A resolution record for cryoEM

Jonathan Ashmore***, Bridget Carragher**, Peter B. Rosenthal, William Weis*
https://doi.org/10.12703/r-01-000002
Published: 2021 July 30

EVALUATION OF

Landmark

Atomic-resolution protein structure determination by cryo-EM.

Yip KM, Fischer N, Paknia E, Chari A, Stark H.
https://doi.org/10.1038/s41586-020-2833-4
Article published: 2020 Nov Nature 587:157-161.

Landmark

Single-particle cryo-EM at atomic resolution.

Nakane T, Kotecha A, Sente A, McMullan G, Masiulis S, Brown P, et al.
https://doi.org/10.1038/s41586-020-2829-0
Article Published: 2020 Nov Nature 587:152-156.

Cryo electron microscopy (cryoEM) is a fast-growing technique for structure determination. Two recent papers report the first atomic resolution structure of a protein obtained by averaging images of frozen-hydrated biomolecules. They both describe maps of symmetric apoferritin assemblies, a common test specimen, in unprecedented detail. New instrument improvements, different in the two studies, have contributed better images, and image analysis can extract structural information sufficient to resolve individual atomic positions. While true atomic resolution maps will not be routine for most proteins, the studies suggest structures determined by cryoEM will continue to improve, increasing their impact on biology and medicine.

EVALUATION BY

Jonathan Ashmore***
University College London
Cellular mechanisms of hearing

Bridget Carragher**
New York Structural Biology Center
Automation and cryo electron microscopy (cryoEM)

Peter B. Rosenthal
The Francis Crick Institute
Structural biology of cells and viruses

William Weis*
Stanford University
Architecture and dynamics of intercellular adhesion junctions and signaling pathways

***Corresponding author:** William Weis (bill.weis@stanford.edu)

****Primarily responsible for drafting the consensus evaluation**

*****Broad perspective** panelist from a different field

Competing interests: The authors declare that they have no competing interests.

How to cite this article: Ashmore et al. A resolution record for cryoEM. Fac Rev 2021, 10:(64) (https://doi.org/10.12703/r-01-000002)

Articles evaluated for landmarks are selected by members of an Advisory Board.

Background

The origins of cryoEM go back more than 40 years, but it is only in the last 6 years that the power of the method has reached its potential. Richard Henderson predicted that cryoEM is theoretically capable of providing atomic resolution of biological macromolecules in a landmark analysis in 1995[1] and this helped to spur on efforts to improve both the instrumentation as well as experimental and computational methods, culminating in the so-called "Resolution Revolution"[2]. Since then, cryoEM has been providing a dazzling array of new structures, many of them of molecular complexes that had been intractable to established methods (like X-ray crystallography and NMR), including very large protein complexes, integral membrane proteins, and highly heterogeneous or conformationally dynamic systems.

The term "atomic resolution" has been used to describe cryoEM maps at about 3 Å resolution. Such maps suffice for building atomic models when additional knowledge about protein geometry is used. However, none of these structures has been solved at a resolution where individual atoms could be discerned. Until now.

Main contributions and importance

The achievement of true atomic resolution using cryoEM is a major landmark for the field. While it may have been predicted and expected, it has not been clear whether the severe challenges associated with imaging radiation-sensitive, frozen-hydrated biological specimens, preserved in very thin vitrified ice films, would have kept this milestone permanently out of reach. This alone is reason to celebrate. Two independent groups have now achieved this goal, and while both teams attribute their success to improvements in instrumentation, their approaches were not identical. Yip *et al.*[3] used a prototype instrument capable of limiting the energy spread of the electron beam with a monochromator and spherical aberration corrector,

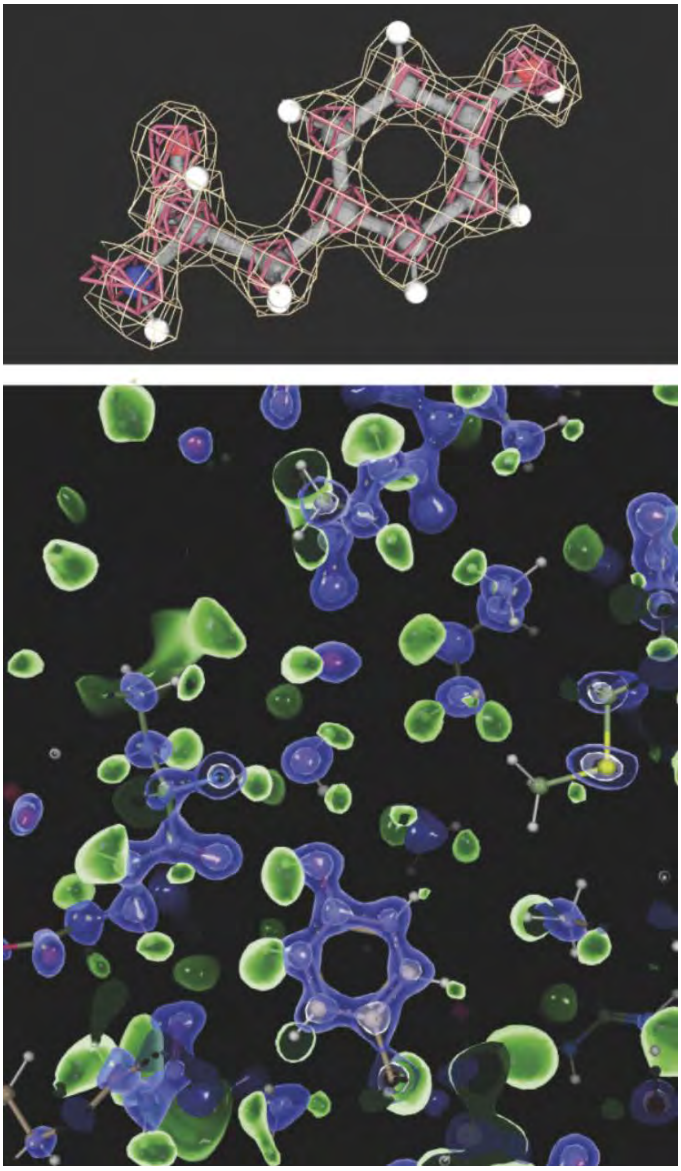

Figure 1. CryoEM maps resolve atoms in a protein assembly
Top: Amino acid side chain (tyrosine) from the atomic resolution map (grey wire mesh) of apoferritin shows resolved peak density (red wire mesh) at locations of carbon atoms (grey spheres), nitrogen atoms (blue spheres), and oxygen atoms (red spheres). Hydrogen atoms are white spheres. Based on data in reference[3]. Image courtesy of Holger Stark.
Bottom: A section of the apoferritin map and atomic model with carbon, nitrogen, and oxygen atoms located at resolved density peaks in the map (blue mesh). The "Difference map" density (green peaks), highlights the differences between the experimental map and the model for the heavy atom positions (carbon, nitrogen, and oxygen), and thus identifies the location of the lighter hydrogen atoms. Based on data in reference[4]. Image courtesy of Sjors Scheres.

while Nakane *et al*.[4] used a new cold field emission electron source, a new direct electron detector, and a new very stable energy filter (Figure 1). These alternatives indicate that there is not a single route to reaching higher resolution, but which set of instrumentation advances provides the most straightforward and economical approach towards this end remains to be determined. To calculate maps of the protein, both groups used the single particle processing software Relion[5] which includes corrections for microscope aberrations that affect images at high resolution. Atomic models were constructed and refined based on the resolved positions of individual carbon, nitrogen, and oxygen atoms and "difference maps" revealed hydrogen atomic positions.

The main significance and likely influence of these studies is that they affirm the current theory of what limits contrast and resolution in cryoEM. A single parameter, the temperature factor or B-factor, describes how structural signal fades due to imaging and computational imperfections and determines the number of particle images needed to reach a specific resolution. In both of these studies the B-factors are impressively low (32 Å^2 and 36 Å^2), with corresponding resolutions of 1.2 Å, and this promises to increase the efficiency of data collection, even for samples that are not capable of going to atomic resolution. This is significant in that electron microscopes are a very expensive and currently scarce resource.

Open questions

The beautiful structures presented at atomic resolution were both of apoferritin (apoF) which is sometimes referred to as the "lysozyme of EM"; it is an ideal test specimen, exhibiting no pathological behavior when vitrified, having high symmetry, and apparently very little flexibility. In fact, a slightly lower resolution 1.35 Å map of apoferritin has since been reported without new instrument advances[6]. This immediately raises the question as to whether atomic resolution can be achieved for more interesting structures – many of which have none of the advantages of apoF. Happily, Nakane *et al*. provide some encouragement on this, presenting a structure of a membrane protein, the β3 $GABA_A$ receptor homopentamer, reconstructed to a resolution of 1.7 Å, a relatively huge leap from the previously best reported resolution of ~2.5 Å. However, for this case it is hard to parse out the relevant factors contributing to this success as the authors combined data obtained using both new and existing technology to produce this map. It remains to be seen if this improvement can be repeated using existing instruments if more effort is devoted to optimizing the sample and correcting for optical aberrations, or if the new technologies are essential to improving the resolution. This leads to further questions as to the relevance of these studies for structure-based drug design (SBDD), an approach to finding new medications dependent on understanding the detailed structure of the biological target of interest. While higher resolution is always an advantage, it is not clear how many structures relevant to SBDD will be amenable to the advantages described in these papers. Targets for drug discovery are frequently flexible and fragile and the obtainable resolution may be more dependent on the limitations of the sample than of the instrument used for imaging. The currently obtainable resolution of these structures is in the range of 3 Å and it is not clear if the described improvements in technology will impact this range or are more suited to structures that are already approaching 2 Å. Nevertheless, the clear improvement in B-factor presents the possibility of improving both the resolution and throughput as fewer images would be needed for a given resolution, and this may also impact those specimens where multiple conformers, dynamics, and heterogeneity are the limiting factors.

One intriguing aspect of these new atomic resolution structures is their potential for improving model building for cryoEM. There are powerful tools for building atomic models based on X-ray diffraction data, which arise

from scattering by electrons in individual atoms and is straightforward to model. In contrast, cryoEM maps describe the electrostatic potential of the specimen, which is sensitive to both electron and nuclear positions and is more challenging to describe. The knowledge obtained of how to optimally build and refine structural models based on cryoEM images may be applicable to more routine structure determinations at lower resolution.

Finally, we would like to draw attention to another notable aspect of this work: both papers were published initially on bioRxiv[7,8] and were widely disseminated and discussed via Twitter and other social media. We also applaud Nakane *et al*. for uploading their raw data to the public database EMPIAR (https://www.ebi.ac.uk/pdbe/emdb/empiar/entry/10424/). This will allow other software developers to reproduce the results and study whether with excellent data there are also other algorithmic paths to atomic resolution. This rapid sharing of critical advances accelerates research, a factor that has been very dramatically highlighted by the crisis of the COVID-19 pandemic in 2020.

Conclusions

This work is a new milestone for cryoEM, definitively fulfilling the predictions made by Richard Henderson 25 years ago. It is clear is that the field is still in a state of rapid evolution and new bars are being set on an almost weekly basis. We anticipate many parallel efforts will be launched to replicate these results and to probe which are the most important aspects of the advances. We also note that other, far less expensive technology developments also hold promise of dramatic improvements in overall resolution and throughput[9]. We are confident that cryoEM is still on a steep arc of improvement and that the future remains bright and full of promise.

References

1. Henderson R 1995. The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological molecules. Q Rev Biophys 28:171–93 doi: 10.1017/S003358350000305X

2. Kühlbrandt W 2014. Biochemistry. The resolution revolution. Science 343:1443–4 doi: 10.1126science.1251652

3. Landmark

Yip KM, Fischer N, Paknia E et al. 2020. Atomic-resolution protein structure determination by cryo-EM. Nature 587:157–61 doi: 10.1038/s41586-020-2833-4

4. Landmark

Nakane T, Kotecha A, Sente A et al. 2020. Single-particle cryo-EM at atomic resolution. Nature 587:152–6 doi: 10.1038/s41586-020-2829-0

5. Scheres SH 2012. RELION: implementation of a Bayesian approach to cryo-EM structure determination. J Struct Biol 180:519-30 doi: 10.1016/j.jsb.2012.09.006

6. Zhang K, Pintilie GD, Li S, Schmid MF, Chiu W 2020. Resolving Individual-Atom of Protein Complex using Commonly Available 300-kV Cryo-electron Microscopes. bioRxiv doi: 10.1101/2020.08.19.256909

7. Landmark

Yip KM, Fischer N, Paknia E et al. 2020. Breaking the next Cryo-EM resolution barrier – Atomic resolution determination of proteins! bioRxiv doi: 10.1101/2020.05.21.106740

8. Landmark

Nakane T, Kotecha A, Sente A et al. 2020. Single-particle cryo-EM at atomic resolution. bioRxiv doi: 10.1101/2020.05.22.110189

9. Recommended

Naydenova K, Jia P, Russo CJ 2020. Cryo-EM with sub-1 Å specimen movement. Science 370:223–6 doi: 10.1126/science.abb7927