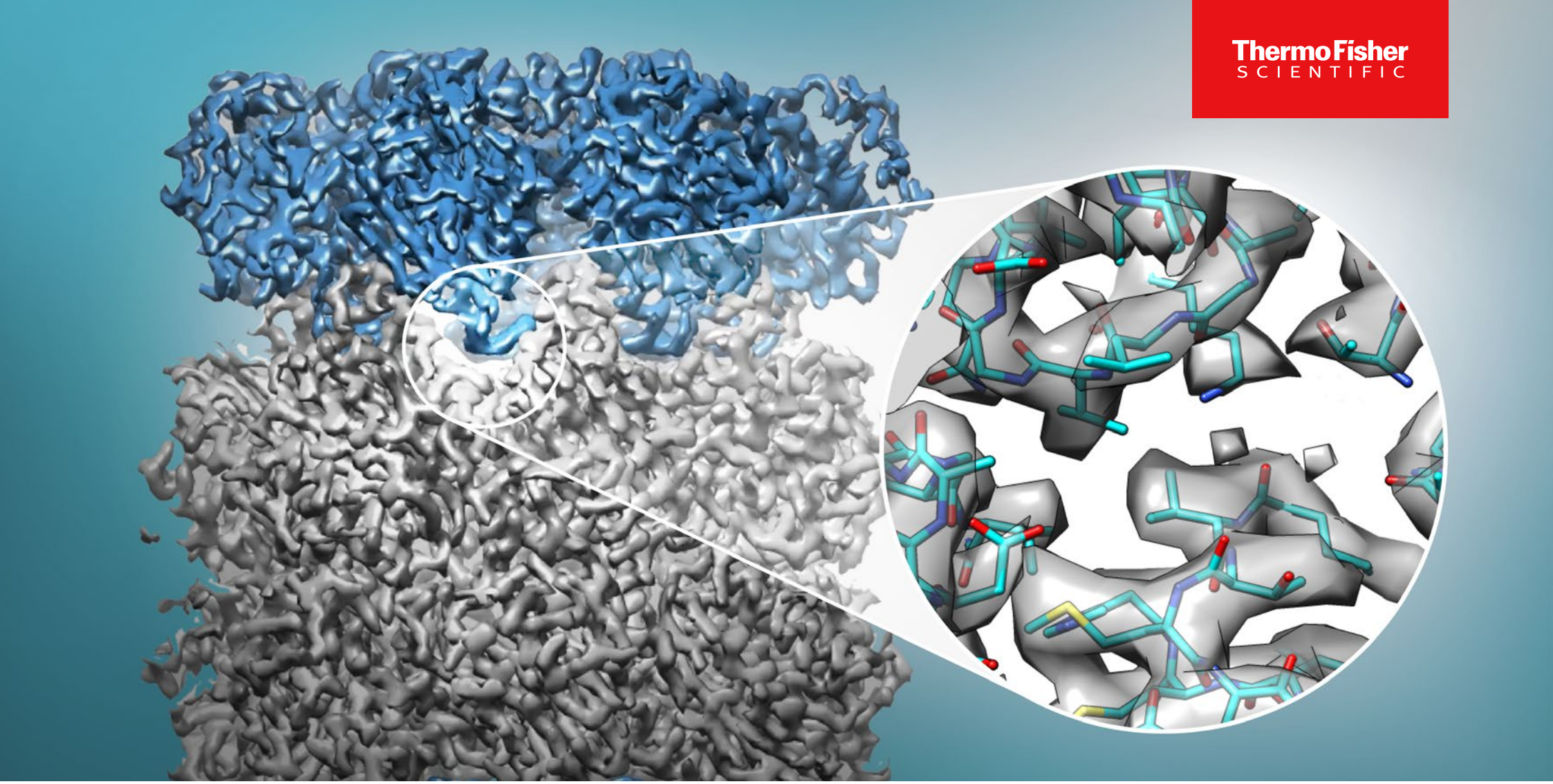


ThermoFisher SCIENTIFIC

Reach the Targets of Drug Discovery

Go beyond structure to understand function with cryo-EM

thermo scientific

The Tundra Cryo-TEM can provide you with a big step forward in terms of ease-of-use and affordability. It offers relevant resolutions that reveal binding sites, conformations, and hit finding, while ensuring that a much broader range of pharmaceutical scientists can operate cryo-TEM with minimal training.

Both X-ray crystallography (XRD) and nuclear magnetic resonance (NMR) spectroscopy are challenged by the size, complexity, and conformational flexibility of membrane proteins. Cryo-EM can determine structures of challenging proteins without the need for crystals, providing unmatched structural insights for pharmaceutical hit generation and lead optimization. Due to its perceived complexity, steep learning curve, and substantial capital investment, cryo-EM has felt out of reach for many drug discovery scientists. The Tundra Cryo-TEM makes cryo-EM more accessible, cost effective, and space efficient without sacrificing capability.

Learn how the Tundra Cryo-TEM offers structural determination at biologically relevant resolutions.

Biologics discovery and development

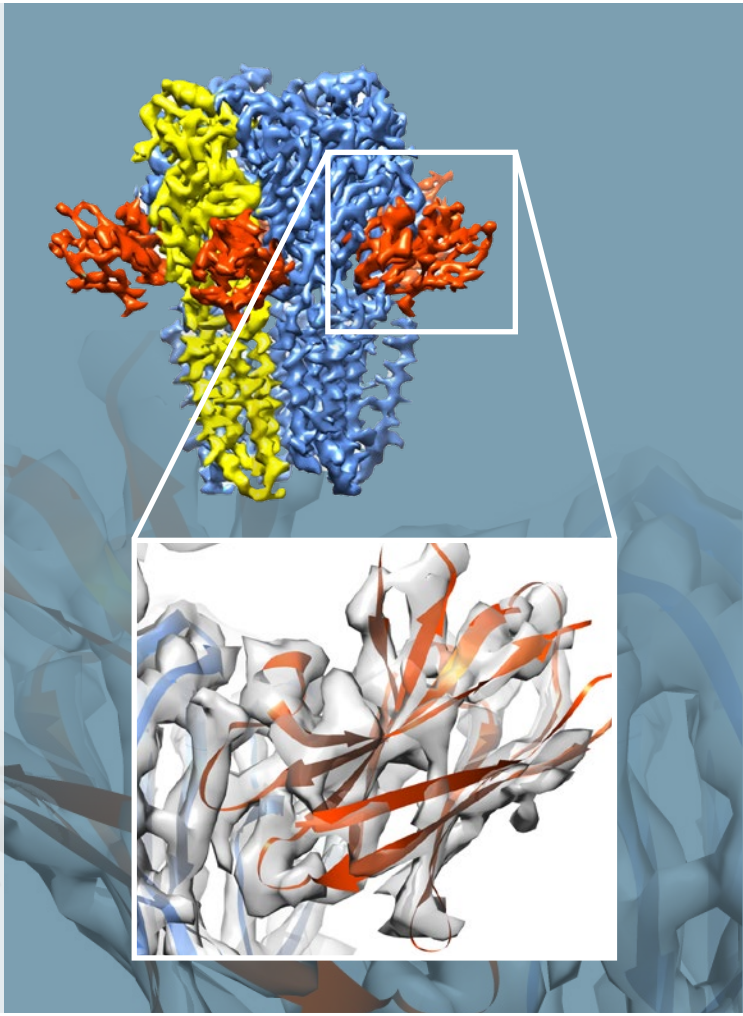
Revolutionize drug discovery with Tundra Cryo-TEM

Biologic drugs range from therapeutic proteins, like peptides and antibodies, to hormones, vaccines, gene therapies, cellular therapies, growth factors, cytokines, insulin, and others.[1] Due to the high molecular complexity and specificity of biologics, knowing the structural information and binding pattern between drug target and discovery candidate can be crucial. An in-depth understanding of a candidate's mechanism of action, binding orientation, epitope-paratope identification alteration, and affinity alteration can guide drug development and increase success rate.

Overcoming challenges

However, many of these critical biological targets are unsuitable for traditional structure determination techniques because of their size, complexity, and, in some cases, conformational flexibility. You may find biologic drug discovery a challenge when relying on the unpredictable success rates of these traditional techniques, which can lead to staggering costs and overextended timelines. In addition, structures from traditional structure determination techniques, such as XRD, do not guarantee full biological relevance due to crystal contacts[2] and the presence of solvents and polymers.

Seeing the Future: Cryo-EM in Drug Discovery
Interviews with Xiayang Qiu, PhD, Seungil Han, PhD, and Philip Dormitzer, MD, PhD, Pfizer Inc.



$GABA_A$ receptor in a lipid nanodisc. Its structure was determined with the Tundra Cryo-TEM to a 3.4 Å resolution. Sample courtesy of Radu Aricescu, Medical Research Council Laboratory of Molecular Biology, Cambridge, as well as Dimple Karia and Abhay Kotecha, Thermo Fisher Scientific.

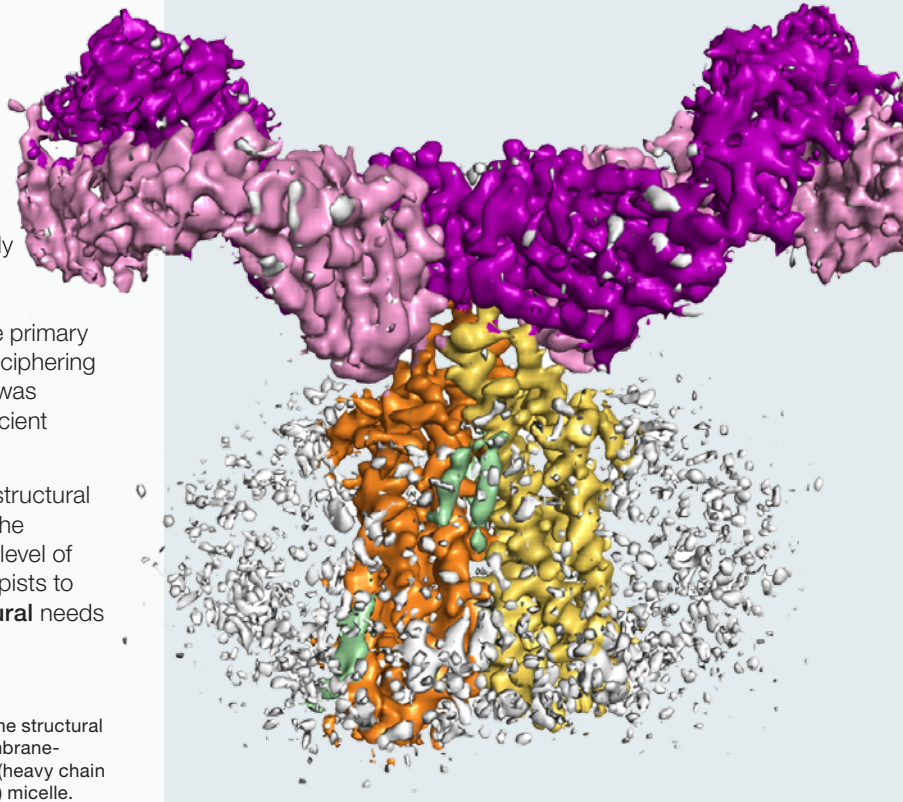
Cryo-EM single-particle analysis, a crucial structural determination tool in drug discovery, can obtain structural insights for a broad range of otherwise intractable biological targets to facilitate the development of new therapeutics. The near-native conditions of the technique avoid disrupting your sample's physiological state and eliminate the length and uncertainty of other traditional methods.

Large macro-complexes, such as whole targets bound to fragment antibodies, which are otherwise not amenable to traditional structural methodologies, can easily be characterized with cryo-EM SPA. Beyond simply extending structural analysis to samples that cannot be crystallized, cryo-EM techniques can explore entirely new questions that pertain to dynamic and heterogeneous assemblies of your biologic sample.

Case study

The antibody rituximab, known to treat certain autoimmune diseases and types of cancer, was recently imaged by cryo-EM with its target, CD20.[3] The derived structure-based model of the CD20-rituximab complex (right), provides an unprecedented view into the structure of the therapeutic target CD20 and hints at the previously unclear mechanism of action of rituximab. These structural insights explain why rituximab has such high affinity for CD20 despite its low affinity for the primary epitope found on one of its extracellular loops. Deciphering the binding mode of the antibody to the receptor was essential for discovering and developing more efficient antibody-based drugs.

This example of **epitope mapping** and biologic structural characterization can easily be accomplished on the Tundra Cryo-TEM, which is designed with a high level of automation. This makes it easy for non-microscopists to use cryo-EM to address biologics related **structural** needs and revolutionize their work.



Right: CD20-rituximab complex determined by cryo-EM. The structural details reveal the interactions between CD20, a B-cell membrane-protein dimer, and two antigen binding fragments, or Fabs (heavy chain in purple and light chain in pink) in a glyco-diosgenin (GDN) micelle. Credit: Model adapted from Rougé, et al by Hans Raaijmakers.

Cryo-EM for small molecule discovery

Tundra Cryo-TEM can expand your scientific discovery

Structure-based drug design provides structural insights on how small molecules interact with their drug targets, revealing information on structure-activity relationships. Fewer compounds need to be synthesized, reducing time, costs, and failure rates in the drug discovery process, resulting in high-efficacy drug candidates.

Overcoming challenges

Accurately predicting the molecular properties of targets that have remained recalcitrant to traditional structural biology techniques is challenging. The absence of timely, detailed structural insights informing your drug design could lead to:

- Lengthy trial and error processes when generating small molecule candidate leads
- Missing more effective options due to tremendous pressure to speed up results

Structural insights can help reduce your lab's time-to-market from early drug discovery to clinical trials.

Cryo-EM is commonly used to generate structures of intractable targets, such as ion channels, transporters, receptors, and membrane–protein complexes. The resolution of cryo-EM can reveal binding pockets of small molecules, making it easier to unambiguously assign the binding mode of these molecules and expanding your structure-enabled projects.

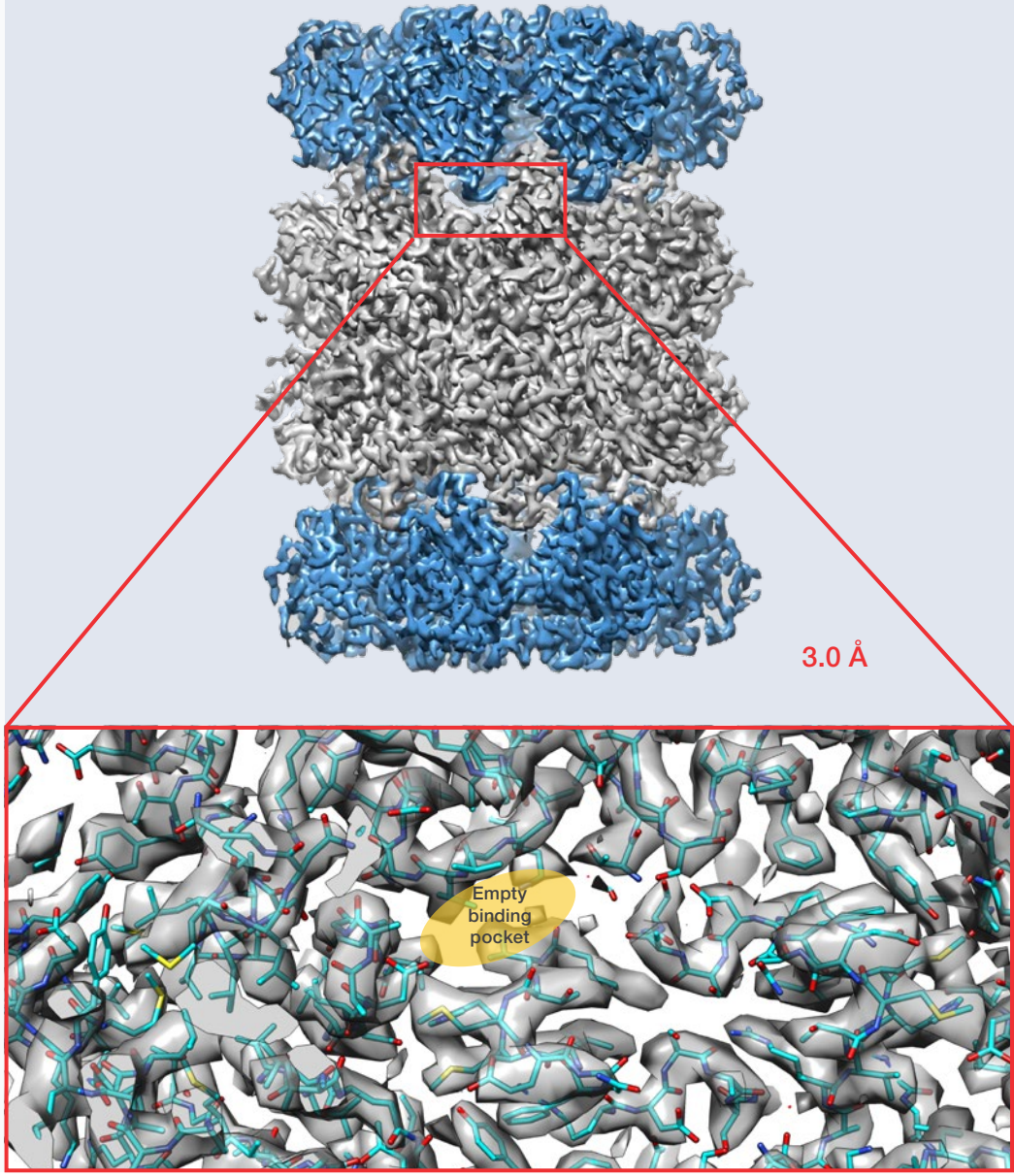
Bring the accessible Tundra Cryo-TEM onsite in order to:

- Expand your structure-enabled projects
- Avoid scheduling waits at cryo-EM imaging services or shared facilities
- Generate high-quality cryo-EM samples that can be sent out for data collection with higher resolution tools (see sample optimization)
- Visualize difficult-to-crystallize macromolecules at a resolution that supports lead discovery projects

Whether you are a structural biologist or a medicinal chemist, the ease-of-use of the Tundra Cryo-TEM can empower you to expand your structural discovery work. Take advantage of rational drug design for major drug target classes, reducing drug discovery costs and bringing better medicines to market faster.

Case study

The reconstruction map of the T20S proteasome was obtained at 3.0 Å resolution using the Tundra Cryo-TEM. These structural insight allowed researchers to determine how ligands bind to the protein: a key component of cell metabolism. By zooming in to a typical binding pocket of the T20S proteasome, researchers could see that the binding position was empty (yellow fill-in), as no density was visible in this area. As an illustration, the binding pocket of bortezomib (a proteasome inhibitor used in cancer treatment) is shown, demonstrating that at this resolution, you can confidently determine whether a ligand is bound to its target.

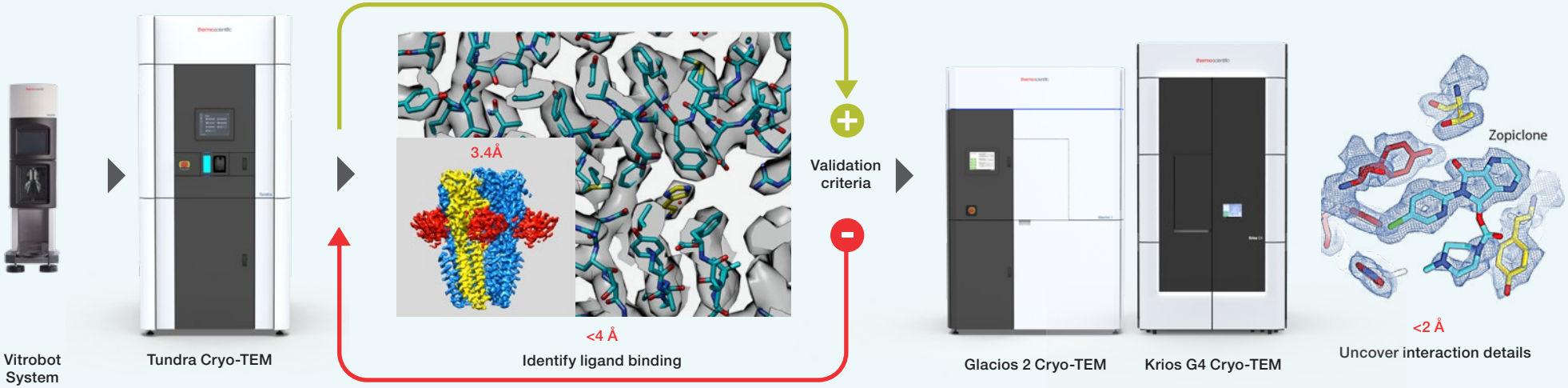


Right: Atomic model of the T20S proteasome fit to the electron density map that was determined with the Tundra Cryo-TEM. The generated structural map shows the binding pocket for bortezomib in yellow. Sample courtesy of Juergen Plitzko, Max Planck Institute of Biochemistry, Martinsried.

Case study

Human $GABA_A$ (gamma-aminobutyric acid type A) receptor is a 200 kDa membrane protein and ligand-gated chloride-ion channel that mediates inhibitory neurotransmission. Neuronal disorders such as epilepsy, insomnia, anxiety, and sleep disorders are a direct result of $GABA_A$ receptor neurotransmitters not functioning properly as chemical messengers in our brains.

As an important therapeutic target, it is vital to understand the molecular mechanism by which these receptors mediate neurotransmission. However, even with decades of effort, only non-physiological forms of $GABA_A$ receptors have been crystallized and structurally resolved, even though physiological forms are needed to explain the receptors' mechanism of action. The Tundra Cryo-TEM was used to optimize the sample, generating an initial structure of the stable conformation of the $GABA_A$ receptor with details that can identify ligand and nanobody binding. The map shows an atomic model fit with all the major side chains visible and a histamine (yellow) ligand in its binding pocket. The protein–protein interaction of mega-bodies (red/orange densities) with their receptors is also visible.



Rapid in-house sample-optimization cryo-EM workflow shows how the Tundra Cryo-TEM is used to determine the right criteria for sample preparation to generate a high-resolution structure. The Tundra Cryo-TEM generated structure of $GABA_A$ receptor shows major side chains, with mega-bodies (red and orange) and a histamine (yellow) in its binding pocket. Sample courtesy of Radu Aricescu, Medical Research Council Laboratory of Molecular Biology, Cambridge.

Tundra introduction

Adding cryo-EM to your structure-based drug design (SBDD) workflow can generate many benefits by providing insights into the near-native states of proteins that are challenging for traditional methods.

The Thermo Scientific™ Tundra™ Cryo-TEM is our latest dedicated structure determination solution to bring cryo-electron microscopy (cryo-EM) single-particle analysis (SPA) to every structure-based biochemistry laboratory. It is designed to be accessible at a lower cost of purchase and maintenance, while offering a high level of automation that makes it easier for non-microscopists to use cryo-EM to address their biological questions.

This eBook describes applications of the Tundra Cryo-TEM, demonstrating the flexibility and capability of this instrument for biology laboratories. The Tundra Cryo-TEM:

- Aids in biologics discovery and development
- Supports small molecule structure-based drug discovery
- Screens samples for improved throughput and success rates on more powerful microscopes
- Provides ease of use

Key Benefits
User-friendly interface with predefined settings to streamline data collection
AI-guided automation with algorithms that allow the microscope to learn over time
Easy, iterative loading and imaging for common biochemistry sample optimization
Structural information at biologically relevant resolutions
Service solutions to deliver success
Cost-effective and space efficient

Tundra Cryo-TEM: focused on ease of use

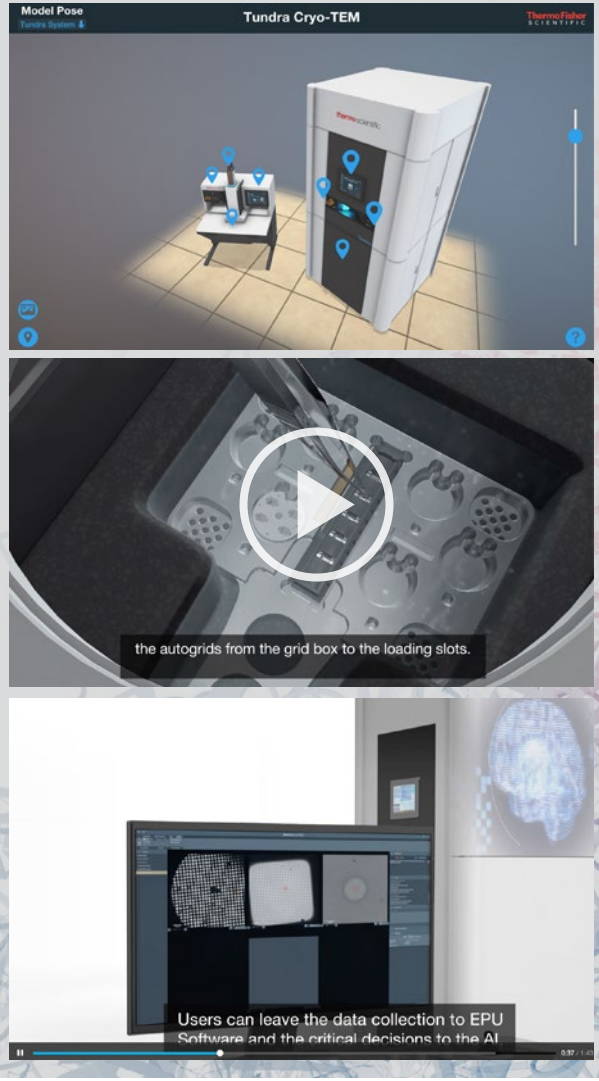
Focus on your science with an intelligent microscope

The Tundra Cryo-TEM offers a big step forward in terms of ease-of-use and affordability while ensuring that cryo-EM can be performed by a much broader range of pharmaceutical scientists with minimal training. It provides:

- An integrated loader that makes it easier for users of any experience level to load samples into the microscope, as compared to conventional systems. Scientists can exchange sample carriers in minutes. Results can be checked immediately, allowing researchers to rapidly optimize biochemistry sample conditions.
- Artificial intelligence and guided automation that help non-experts quickly identify the quality of their samples and easily navigate an otherwise complex workflow. As the sample moves through the cryo-EM process, the results are displayed in a traffic light style that helps operators quickly determine if their sample is viable.

3D structures to answer your biological questions

With Tundra Cryo-TEM, your biochemistry work can be validated at biologically relevant resolution to show the structural details that underlie interactions between proteins, small molecules, and post-translational modifications in large and dynamic protein systems at near-native conditions. These molecular details confirm the mechanism of action by which complex biological systems (e.g., membrane proteins, protein complexes, and macromolecular machines such as viruses, ribosomes, and proteasomes) contribute to human health and disease. The Tundra Cryo-TEM can produce structures at resolutions as high as 3.4 Å for $GABA_A$ receptors and 3.0 Å for T20S proteasome. At this level of detail, many important biological details can be visualized, such as the binding of antibodies or nanobodies (which are critical for epitope mapping), the molecular details of protein-protein complex formation and interaction, binding of small molecules, or virus-receptor interactions. Such high-resolution maps allow *de novo* model building and can help scientists understand how proteins function, how to modify genes, and how to design drugs accordingly.



Interactive model that lets you view the sample loading station, software user interface, and Tundra Cryo-TEM instrument. Rotate your view by clicking and dragging the mouse. Click on pin icons to get more information and view videos.

Service solutions to deliver success

In addition to state-of-the-art-hardware, comprehensive service enables your success on the Tundra Cryo-TEM. The Accelerate service portfolio, which runs through the warranty period, provides workflow validation and application support to train your users in all aspects of your workflow. Your Customer Success Manager will work with you to ensure you achieve your desired results. Keep resources at your fingertips with the step-by-step Scientific Workflows App. View system health and performance metrics through the secure Connected Care portal. Our technical team will monitor your system's performance and respond on-site if maintenance is needed. Accelerate services were created for customers with a range of budgets and buying cycles. You can be confident that our team will give you the training, tools, and resources to be successful using the Tundra Cryo-TEM to optimize your investment.

Cost-effective and space-efficient cryo-EM within your reach

The new hardware architecture of the Tundra Cryo-TEM was purposely designed with a smaller footprint and an easier access path without sacrificing performance. In many cases, this allows you to avoid the additional investment and unwanted downtime that comes with modification of existing laboratory infrastructure (or even the need for a new, purposely built lab) to accommodate the instrument. The Tundra Cryo-TEM is offered at a lower price-point, making it possible for more pharmaceutical companies to obtain structural insights at a biologically relevant resolution.

Workflow Validation

Ensures that your sample achieves a 3.5 Å resolution on a relevant biological system.

System Remote Monitoring

Technical experts will monitor key system parameters and will proactively notify your Field Service Engineer if support is needed.

Scientific Workflows Application

Offers a step-by-step guide through the Cryo-EM SPA workflow, enabling users of all experience levels to optimize their results.

Connected Care Portal

Secure, cloud-based portal delivers insights into system health and performance. Track uptime and utilization, view system health by module, and conveniently access system reports.

Customer Success Manger

A dedicated technical expert will support you during installation, connect you with support and resources, and regularly meet with you to discuss system performance and productivity.

On-site and Remote Applications Support

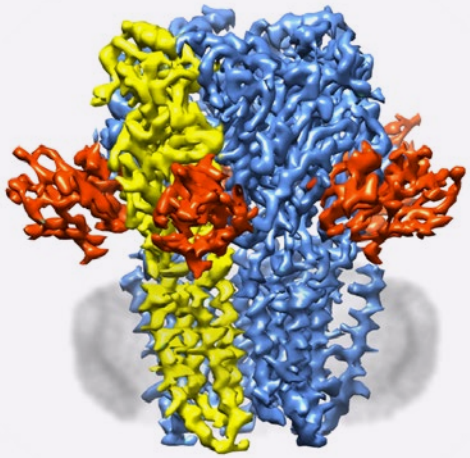
Provides training and support on all aspects of your sample and workflow, including sample loading, microscope and camera operation, microscope optimization, and more.

Targeted technology to support various workflows

Tundra, Glacios, and Krios Cryo-TEM: solutions to answer different biological questions

Example: Human $GABA_A$ receptor

4.3 Å resolution



Tundra Cryo-TEM

<5 Å

Epitope Mapping

2.4 Å resolution

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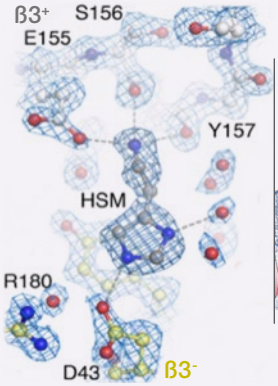
Glacios 2 Cryo-TEM

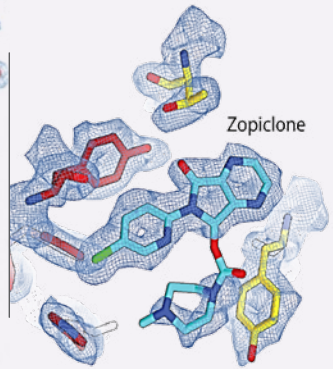
<2.5 Å

H_2O molecules/ Model building

1.7 Å resolution



1.9 Å resolution



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Krios G4 Cryo-TEM

<2 Å

H atoms/ ligand identification / Interaction

Our full cryo-EM portfolio for your drug discovery and development needs

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

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 6 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

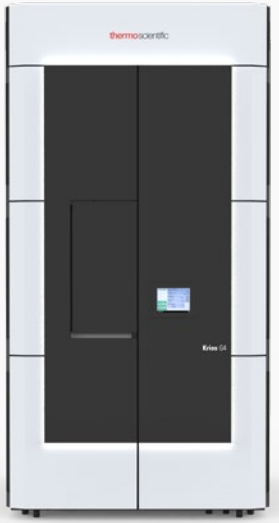
High-resolution SPA	200 kV, <2.5 Å*
High throughput	dataset in 30 minutes
Sample type	proteins, crystals, cells
Applications	SPA, MicroED, tomography

Thermo Scientific Krios™ G4 Cryo-TEM: Powerful and Productive

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

Ultra-high-resolution SPA	300 kV, <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.



		Thermo Scientific Tundra Cryo-TEM (100 kV)	Thermo Scientific Glacios 2 Cryo-TEM (200 kV)	Thermo Scientific Krios G4 Cryo-TEM (300 kV)
Sample type	Proteins (single particle analysis)	✓✓✓	✓✓✓	✓✓✓
	Crystals (microcrystal electron diffraction)	×	✓✓✓	✓✓✓
	Cells (tomography)	×	✓	✓✓✓
Sample preparation	Iterative sample optimization	✓✓✓	✓	✓
	Automated sample optimization	✓	✓✓	✓✓✓
Performance	Ability for routine biology resolution (3.5 Å)	✓	✓✓	✓✓✓
	Ability for near-atomic resolution (2.5 Å)	×	✓✓	✓✓✓
	Ultimate published resolution	2.6 Å*	1.6 Å**	1.22 Å
Throughput and productivity	Time to get to 3.5 Å (apoferritin)***	6 hours	30 minutes	A few minutes
	Time to get to 2.5 Å (apoferritin)***	×	2 hours	10 minutes
Track record	Publications (2014–2019)	0	>35**	>1570
	Install base	0	>60**	>225
Single-vendor support	One stop shop	✓✓✓	✓✓✓	✓✓✓
Ease of use	For new users	✓✓✓	✓	✓✓
	For pushing near-atomic resolution	×	✓✓	✓✓✓
Cost	Average sell price	$	$$	$$$
	Cost of ownership	$	$$	$$$
	Cost per structure	$$$	$$	$

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

Why should I consider bringing Cryo-EM in-house?

Thermo Fisher Scientific offers cryo-EM solutions that allow easy adoption, on-site training, full workflow support and customized financial packages that fit any budget.

Scientifically powerful

- Enables immediate access to cryo-EM for structural insights
- Enhance your SBDD outcomes with fast in-house cryo-EM integration

Easily adoptable

- Hands-on training in the first year to quickly acquire cryo-EM skills and knowledge
- Burden-free site preparation for seamless room remodeling
- Full solution: Thermo Scientific Vitrobot™ System and complete sample preparation toolkit

Financially favorable

- Increase your productivity and leverage costs of outsourced services
- Choose an attractive time-bound price offering
- Accelerate return on investment by generating income quickly through payment plans
- Lower investment barrier with affordable and flexible finance option

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. See how scientists use cryo-EM to access the structures of macromolecular complexes for better drug design.

References:

1. US Food and Drug Administration. “What are ‘biologics’ questions and answers.” https://www.fda.gov/about-fda/center-biologics-evaluation-and-research-cber/what-are-biologics-questions-and-answers (2018) [Accessed May 27, 2021].
2. Luo J, et al., A structural dissection of large protein-protein crystal packing contacts. *Sci Rep* (2015). 474 2015. 5: p. 14214.
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4. Wasilko DJ, Johnson ZL, Ammirati M, et al. Structural basis for chemokine receptor CCR6 activation by the endogenous protein ligand CCL20. *Nature Communications* (2020).

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