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Nomination

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Democratizing cryo-EM: Broadening access to an expanding field

Cryo-electron microscopy (cryo-EM) yields atomic-level structures of megacomplexes and tiny compounds. How can your lab get access to this versatile method? By Chris Tachibana

For decades, says Bridget Carragher, cryo-EM was a “niche, hole-in-the-wall” field. But in 2017, cryo-EM passed nuclear magnetic resonance (NMR) spectroscopy for number of annual entries in the Protein Data Bank, the world’s sole repository for 3D structural data on proteins, nucleic acids, and large biological molecules. And now it’s gaining on the grandaddy of structural methods, X-ray crystallography.

Carragher leads a cryo-EM facility at the New York Structural Biology Center, which is supported by the U.S. National Institutes of Health (NIH) and the Simons Foundation. Two other NIH-funded centers are at Stanford University and Oregon Health & Science University (OHSU). “The trend everywhere is for national cryo-EM facilities,” says Poul Nissen, structural biologist at Aarhus University, which is the Danish national facility, together with the University of Copenhagen.

National centers serve a cryo-EM community that is rapidly expanding as software and hardware breakthroughs, especially in electron detectors, demonstrate how cryo-EM can advance basic research, drug development, and even solar-cell technology (1,2).

Crystallographic resolution — without crystals, but at a cost

Unlike X-ray crystallography, cryo-EM does not require crystallized samples. This eliminates a time-consuming step and allows atomic-level reconstructions of lumpy complexes and integral membrane proteins that have resisted crystallization. It can show conformational changes, such as ribosomes flexing their structure as they go through protein synthesis (3).

Cryo-EM works with unstained, aqueous samples. For single-particle analysis (SPA), its most common application, researchers drop samples onto a grid that is flashcooled by being plunged into liquid ethane. This freezing—or rather vitrifying—is so rapid that sample molecules are immobilized with their structure preserved and without ice crystals that interfere with transmission electron microscopy (TEM). Researchers then take thousands of TEM images by beaming electrons through the sample. Molecules caught in random orientations scatter the electrons, creating patterns used to generate 3D models.

Craig Yoshioka, codirector of the NIH cryo-EM center at OHSU, points out a promising development: Crystallographers who had truncated or mutated proteins to coax them into crystals can now study full-length wildtype proteins using cryo-EM. “This should better represent targets in their native states,” he says, “including with posttranslational modifications like glycosylation.”

Currently, SPA works best with large samples around 200 kDa, so researchers with smaller proteins might turn to microcrystal electron diffraction (microED), a cryo-EM method with a larger size range. Another issue with SPA is that it uses cell extracts; but inside cells, says University of California, San Diego biophysicist Elizabeth Villa, “proteins aren’t floating in water. They’re packed with other components, interacting with them, or forming networks that break up during extraction.” Villa uses cryo-electron tomography (cryo-ET), which images sections of cells or even tissues, to visualize components in situ.

And cryo-EM has an overarching drawback: cost. Top-of-the-line, 300-kiloelectron volt (keV) cryo-EM machines are around USD 5–7 million, with added costs for space, service contracts, and experienced staff. Pharmaceutical companies may have in-house facilities or use a company like Nanolmaging Services. Most cryo-EM clients are from pharma or biotech, says Carragher, a cofounder. Example projects include analyzing vaccines, antibodies, and drug targets. The company is rare among cryo-EM contractors in owning its own equipment, with others often using instruments at partner institutes.

Major research institutions also invest in cryo-EM facilities, but smaller universities can’t afford them. However, scientists including Gabriel Lander’s group at Scripps Research have revealed single-angstrom (Å) structures of proteins using less-powerful 100-keV or 200-keV microscopes. cont.>
(4) that cost USD 1–2 million. These results encourage scientists who call for
democratizing cryo-EM with more affordable, workhorse instruments (5).

Community service
Cryo-EM access should increase thanks to the new NIH centers, which have
cutting-edge equipment and a focus on service and training—center personnel
are not allowed to be coauthors on users’ publications. At full capacity, the
OHSU site will be collecting data 24/7 on 200–300 active projects at a time and
training 50–plus visiting scientists a year, Yoshioka estimates. He expects up to
hundreds of reconstructions per year per center.

And services are free. “You write a proposal,” Carragher says, “and if it’s
accepted based on criteria, such as scientific merit, feasibility, and need, you
get cryo-EM time.” This model is similar to national synchrotron facilities,
and many, such as the United Kingdom’s Electron Bio-Imaging Centre at the
Diamond Light Source (Oxfordshire) and the Brazilian Nanotechnology
National Laboratory (LNNano) of the Brazilian Center for Research in Energy
and Materials (Campinas), are located close to synchrotrons.

LNNano is the only cryo-EM facility in Latin America, and is supported by
government and State of São Paulo funding. Industrial clients are charged for
services, but service and training are free for academic researchers after project
evaluation, says LNNano researcher Rodrigo Portugal.

LNNano Senior Scientist Marin van Heel says cryo-EM is a powerful tool for
structure-based drug and vaccine design, so it is essential in the region because
of “big needs, like in neglected diseases such as Zika.” SPA research is underway
at LNNano with collaborators in Brazil, Peru, Uruguay, and Argentina.

Besides cost, the major burden at LNNano facilities is brain drain. Despite
holding multiple workshops and the annual Brazil School for Single Particle
Cryo-EM, “people get headhunted away to a center or pharma company in
another country,” van Heel says.

“It’s THE issue”
Cryo-EM software and hardware have “advanced amazingly,” Yoshioka
says, “but it can still be difficult to reliably take any protein from a gene
to a structure.” Cryo-EM doesn’t require large crystals, but sample purity, heterogeneity, and concentration are still important.

“Sample prep isn’t an issue,” says Carragher, “it’s THE issue.” During
vitrification, “particles glue themselves together, stick to the
air-water interface, adopt sulky
conformations, or fall apart.” Commercial automated systems make sample
preparation more reliable. However, a downstream challenge is caused by
terabytes of data that require dedicated workstations.

Digital developments
Cryo-EM users uniformly praise software groups for advancing data analysis
and structure resolution. Open-source software, such as RELION from Sjors
Scheres at the UK’s Medical Research Council Laboratory of Molecular Biology,
and work by others including Niko Grigorieff at Janelia Research Campus and
the University of Massachusetts, have been instrumental to the field, says
Yoshioka. An up-and-coming computational advance, he notes, is real-time
processing and reconstruction as data are collected.

That’s what cryoSPARC Live does. Currently in beta testing, the software
comes from University of Toronto spinout Structura Biotechnology, run by
brother-and-sister team Ali Punjani and Saara Virani. CryoSPARC Live adds
to the cryoSPARC package of SPA tools, including 2D image curation and 3D
reconstruction without prior structural knowledge.

CryoSPARC Live, Virani says, shows initial images after a few minutes,
6-Å to 8-Å 3D structures in about an hour, and refined high-resolution
structures a few hours later. Researchers can make real-time adjustments, such
as moving the sample to focus on the best areas and deciding how much data
to collect, saving time and money, she says. With demand for cryo-EM growing
rapidly, the field is wrestling with commercialization issues. Punjani explains
that cryoSPARC is free for academic users, while commercial clients such as
pharma companies must buy a license.

A computational angle on cryo-EM democratization, Punjani says, is to
modify algorithms to get better images from lower-end microscopes. Also,
cloud-hosted computation would let labs rent processing time as needed
instead of investing in dedicated hardware.

Full tilt on innovations
“Single-particle will be the bread-and-butter method for high-resolution
cryo-EM for a while,” Yoshioka says. Advances in other areas extend the size
range for resolving structures and allow views of the cell interior.

Getting high-resolution images of proteins smaller than 100 kDa pushes the
limit of current SPA. MicroED, developed by Tamir Gonen’s group, achieves
atomic resolution for size ranges of complexes larger than 200 kDa to organic
molecules under 10 carbon atoms. MicroED uses crystals one-billionth the size
needed for X-ray crystallography, explains Gonen, now at the University of
California, Los Angeles (UCLA). In microED, vitrification protects samples
so that diffraction patterns are generated by rotating a single microcrystal
through an electron beam, capturing all angles for 3D reconstruction of its
molecules.

Gonen used microED to visualize structural changes in a channel as a sodium
ion passed through. “Because we used crystals containing only about 1,000
units,” he says, “we could tease out smaller differences and capture a transition
state” (6).

Medicinal chemists, forensic scientists, and drug developers are excited
about the “powder-to-structure” application of microED. Gonen’s group and
others published methods for 30-min identification of small molecules such as
ibuprofen or biotin by structure, including in mixtures (7).

Gonen has worked with Thermo Fisher Scientific to develop relatively
easy-to-use microED hardware and software. “You don’t need to know much
now to get a sample into a ‘scope and collect data. It could make microED more
available to the community,” he says. Steve Reyntjens, Thermo Fisher’s director
of product marketing, says the microED package is easy to add as an optional
item on new microscopes or as a retrofit to existing instruments.

The David Geffen School of Medicine at UCLA has a microED center that
works with academic and industry scientists and offers microED training,
including at an annual summit coming up in October 2020 (8).

Cryo-ET reveals cellular contents not as they appear in textbooks or
videos “with empty space, a particle, then empty space,” says
Villa. It shows cells jam-packed with molecules.
Researchers using specialized TEM grids for growing cells before inducing a change, such as stimulating neurons or exposing human cells to medicines. Villa says, "to see at high resolution what happened at the point you did something to the cell."

Sample preparation for cryo-ET is low throughput, but a variation of CLEM with multiplexed fluorescent markers, developed by John Briggs’ group at the European Molecular Biology Laboratory in Germany, could more quickly identify cells for cryo-ET. Cryo-ET will allow observation of molecules in their native environment and in whole tissues, such as molecular views of “the connectome” of neuron-to-neuron interactions, Nissen says. The Thermo Fisher second-generation FIB instrument, notes Reyntjens, has cryo-liftoff capability for manipulating miniscule samples cut from vitrified tissue thinned to 100 nm–150 nm and transferred to a cryo-EM instrument for tomography.

Nikhil will soon launch national cryo-ET centers. The current cryo-EM centers, Carragher explains, collect tomograms if users have samples ready for cryo-ET. The national centers will provide access to equipment, plus assistance with tricky cryo-ET specimen preparation.

In addition to developments like CLEM, structural analysis that combines cryo-EM with data from multiple sources is on the rise. “Increasingly, people use crystallography, NMR, CLEM, mass spec—everything out there—to get an answer,” Carragher says. “But if we want these tools in everyone’s toolkit, they need to be more accessible.”

New ambitions

Along with solving the access problem, Nissen observes, the field should shift its perspective from focusing only on structures to “asking what the structure is doing in the cell in its native state. Getting label-free, time-resolved structures in natural contexts is the ultimate goal and also a new level of ambition to instill in students and postdocs.”

Nissen and others predict increasing industry use of cryo-EM for developing antibody therapeutics, small molecule drugs, and diagnostics. “We should also work with the medical community on unmet diagnostic needs,” he says, “where histology doesn’t show good differences between disease and healthy tissue. We might find molecular differences in tissues [by also] using cryo-ET.”

van Heel, who helped develop cryo-EM and has watched its use grow, says about working in the field, “It’s challenging at the moment, but it’s a great time to be alive. There’s no time for vacation.”

References


Chris Tachibana is a freelance writer who specializes in life sciences.
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