

Cryo-EM and X-Ray Crystallography: Complementary or Alternative Techniques?

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Since its virtual establishment in 1912 with the first diffraction photograph from a crystal of copper sulfate [1], X-ray crystallography has been a tremendously invaluable tool in understanding not only the chemical nature of crystals, but also the three-dimensional structure of molecules. The latter is testified in the Cambridge Structural Data Base (CSD, <http://www.ccdc.cam.ac.uk>) with more than 800,000 structures deposited, and by the Protein Data Bank (PDB, <http://www.rcsb.org/pdb>), that nowadays count the structure of more than 120,000 biological macromolecules. The application of crystallography to life sciences has played a fundamental role in the field, in particular in biochemistry and in molecular biology, where the knowledge of the structure of macromolecules at atomic or nearly-atomic resolution allows to interpret the mechanisms underneath the biological processes in terms of the disposition of atoms in space. It is impossible in this short space to list even part of the achievements of structural biology that has taken place since the '60, when the structures of the first proteins became available [2], to the present times.

Despite X-ray crystallography can in principle reach very high resolution, only about 700 structures of biological macromolecules have been refined at a resolution higher than 1.0 Å, whilst the large majority of them is in between 1.5 Å and 2.5 Å (more than 60,000 deposited structures), and quite a few at a resolution worse than 3.5 Å (less than 1,500, about 1.2% of the total).

Third generation synchrotrons and, more recently, X-ray free electron lasers (XFEL) have allowed to push the size of useful crystals to the limit of nanometers [3-5]. Structures can be obtained merging together diffraction data from several nano-crystals [6,7].

Classical transmission electron microscopy (EM) is also a relatively old technique but, even if the energy of electrons used in a commercial electron microscope (100 keV – 300 keV) allows in principle imaging at sub-Angstrom resolution, this does not happen in practice. Owing to the technical limitations imposed by the instrument, until few years ago the resolution of the images was rarely better than 10 Å, and a 5 Å resolution was reached in a limited number of cases. A revolution in the field came into sight around 2012, with the advent of new electron detectors, phase plate devices, beam-induced motion correction and other useful devices (see, for example, [8-11]). These tools have allowed an improvement of the resolution limits till 3 Å or, in the most favorable cases, 2.2 Å - 2.5 Å [12]. Consider that even in crystallography a resolution of 3 Å or 3.5 Å is usual for crystals of very large molecular complexes, so for structures with a huge number of atoms EM and crystallography give comparable results.

In the technique called “cryo-EM” a small drop of solution containing the macromolecule is frozen instantaneously, in order to avoid the formation of ice crystals, at 100 K or less. Single molecules or particles of large biological complexes

are present in the solution in a random orientation, and a large number of two-dimensional projections of the same molecule can be observed in each single picture. The use of a very large number of particles (of the order of 100,000 or similar) allows the reconstruction of a three-dimensional electron density map, analogous to that deriving from the X-ray diffraction of a crystal. At this point the interpretation of the map can be carried out using methodologies typical of X-ray diffraction, making use of already existing software.

The main advantage of cryo-EM is the fact that, instead of crystals, single molecules in solution are used. In fact, the growth of crystals is still the rate determining step in an X-ray structure determination: despite several technical improvements, crystal growth retains a component that is more close to an art than to a science. In the case of large molecular complexes, often labile and difficult to obtain in crystal form, cryo-EM opens the possibility of determining their three-dimensional structure directly in solution. A second advantage is that the phase problem does not exist at all in cryo-EM and the electron density map is not affected by the bias introduced by the use of phases calculated from the model.

Of course drawbacks are also present in cryo-EM. The first is that only large molecules or molecular complexes (the present limit is around 200,000 Da) can be clearly distinguished in the pictures, and for the moment small proteins (although a protein of 100,000 Da cannot really be considered small) are out of the reach. Nevertheless, in cryo-EM there is still space for a lot of technical improvements [13].

Finally, in cryo-EM, as it happens in crystallography, objects must be structurally identical in order to be mediated them and to give rise to a unique molecular model. Eventually, two or three different conformations can be distinguished and ordered in classes [14]; despite that, fully flexible molecules can be studied using a different technique based on EM, called cryo-electron Tomography (cryo-ET) [15]. For the moment the resolution of this technique is still definitely lower.

Will cryo-EM replace crystallography for the structural analysis of biological macromolecules? At present, the two appear more as complementary than alternative techniques: crystallography can reach higher resolution and a better definition of the atomic positions, whilst cryo-EM works probably better for large complexes or aggregates. Applications of cryo-EM are developing at a very fast rate, limited mostly by availability of the microscopes equipped with all the necessary tools for the determination of structures at nearly-atomic resolution [16]. In addition, the processing of data requires specific know-how and the availability of computer clusters to process the very large amount of data [17-19].

The *NanoWorld Journal* appears as an ideal forum for such kind of studies and encourages the submission of *research articles*, *reviews* or *commentaries* both in nano-crystallography and in cryo-EM or cryo-ET techniques.

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