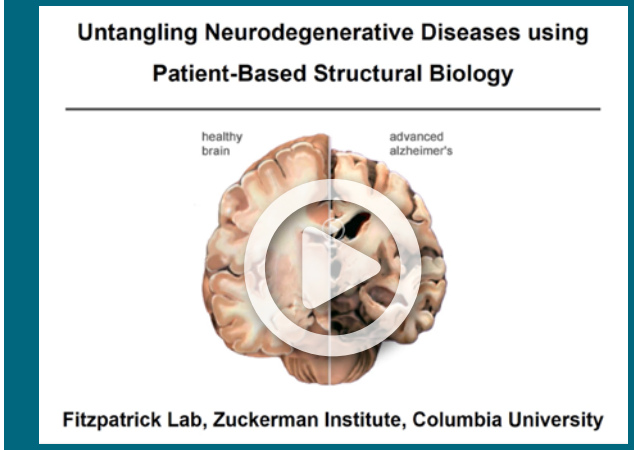


GABAA receptor in complex with a Fab115 antibody. PDB ID: 7T0W.

Revealing the mechanisms underlying encephalitis

Noviello *et al.* identified the structures of antibodies bound to the γ-aminobutyric acid type A (GABAA) receptor of patients with encephalitis. These structural insights help to reveal the mechanisms of GABAA inhibition and the pathology underlying autoimmune encephalitis.

Noviello, MN, *et al.* **Structural mechanisms of GABA$_A$ receptor autoimmune encephalitis.** *Cell* 185:14, 2022. doi.org/10.1016/j.cell.2022.06.025



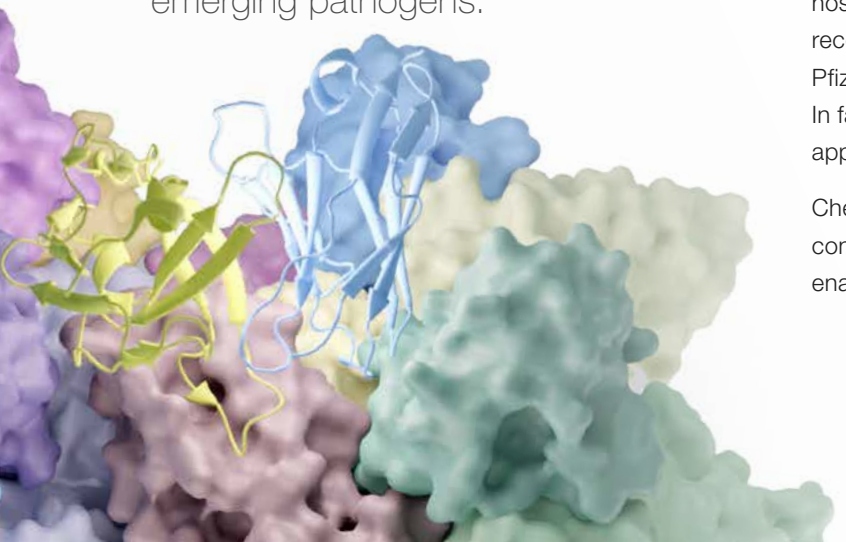
Learn more by watching the related webinar, "Untangling neurodegenerative diseases using cryo-electron microscopy"
Prof. Anthony Fitzpatrick, Columbia University, discusses how cryo-EM is used to solve the structure of amyloid fibrils from a range of neurological disorders and elucidates the molecular/structural basis of neurodegeneration.

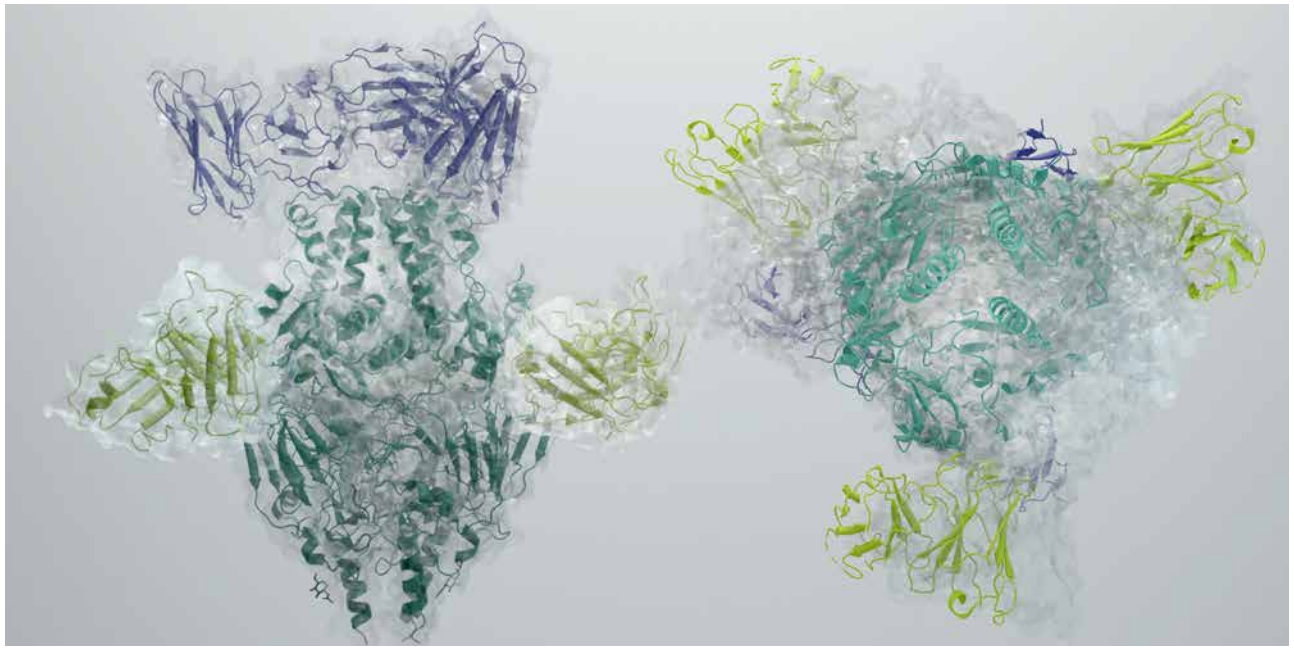
Untangling Neurodegeneration

Cryo-EM is being 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 by downloading our Neurodegeneration eBook.

Virology

In the last decades, numerous viruses have emerged with significant impacts on human health and society. In the wake of these outbreaks (such as HIV, Ebola, Zika, SARS-CoV-2, and mpox) there has been an increased interest in quick, molecular-level analysis of virus behavior in order to guide the rapid development of effective treatments. Cryo-EM has increasingly played a critical role in the characterization of viruses and the corresponding host immune response. These insights have helped to identify and develop therapeutics and vaccines, revolutionizing anti-viral drug discovery and vaccine design for newly emerging pathogens.





Structure of Human RSV F variant (construct pXCS847A), as described by Che *et al.* PDB ID: 7UJA

Identifying an effective vaccine to combat RSV

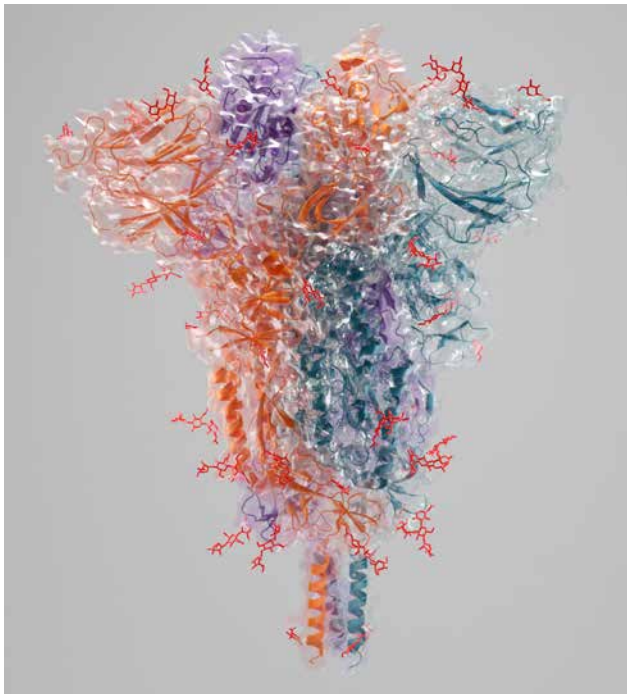
Respiratory syncytial virus (RSV) is a common respiratory virus where at-risk populations such as infants and older adults can develop severe RSV infections that require hospitalization. Promising vaccine candidates have recently been developed by GlaxoSmithKline (GSK) and Pfizer with the aid of cryo-EM and rational drug design. In fact, the very first vaccine by GSK has recently been approved by the Food and Drug Administration.

Che *et al.* describe the development of RSV prefusion F constructs for the optimization of vaccine immunogenicity, enabling enhanced protective immunity against RSV.

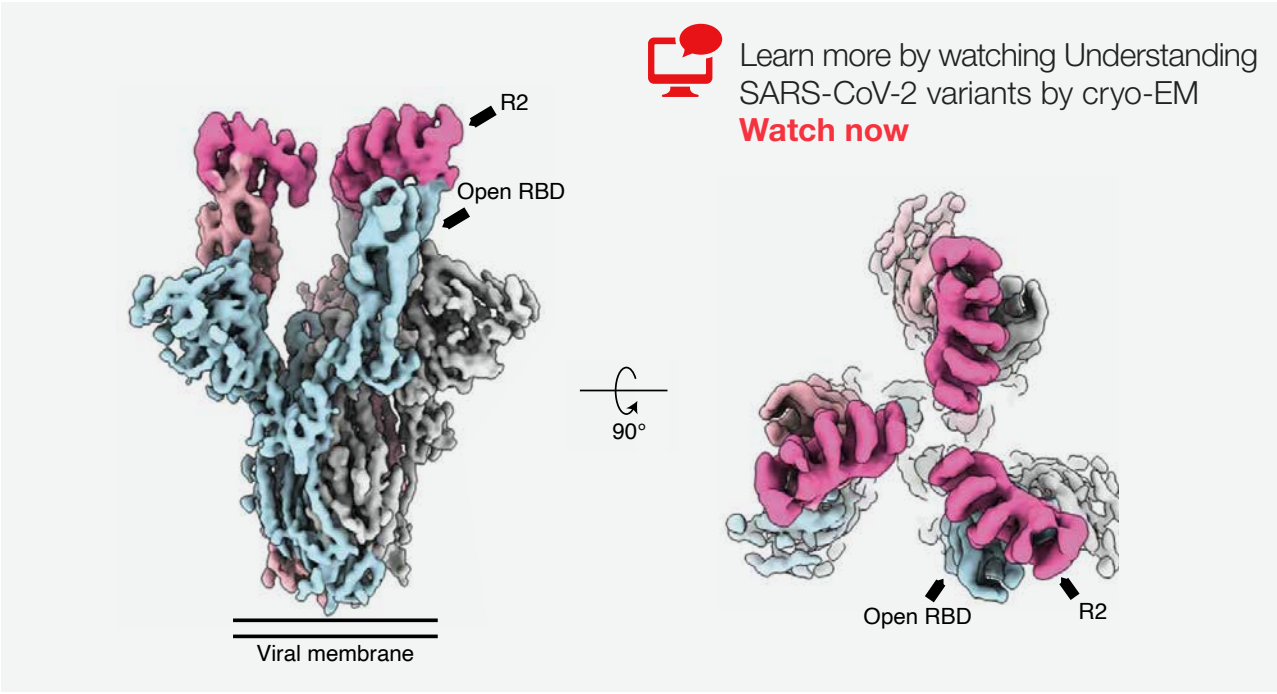
Harshbarger *et al.* used detailed maps of the AM14 epitope on DS-Cav1 to gain a comprehensive understanding of RSV F trimer specificity that impacted both vaccine design and the quality assessment of PreF-based immunogens.

Che, Y, *et al.* **Rational design of a highly immunogenic prefusion-stabilized F glycoprotein antigen for a respiratory syncytial virus vaccine.** *Science Translational Medicine* 15: 693, 2023. doi/10.1126/scitranslmed.ade6422

Harshbarger W. *et al.* **Improved epitope resolution of the prefusion trimer-specific antibody AM14 bound to the RSV F glycoprotein.** *MAbs* 13(1) doi: 10.1080/19420862.2021.1955812



SARS CoV-2 spike protein structure with single residue substitution at D614G. PDB ID: 7KRQ.



Orthogonal views of a SARS-CoV-2 spike protein with an open receptor binding domain (RBD). Figure reproduced under CC BY 4.0.

Powerful insights in the battle against SARS-CoV-2

Structural biology has contributed to our understanding of SARS-CoV-2 since the beginning of the COVID-19 pandemic, providing critical information on the viral spike protein. In this study by Jun Zhang and Bing Chen, several structures of monoclonal neutralizing antibodies in complex with the spike protein were determined. This provided a broader mechanistic understanding of the neutralization mechanisms of spike antibodies, guiding the development of future therapeutics.

Zhang, J, and Chen, B. **Fighting SARS-CoV-2 with structural biology methods.** *Nat Methods* 19 p381–383, 2022. doi.org/10.1038/s41592-022-01448-9

Inhibiting diverse SARS-CoV-2 variants

A continuing area of concern is SARS-CoV-2 variants that evade conventional treatment and vaccination. Rothenberger *et al.* used single particle analysis to demonstrate how a clinical candidate antiviral (DARPin) can bind the spike protein of a range of SARS-CoV-2 variants, successfully inhibiting infection.

Rothenberger, S, *et al.* **The trispecific DARPin ensovibep inhibits diverse SARS-CoV-2 variants.** *Nat Biotechnol* 40 p1845–1854, 2022. doi.org/10.1038/s41587-022-01382-3

The Rise of Structural Virology

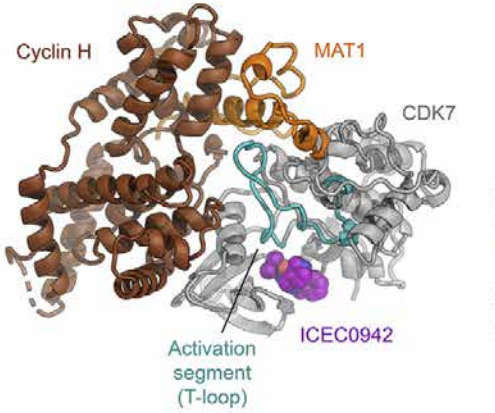
Learn more about the use of single particle analysis in virology, including antiviral drug discovery and vaccine design, by downloading our Virology eBook

Cancer research

Cancer encompasses a wide range of diseases that are mediated by multiple protein signaling pathways. While it is known that genetic mutations can alter how these pathways function, the precise mechanisms and potential countermeasures (i.e., therapeutic targets) are not always clear. Using single particle analysis, it is possible to characterize how mutations affect protein conformations and structures, and subsequently, how these changes impact signaling and function.

Uncovering the structural basis of inhibitor selectivity

CDK-activating kinase (CAK) has been identified as a promising target for cancer chemotherapy; structural information has the potential to improve drug development, reducing treatment side effects. Greber *et al.* determine the structures of human CAK bound to several nucleotide analogues and inhibitors. With cryo-EM, this could be accomplished without protein crystallization, providing detailed insight into inhibitor binding and a potential mechanism for target selectivity.



Structure of the human CAK-ICEC0942 complex determined with single particle analysis. Figure reproduced under CC BY 4.0.

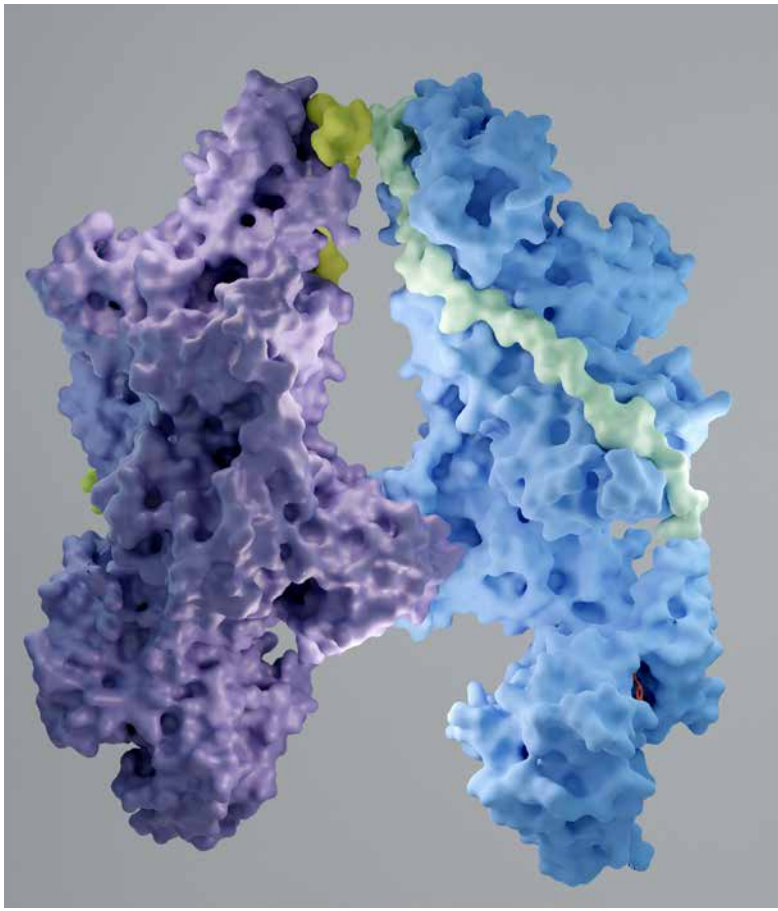
Cushing, VI, *et al.* **High-resolution cryo-electron microscopy of the human CDK-activating kinase for structure-based drug design** *bioRxiv,* 2023. doi.org/10.1101/2023.04.07.536029

Basil Greber, Eva Nogales *et al.* **2.5 Å-resolution structure of human CDK-activating kinase bound to the clinical inhibitor ICEC0942.** *Biophysical Journal* 120:4, 2021. doi.org/10.1016/j.bpj.2020.12.030

Understanding the activation of JAK

Glassman *et al.* report the cryo-EM structure of a full-length Janus kinase (JAK), which is an essential component of cytokine signaling. Mutations to this kinase have led to immunodeficiency and myeloproliferative disorders. Single particle analysis provided insights into how a range of disease mutations impact the function of JAKs.

Glassman, CK, *et al.* **Structure of a Janus kinase cytokine receptor complex reveals the basis for dimeric activation.** *Science* 376:6589, 2022. doi.org/10.1126/science.abn8933



Structure of active Janus Kinase (JAK) dimer complexed with cytokine receptor intracellular domain. PBD ID: 7T6F

Understanding the Complexity of Cancer with Cryo-EM

Structural insights can help deconvolute the conditions that lead to cancer-cell growth and identify new ways to treat these debilitating diseases. Learn how cryo-EM is unraveling the molecular drivers of cancer and revolutionizing cancer research in our Cancer research eBook

Agriculture

Structural biology is an important tool for plant research, as molecular insights can lead to improvements in agricultural or bioenergy techniques, producing crops that are more efficient and have higher yields of food or energy.

Exploring cellular respiration in mung beans

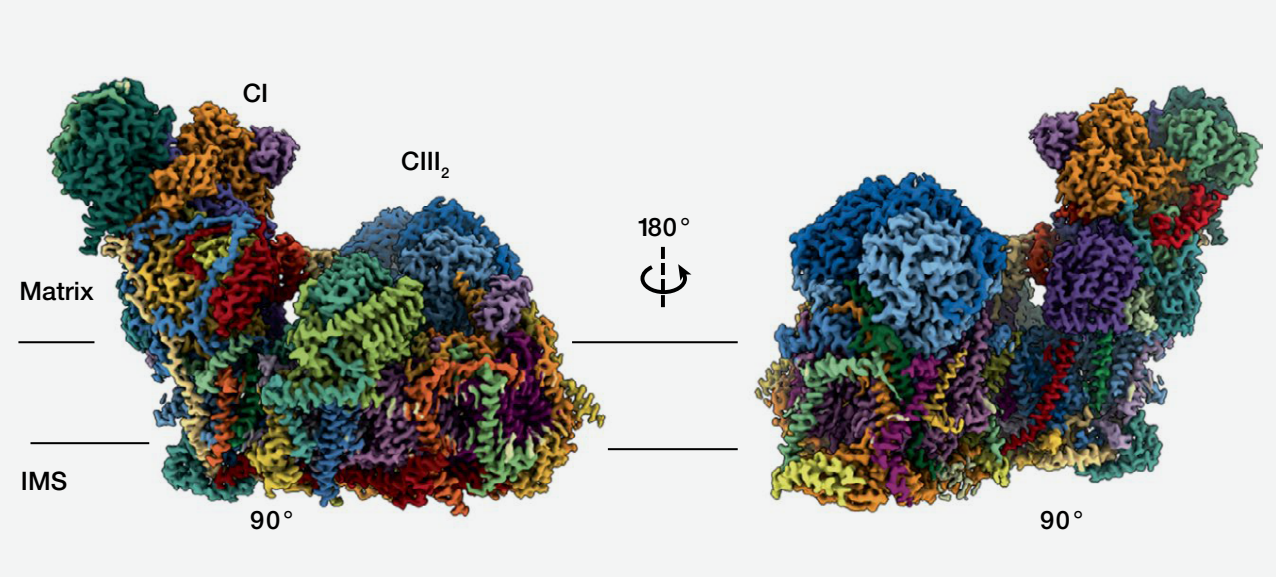
Oxidative phosphorylation (OXPHOS) is the final step in the cellular respiration of plants, and is carried out by protein complexes in the inner mitochondrial membrane. Maldonado *et al.* utilize cryo-EM structures to deconvolute the physiological function of a supercomplex involved in the OXPHOS electron transport chain. This structural analysis provides critical details toward a greater understanding of cellular respiration in plants.

Maldonado, M, *et al.* **Plant-specific features of respiratory supercomplex I + III2 from *Vigna radiata*.** *Nat. Plants* 9 p157–168, 2023. doi.org/10.1038/s41477-022-01306-8

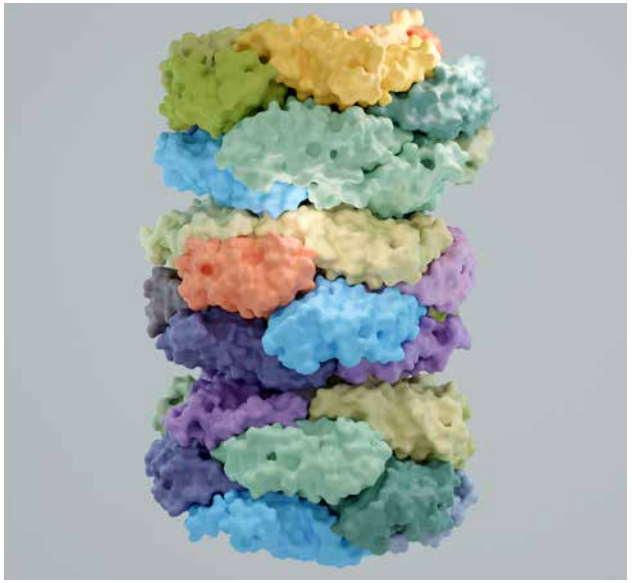
Illuminating light-harvesting mechanisms in cyanobacterium

Domínguez-Martín *et al.* provide detailed insights into the biophysical underpinnings of cyanobacterial light harvesting. The results can help drive further bioengineering of phycobilisome (PBS) protein complexes in natural and artificial light-harvesting systems.

Domínguez-Martín, MA, *et al.* **Structures of a phycobilisome in light-harvesting and photoprotected states.** *Nature* 609 p835–84, 2022. doi.org/10.1038/s41586-022-05156-4



Cryo-EM density map of the I + III2 oxidative phosphorylation supercomplex, taken from mung beans (*Vigna radiata*). Figure reproduced under CC BY 4.0.

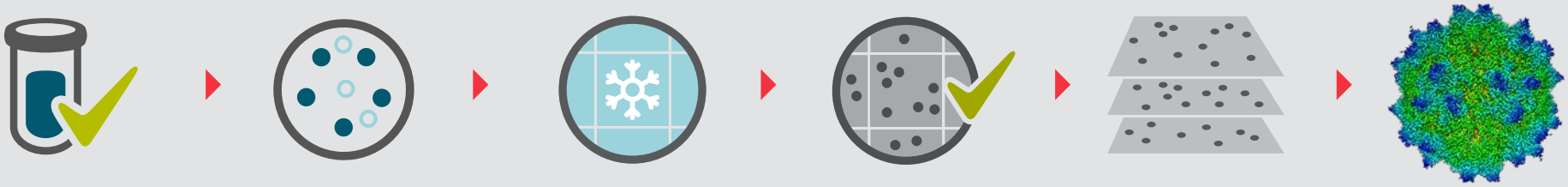


Phycobilisome (PBS) protein complex determined with cryo-EM. PDB ID: 7SC8.

Appendix

The single particle analysis workflow

Single particle analysis enables the near-atomic structural determination of challenging proteins and protein complexes, without the need for crystallization. Samples can be studied directly in solution, thereby keeping the protein structures closer to their native conformations. High-quality data collection is now more accessible than ever before, thanks to recent advances in sample preparation and data processing. What follows is a general description of the single particle analysis workflow, taking you from your sample of interest to its high-resolution structure.



Sample preparation

The quality of structural analysis is directly related to sample preparation: purified, homogeneous, and biochemically active proteins/macromolecules in a stable buffer typically provide the best results.

Negative-stain screening

Negative-stain electron microscopy is an easy and cost-effective method for the quality assessment of purified biological specimens at room temperature. This screening allows you to qualitatively assess particle composition and conformational homogeneity, which can only be done at the microscopic scale.

Vitrification

Once sample purity has been verified, the sample is vitrified (i.e., rapidly frozen to suspend the specimens in a layer of amorphous (vitreous) ice). 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.

Cryo-EM grid screening

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

Data acquisition

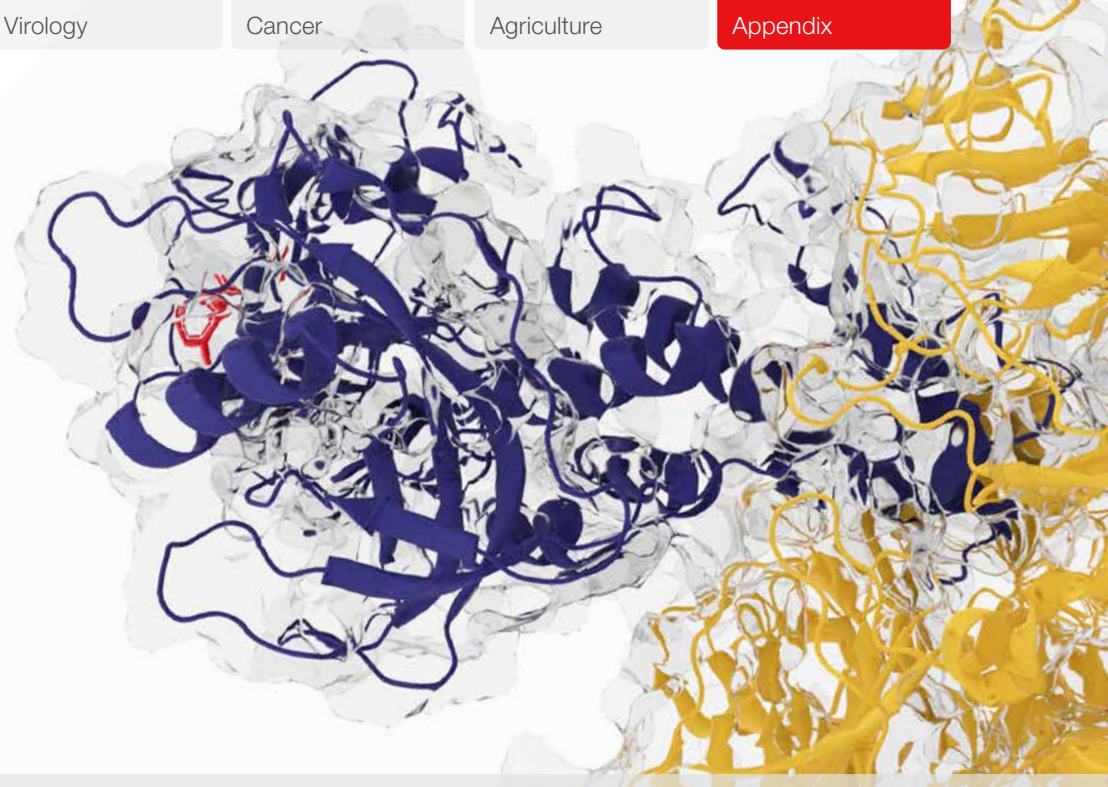
Data collection consists of high-resolution imaging with a specifically designed cryo-TEM. With advances in data collection software, individual particles can be automatically identified in the TEM image and grouped according to particle orientation. For every sample, imaging and identification can be simplified by robust, reliable automation.

Structure visualization

Once sufficient particle data is collected (ideally representing the sample from as many different orientations as possible), it can be recombined into a 3D representation of the protein/macromolecule. 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.

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 options for financing, leasing, lease-to-own, 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™ 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**

Additional resources

Curious to know what else is possible with single particle analysis? Below is a selection of additional articles and webinars that showcase the power of this revolutionary technique.

Learn more about scientists like Dr. Eva Nogales who are behind cutting edge cryo-EM research with our Cryo-Talk series. **Watch now**

Cancer

Nadezhdin KD, *et al.* **Structural mechanisms of TRPM7 activation and inhibition.** *Nature Communications* 14(1):2639, 2023. doi: 10.1038/s41467-023-38362-3

Metabolic diseases

Cho, YK, *et al.* **Adipocyte lysoplasmalogenase TMEM86A regulates plasmalogen homeostasis and protein kinase A-dependent energy metabolism.** *Nat Communications* 13:4084, 2022. doi.org/10.1038/s41467-022-31805-3

Membrane protein

Huang, W, *et al.* **Structure of the neurotensin receptor 1 in complex with β-arrestin 1.** *Nature* 579 p303–308, 2020. doi.org/10.1038/s41586-020-1953-1

Learn more by watching this on-demand webinar and other drug discovery talks **Watch now**

Neuroscience

Stern, A, *et al.* 2022. **Abundant Aβ fibrils in ultracentrifugal supernatants of aqueous extracts from Alzheimer's disease brains.** *bioRxiv pre-print*, 2022. doi.org/10.1101/2022.10.18.512754

Yang, Y, *et al.* **Cryo-EM structures of amyloid-β filaments with the Arctic mutation (E22G) from human and mouse brains.** *Acta Neuropathol* 145 p325–333, 2023. doi.org/10.1007/s00401-022-02533-1

Virology

Mukhamedova, M, *et al.* **Vaccination with prefusionstabilized respiratory syncytial virus fusion protein induces genetically and antigenically diverse antibody responses.** *Immunity* 54:4, 2021. doi.org/10.1016/j.immuni.2021.03.004

Malhi, H, *et al.* **Immunization with a self-assembling nanoparticle vaccine displaying EBV gH/gL protects humanized mice against lethal viral challenge.** *Cell Reports Medicine* 3:6, 2022. doi.org/10.1016/j.xcrm.2022.100658

Sun, C, *et al.* **Induction of Broadly Cross-Reactive Antibody Responses to SARS-CoV-2 Variants by S1 Nanoparticle Vaccines.** *Journal of Virology* 96:13, 2022. doi.org/10.1128/jvi.00383-22

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

Explore our integrated solutions and support for the entire single particle analysis workflow, from sample preparation to data analysis.

Optimizing your sample preparation

Thermo Scientific™ VitroEase™ products are specifically designed to facilitate cryo-EM sample preparation, helping you optimize your samples and prepare grids that will yield high-resolution cryo-EM structures. You can optimize samples with the **VitroEase Buffer Screening Kit** and use the **VitroEase Apoferritin Standard** to validate workflows. The **VitroEase Cryo-EM Training Kit** facilitates training for the next generation of cryo-EM users.

The **VitroEase Methylamine Vanadate** and **Methylamine Tungstate Negative Stains** are ready-to-use solutions that are designed to help you prepare samples for negative stain assessment. Often, this is done on a simple side-entry microscope (e.g., a **Thermo Scientific Talos™ L120C TEM**), since screening is usually done one grid at a time, and the actual time spent on the microscope is short.

Simplifying the vitrification process

The entire vitrification procedure can be simplified using semi-automated plungers such as the **Thermo Scientific Vitrobot System**, which can be combined with the **VitroEase Cryo-EM Training Kit** to help anyone learn the intricacies of the vitrification process.

Cryo-transmission electron microscopes

Thermo Fisher Scientific offers a range of cryo-electron microscopy instruments suited to a variety of analytical needs. With the Thermo Scientific Tundra™ Cryo-TEM, you can expand the possibilities of your biochemical research without prior microscopy experience and at a more affordable price point. This offers your laboratory a cost-effective, easier-to-use cryo-EM solution optimized for single particle analysis. The Thermo Scientific Glacios™ 2 and Krios™ G4 Cryo-TEMs are capable of producing higher resolution results and have the ability to perform additional cryo-EM methods such as MicroED and cryo-electron tomography.

In addition, our solution for 60°C heat decontamination allows the Krios Cryo-TEM to be installed in higher biosafety-level containment facilities (e.g. BSL-3).

Tundra Cryo-TEM: accessible & smart

- Fully automated and requires minimal expertise to use
- Cost-effective platform for labs that are new to cryo-EM
- Ideal for sample optimization for analysis on higher resolution platforms

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

Glacios 2 Cryo-TEM: powerful & versatile

- Automated sample assessment and acquisition of large data sets for higher throughput
- Improved detector and AI-enabled software work together to provide rapid, high-quality results

High-resolution SPA	200 kV, <2.5 Å*
High throughput	dataset in 30 minutes
Sample type	proteins, crystals, cells, macromolecules
Applications	SPA, MicroED, tomography

Krios G4 Cryo-TEM: Unparallel performance

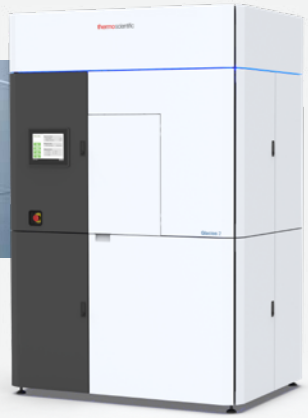
- Designed for true atomic-resolution cryo-EM and speed
- Highest level of automation from sample vitrification to data analysis

Ultra-high-resolution SPA	300 kV, <1.5 Å*
Highest throughput	dataset in minutes
Sample type	proteins, crystals, cells, macromolecules
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.

See a full demo of the
Tundra Cryo-TEM in action

Watch to learn more about the
Glacios 2 Cryo-TEM



Watch to learn more about the
Krios G4 Cryo-TEM

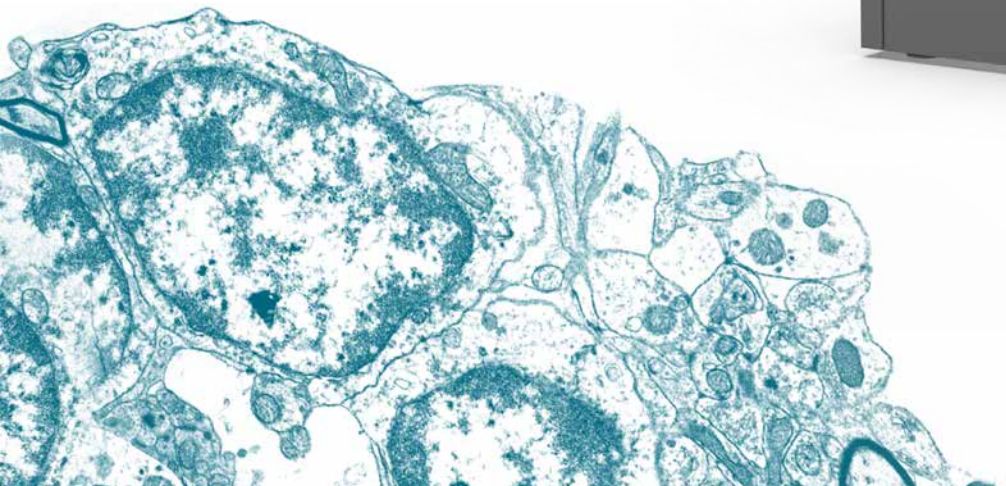
A multidisciplinary tool to visualize biological samples and more

The Thermo Scientific Talos L120C G2 (S)TEM can help you visualize sections of resin-embedded cells and tissues, isolated particles of protein complexes and viral assemblies, as well as a wide variety of other materials, down to sub-nanometer resolutions.

The flexible base configuration allows you to adapt the system to your needs. It can be configured for multiple applications and different imaging and detection techniques. With continual system and software upgrades, you can even implement new applications, access new technologies, and improve ease of use with new features and better automation.

"The Talos L120C is installed in the regular biochemistry lab next to other sample preparation equipment. Users can prepare samples there and directly load them in the microscope. We have found a good correlation between samples imaged in negative stain EM and in cryo-EM. The microscope is also a good training tool for new users as the working and user interface are very similar to the Krios G3 in the lab."

Dr. Dennis Thomas
Facility Manager, CSHL, Cold Spring Harbor, NY



Learn more at **thermofisher.com/Talos-L120C-TEM**

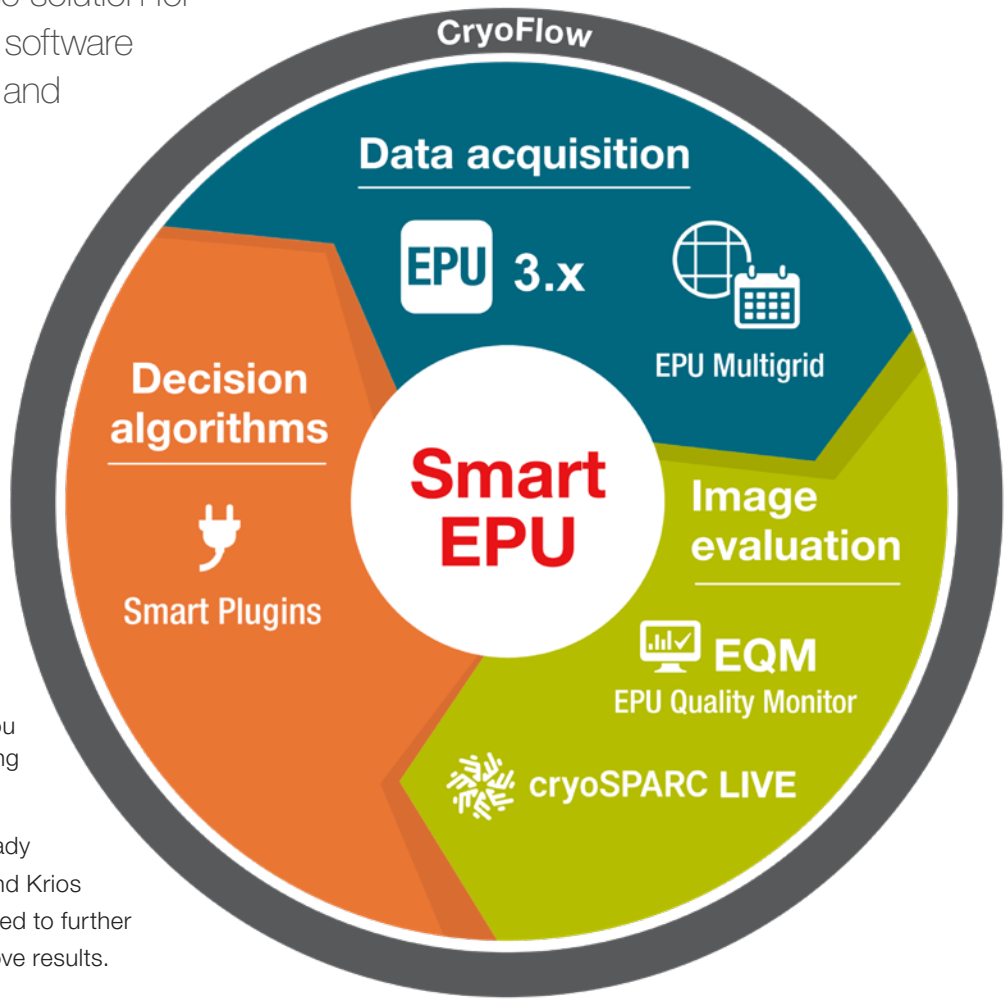
Work more efficiently with Smart EPU Software

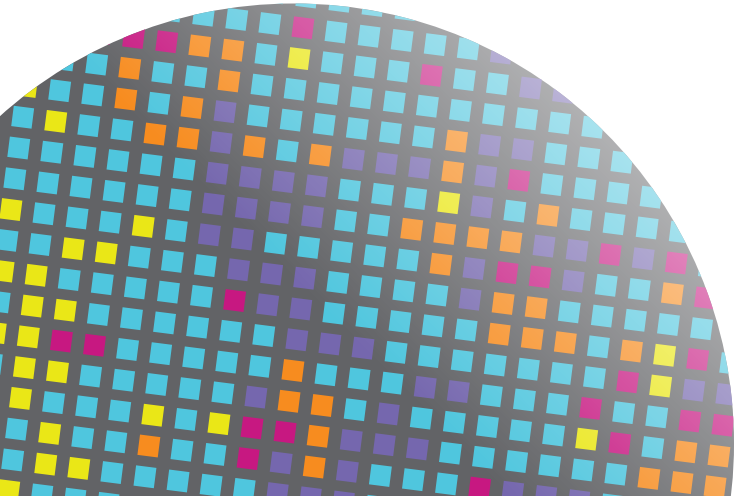
Thermo Scientific Smart EPU Software is an innovative and easy-to-use solution for streamlined and accurate single particle analysis data acquisition. This software suite combines automation and user guidance to increase throughput and deliver reproducible results. With Smart EPU Software, you can make efficient use of the microscope and focus more on your research.

Smart EPU Software is comprised of interactive components that automate multiple image acquisition steps, as well as AI-driven technology that helps you make decisions, including:

- The user-friendly **EPU 3 Interface**, which helps you set up experiments quickly and easily, regardless of experience level
- **EPU Multigrid**, which sets up a queue of automated acquisitions across multiple grids to maximize efficiency
- **EPU Quality Monitor** analyzes images in real-time to determine the quality of your data as it is generated
- **Embedded CryoSPARC Live™** processes images on the fly to assess sample quality and accelerate structural determination
- **Smart Plugins** automatically adjust data collection settings based on output from real-time image analysis
- **Thermo Scientific™ CryoFlow™ Software** provides easy access to your results through a web portal, allowing you to manage your data and reporting anywhere

Some of these components are already included with the Tundra, Glacios, and Krios Cryo-TEMs, while others can be added to further enhance your productivity and improve results.





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

Service, support, and financial options

Our **Dedicated Support Network** is committed to your success when you purchase our cutting-edge cryo-electron microscopes with complete end-to-end workflow solutions

- Remote fleet monitoring for maximum uptime
- Local technical experts just a call away
- Financing to meet changing needs, including construction and innovation
- Dedicated Program Manager to ensure success
- Full remote operation from any site

The **Electron Microscopy Funding Support Center** offers technical content, resources, and proposal writing tools to help you acquire the research instrument you need.

Further reading

Getting started with Cryo-EM eBook

Explore how cryo-EM can overcome the current limitations of traditional techniques such as X-ray crystallography (XRD). Learn about key methods, including single particle analysis, microcrystal electron diffraction (MicroED), and cryo-tomography, and how these techniques are used to answer important scientific questions. Discover how cryo-EM has become easier to adopt and more affordable than ever before.

Cryo-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.

Integrative structural biology: high-precision 3D analysis from structure to function

Learn more about integrative structural biology, which combines multiple structural determination techniques to enable the full characterization of macromolecular systems. Advances in mass spectrometry, in combination with cryo-EM and other structural tools, are revolutionizing our understanding of protein structure, function, and dynamics. Learn more about tools for integrative structural biology, the benefits of combining cryo-EM, cryo-ET, and mass spectrometry, and the advantages that integrated modeling provides.

Tundra Cryo-TEM brochure

Discover how the Tundra Cryo-TEM allows you to perform structural analyses on challenging proteins and macromolecules with unprecedented ease of use, making cryo-EM more accessible and enabling discoveries in infectious and neurodegenerative diseases, cancer, and more. Validate ligand/protein binding, perform particle characterization, prepare cryo-EM grids, and reveal high-resolution structures.

Glacios 2 Cryo-TEM Brochure

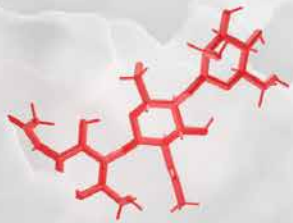
Explore how the Glacios 2 Cryo-TEM can provide deeper insights into protein structures, transform drug discovery and development, uncover the interactions of proteins/complexes, and overcome the challenges of small molecule visualization. Discover cryo-EM that is accessible and versatile with improved ease-of-use and productivity for both new and experienced users.

Krios G4 Cryo-TEM Brochure

Discover how the award-winning 300 kV Krios G4 Cryo-TEM offers unmatched biological insights through the 3D visualization of proteins and molecular machines, their localization, and dynamics within the architecture of the biological cell.

Talos L120C (S)TEM Brochure

Learn how the flexible Talos L120C (S)TEM can be used to visualize sections of resin-embedded cells and tissues, isolate particles of protein complexes and viral assemblies, as well as other materials down to sub-nanometer resolutions. With a flexible base configuration, the system can be adapted to multiple applications and use different imaging and detection techniques, including cryo-EM single particle analysis.



Cryo-EM Trends for Drug Discovery eBook

Discover the numerous advancements in cryo-EM that have increased the efficiency, ease-of-use, and throughput of the workflow, allowing this technique to become an invaluable tool for the pharmaceutical industry. Learn more about the applications and supporting case studies that utilized cryo-EM as part of the drug discovery process.

Reach the Targets of Drug Discovery eBook

Created specifically for pharmaceutical scientists, this eBook provides information, videos, and supporting case studies about biologics discovery and development, small-molecule structure-based drug design, efficient sample optimization workflows, and in-house cryo-EM adoption plans. Explore how the easy-to-use Tundra Cryo-TEM help to accelerate drug discovery.

"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

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.

Learn more about the fundamentals of cryo-electron microscopy at our **EM Resource Center**

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