Cryogenic Electron Microscopy in the United States

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Abstract

Cryogenic Electron Microscopy (cryo-EM) has led to a revolution in the field of biomolecular research, including research into harmful viruses such as HIV and Tuberculosis, as well as cancer and bacteria. There has been an explosion of research with these technologically advanced instruments within the United States, which now houses over 100 cryo-EM throughout the country. These instruments are now able to generate movies of the experiments, providing an unprecedented view of the specimen. One observing session can generate anywhere between 1-10 TB of raw data. However, countries such as those within the entirety of the African continent don't have one cryo-EM, even though there are African researchers learning how to use these powerful instruments, and are developing cutting edge research projects that involve the use of cryo-EM. There is currently an untapped potential for remote collaboration between US scientists and African scientists, especially with the research and education networks (RENs) that are primed to support the data volumes generated by the newest cryo-EMs. The potentially beneficial collaboration between the AmLight—ExP network, SANReN and TENET with the University of Cape Town, and the U.S. NSF and NIH supported researchers, to expand remote observation pilot programs to African researchers should be explored.

Background and Introduction to Cryo-EM

The understanding of macromolecular complexes within living cells is the cornerstone of molecular biology. The aim of structural biology is to deduce how macromolecular complexes function by studying their 3D arrangement. Imaging precursors to cryogenic electron microscopy (cryo-EM) include X-ray crystallography and nuclear magnetic resonance (NMR). These methods are powerful, however they have their own drawbacks. X-ray crystallography is a formidable imaging technique that yields atomic level resolution and is independent of sample size, with the caveat that the complex of interest must be crystallized. NMR has the capability of providing dynamics and interaction information and is restricted to small complexes with molecular weights below 40-50 kDa (see glossary). Both of the aforementioned techniques also require relatively large and homogeneous samples on the order of several mg. Cryo-EM instruments have the capability of producing 3D images with smaller samples (on the order of 0.1 mg). Cryo-EM also imposes less restrictions on purity of samples, and does not require crystallization.

EMs were employed to investigate bacteriophages as early as the first half of the 20th century (Ruska). One of the largest problems for observing biological samples with EMs is the degradation of the structural integrity of the sample with time. EM requires close to a vacuum within the beam path otherwise air molecules will scatter the electrons, which compromises preservation of liquid aqueous samples. Biological macromolecules are also susceptible to damage from the radiation of the electron beam of the instrument. Early EM studies of biological samples used fixation techniques or dehydrated samples, which introduced artifacts in the images of the structures. Negative staining (Brenner and Horne) became popular in the 60s and is still widely used, however this technique has the aforementioned issue with imaging.

Building off the previous work of Henderson and Unwin (Henderson and Unwin, Unwin and Henderson) as well as Taylor and Glaesor (Taylor and Glaeser, Hayward and Glaeser), Dubochet et al. (Adrian et al., Dubochet et al. (1988), Dubochet et al. (1981)) showed how fully preserved samples could be imaged by freezing them in a thin layer of a noncrystalline form of solid water, called amorphous or vitreous ice (see glossary). Considering vitreous ice needs to be maintained around the temperature of liquid nitrogen, this technique was termed “cryo-EM”. Combining cryo-EM imaging and 3D reconstruction techniques demonstrated potential to identify individual atoms.

In 1997, single particle reconstructions of the hepatitis B virus core particle resolved a helices for the first time (Bottcher, Conway); in 2008, the amino acid backbone could be traced in cryo-EM maps of epsilon-15 virus, polyhedrosis virus, and the rotavirus inner capsid particle (Jiang, Yu); and in 2010, maps with sufficient details for de novo atomic model building were obtained for aquareovirus and adenovirus (Zhang, Liu).

EM imaging previously relied on film or charge-coupled devices (CCDs), which have resolution issues and indirect imaging complications respectively. With the advent of direct electron detectors, the direct quantum efficiency (DQE) (see glossary) has improved drastically. Direct electron detectors have a much faster readout, enabling images to be acquired as a series of movie frames at 17–400 frames per second (see Figure 1). These technological advances, which were introduced into the field around 2012 led to a revolution in cryo-EM single particle analysis. The movies that are now taken during a cryo-EM experimentation session have increased the resolution and the amount of data generated, and are able to show a dynamic view of the samples that simply was not possible with the previous generation of detection that only provided a still shot.

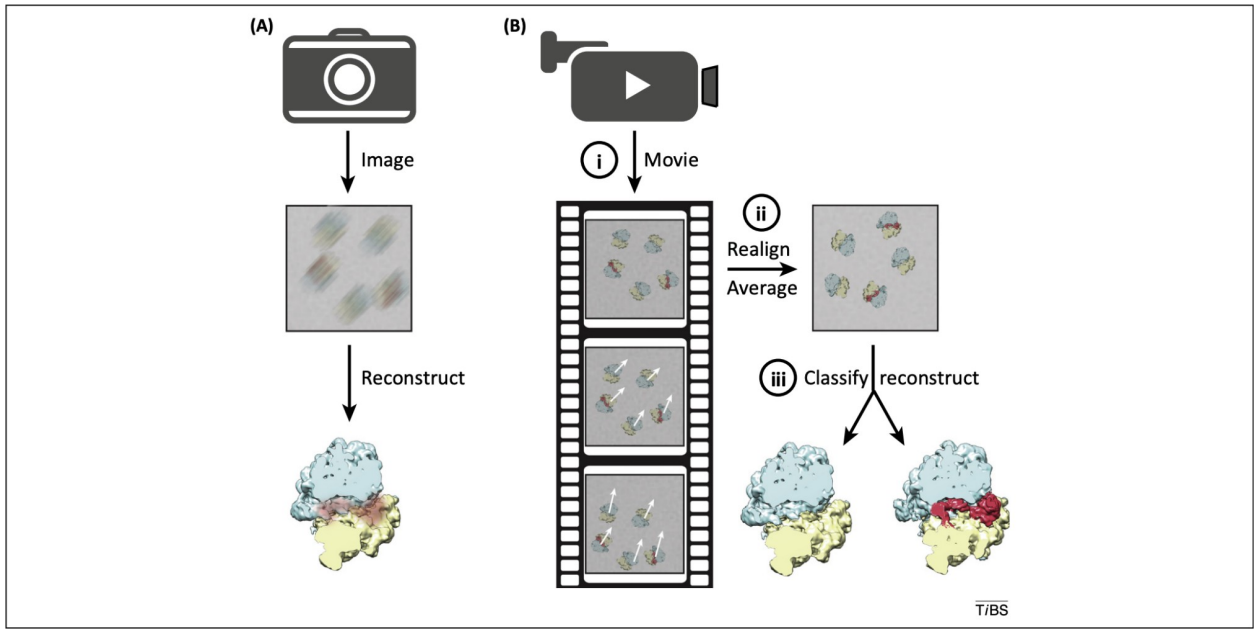

Figure 1. Recent technological advances (A) Previously, noisier images were recorded on photographic film, beam-induced sample motion led to image blurring, and structurally different particles were often mixed in a single reconstruction. (B) Three recent advances yield better reconstructions: (i) digital direct-electron detectors (see glossary) yield data of unprecedented quality and allow recording movies during exposure; (ii) computer programs to realign the movie frames may correct for sample movements that are induced by the electron beam; and (iii) powerful classification methods lead to multiple structures from a sample mixture. Figure and caption taken from (Xiao-Chen et al.)

Cryo-EMs are now powerful tools used to research viruses (e.g. Malaria, HIV-1 GP140, Tuberculosis, and HPV), cancers, bacteria, DNA, RNA, proteins, and enzymes such as the Angiotensin converting enzyme, which helps regulate blood pressure. A single cryo-EM experiment run can capture thousands of pictures and will generate 1-10 TB of raw data.

Cryo-EM in the US

There are over 100 Cryo-EM facilities privately and publicly (e.g. National Institute of Health (NIH)) funded in the United States alone. For a working list of Cryo-EM centers in the United States and internationally, please reference cryo-EM Service Centers, a workinglist.

The national science foundation alone has provided tens of millions of dollars for projects involving cryo-EM research and instrumentation within the last decade (see Figure 2)

AwardNumber	Title	LastAmendmentDate	PrincipalInvestigator	Organization	EndDate	AwardedAmountToDate	PIEmailAddress
1549132	Center for Bright Beams	2023-08-09	J. Ritchie Patterson	Cornell University	2026-09-30	34899149.0	jrp3@cornell.edu
1539918	MIP: Platform for the Accelerated Realization,...	2021-09-20	Darrell Schlom	Cornell University	2022-02-28	27816072.0	schlom@cornell.edu
1541079	RII Track-1: Louisiana Consortium for Innovati...	2019-07-31	Michael Khonsari	Louisiana Board of Regents	2021-06-30	20000000.0	khonsari@lsu.edu
2039380	MIP: Platform for the Accelerated Realization,...	2023-12-20	Darrell Schlom	Cornell University	2026-05-31	14404166.0	schlom@cornell.edu
1707356	NeuroNex Technology Hub: Enhanced resolution f...	2023-07-18	Kristen Harris	University of Texas at Austin	2024-08-31	9650000.0	kharris@mail.clm.utexas.edu
1542015	NNCI: North Carolina Research Triangle Nanotec...	2019-07-12	Jacob Jones	North Carolina State University	2021-08-31	5500000.0	jacobjones@ncsu.edu
1345219	CREST Phase II: Computational Center for Funda...	2017-07-07	Branislav Vlahovic	North Carolina Central University	2021-01-31	5409302.0	vlahovic@nccu.edu
0932300	NEXT GENERATION COMPOSITES CREST CENTER, NextG...	2016-01-12	Patrick Mensah	Southern University	2016-08-31	5100000.0	patrick_mensah@subr.edu
1542205	NNCI: Soft and Hybrid Nanotechnology Experimen...	2019-09-23	Vinayak Dravid	Northwestern University	2021-08-31	5098461.0	v-dravid@northwestern.edu
1542153	NNCI: Mid-Atlantic Nanotechnology Hub (MANTH) ...	2019-07-12	Mark Allen	University of Pennsylvania	2021-08-31	5000000.0	mallen@seas.upenn.edu

Figure 2. Top ten NSF awards for projects involving cryo-EM research and instrumentation within the last decade sorted by Award Amount to Date

Discussion

The use cases for cryo-EM are so varied and prolific that the 2017 Nobel prize in Chemistry was awarded “for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution”. Research using cryo-EM has expanded rapidly over the last decade, and the instrument is critical for research of diseases such as HIV and cancer.

The United States has many resources for cryo-EM research including training, national funding, private funding, and the instruments themselves. In stark contrast, the entirety of Africa does not have a single cryo-EM. The NSF has funded over 3,000 proposals for cryo-EM

research and instrumentation, and the NIH funds on the order of 10 cryo-EM in the US. The cost to build, house, and maintain a cryo-EM can easily add up to seven figures conservatively. There is currently a push for the training and use of cryo-EM in Africa, especially the electron microscope unit (EMU) at the University of Cape Town (UCT) in South Africa headed by Dr. Robert Knutsen. As per Dr. Jeremy Woodward of the EMU, the entirety of South Africa currently has only 8 observing spots at the eBIC, a cryo-EM in the UK, this year.

Next Steps

EMU currently has a heavy collaboration with the Carnegie Corporation, as well as the Chan Zuckerberg Initiative in the United States. This collaboration is primarily to provide African researchers the tools they need in order to learn to perform cryo-EM experiments. There is high potential for cryo-EM research collaboration between African and American scientists as more and more African scientists learn to use the powerful instrument, especially with the growing potential for remote experimentation. The ecosystem for research networking has piloted a remote access project funded by the NSF using Internet2, which could be scalable internationally using AmLight.

A place to start would be to research Rutgers (part of the remote pilot program for universities in the Northeast US), other universities, and the OSG to gauge their interest in collaborating with the EMU at UCT. A point of focus would be on Dr. Jeremy Woodward and his associates at EMU, who has already expressed the desire for more international collaboration, from the science point of view. Another point of focus would be to define collaborations with other university research programs in SW and SE Africa, as well as the US. From an international network point of view, it's important to include TENET and SANReN, and any collaborators outside of S. Africa that have connections with their NREN and Regional networks (e.g. Ubuntunet), which is directly connected to the Cape Town TENET Point-of-Presence by default.

Glossary

3D reconstruction: the mathematical operation to calculate a 3D density map from a collection of 2D projection images.

Charge-coupled device (CCD): an older generation of digital cameras for cryo-EM was based on this technology. Given that the CCD chip is sensitive to photons, an extra layer on top of the chip is used to convert electrons into photons. This conversion is a major source of additional noise in cryo-EM images.

Dalton (Da): an alternate name for the atomic mass unit, and kilodalton (kDa) is 1,000 daltons. Thus a protein with a mass of 64kDa has a molecular weight of 64,000 grams per mole.

Detective quantum efficiency (DQE): a measure for how much noise is added relative to the incoming signal due to errors in the detection process. A perfect detector does not add any noise and has a DQE of 1. In practice, all detectors add noise and have DQEs less than 1.

Direct electron detector: the latest generation of digital cameras for cryo-EM. 'Direct' refers to the fact that electrons are detected directly, in contrast to CCD cameras, where electrons are first converted into photons.

Movie mode: a mode of operation for the new detectors where multiple images are recorded during exposure of the sample to the electron beam.

Resolution: a measure of the smallest detail that is discernible in a density map or image. Resolution is often measured in Å (1 Å = 0.1 nm). Maps with better resolutions resolve smaller features. At resolutions better than 3.5 Å , many amino acid side-chains are resolved; at resolutions better than 4.8 Å, individual b strands are resolved; at resolutions better than 9 Å, a helices are resolved; and at worse resolutions only protein domains are resolved.

Single-particle analysis: a cryo-EM procedure where individual macromolecular complexes that are frozen in a thin layer of vitreous ice are imaged. The 3D structure of the complex is reconstructed from projection images of individual complexes (called particles) in different relative orientations.

Vitreous ice: an amorphous form of solid water. The fact that this form of ice is not crystalline renders it particularly suitable for cryo-EM single-particle analysis.

Works Cited

Adrian, M. “Cryo-electron microscopy of viruses.” *Nature*, vol. 308, 1984, pp. 32-36.

Bottcher, B. “Determination of the fold of the core protein of hepatitis B virus by electron cryomicroscopy.” *Nature*, vol. 386, 1997, pp. 88-91.

Brenner, S., and R. W. Horne. “A negative staining method for high resolution electron microscopy of viruses.” *Biochim. Biophys. Acta*, vol. 34, 1959, pp. 103-110.

Conway, J. F. “Visualization of a 4-helix bundle in the hepatitis B virus capsid by cryo-electron microscopy. Nature 386, 91–94.” *Nature*, no. 386, 1997, pp. 91-94.

Dubochet, J. “Cryo-electron microscopy of vitrified specimens.” *Q. Rev. Biophys*, vol. 21, 1988, pp. 129-228.

Dubochet, J. “Low temperature electron microscopy.” *Annu. Rev. Biophys. Bioeng.*, vol. 10, 1981, pp. 133-149.

Hayward, S. B., and R. M. Glaeser. “Radiation damage of purple membrane at low temperature.” *Ultramicroscopy*, vol. 4, 1979, pp. 201-210.

Henderson, R., and P. N. Unwin. “Three-dimensional model of purple membrane obtained by electron microscopy.” *Nature*, vol. 257, 1975, pp. 28-32.

Jiang, W. “Backbone structure of the infectious epsilon15 virus capsid revealed by electron cryomicroscopy.” *Nature*, vol. 451, 2008, pp. 1130–1134.

Liu, H. “Atomic structure of human adenovirus by cryo-EM reveals interactions among protein networks.” *Science*, vol. 329, 2010, pp. 1038–1043.

Ruska, H. “Uber ein neues bei der bakteriophagen Lyse auftretendes Formelement.” *Naturwissenschaften*, vol. 29, 1941, pp. 367-368.

Taylor, K. A., and R. M. Glaeser. “Electron diffraction of frozen, hydrated protein crystals.” *Science*, vol. 186, 1974, pp. 1036-1037.

Unwin, P. N., and R. Henderson. “Molecular structure determination by electron microscopy of unstained crystalline specimens.” *J. Mol. Biol.*, vol. 94, 1975, pp. 425-440.

Xiao-Chen, Bai, et al. “How cryo-EM is revolutionizing structural biology.” *Trends in Biochemical Sciences*, vol. 40, no. 1, 2015, pp. 49-57.

Yu, X. “3.88 A structure of cytoplasmic polyhedrosis virus by cryo-electron microscopy.” *Nature*, vol. 453, 2008, pp. 415-419.

Zhang, X. “3.3 A˚ cryo-EM structure of a nonenveloped virus reveals a priming mechanism for cell entry.” *Cell*, vol. 141, 2010, pp. 472–482.