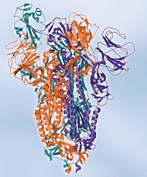
VIROLOGY

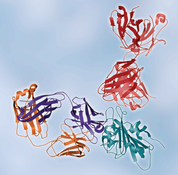
SARS-COV-2

First Spike Protein Structure, PDB 6VSB



SARS-CoV-2 binds to host cells via a trimeric spike glycoprotein. Researchers produced the first 3D structure of the multimeric protein by cryo-EM in mid-February 2020. The determination of the spike protein atomic structure was essential for developing diagnostics, antibody therapeutics, and vaccines.[2]

Monoclonal Antibodies Binding Spike RBD, PDB 6XDG



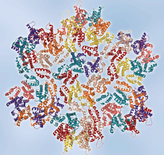
Treatment with a single monoclonal antibody may create selective pressure for resistant mutants, so researchers are testing the efficacy of antibody cocktails that interact with the spike protein simultaneously. The cryo-EM structure showed REGN10933 (orange and purple) and REGN10987 (red and pink) antibodies binding the spike protein receptor-binding domain (RBD; turquoise).[5]

3a Ion Channel, PDB 7KJR

Researchers have implicated the SARS 3a ion channel in processes such as viral release[4] and cell death.[5] In mice with a SARS 3a deletion mutation, viral titer and morbidity are reduced,[6] making 3a an effective vaccine and therapeutic target. Traditional structural analysis techniques are difficult to perform on membrane proteins such as ion channels; however, cryo-EM allowed researchers to view the SARS-CoV-2 3a dimer in its native conformation.[7]

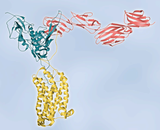
HUMAN IMMUNODEFICIENCY VIRUS (HIV-1)

HIV-1 Capsid, PDB 3J34



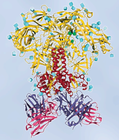
X-ray crystallography determined only the structure of assembled HIV-1 capsid protein hexamers. Cryo-EM showed the structure and distribution of penton regions that facilitate capsid curvature and genome enclosing, and residues important for assembly and infectivity.[7]

HIV-1 gp120 Bound to Host Receptors, PDB 6MET



The HIV-1 envelope fusion protein gp120 (turquoise) interacts with host CD4 (pink) and a co-receptor such as CCR5 (yellow). Cryo-EM helped scientists understand the conformational changes that occur during these interactions and the role of CCR5 in fusing the virus to the host membrane.[9]

HIV-1 Envelope Glycan Shield, PDB 6X9R

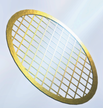


The HIV-1 envelope (Env; yellow and red) is surrounded by a dense array of N-linked glycans (turquoise and green) that mask the protein from immune recognition. Using cryo-EM, researchers localized this "glycan shield" and discovered features of its structure and function that will aid in vaccine development.[10]

SOLVING STRUCTURES IN A FLASH

SCIENTIFIC BREAKTHROUGHS WITH CRYOGENIC ELECTRON MICROSCOPY

Dramatic improvements to cryogenic electron microscopy (cryo-EM) technology have led to an explosion of research using this technique in diverse research areas. As cryo-EM protein structures increase in resolution, scientists learn more about molecular interactions than ever before, revolutionizing therapeutic development in virology, neurology, cancer, and a range of other diseases. Cryo-EM reveals key insights into the function, structure, and molecular basis for disease-associated mutations.



Traditional electron microscopy (TEM) lagged behind X-ray crystallography as the main choice for protein structure research. Although protein crystallization is challenging and time-consuming, traditional TEM techniques such as negative staining limit the determination of proteins to low-resolution structures that do not resolve the internal protein conformation, the interaction of amino acid side chains, and the atomic structure. That changed once Jacques Dubochet developed a method to vitrify (flash-freeze) electron microscopy samples; this approach prevents dehydration, avoids ice crystal formation, and captures proteins in their native state.[1] Vitrified cryo-EM specimens are illuminated with high-energy electrons (100-300 keV) and individual protein molecules are visualized in captured raw images. From these imaged molecules, scientists reconstruct high-resolution 3D maps and build atomic models of the protein structure.

Compared to X-ray crystallography, cryo-EM

- Does not require protein crystals
- Uses small amounts of purified protein
- Captures multiple protein conformations in their native state

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NEUROSCIENCE

C-terminal Portion of LRRK2, PDB 6VNO

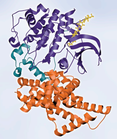
Leucine-rich repeat kinase 2 (LRRK2) protein is linked to genetically-inherited Parkinson's disease, but its mechanism and structure have been unknown for almost 20 years. Researchers finally visualized the protein in its natural environment with cryo-EM[11] and cryo-electron tomography (cryo-ET),[12] providing information that is crucial for future kinase inhibitor drug design.

Hallucinogen Complexed with a GPCR, PDB 6WHA

Psychedelics are used as therapeutics for neuropsychiatric disorders such as depression, anxiety, and substance abuse.[13] These drugs activate the 5-HT_{2A} serotonin receptor (HTR2A)—a membrane G protein-coupled receptor (GPCR). To understand the mechanism of action, researchers performed cryo-EM to obtain the structure of a prototypical hallucinogen ligand (yellow) complexed within HTR2A (pink) and coupled G proteins (not shown).[14]

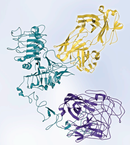
CANCER

Human CAK in Complex with Small Molecule Inhibitor, PDB 6XD3



Human CDK-activating kinase (CAK) is an important regulator of transcription initiation and the cell cycle. When CAK misregulates gene expression and cell division, cancer can occur, making it an attractive target for cancer treatment.[15] Using cryo-EM, scientists viewed how a small molecule inhibitor (yellow) binds to the human CAK complex (purple, turquoise, orange).[16]

Monoclonal Antibodies Simultaneously Binding to HER2, PDB 6OGE



Human epidermal growth factor receptor 2 (HER2) associates with aggressive breast cancers and cancer reoccurrence. Interestingly, HER2-positive tumors are more likely to respond to treatment, especially combination antibody therapy.[17] To understand the synergy between two monoclonal antibodies, scientists used cryo-EM to image the simultaneous binding of trastuzumab (purple) and pertuzumab (yellow) antibodies to different HER2 subdomains (turquoise).[18]

THE HISTORY OF CRYO-EM[19–22]

1968
First 3D EM reconstruction; DeRosier and Klug

1974
First cryo-EM diffraction patterns; Taylor and Glaeser

1982
Vitrification developed; Dubochet et al.

1987
Birth of single particle reconstruction; van Heel, Radermacher, and Frank et al.

2007
Maximum-likelihood 3D software developed; Scheres et al.

2012
Direct electron-detection cameras first used; Grigorieff et al.

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

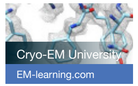
2017
Jacques Dubochet, Joachim Frank, and Richard Henderson win the Nobel Prize in Chemistry for cryo-EM

2020
Atomic level resolution achieved; Yip et al., Nakane et al.

2021 and beyond
Cryo-EM is poised to become the dominant technique for structure determination. New cryo-EM platforms are increasing ease-of-use and access.

thermoscientific

Getting started in cryo-EM



Cryo-EM University is an online learning platform that features over 70 hours of theoretical lectures and videos. Enroll in our free EM-learning.com online courses to learn cryo-EM essentials, including our single particle analysis (SPA) workflow, via step-by-step instructions and expert demonstrations.

scientific workflows

The Scientific Workflows App (available in the iOS App Store and Google Play Store) puts the Cryo-EM University videos and content at your fingertips. The lite version (free) includes a lab notebook, lists of consumables and accessories, plus all of the rich useful content available at em-learning.com. The full version (part of the Accelerate service offering) also includes updated research articles every quarter, troubleshooting tips, and supervisor information.

Practical paths to accessing cryo-EM

Thermo Fisher Scientific provides accessible ways to leverage the advantages of cryo-EM without making a full investment. Our Cryo-EM Access program connects biopharma to world-class facilities where you can advance your research by obtaining cryo-EM structures and proof of concept data for cryo-EM using your own samples.

- ▶ Find facilities offering cryo-EM instrument access for COVID-19 related research
- ▶ Find facilities offering cryo-EM instrument access for academics

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Thermo Fisher Scientific offers the expertise to support both your investment and your research endeavors. We can help you precisely plan every aspect of your laboratory to control building costs and limit downtime. Our cryo-EM application specialists can assist with everything from sample preparation to data processing. We provide long-term ongoing support, as well as flexible financing options to get you in the game.

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Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner, and safer. We serve life science researchers from academic institutions and the pharmaceutical industry, providing an unmatched combination of complete workflow solutions ranging from cryo-EM structural determination of macromolecular complexes and protein sociology in the native state to reconstruction of 3D architecture of tissues and cells. Our solutions help researchers unlock the mysteries of underlying protein function and cellular processes and bridge the gap between basic scientific research to design of new therapies.

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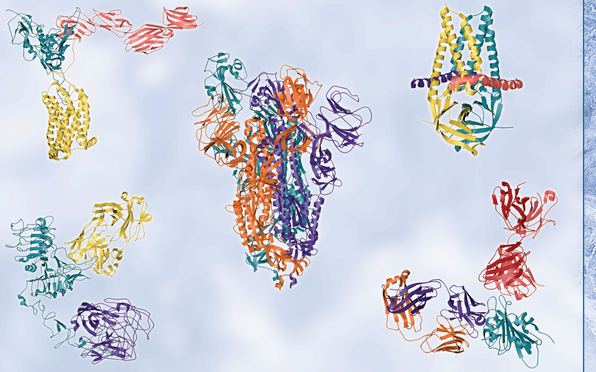

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