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Title:

Why cryogenic electron microscopy matters?

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Why cryogenic electron microscopy matters?

Abstract:

In this paper, the author will start with some basic concepts of cryogenic electron microscopy, including the fundamental setup, sample preparation, and the history of invention. Then theoretical principles and instrumentation will be discussed, which comprises of electron diffraction, TEM, followed by the uniqueness of cryo-EM. Next, two aspects (biological science and material science) of some recent application using cryo-EM will be introduced. At the end of the paper, author's conclusion will be presented.

1 Introduction

Cryo-electron microscopy (cryo-EM) is a kind of transmission electron microscope (TEM) where the sample is examined at cryogenic temperature (generally lower than -160℃). [1]It has become a mainstream tool for determining the structures from cells to macromolecular complexes at the atomic resolution. It has many advantages over other techniques such as X-ray crystallography and nuclear magnetic resonance (NMR). [2]Thanks to its powerful functions, lots of researchers consider this as an ultimate means to discover fundamental structures of different materials, allowing them to understand the mechanisms as well as principles from the very basic level.

The Nobel Prize in Chemistry 2017 was awarded to Professor Jacques Dubochet, Dr. Joachim Frank and Dr. Richard Henderson who contributed to this invention *"for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"*. Back to last century, electron microscopes were long believed to only be suitable for imaging dead matter, because the powerful electron beam destroys biological material. But in 1990, Richard Henderson succeeded in using an electron microscope to generate a three-dimensional (3D) image of a protein at atomic resolution. This breakthrough proved the technology's potential. Joachim Frank made the technology generally applicable. Between 1975 and 1986 he developed an image processing method in which the electron microscope's fuzzy

two-dimensional (2D) images were analyzed and merged to reveal a sharp 3D structure. Jacques Dubochet added water to electron microscopy. Liquid water evaporates in the electron microscope's vacuum, which makes the biomolecules collapse. In the early 1980s, Dubochet succeeded in vitrifying water – he cooled water so rapidly that it solidified in its liquid form around a biological sample, allowing the biomolecules to retain their natural shape even in a vacuum. Following these discoveries, the electron microscope's every nut and bolt have been optimized. The desired atomic resolution was reached in 2013, and researchers can now routinely produce 3D structures of biomolecules. In the past few years, scientific literature has been filled with images of everything from proteins that cause antibiotic resistance, to the surface of the Zika virus. Biochemistry and material science are now facing an explosive development and are all set for an exciting future.[3]

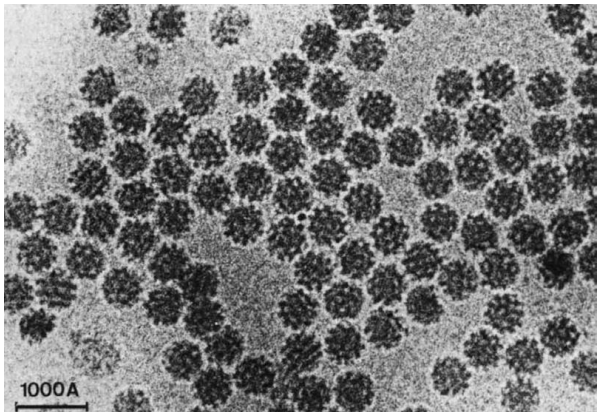

Fig.1 Dubochet generated the first images of viruses surrounded by vitrified water in 1984. Image from Nature **308**: 32-36[4]

However, cryo-EM also entails several challenges. In Li's recent review paper, his group identifies six major areas in materials science that benefit from the interdisciplinary application of cryo-EM, pointing out a grand picture for the future of these challenges we are faced with. [5]

In this paper, the author will present a general discussion about the theoretical principles of this novel technology, the core of the instrumentation set-up, as well as some recent inspiring discoveries published online regarding biological and material science. At the end of the paper, several conclusions will be discussed.

2 Theoretical Principles and Instrumentations of cryo-EM

As mentioned above, to get to know about cryo-EM, we should first start to learn about the principle of electron diffraction, as well as TEM. Then the optimized method for the “cryogenic” type of TEM will be discussed.

2.1 Electron Diffraction

The electron is a low mass, negatively charged particle. It can easily be deflected by passing close to other electrons or the positive nucleus of an atom. These Coulombic (electrostatic) interactions cause the electron scattering which is the process that makes TEM feasible.

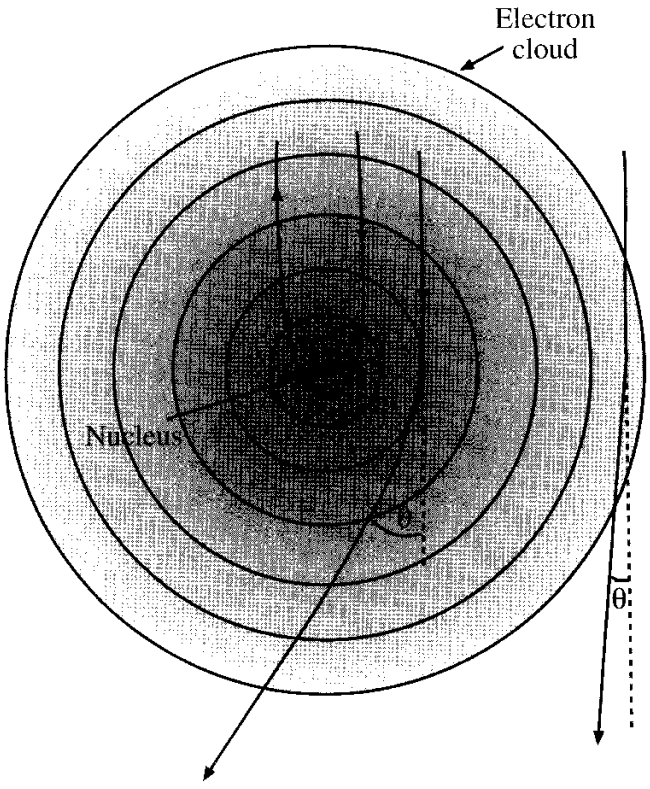

Fig.2 An idealized model of the atom, which consists of the positive charged nucleus and the surrounding negatively charged electron cloud

Generally the scattering conditions of the electrons can be separated into two parts: elastic scattering and inelastic scattering. The former one is mainly used in electron diffraction and TEM imaging, while the latter one is preferable for EDS and EELS. At this time, we shall focus on the imaging part, so elastically scattering takes the lead, which usually occurs at relatively low angles (1-10°).

According to Bragg's Law, electron beam incident on a set of crystal planes is only reflected or diffracted at the specific angles θ.

$$2d\sin\theta = \lambda$$

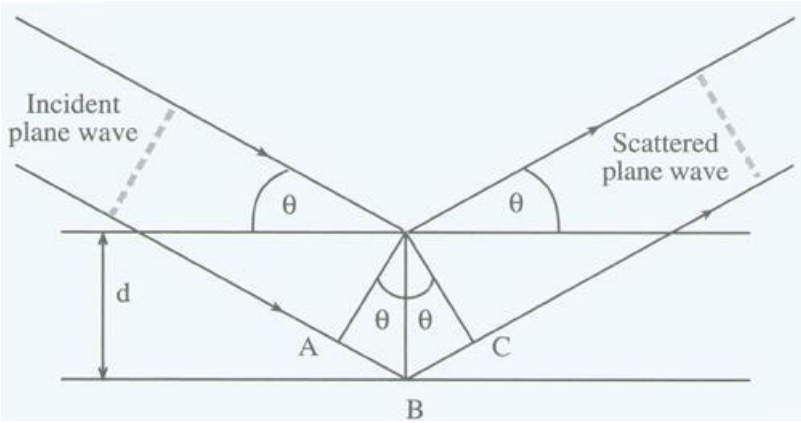

Fig.3 The incident angle is equivalent to the scattered angle, given the normal plane as the perpendicular direction to the parallel lattice plane

In TEM, Bragg's angle tends to be very small. Using the Bragg's Law equation above, we can conclude that only the planes almost parallel to the incident beam can generate Bragg diffraction.

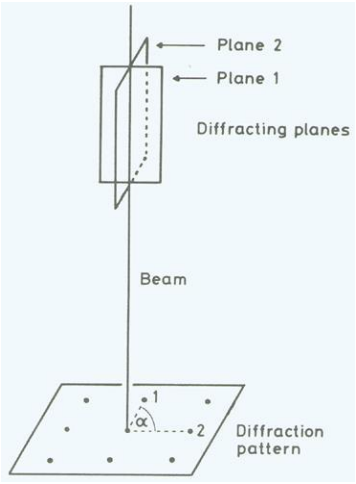

Fig.4 A demonstration of how the incident beam connects diffracting planes with specific diffraction pattern

Even if the Bragg's Law is satisfied, electron diffraction may still not occur due to the systematically absent. That's why structure factor is introduced to deal with this problem.

The structure factor F_{hkl} is a measure of the amplitude scattered by all the atoms in a unit cell into the reflection hkl, where n is the total number of atoms in the unit cell, the subscript j denotes the j^{th} atom, f_j is the atomic scattering factor. $(\vec{K}_g - \vec{K}_0)\cdot\vec{r}_j$ is the pass difference. The intensity diffracted by the unit cell into the hkl reflection is $I_{hkl} \propto |F_{hkl}|^2$. When F=0, I=0. Systematically absence occurs. Bragg diffraction does not occur under this circumstance.

$$F_{hkl} = \sum_{j=1}^{n} f_j \exp 2\pi i(\vec{K}_g - \vec{K}_0)\cdot\vec{r}_j$$

The full condition for Bragg diffraction should meet both the conditions below: satisfying the Bragg's Law as well as a non-zero structure factor.[8][9]

2.2 Basic features for a TEM

Transmission electron microscope (TEM) is a microscopy technique that functions similar to a light microscope, which uses a beam of exited electrons as a light source to provide structure information of an ultra-thin specimen. The uniqueness of TEM is the ability to obtain full morphological (grain size, grain boundary and interface, secondary phase and distribution, defects and their nature, etc.), crystallographic, atomic structural and microanalytical such as chemical composition (at nanometer scale), bonding (distance and angle), electronic structure, coordination number data from the sample. TEM is most efficient and versatile technique for the characterization of materials. The image is formed by the interaction of the electrons transmitted through the specimen, which is then magnified and focused on the sensor.[6]

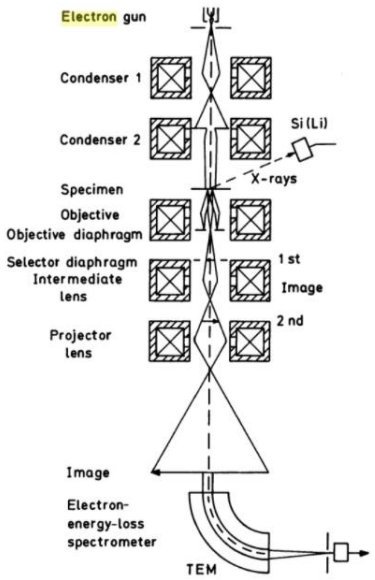

Fig.5 Schematic ray path for a transmission electron microscope equipped for additional x-ray and electron energy-loss spectroscopy (EELS)

In a conventional transmission electron microscope, a thin specimen is irradiated with an electron beam of uniform current density; the electron energy is in the range 60-150keV (usually 100keV) or 200keV-3MeV in the case of the high-voltage electron microscope (HVEM).

Electrons are emitted in the electron gun by thermionic emission from tungsten hairpin cathodes or LaB_6 rods or by field emission from pointed tungsten filaments. The latter are used when high gun brightness is needed. A two-stage condenser-lens system permits variation of the illumination-intensity distribution behind the specimen is imaged with a three- or four-stage lens system, onto a fluorescent screen. The image can be recorded by direct exposure of a photographic emulsion inside the vacuum.

The lens aberrations of the objective lens are so great that it is necessary to work with very small objective apertures, of the order of 10-25 mrad, to achieve resolution of the order of 0.2-0.5 nm. Bright-field contrast is produced either by absorption of the elections scattered through angles larger than the objective aperture (scattering contrast) or by interference between the scattered wave and the incident wave at the image point (phase contrast). The phase of the electron waves behind the specimen is modified by the wave aberration of the objective lens. This aberration and the energy spread of the electron gun, which is of the order of 1-2 eV, limits the contrast transfer of high spatial frequencies.

Electrons interact strongly with atoms by elastic and inelastic scattering. The specimen must therefore be very thin, typically of the order of 5nm-0.5 μm for 100keV electrons, depending on the density and elemental composition of the object and the resolution desired. Special preparation techniques are needed for this. Thicker specimens can be investigated in a high-voltage electron microscope.

TEM can provide high resolution because elastic scattering is an interaction process that is highly localized to the region occupied by the screened Coulomb potential of an atomic nucleus, whereas inelastic scattering is more diffuse; it spreads out over about a nanometer.

A further capability of the modern TEM is the formation of very small electron probes, 2-5 nm in diameter, by means of a three-stage condenser-lens system, the last lens field of which is the objective pre-field in front of the specimen. This enables the instrument to operate in a scanning transmission mode with a resolution determined by the electron-probe diameter; this has advantages for imaging thick or crystalline specimens and for recording secondary electrons and backscattered electrons, electron-beam-induced currents. [7]

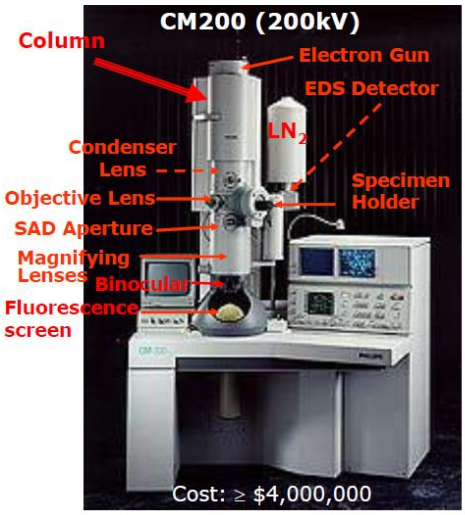

Fig.6 An overall appearance of a TEM and the names of different parts[6]

2.3 What's special for the cryo-EM?

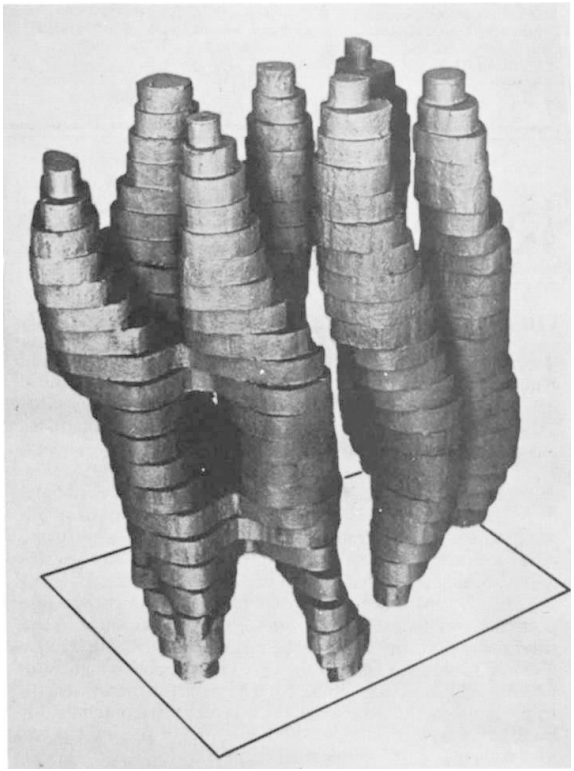

Fig.7 The first rough model of bacteriorhodopsin, published in 1975. Image from Nature 257: 28-32[8]

This was the best picture of a protein ever generated using an electron microscope. Many people were impressed by the resolution, which was 7 Å, but this was not enough for Richard Henderson. His goal was to achieve the same resolution as that provided by X-ray crystallography, about 3 Å, and he was convinced that electron microscopy had more to give.

Joachim Frank was working at the New York State Department of Health. He devised a method for the 3D reconstruction of the tilted pictures obtained from the cryo-EM, making it possible to give out the 3D models of different proteins, which greatly benefited the cellular level of research.[11]

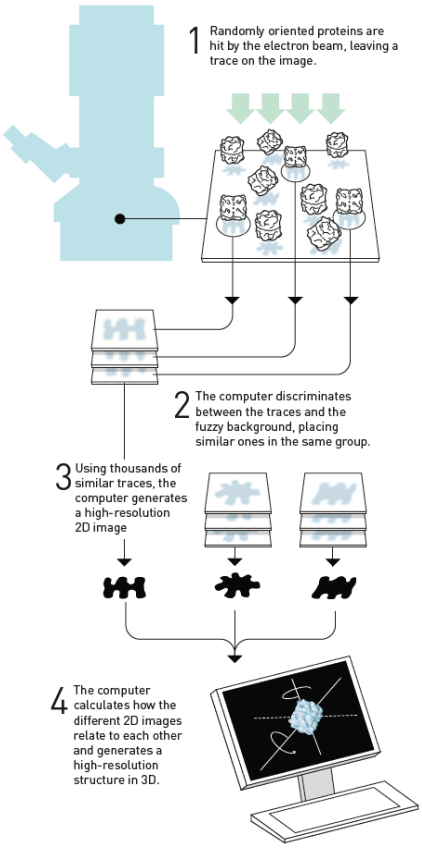

Fig. 8　Frank’s image analysis for 3D structures

The preparation and observation of frozen-hydrated biological particles involved the following operations: (1) forming a thin layer of the suspension; (2) cooling it into the vitreous state; (3) transferring it into the microscope without rewarming above the devitrification temperature ($T_d \approx 140K$); and (4) observing it below T_d and with an electron dose low enough to preserve the structure of the specimen.[4] The method perfectly enhanced the resolution for the fragile sample, bringing them to the Å level. These three major breakthroughs in cryo-EM makes it a possible way for the sub-nanometer analysis for the cells as well as proteins.

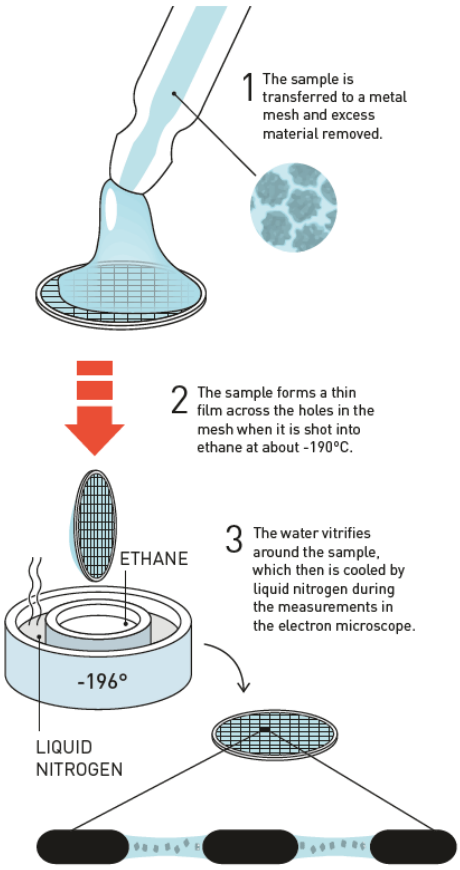

Fig.9 Dubochet's vitrification method

3 Recent applications of cryo-EM

Improvements in the electron microscope have been matched by great advances in technology for ancillary equipment such as image and probe correctors, spectrometers, and detectors, which now allow the recovery of unprecedented levels of information on the 3D structures of proteins and nucleic acids in biological applications.[12]

3.1 Biological science

Cryo-EM is flourishing as a popular method of choice in structural biology and is rapidly developing as the key approach to determining the 3D structures of large macromolecular assemblies. In Williams' review article, the author introduces some of the developments in instrumentation and methods that have led to the rise of cryo-EM in the life science community. [12]

In 2017, Shi's team in Tsinghua University proposed a method on sample preparation and electron microscopy of human γ-secretase, which showed the structure, and further gave some insights on the function revealed by the cryo-EM structures of this protein.[13]

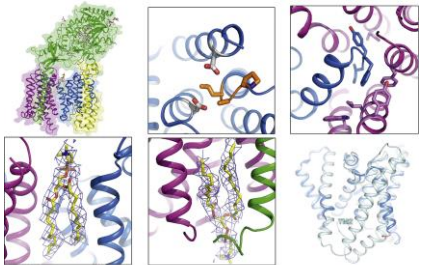

Fig.10 Structural features of human γ -secretase[13]

The latest breakthrough from Holger Stark's team was published on Nature this October, pushing the limiting resolution to 1.25 Å, owing to the newly developed EM technology, which ensures only the electrons with a relatively small distribution of energy can interact with the samples, thus promoting the final image quality. They select a kind of protein called apoferritin because of it rigidity. In the end, Stark reconstructs the protein with almost twice the data that could be inferred before. Their efforts greatly elevate the standard to a brand-new level, and this effort promotes its usage in drug design.[14]

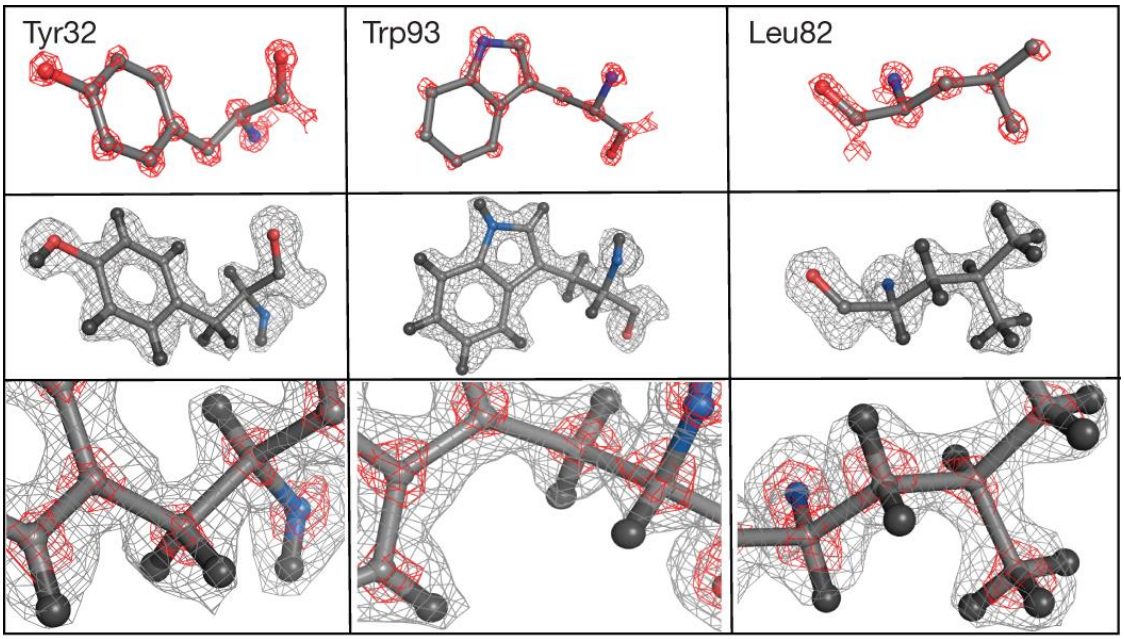

Fig. 11 Visualization of individual atoms and hydrogens at 1.25 Å resolution.

3.2 Material science

Beyond life science, the development of cryo-EM for the physical sciences may offer access to previously inaccessible length scales for materials characterization in systems that would otherwise be too sensitive for high-resolution electron microscopy and spectroscopy. Weakly bonded and reactive materials that typically degrade under electron irradiation and environmental exposure can potentially be stabilized by cryo-EM, opening up exciting opportunities to address many central questions in materials science.[5]

Li *et al.* applied cryo-EM techniques commonly used for studying biological samples to examine batteries. They identified the solid electrolyte interphase that forms, observed the interactions of Li with interphase, and captured the formation of dendrites that can be detrimental to the lifetime of a battery.[15]

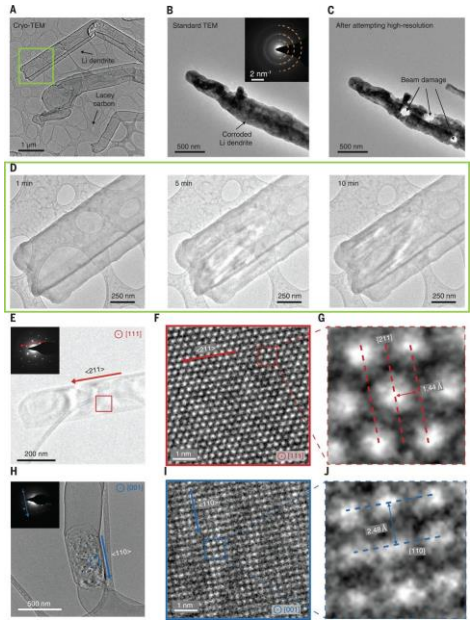

Fig.12 Atomic-resolution TEM of Li metal dendrites[15]

Using cryo-ET, Weber *et al.* revealed the first 3D reconstructions of the hydrated Nafion nanostructure, which exhibits an interconnected channel-type network with a domain spacing of about 5 nm. This direct imaging approach may provide insights for molecular dynamics simulations of proton transport in this ionomer, enabling mechanistic understanding of ion transport and design principles for future ionic polymers.[16]

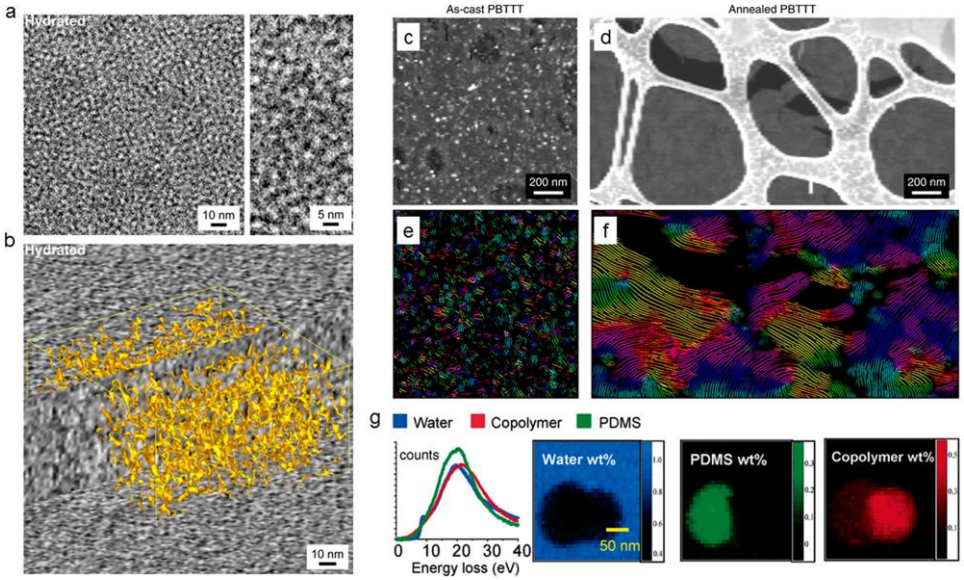

Fig.13 Cryo-EM for soft polymer materials[16]

Initial work by Cui's research group from Stanford shows that instabilities and metastable states may be stabilized at cryogenic temperatures and low-electron-dose conditions. Using cryo-EM, they froze and preserved CO_2 guests adsorbed within a MOF framework to enable direct imaging of the gas molecules with atomic resolution.[17] Later, using time-resolved cryo-EM, Patterson and colleagues probed the structural evolution of protein−MOF

hybrid systems and reported the nonclassical pathways via dissolution−recrystallization of highly hydrated amorphous particles and solid-state transformation of a protein-rich amorphous phase.[18] The atomic resolution achieved in this work compared to that in their previous study using LP-TEM[19] and again demonstrates cryo-EM to be a promising alternative to LP-TEM. With cryo-EM, it is possible to reveal the structural evolution of MOFs during guest intercalation and to resolve the conformation and chemistry of such guests spatially within the framework to develop a complete picture of MOF adsorption kinetics at the single-particle level.

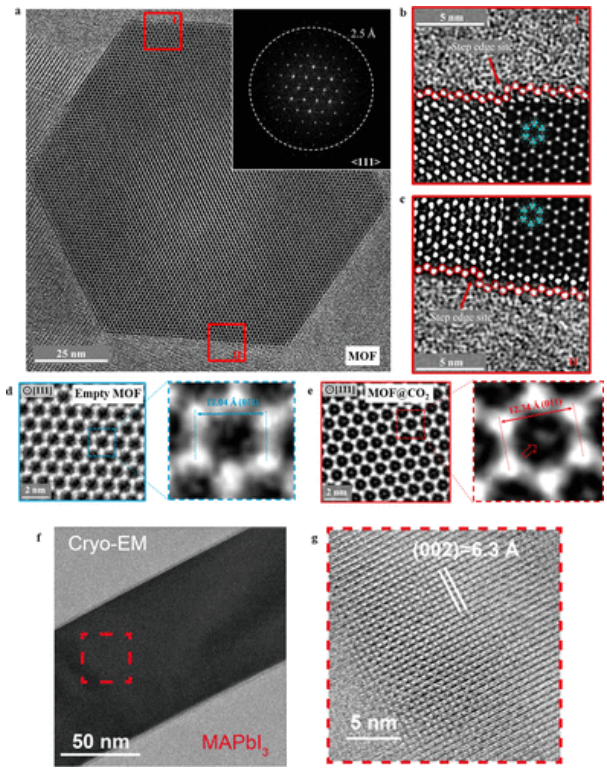

Fig.14 Cryogenic electron microscopy (cryo-EM) for metal−organic frameworks (MOFs) and perovskite solar cell materials

More recently, the ever-improving spectral and spatial resolution of analytical TEM promises the simultaneous access of chemistry and structure in quantum materials. For example, picometer-scale shifts in atomic position are sufficient to break inversion symmetry and to produce polarization in oxide heterostructures, resulting in ferroelectric polarization.[20] Although measuring these picometer shifts is possible using STEM imaging equipped with aberration correctors, sample drift at cryogenic temperatures is problematic for atomic-resolution imaging and spectroscopy. The Kourkoutis group was able to overcome this challenge by decreasing the dwell time to 0.5 μs per pixel, enabling sub-angstrom resolution (0.78 Å) and picometer precision at low temperatures (~93 K). With these measurements, they were able to discover and to map how charge ordering changes between room temperature and cryogenic temperature in a Manganite model system.[21]

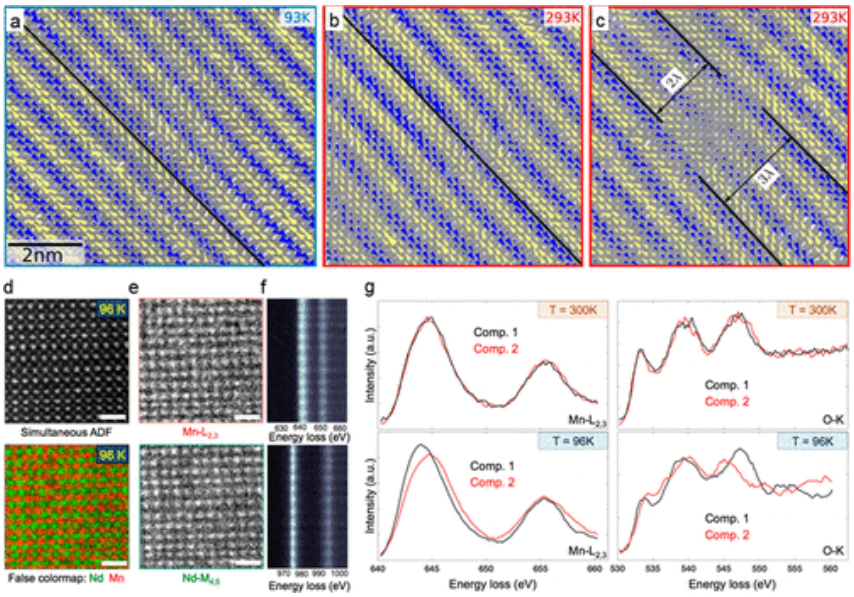

Fig.15 Cryogenic electron microscopy (cryo-EM) for quantum materials[21]

4 Conclusion

Since the very beginning of human history, we have been trying to understand the basic principles that master our universe. The invention of the electronic microscopy opens a window for us to take a look at the micro scale things we have never seen before, and the cryo-EM makes its development one step further. There has been numerous grand advances at present, but there are still many grand challenges to be solved. Hopefully this paper represents the mainstream of this area. During the draft of this term paper, I searched for a plethora of reviews and papers on topic related to bioscience as well as material science, which really helped me to understand "Why cryo-EM matters" even better and deeper.

At the end of the paper, I would like to thank Prof. Chundong Wang for his wonderful lectures delivered this semester, and the meaningful SEM experiment he proposed in order to let us understand the theoretical analysis more easily. I believe this course would be deeply embedded in my mindset, and it would be of great benefit during my upcoming postgraduate career.

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