Nogales, E., and Scheres, S. (2015). Cryo-EM: a unique tool for the visualization of macromolecular complexity. Mol. Cell *58*, 677-689.

This review covers the history of this technique and many of the critical breakthroughs in recent years. It discusses which types of compounds can be visualized using this method, which is mostly large macromolecules that may exist in multiple states, conformations, or orientations. The image processing/analysis has been the main limiting factor for the past two decades, but more powerful equipment has been and will continue lowering that barrier. The rest of the review gave examples of cryo-EM’s capability to image complex protein assemblies, multiple states, heterogeneous samples, and small complexes (somewhat).

Unwin, N. Acetylcholine receptor channel imaged in the open state. Nature 373, 37–43 (1995).

This is one of the earliest uses of this technique to determine the structure of a ligand gated ion channel. The structure of the closed channel had been previously imaged and this one focused on its open state and how it differed from the closed state. It determined where the presumed binding sites for ACh were and how its binding caused rotation of the subunits (primarily the alpha subunits) and how that rotation propagated down to the membrane spanning region of the pore. This rotation disrupted the leucine ring that linked all five subunits together and normally would keep the channel shut.

Murata, K.; Mitsuoka, K.; Hiral, T.; Walz, T.; Agre, P.; Heymann, J.B.; Engel, A.; Fujiyoshi, Y. Structural determinants of water permeation through aquaporin-1. Nature 2000, 407, 599–605.

This is another early example of using EM to determine the structure of AQP1, except it was done with electron crystallography, which is sort of halfway between x-ray crystallography and cryo-EM. They were able to find the order and arrangement of the alpha helices in the subunits forming the tetramer and suggested how these arrangements could stabilize the channel. At the narrowest section of the pore, they determined the residues that line this region that would allow for permeation of water but not of ions or larger molecules, which aligns with experimental results.

Tao, X., Hite, R. K. & MacKinnon, R. Cryo-EM structure of the open high-conductance Ca2+-activated K+ channel. Nature 541, 46–51 (2017).

This is a recent example of cryo-EM being used to determine the structure of the BK channel. The selectivity filter in BK is very similar to what has already been found in other K+ channels. However, differences arise in the voltage sensor of BK. The S4 segment of BK channels has half the number of positively charged residues that function as the voltage sensor in other K+ channels. How this affects the voltage dependence of the channel isn’t entirely clear, but they suggest that it might limit the voltage sensor movements. Otherwise, much of the structure was not surprising because it lined up with many electrophysiological predictions.

Basak, S. et al. Cryo‑EM structure of 5‑HT3A receptor in its resting conformation. Nat. Commun. 9, 514 (2018).

This article is a recent attempt at characterizing the structure of the 5‑HT3AR without prior activation or inhibition, so that it will be in its resting state. Compared to the early paper on the nAChR, this one gives a far more detailed view of the pentameric receptor beyond just its subunits. It resolved the subunits enough to determine the arrangement of the alpha helices, and the distance between the ones lining the pore, as well as finding the serotonin binding sites. Additionally, this article compared the cryo-EM structure to that from x-ray crystallography and found that there were subtle but important differences in their structure.

Y. Cheng, Single-particle cryo-EM-How did it get here and where will it go, Science. 361 (2018) 876–880. doi:10.1126/science.aat4346

This review offers a more succinct and digestible view of cryo-EM and covers the major breakthroughs and limitations that have led to its introduction and it also offers suggestions on where the field will go. Time resolved cryo-EM would be the next step in the process in addition to improving throughput to determine the structure of more molecules.

Liu, Z., Guu, T., Cao, J., Li, Y., Cheng, L., Tao, Y. J., & Zhang, J. (2016). Structure determination of a human virus by the combination of cryo-EM and X-ray crystallography. *Biophysics reports*, *2*(2), 55-68.

This is an example of how two different techniques can be used to determine the structure of, in this case, the hepatitis E virus (HEV). Both x-ray crystallography and cryo-EM have limitations in the types of molecules they can be used on and viruses are one such example that both have difficulty resolving (mostly due to insufficient sample size). Here, they used cryo-EM to get a low-resolution structure of HEV and then used this reference information to get the final higher resolution structure from x-ray crystallography. The combined techniques they used in this could be helpful for determining the structures of other viruses (or small proteins) that haven’t been able to be used for either technique.

Gonen, T., Cheng, Y., Sliz, P., Hiroaki, Y., Fujiyoshi, Y., Harrison, S. C., & Walz, T. (2005). Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. *Nature*, *438*(7068), 633-8.

This is the highest (or one of the highest) resolution structure that has been detailed with cryo-EM, (1.9 Å resolution). AQP0, another aquaporin channel, forms junctions between fibre cells and these junctions require interactions with surrounding lipids. They determined the structure of a junctional AQP0 and compared it with non-junctional AQP0 to determine how lipids interact with the protein. Crystal structures of some other proteins contained lipids with high binding affinity for the protein, which they found for the junctional AQP0 proteins. Based on this, they suggest roles for how lipid interactions affect how AQP0 subunits interact laterally.

Unwin, N., & Fujiyoshi, Y. (2012). Gating movement of acetylcholine receptor caught by plunge-freezing. *Journal of molecular biology*, *422*(5), 617-634.

This is a more recent article that highlights the differences and improvements in resolution that cryo-EM has made in the 17 years since the paper capturing the structure of the open ACh receptor had been published. For instance, this paper demonstrated that the subunits aren’t symmetrical as the original paper had presumed. Additionally, this article showed how having a ligand binding site far away from the gate would allow for opening almost instantaneously. This was possible to do because they could resolve the individual helices in far more detail than they were able to in the original article.

Razinkov, I., Dandey, V., Wei, H., Zhang, Z., Melnekoff, D., Rice, W. J., Wigge, C., Potter, C. S., … Carragher, B. (2016). A new method for vitrifying samples for cryoEM. *Journal of structural biology*, *195*(2), 190-198.

Most of the techniques used for cryo-EM have improved in the decades since its introduction. One of the ones that hasn’t changed much is the vitrification process. The purpose of this article is to describe a way to automate sample vitrification in order to have more consistency from sample prep to sample prep. They developed a self-blotting grid would spread the sample to a thin layer of liquid that ideally would reduce the amount of sample required. This would be accomplished if there was more consistency in how the ice is distributed evenly across the surface. Having samples spread evenly throughout the ice would also reduce the empty spaces that would need to be sorted through after imaging.

Web resources

* <https://www.rcsb.org/>
  + Protein data bank containing archives of the 3D shapes of proteins, nucleic acids, and complex assemblies
* <https://www.ebi.ac.uk/pdbe/emdb/>
  + The Electron Microscopy Data Bank (EMDB) is a public repository for electron microscopy density maps of macromolecular complexes and subcellular structures. It covers a variety of techniques, including single-particle analysis, electron tomography, and electron (2D) crystallography.
* <https://www.ebi.ac.uk/pdbe/emdb/empiar/>
  + EMPIAR, the Electron Microscopy Public Image Archive, is a public resource for raw, 2D electron microscopy images. Here, you can browse, upload and download the raw images used to build a 3D structure.
* <https://www.ebi.ac.uk/>
  + The European bioinformatics institute maintains a search engine for many compounds (sequences, molecules, proteins, etc.) and how they are researched in many areas (interactions, expression, structure, pathways, etc.).
* <https://www.nature.com/subjects/cryoelectron-microscopy>
  + This page is frequently updated with the latest news and research for cryo-EM.
* <https://bsir.bio.fsu.edu/>
  + FSU has several electron microscopes and the capability to do cryo-EM here.
* <http://ncmi.bcm.tmc.edu/ncmi/>
  + National center for macromolecular imaging is another site that offers information and training opportunities for individuals who wish to learn how to do cryo-EM.
* <http://www.crystallography.net/cod/>
  + Open-access collection of crystal structures of organic, inorganic, metal-organics compounds and minerals, excluding biopolymers.
* <http://www.bmrb.wisc.edu/>
  + A repository for data from NMR spectroscopy on proteins, peptides, nucleic acids, and other biomolecules.
* <http://challenges.emdataresource.org/>
  + EMDataBank is hosting community-wide challenges to critically evaluate 3DEM methods that are coming into use, with the ultimate goal of developing recommendations for validation criteria associated with every 3DEM map deposited to the EM Data Bank (EMDB) and map-derived model deposited to Protein Data Bank (PDB).