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Cryo-EM for nanomaterials: Progress and perspective

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ABSTRACT **Cryogenic electron microscopy (cryo-EM) has extensively boosted structural biology research since the "resolution revolution" in the year of 2013 which was soon awarded the Nobel Prize in Chemistry in 2017. The advances in camera techniques and software algorithms enabled cryo-EM to routinely characterize the three-dimensional structures of biomolecules at near-atomic resolution. Biomolecules are basically sensitive to electron irradiation damage, which can be minimized at cryo-temperature. This principle has inspired material scientists to characterize electron beam- or air-sensitive materials by cryo-EM, such as the electrodes in the lithium-ion battery, metal-organic frameworks (MOFs), covalent-organic frameworks (COFs) and zeolites. In addition, the reaction systems can be fast-frozen at vitreous ice in cryo-EM, which correspondingly preserves the materials at the close-to-native state. Herein, we summarized the development and applications of both the cryo-EM technique and other emerging cryo-techniques in materials science, and energy storage and conversion. Cryo-EM techniques, capable of the direct observation of sensitive materials and electrochemical reaction processes, will greatly renew our understanding of materials science and related mechanisms.**

Keywords: cryogenic electron microscopy, structural biology, 3D reconstruction, atomic resolution, nanomaterials

INTRODUCTION

Cryogenic electron microscopy (cryo-EM) has been widely used in structural biology, materials science, chemistry, physics, etc. The 2017 Nobel Prize in Chemistry has awarded three scientists (i.e., Jacques Dubochet, Joachim Frank and Richard Henderson) who have made outstanding contributions to the development of cryo-EM.

As shown in Fig. 1, cryo-EM techniques have overcome a series of key challenges and gradually approached wide applications. In the 1970s, in order to solve the radiation damage problem of samples [1], Glaeser's group [2,3] firstly proposed directly freezing samples with liquid nitrogen (cryogenic technique). Thereafter, McDowall's group [4] optimized this freezing method by replacing liquid nitrogen with ethane, which can transfer water in the samples into vitreous ice and subsequently avoid damaging the samples due to crystalline ice. These studies promote the birth of cryo-EM, which is the combination of cryogenic technique and transmission EM (TEM).

In recent years, with the development of computer sciences and other technologies, cryo-EM has been further developed from the points of both hardware facilities and algorithm methods. In particular, these breakthroughs have boosted the development of data processing and three-dimensional (3D) reconstruction techniques for cryo-EM. In 2013, Cheng's group [5] solved the structure of 20S protein at 3.4 Å for the first time by employing the direct-electron detector (DDD), which was afterward viewed as the "resolution revolution" of the cryo-EM technique. The DDD camera, compared with previous cameras, is capable of directly detecting the electronic signals without the electron-photon-electron transfer process which is generally applied in the charge-coupled devices. DDD camera can read electronic signals fast and transfer more than dozens of frames in one second. The fast response of the DDD camera allows the tracking of sample shaking. Thus, by the subsequent motion correction on the sample shaking, the high-frequency signals can be restored to get clear pictures. In addition, data processing methods of cryo-EM have also been significantly developed. Frank [6] proposed that superimposing a large number of low-dose imaging data of the same biological molecule can improve the signal-to-noise ratio in 1975. Significantly, they proposed that using different perspective orientations is beneficial for the reconstruction of final 3D structures. Thereafter, in 2012, Scheres *et al.* [7] developed the Relion, where the Bayesian prior probability analysis algorithm has been used to improve the 3D reconstruction performance.

In the field of materials science, TEM is one of the most widely used characterization methods [8–12]. Normally, materials can be directly imaged by room-temperature TEM. However, some materials are sensitive to electron beam irradiation or high temperature. Due to the vitreous ice state, cryo-EM can not only inhibit the irradiation damage of materials due to the electron beam or high temperature, but also effectively keep the intrinsic structures of the sensitive nanomaterials. Recently, researchers have applied cryo-EM to the field of materials science and chemistry. Compared with other common electron

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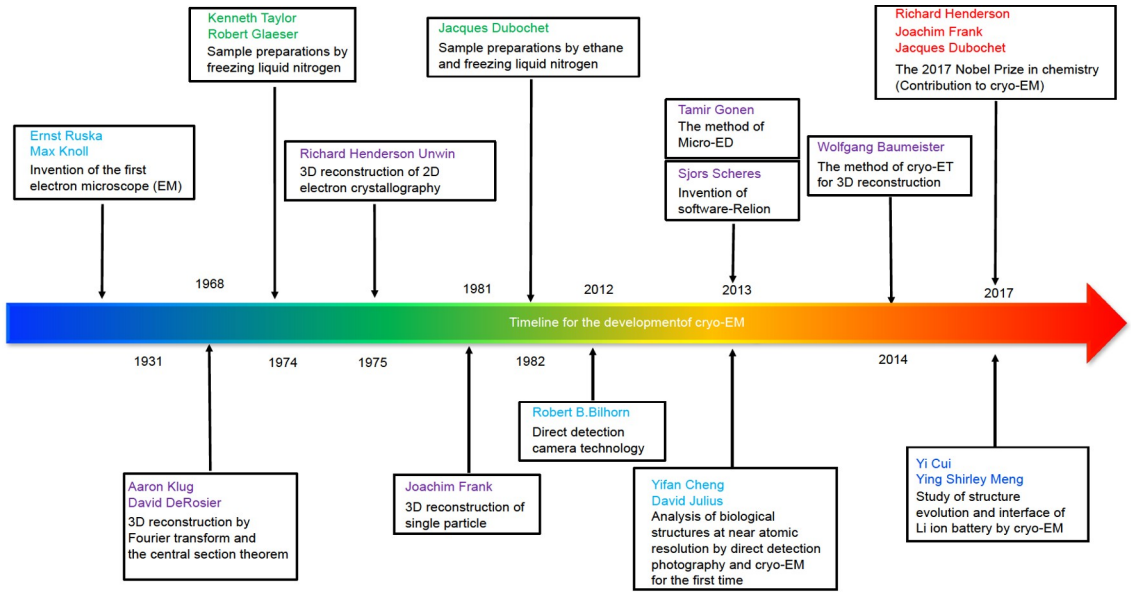

Figure 1 Timeline for the development and application of cryo-EM.

Table 1 The difference between cryo-EM and standard TEM

Cryo-EM	Standard TEM
Ultra-low temperature (liquid nitrogen temperature)	Room temperature
Low electron dose	High electron dose
Characterization of electron beam-sensitive materials	Characterization of stable materials
No contact with air during transferring samples	Contact with air during transferring samples
Intrinsic structures of samples	Unnatural states of samples

microscopes, such as room-temperature TEM, the cryo-EM shows three outstanding advantages (as shown in Table 1 and Fig. 2): (1) the samples can be frozen rapidly and thus kept in the physiological state or intermediate state which can also be regarded as *in situ* observations during reactions; (2) the samples are frozen in liquid nitrogen and then transferred under cryogenic temperature, which can correspondingly prohibit them from contacting air; (3) the low-dose electron beam irradiation can be applied in cryo-EM, which can impressively reduce the irradiation damage on materials. These advantages enable the cryo-EM a promising alternative for characterizing electron beam- and air-sensitive materials. Recently, several high-quality reviews covering the analysis of nanomaterials for energy storage and conversion *via* cryo-EM have been published [13–17]. However, comprehensive discussion focusing on the characterization of various environment-sensitive materials *via* cryo-EM and other related technologies (e.g., cryogenic focused ion beam (cryo-FIB), cryogenic electron tomography (cryo-ET), and cryogenic electron energy loss spectroscopy (cryo-EELS)) is still rare. Therefore, in this review, we also highlight the discussion of the aforementioned part of the blank.

APPLICATION OF CRYO-EM IN MATERIALS CHARACTERIZATIONS

As shown in Fig. 3, by installing a freezing device on common EM equipment, cryo-EM is established. The cryo-EM includes cryo-scanning EM (cryo-SEM) and cryo-TEM, which can be widely used in the field of material characterization. In cryo-EM, the sample can be cooled down to the liquid nitrogen temperature (i.e., 77 K).

Moreover, cryo-SEM enables the observation of the microscopic morphology of electron beam-sensitive aqueous (solvent) samples. It should be noted that pre-treatments of samples, including low-temperature freezing fixation, fracture, coating (i.e., gold-spraying and carbon-spraying), and refrigerating sample transportation, are necessary before cryo-SEM observation. The rapid freezing and refrigerating sample transportation technology can keep ice in glass state, which can avoid the generation of ice crystals and guarantee *in situ* cryogenic observation.

Application of cryo-EM in batteries

The key factors for battery design are the anode, cathode, and electrolyte materials, which determine the storage capacity and charge/discharge rate of batteries. However, the lack of high-resolution characterization methods limits the further understanding of internal microstructure evolution during battery charging and discharging. Cryo-EM can not only realize the characterization of electrode materials at atomic scale, but also assist the investigation of battery fabrication procedures, including sample preparation, chemical analysis, and spectroscopy. In recent years, cryo-EM has achieved important progress in the study of the lithium state and interface composition of lithium batteries (Fig. 4) [26].

Anode interface of batteries

In 2017, Li *et al.* [19] firstly observed the atomic interfacial structure of Li-battery by cryo-TEM, which successfully preserved the original state of Li and avoided electron beam irradiation damage. They have developed a new cryo-transfer technology that can directly transfer active materials of batteries into the TEM cavity without exposing them to air and moisture. Subsequently, it successfully retained the state of the material under working conditions. Li *et al.* [19] distinguished the lattice

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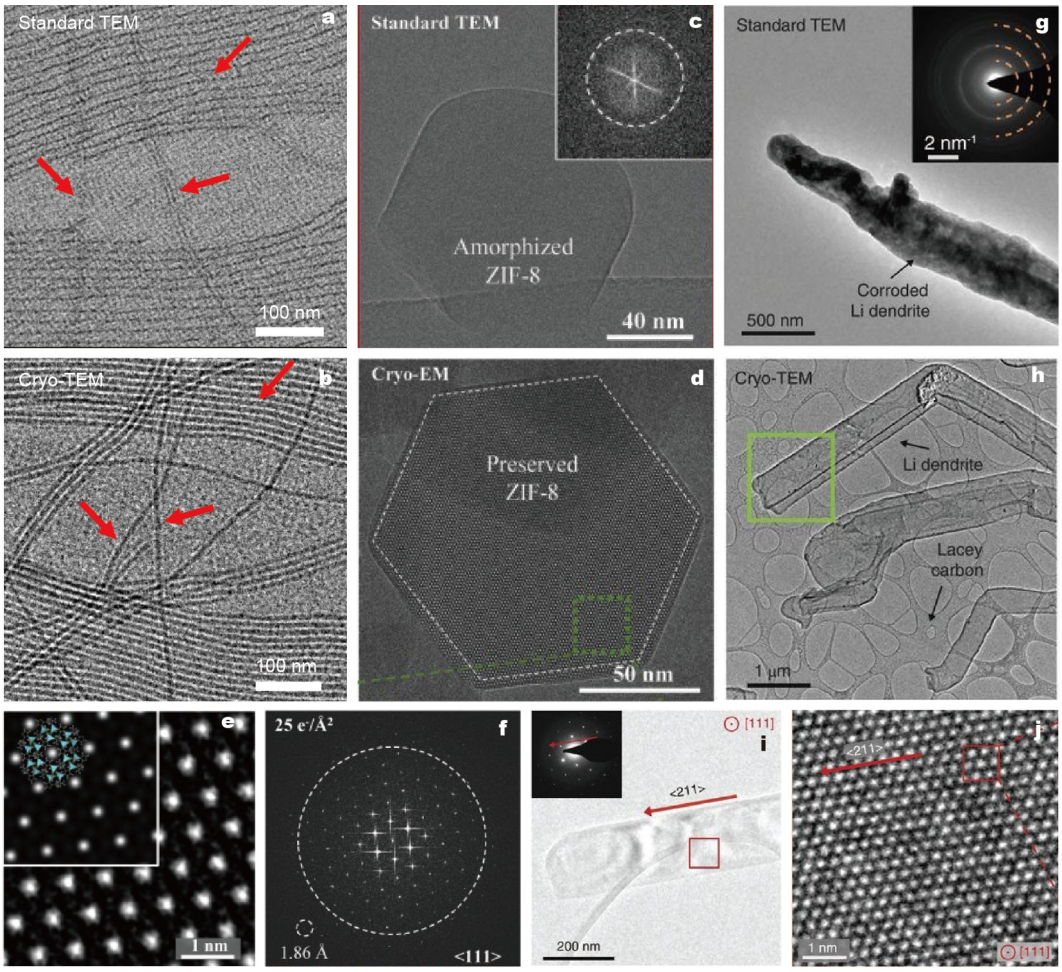

Figure 2 Comparison of material characterizations using cryo-EM and standard TEM. (a) Standard TEM and (b) cryo-EM images of ultrathin Au nanowires. (c) Standard TEM and (d) cryo-EM images of ZIF-8. (e) Magnified TEM image marked in green dash square of (d) and (f) the corresponding fast Fourier transform (FFT) pattern. Reprinted with permission from Ref. [18]. Copyright 2019, Elsevier Inc. (g) Standard TEM and (h) cryo-EM images of Li metal dendrites. (i) TEM image of Li metal dendrites with the direction of <211>. (j) Magnified high-resolution TEM image of Li metal dendrites marked in red square of (i). Reprinted with permission from Ref. [19]. Copyright 2017, American Association for the Advancement Science.

space matching with the {110} planes of metallic Li at atomic resolution, confirmed the crystallinity of electroplated Li, and clearly characterized that the solid electrolyte interphase (SEI) shows a double-layer structure. Impressively, the outer layer of SEI is highly ordered crystalline Li_2O and the inner layer is the amorphous matrix, which is in sharp contrast to the previously reported SEI mosaic model in the pristine ethylene carbonate/diethyl carbonate (EC/DEC) electrolyte. It is also different from the previously reported double-layer SEI structure formed by fluoroethylene carbonate (FEC) additives. Actually, it will form spherical Li nuclei which can significantly improve the uniformity of SEI, inhibit dendrites and effectively protect the Li surface. The observation of these structures benefits from the excellent maintenance of their initial structures by cryo-EM (Fig. 4a–c).

Wang *et al.* [27] carried out the surface characterization of wrinkled graphene cage (WGC) by cryo-EM, which is different from recently reported amorphous carbon spheres and can provide continuous robust Li metal protection. When metal Li is exposed to high-energy electron beam irradiation at room temperature, it will transmute due to its low melting point. Therefore, a room-temperature electron microscope is unfavorable for the characterization of the original structure. At the same time, the uniformity of SEI is closely related to the uniformity of Li deposition. The use of cryo-TEM with a low electron dose and the sample preparation in vitreous ice can maintain the original structures, which plays an important role in the study of Li deposition process and SEI film. In addition, due to the ultra-high salt concentration, the graphene surface is exposed to anions rather than solvent molecules, which further reduces the SEI thickness. Meanwhile, the nucleation overpotential on Au nanoparticles embedded on the inner surface is reduced, which makes Li deposition very uniform and dense. Accordingly, Li metal can be preferentially plated into the cages through the defects/inholes on the graphene shell to form Li deposition. Therefore, the expansion of the Li graphene shell is greatly reduced. At the same time, thinner and more stable SEI was clearly observed (Fig. 4d–f). Wang *et al.* [28] simultaneously stabilized the electrochemical deposition of active Li metal using cryo-TEM and reduced the electron beam irradiation damage. Correspondingly, they studied the nanostructure and chemical composition of electrodes, and solid electrolyte interfaces (Fig. 4g–i).

Huang *et al.* [29] characterized the SEI on the negative elec-

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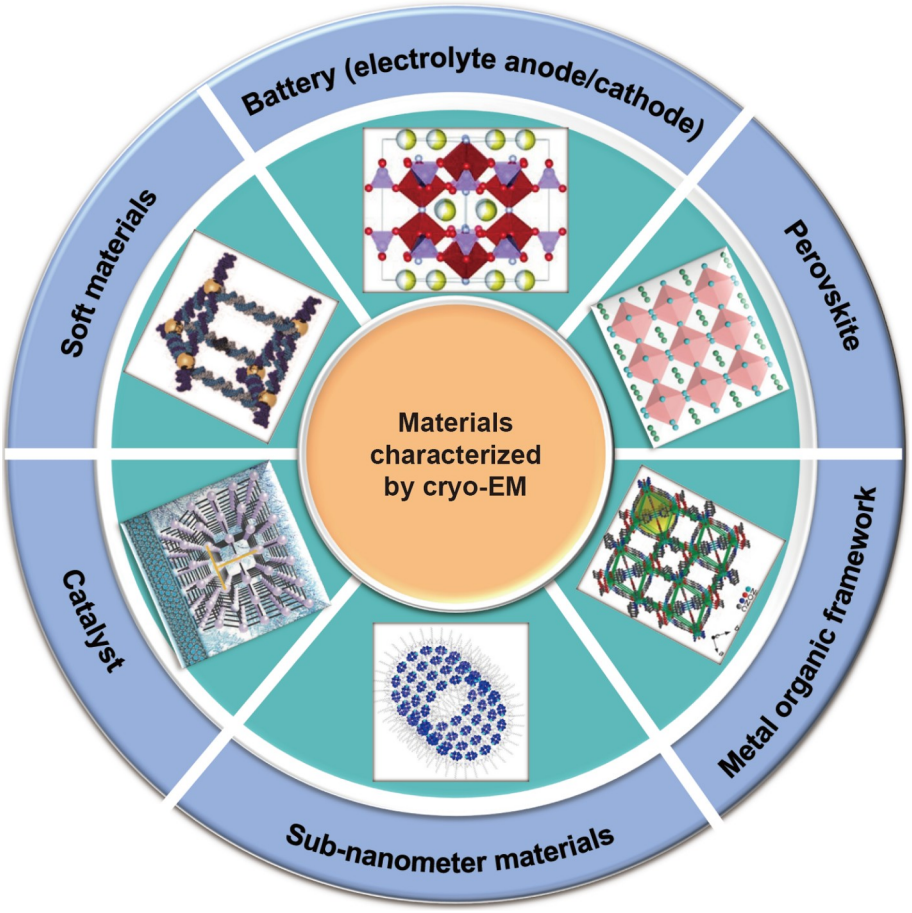

Figure 3 Cryo-EM characterization for different materials, including metal-organic frameworks (MOFs) [20], nanocatalysts [21], soft materials, perovskites [22,23], sub-nanometer materials [24], and batteries [25]. Reprinted with permission from Refs [20]. Copyright 2015, American Association for the Advancement of Science; [21]. Copyright 2022, Wiley-VCH; [23]. Copyright 2020, Wiley-VCH; [24]. Copyright 2019, American Association for the Advancement of Science; [25]. Copyright 2014, ACS publications.

trode of the battery through cryo-TEM. They proved that SEI can evolve into both compact SEI and extended SEI, which spans hundreds of nanometers during the cycle. They further discovered that the original electrode surface and electrolyte components were modified to facilitate a compact SEI reaction in the first cycle, which can extend the cycling life of the battery. They also graphed the gradual evolution of SEI nanostructures on copper oxide surface by cryo-TEM to illustrate the strong influence of SEI nanostructures during Li-ion migration [30]. Li *et al.* [31] used cryo-TEM to study the mechanism of triggering failure modes of SEI nanostructures with various layers in Li metal anodes for the first time to figure out the structure-function relationship.

Through quantitative analyses and the assistance of cryo-EM, Fang *et al.* [32] observed and analyzed the content of the local microstructure and nanostructure of the reactive metal LiO_2, and verified that the non-reactive metal LiO_2 is the main reason for both inactive Li and the capacity decay. Subsequently, the formation mechanism of inactive Li in different types of electrolytes was established, and the possible explanation for low coulombic efficiency of Li metal anodes during charging and discharging was provided as well.

Moreover, cryo-EM has also been widely used in the study of other types of battery interfaces. Huang *et al.* [33] used cryo-EM technology to observe the original SEI structure on the surface of Sn anodes in carbonate and ether electrolytes, and explained the mechanism for the superiority of ether electrolytes in sodium-ion batteries.

Cathode electrolyte interphase (CEI)

At the same time, the structure and composition of CEI produced during the cycle of the battery cathode have a significant impact on the coulomb efficiency and cycle performance of the cathode. During the observation of cryo-EM, the original structure of CEI can be maintained very well, which is of great significance to understanding the formation mechanism of CEI and its relationship with electrical properties.

Alvarado *et al.* [34] proposed a new type of non-carbonate electrolyte system (i.e., a non-carbonate electrolyte system with a single sulfone solvent) and thoroughly investigated it *via* cryo-TEM combined with EELS. In their observation, the CEI formed in non-carbonate electrolytes showed less oxygen vacancy or cation interstitials after 50 cycles. Therefore, the material structure was stable and the transition metal migration was reduced. Combined with X-ray photoelectron spectroscopy (XPS) analysis, the reduced Mn^{3+} was found to show a high surface concentration, resulting in local Jahn-Teller distortion. The synergistic effect of the newly discovered solvents and salts

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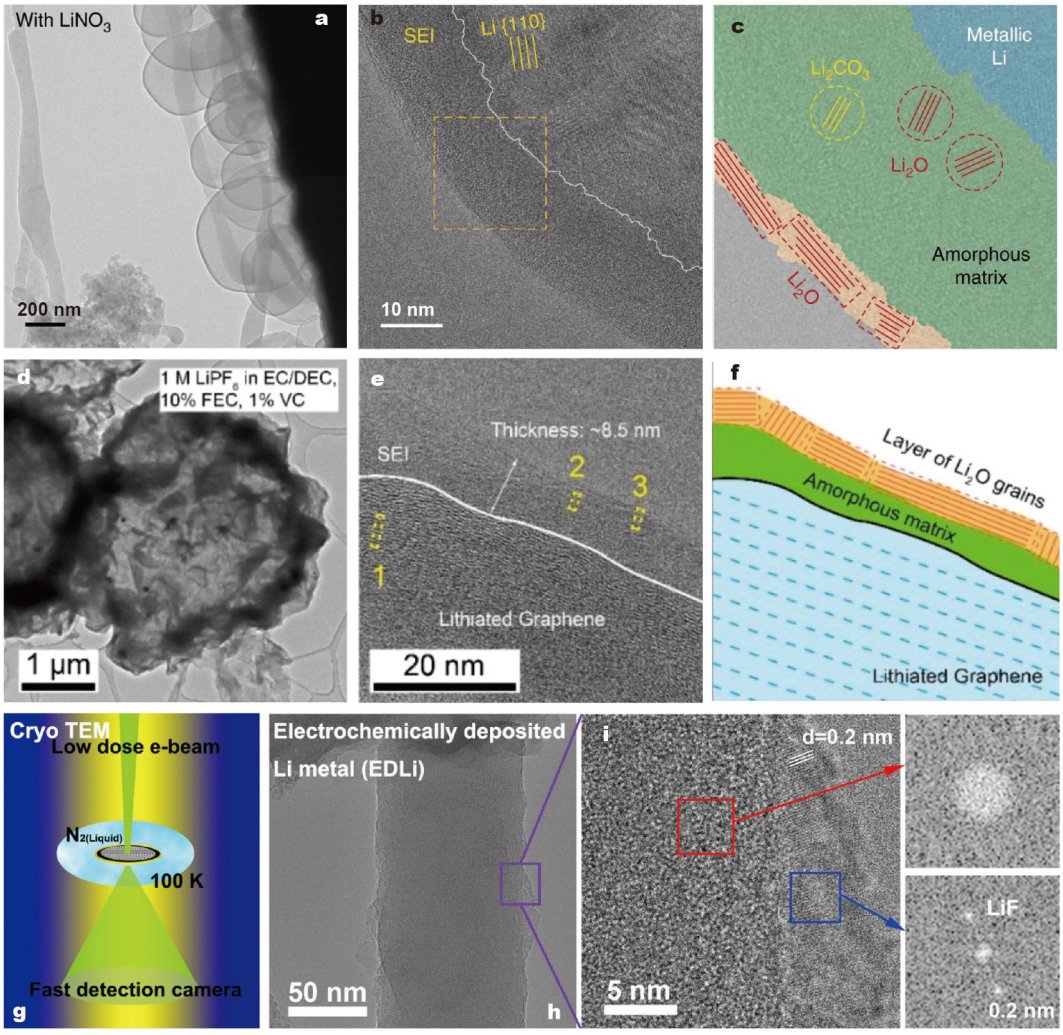

Figure 4 (a) Cryo-EM and (b) high-resolution cryo-EM images of the SEI structures in the presence of nitrate. (c) Schematic illustration of the area marked with orange square in (b). Reprinted with permission from Ref. [38]. Copyright 2018, Nature publishing group. (d) Cryo-EM and (e) high-resolution cryo-EM images of WGC after low areal capacity Li metal deposition. (f) Schematic illustration of SEI nanostructure on the surface of WGC. Reprinted with permission from Ref. [27]. Copyright 2019, ACS publications. (g) Schematic illustration of cryo-TEM. (h) Cryo-EM image and (i) high-resolution cryo-EM image and the corresponding FFT patterns for the selected regions of the electrochemically deposited Li metal (EDLi). Reprinted with permission from Ref. [28]. Copyright 2017, ACS publications.

meets the interface requirements of graphite anode and high-pressure cathode (i.e., $LiNi_{0.5}Mn_{1.5}O_4$ and LNMO) well, which is due to the formation of a layer of CEI with uniform thickness and coverage on the surface of LNMO particles. In addition, Liu's group [35] also observed the sulfurized polyacrylonitrile (SPAN) cathode surface of Li-S battery with high-concentration ether-based electrolyte by cryo-EM for the first time, which showed span crystal CEI of LiF and $LiNO_2$. This can well explain the cycling stability of SPAN in ether-based electrolytes.

The above discussion proves that cryo-EM has broad application prospects in studying the interface process of functional materials and devices, which is of great significance for their practical application. For instance, Zachman *et al.* [36] obtained the natural-state interface structure of the Li metal battery through the rapid-freezing of the liquid component (i.e., vitrified liquid electrolyte) with cryo-scanning transmission EM (cryo-STEM). In addition, Yu *et al.* [37] further used the cryo-EM technique to characterize the Li formation in a single layer MoS_2 to reveal the reaction processes.

Application of cryo-EM in perovskites

Perovskite is a general term for a class of crystalline materials with "ABX_3" composition, where the "A" is a large cation, "B" is a small cation and "X" is an anion. Each "A" ion is surrounded by an octahedron composed of "B" and "X" ions. Perovskites can be divided into organic perovskites (without metal elements), inorganic perovskites (with metal elements), and inorganic-organic hybrid perovskites. However, perovskites are sensitive to air, water and electron beams. This affects the corresponding material structure characterization by room-temperature EM.

Recently, Li *et al.* [22] applied cryo-EM technology to characterize the structure and decomposition process of the perovskites (Fig. 5). Compared with conventional TEM, cryo-EM can not only prevent the decomposition of perovskite deriving from air exposure but also increase its tolerance to electron beam irradiation by preventing vacuum-induced methylamine molecules from escaping. Subsequently, the atomic-resolution TEM images of $MAPbI_3$ nanowires were successfully obtained by cryo-EM for the first time. Moreover, they used the freezing

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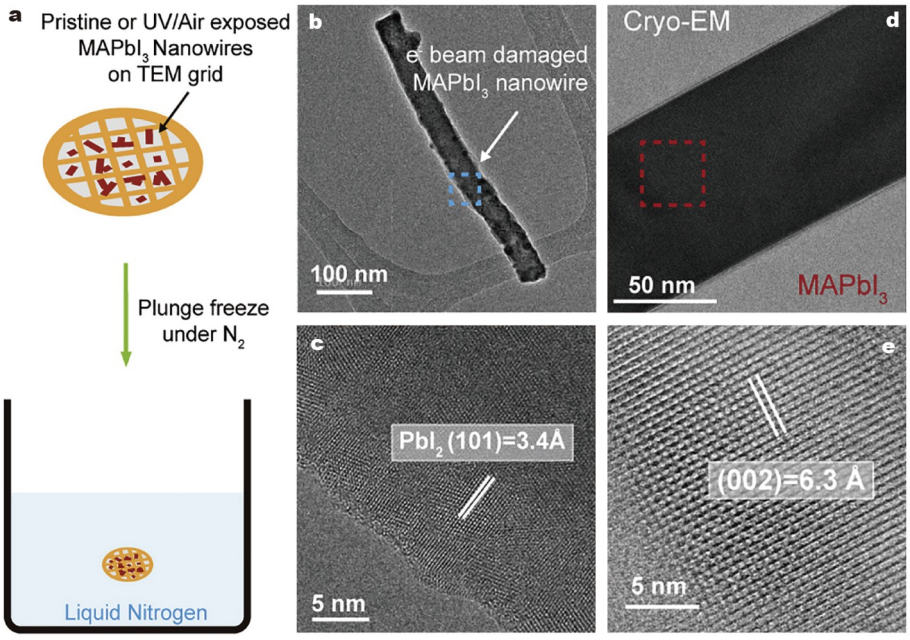

Figure 5 (a) Schematic illustration of the sample preparation process of $MAPbI_3$ for cryo-EM. Characterization of $MAPbI_3$ using (b, c) standard TEM and (d, e) cryo-EM. Reprinted with permission from Ref. [22]. Copyright 2019, Elsevier Inc.

method to observe the decomposition process of perovskite materials under the condition of ultraviolet light and water vapor. Most importantly, the authors found that different materials have different tolerances to electron beam exposure. By the electron dose tolerance test, the electron dose rate of different perovskites is minimized and the standard of electron dose accumulation is established as well. Note that the ideal electron dose exposure of each material is specified in this study, and the evaluation of electron beam irradiation damage is also extended from biological cryo-EM to the beam-sensitive hybrid perovskite.

Application of cryo-EM in soft materials

Soft materials are a class of substances between solid and ideal fluid with macromolecular structures, such as metal-biomolecular composites, which are sensitive to electron beams and difficult to be fully characterized. In such conditions, cryo-EM provides an effective platform for their structural characterization and analysis.

As early as 2015, Tian *et al.* [39] studied the octahedral 3D mesoscale clusters composed of self-assembled DNA on Au nanoparticles through cryo-TEM, and proposed a detailed understanding of the structure-construction process. Li's group [40] used cryo-STEM to characterize the tetrahedral unit/superlattice of Au-DNA self-assemblies. Assemblies in aqueous solutions are an integral part of nanotechnology, and some stimuli may cause structural changes. Through cryo-EM, Gao *et al.* [41] observed the morphologies of the C16K2 amphiphilic polypeptide chain assemblies assembled under different pH conditions. It was found that they can easily self-assemble into the spherical gel structure at pH 7.5. Meanwhile, slender nanofibers can be formed at pH 8. While the pH is greater than 8.3, the self-assembled structure is a bilayer nanobelt. Huo *et al.* [42] used cryo-TEM to characterize the poly(2-dimethylaminoethyl methacrylate)-*b*-poly(benzyl methacrylate)-*b*-poly(2-perfluorooctylethyl methacrylate) (PDMA-*b*-PBzMA-*b*-PFMA) assemblies, (e.g., D33-B58-F19, D33-B58-F30, D33-B58-F62, and D33-B58-F120 assemblies, where D, B, and F stand for PDMA, PBzMA, and PFMA, respectively) and determined the original assembly morphology. Mirabello *et al.* [43] characterized the different stages of magnetite formation by cryo-TEM and found that the crystallization by particle attachment is a colloidal assembly process which can be controlled to achieve desired crystal sizes and properties (Fig. 6).

Application of cryo-TEM in MOFs

MOFs are a large class of crystalline porous materials, constructed by the coordination of metal ions or clusters with polytopic organic ligands. Meanwhile, MOFs possess versatile promising features, such as tunable structure and function, large surface area, and highly ordered pores, which could be significant for catalytic, sensing, and electrochemical applications. However, MOF materials are highly sensitive to electron irradiation and high-resolution TEM images are difficult to be collected for clear structure characterization.

In 2012, Wiktor *et al.* [44] tried to overcome the instability of MOFs by low-dose electron beam technology and liquid nitrogen freezing in cryo-TEM. For the first time, they observed a complete nanohole structure of the MOF-5 crystal. The technology can also be applied to characterize other MOF nanomaterials in detail, including MOF interface, MOF-nanoparticle interaction, and 2D MOF nanostructures. Taking advantage of cryo-EM, Li *et al.* [18] observed the atomic surface structure of the zeolite imidazolium salt framework (ZIF-8) and its interaction with guest carbon dioxide molecules, which evidenced the capability of cryo-EM in stabilizing MOF structures and preserving its host-guest chemistry (Fig. 7).

APPLICATIONS OF OTHER RELATED CRYO-EM TECHNOLOGIES IN MATERIALS

With the development of the cryo-EM technique, other techniques related to cryo-EM, such as cryo-FIB, cryo-ET, cryo-EELS,

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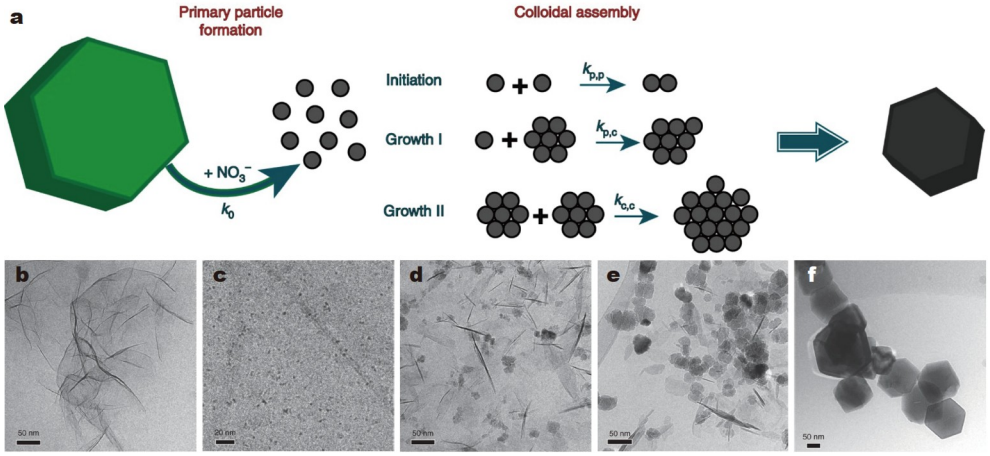

Figure 6 (a) Schematic illustration of the formation of magnetite by the colloidal assembly. (b–f) Cryo-EM characterization of different stages of magnetite formation: solutions (b) before and (c) after adding NO_3^-, products after the addiction of (d) 35% and (e) 40% of the total amount of NO_3^-, and (f) final product. Reprinted with permission from Ref. [43]. Copyright 2020, Nature publishing group.

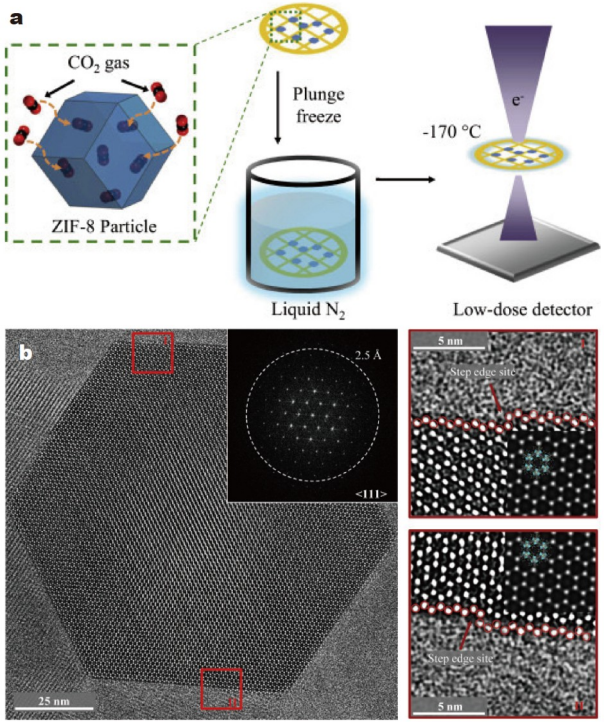

Figure 7 (a) Schematic illustration of sample preparation process of ZIF-8 under cryogenic conditions. (b) Denoised cryo-EM images of ZIF-8 and the corresponding magnified zones I and II. Reprinted with permission from Ref. [18]. Copyright 2019, Elsevier Inc.

single particle, and microcrystal electron diffraction (micro-ED), are also developed, which have made an important contribution to material characterizations as well.

Cryo-ET

Cryo-ET technique is mainly adopted to analyze the 3D structures of a variety of amorphous biological samples (including tissues, cells, subcellular cells, and even bio-macromolecular complexes). The *in-situ* rapidly frozen samples, with the structures preserved at the near-physiological state, are observed at different deflection angles, where a series of 2D projection images are collected. Subsequently, the 3D structures can be reconstructed *via* the back-projection method. At present, the application of cryo-ET in the field of materials is mainly focusing on the study of the 3D structure of soft materials.

Parry *et al.* [45] revealed the complex morphology of amphiphilic double-comb double-resistance copolymers which contain tripeptides through cryo-ET and cryo-TEM techniques (Fig. 8a–j). Wirix *et al.* [46] studied the 3D structure of a poly(3-hexylthiophene) (P3HT) assembly, through combining cryo-TEM and cryo-ET (Fig. 8k–n). Liu *et al.* [47] reconstructed the ice layer and gas-liquid interface between graphene-based functionalized graphene membrane (FGM) and holey carbon membrane without graphene *via* cryo-ET. The result suggests that the FGM grid not only shows more adsorption of the protein but also can reduce the protein denaturation damage caused by the gas-liquid interface. Moreover, the thinner ice layer makes it easier to observe the proteins and obtain high-resolution characterizations.

Micro-ED technology

Micro-ED technology was firstly proposed by Gonen's group [48] for 3D electron crystallography of protein microcrystals, which mainly uses electron diffraction to characterize the 3D shape and morphology of tiny crystals (Fig. 9) [49]. Currently, it has been applied in sensitive material characterization [50].

The atomic structure of a metal cluster is the basis for understanding its optical, electronic, and chemical properties. Vergara *et al.* [51] analyzed the subatomic resolution structure of the largest water-bearing Au cluster, i.e., Au_{146}(p-MBA)$_{57}$, by micro-ED. This is the first sub-atomic resolution (i.e., 0.85 Å) metal cluster structure analyzed by micro-ED through freezing the sample at cryogenic temperature. Micro-ED is used as a novel and important tool for the characterization of nanomaterials. It should be noted that during micro-ED characterization, only low-dose electron beam irradiation is applied for diffraction data collection to minimize the sample damage. With further development of the micro-ED technique, it can be further used for structural characterization of complex molecules, such as porous organic polymers (POPs), covalent organic fra-

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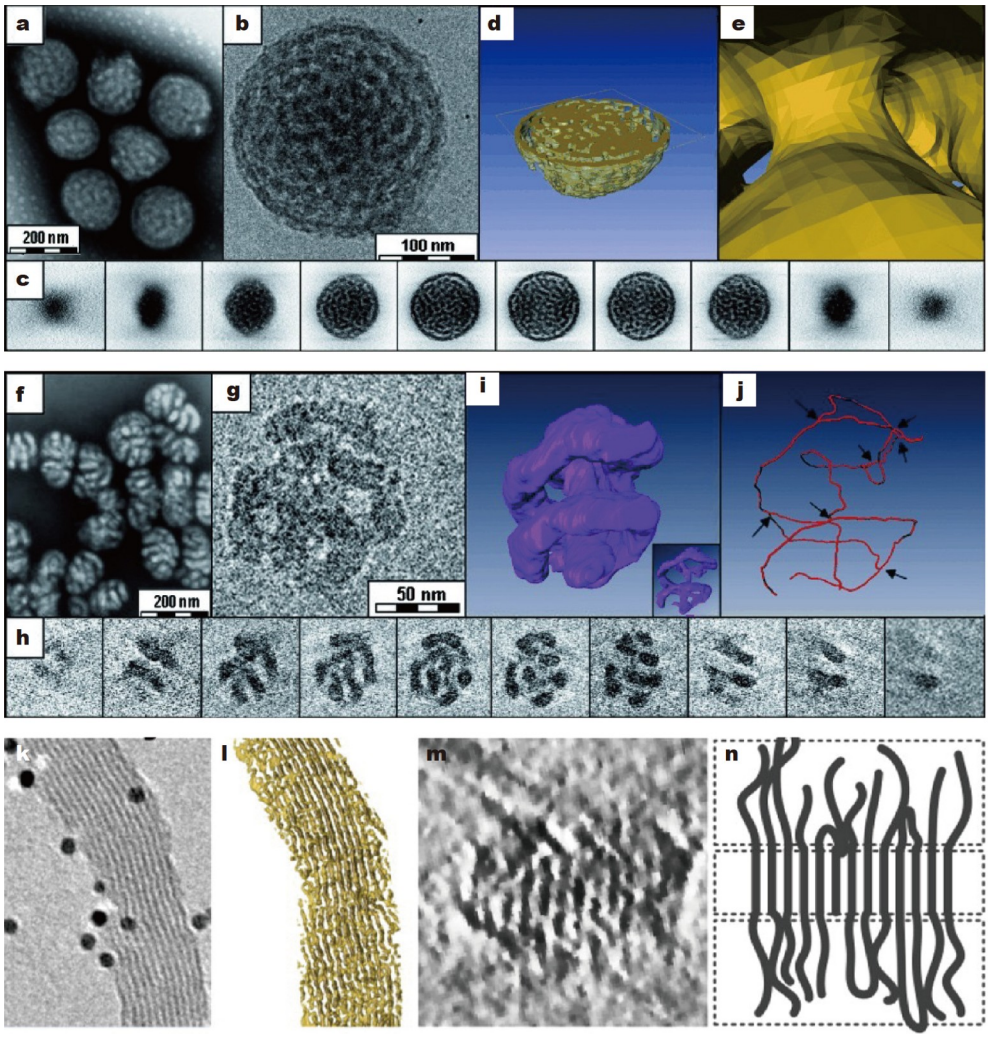

Figure 8 (a) Standard TEM and (b) cryo-TEM analysis, and (c–e) reconstructions for aggregates of PNOEG-PNGLF. (f) Standard TEM and (g) cryo-TEM analysis, and (h–j) reconstructions for aggregates of PNOEG-PNLVL by cryo-ET technique. Reprinted with permission from Ref. [45]. Copyright 2008, Wiley-VCH. (k–n) Cryo-ET technique reveals local structures of P3HT. Reprinted with permission from ref. [46]. Copyright 2014, ACS publications.

meworks (COFs), and MOFs in the future, which will greatly promote our understanding of the above materials.

Single particle 3D reconstruction technology (SPRT)

SPRT uses the calculation average method to compare and superimpose a large number of low-dose 2D imaging data of the same molecule from different directions, so as to obtain the final 3D reconstruction through the central section theorem (Fig. 10). The combination of this technology with cryo-EM can *in situ* reveal the real 3D structure of the materials effectively.

Tian *et al.* [39] uncovered the structure of the DNA frame and revealed that the nanoparticles are spatially coordinated in the prescribed manner by combining cryo-EM with SPRT (Fig. 10a). In addition, Ma *et al.* [52] used a combination of cryo-EM and SPRT to reveal the existence of an isolated ultrafine silica cage (less than 10 nm) with a dodecahedral structure. Such a highly symmetrical self-assembled cage was formed by arranging primary silica clusters on the surface of the surfactant micelle in an aqueous solution. This finding paved the way to fabricate nanocages using silica and other inorganic materials as advanced functional materials (Fig. 10b).

Cryo-FIB

Cryo-FIB technology uses accelerated ion beam under low-temperature conditions (i.e., liquid nitrogen temperature) and focuses on observing the surface of samples. The strong ion beam can strip the surface atoms of the samples and characterize micro-/nano-scale surface tomography. Normally, during this process, a physical sputtering reaction of a chemical gas can selectively strip a metal, silicon oxide layer or deposited metal layer. Subsequently, an electronic image by generating a secondary electron signal can be obtained.

TEM sample preparation *via* conventional room-temperature FIB techniques can introduce artificial hydrides, which can affect the material characterization and structure analysis. Meanwhile, the cryo-FIB technology can use freezing technology to prevent the generation of artificial hydrides, and in turn, allows more accurate material analyses [53].

Stephenson *et al.* [54] proposed a new experimental protocol for the characterization of beam-/temperature-sensitive materials, where a plasma FIB is installed with a solid-state cooling stage to reduce beam damage/contamination. By using the aforementioned protocol, the surface oxidation of samples can

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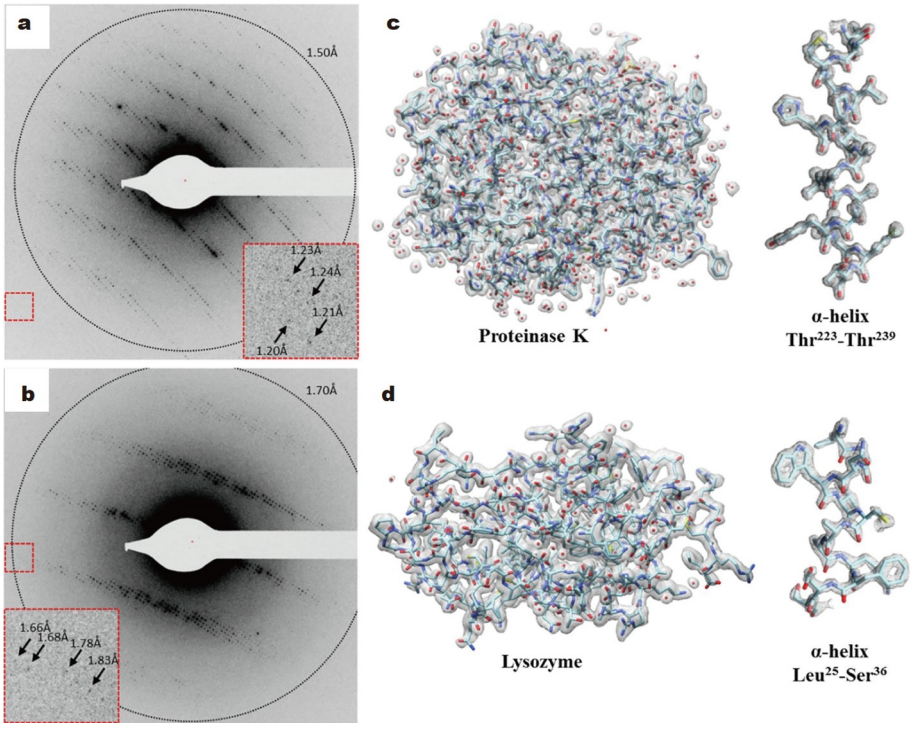

Figure 9 Micro-ED diffraction patterns of (a) proteinase K and (b) lysozyme. Models and density maps of (c) proteinase K and (d) lysozyme. Reprinted with permission from Ref. [49]. Copyright 2019, Elsevier.

be suppressed effectively. For example, a frozen water sample is prepared and transferred by cryo-FIB technology, which avoids obvious melting or sublimation. Moreover, the cryo-FIB can also assist nanostructure characterization of other liquid or soft materials. Chang *et al.* [55] proved that the electrochemical polishing and room-temperature FIB sample preparation technology can introduce a large amount of hydrogen into titanium and titanium alloys, which leads to hydride formation through TEM and atom probe tomography (APT) characterization. By contrast, the cryo-FIB milling, conducted below 150 K, can effectively protect the samples from exposure to hydrogen in the environment. Zachman *et al.* [36] performed a complete solid-liquid interface cross-section characterization at the nanoscale by cryo-FIB/SEM, which has extended its application in other fields.

Li metal in lithium-ion batteries is very sensitive and can be easily damaged by external probes, resulting in morphological and chemical changes during characterizations. To avoid such damage, Meng's group [56] characterized the morphology of electrochemical Li-ion deposition at the atomic scale by cryo-FIB and performed 3D reconstruction (Fig. 11a, b). Impressively, the results show that Li films deposited by Li salt electrolyte are obviously thinner and denser [56,57]. They also characterized the growth process of Li nucleus by cryo-FIB and cryo-EM [58]. Furthermore, Cui's group [59] used cryo-EM and SEM-FIB to *in situ* characterize the morphological evolution of Li deposition. They confirmed the solid nature of Li deposition and found that Li deposition can corrode the surface of copper. Tu *et al.* [60] studied the Li-Al_2O_3/Cu electrode by cryo-SEM-FIB, revealing that Li electrodeposits anchored by the Al_2O_3 interphase and greatly enhanced the Li depositing stability (Fig. 11c–e). Moreover, the rapid development of cryo-FIB has also been achieved. Recently, Hoffman *et al.* [61] combined frozen super resolution with FIB and SEM (cryo-SR/EM). The cryo-SR/EM can be used to visualize the protein-superstructure relationships. This cutting-edge technology can characterize sensitive materials and analyze the tomography of complicated structures in the near future.

Low-dose technology

In cryo-EM, other than the low temperature applied for photographing, a low electron dose is also applied for illumination. Meanwhile, high-resolution TEM, requiring a high electron beam dose, is an important tool for the characterization of crystal nanostructures. However, the electron beam-sensitive materials, e.g., MOFs, COFs, and hierarchical zeolites, can be easily damaged by a high-electron-dose beam when using standard TEM. Currently, the key challenges for high-resolution imaging of electron beam-sensitive materials lie in obtaining images under extremely low electron dose, time-limited research on the ribbon axis, accurizing image alignment, and accurizing the calculation of over-focus values.

Han's group [23,62,63] analyzed a series of electron beam-sensitive materials (including MOFs, COFs, and hierarchical zeolites) using a direct observation electronic computing (DOEC) camera with a reduced overall electron dose. Correspondingly, the coexistence of benzene rings, surface ligand-free, and surface ligand capping in UiO-66 can be observed (Fig. 12) [64]. They also observed the subunit resolution structural defects of UiO-66 catalytic system [62], and the coherent interface between the armchair surface termination and the assembled ZIF-8 crystals [65]. Moreover, low-dose imaging and ET are efficient to characterize the structural heterogeneity and 3D morphology of carbohydrate nanoparticles, such as nanocelluloses, chitin, and starch nanocrystals, which present great potential as building blocks of functional materials in various

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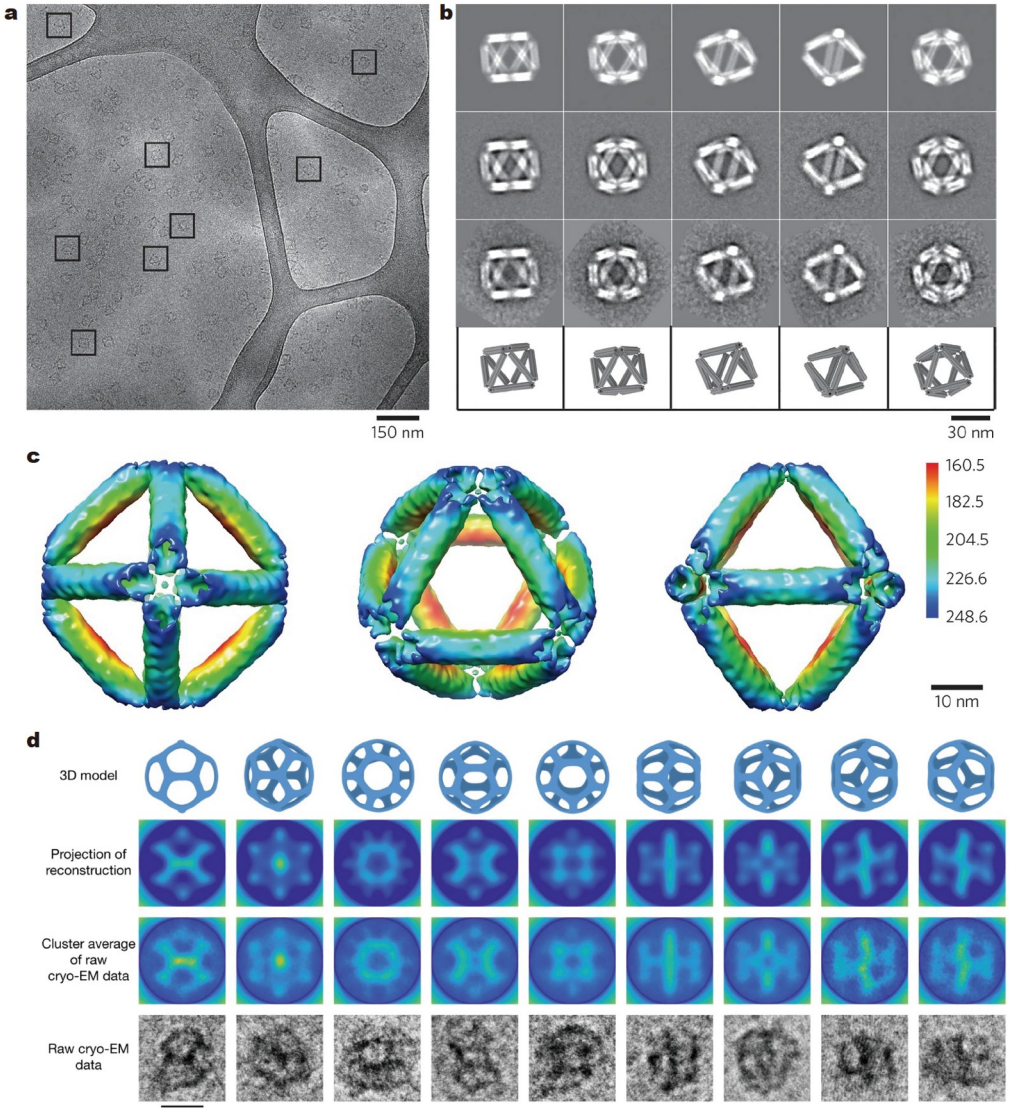

Figure 10 (a) Cryo-EM image, (b) reconstructions, and (c) surface-rendered 3D density maps at various directions of the self-assembled DNA origami octahedron by cryo-EM technique and SPRT. Reprinted with permission from Ref. [39]. Copyright 2015, Nature publishing group. (d) Single-particle reconstruction of the dodecahedral silica cage. Reprinted with permission from Ref. [52]. Copyright 2018, Nature publishing group.

fields, ranging from biomedical and cosmetic applications to optical and load-bearing materials [66,67].

CONCLUSION AND PERSPECTIVE

Cryo-EM and corresponding technologies (i.e., cryo-FIB and cryo-ET) are emerging as important tools in the fields of materials science. The fine structures of electron beam- and temperature-sensitive materials, such as perovskites, soft materials, MOFs, COFs, and battery electrodes, have been successfully captured by cryo-EM, owing to the advantages of cryo-freezing and low-dose irradiation strategies. Moreover, we also reviewed some arising methods, such as cryo-FIB and micro-ED. Since a large number of materials are natural crystals with limited size, micro-ED is highly feasible to characterize these structures at atomic resolution. In addition, the low-dose imaging and micro-ED are efficient and promising for *in situ* characterizing the structural heterogeneity and 3D morphology of soft materials, such as ligand-induced chiral materials and carbohydrate nanoparticles.

Impressively, structural biologists practically reconstruct the high-resolution 3D structures of biomolecules by averaging signals and visualizing the residues interactions. We envision that such 3D reconstruction methods show extensive potential to be generalized for characterizing the structures of sensitive materials.

In addition, we boldly suspect that some other electrochemical materials with outstanding electrochemical properties [12,68–73] can be further refined using cryo-EM to get a deeper understanding of their reaction mechanisms and outstanding performance. For example, Chen's group [74–76] synthesized electrocatalytic materials with dense defects and excellent performances in an ultrafast way by using high-temperature thermal shock technology. This technology is expected to synthesize materials with metastable structures which are unstable under electron beam irradiation. In this regard, cryo-EM shows great potential to directly observe the metastable phase, detect the atomic arrangement, and even *in situ* record the defect evolution process.

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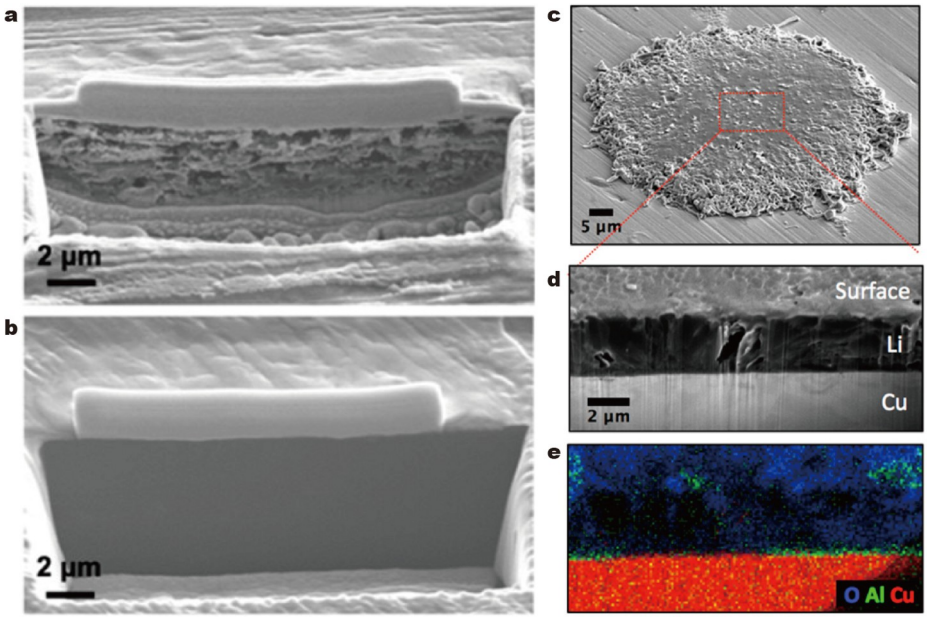

Figure 11 The comparison of (a) conventional FIB and (b) cryo-FIB. Reprinted with permission from Ref. [56]. Copyright 2019, ACS publications. (c) SEM image, (d) magnified cross-sectional SEM image and (e) energy dispersive X-ray spectroscopy (EDX) elemental mapping of an electrodeposit on thick Al_2O_3-coated interphases by cryo-FIB. Reprinted with permission from Ref. [60]. Copyright 2018, ACS publications.

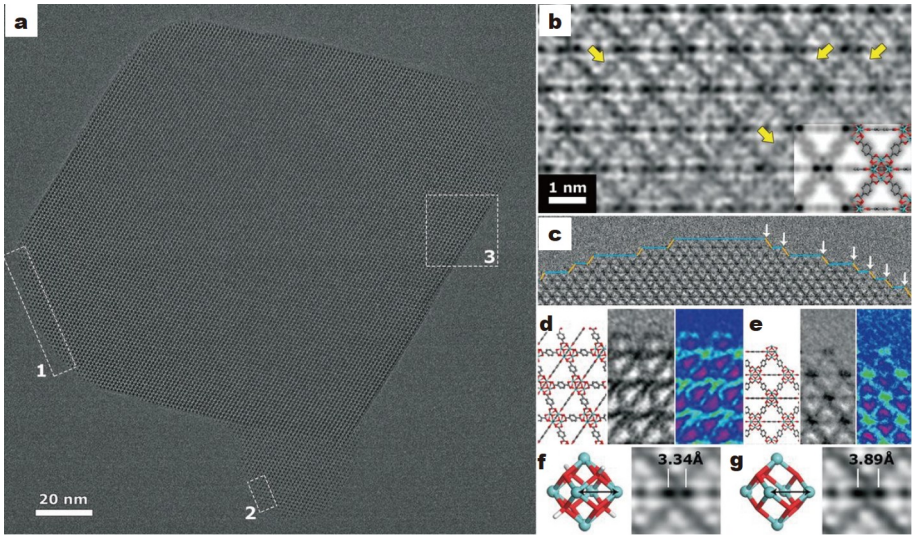

Figure 12 (a) High-resolution TEM of thermally treated UiO-66 using low electron dose. (b) Denoised image of zone 2. (c) Truncation surface of zone 1. Structural models, high-resolution TEM images, and the average images from zones 3 (d) and 1(e). Structural models and high-resolution TEM images of (f) hydroxylated and (g) dehydroxylated Zr clusters. Reprinted with permission from Ref. [64]. Copyright 2018, American Association for the Advancement of Science.

Moreover, liquid cells, made of silicon nitride, have also been widely used in nanoparticle formation, electrochemical reaction revolution, biomineralization, and corrosion [77]. Nevertheless, the thickness (~10 nm) and conductivity of silicon nitride used for liquid cells are still not enough for soft materials with low contrast in TEM [78]. Graphene liquid cell (GLC) is recently developed due to its ultrathin structure and superior conductivity. Other than nanoparticle growth evolution, GLC can also be used to wrap bacteria or proteins, so as to observe their shape and living status [79]. Note that GLC is sealed by π-π stacking between the two graphene layers, which is largely different from the silicon nitride liquid cell blocked by glue or clamping [80]. Nonetheless, the contrast and electron beam irradiation damage in GLC remain key challenges, which are supposed to be the future research directions for liquid cell technique in cryo-EM. If such problems can be solved, a more dynamic process of proteins and electrochemical reaction process can be studied in GLC, which is significant in both structural biology and chemistry.

On the other hand, with the capacity of retaining the original structures, cryo-EM is of great significance for studying the relationship between the real structure and electrochemical performance of the Li-ion batteries. For example, Cui's group [81] studied the transformation between SEI and CEI by using

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cryo-EM and pushed forward the idea of designing CEI by using SEI principles and recipes, which greatly broadened the idea of CEI and electrolyte design. In lithium-ion batteries, defect (i.e., vacancies and doping) engineering is an effective strategy to design novel nanostructures and improve electrochemical performance. However, such defect structures are generally vulnerable to electron beam irradiation. We believe that the use of cryo-EM and its related 3D reconstruction techniques can maximally detect the sensitive structures, especially at the interface, so as to provide a new platform to further investigate their structure-property relationships.

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Conflict of interest The authors declare that they have no conflict of interest.

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冷冻电镜在纳米材料研究中的应用: 进展与展望

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摘要 自2013年“分辨率革命”以来, 冷冻电子显微镜(cryo-EM)广泛推动了结构生物学研究的发展, 并很快在2017年荣获诺贝尔化学奖. 相机技术和软件算法的进步使cryo-EM得以实现对生物分子三维结构的近原子分辨率表征. 生物分子通常对电子辐照损伤较为敏感, 而低温可以将辐照损伤最小化. 这一原理启发了材料科学家利用cryo-EM表征电子束或空气敏感型材料, 例如锂离子电池中的电极、金属有机框架(MOFs)、共价有机框架(COFs)和沸石. 此外, 在cryo-EM中, 反应体系可在玻璃冰状态快速冻结, 从而保存材料的近初始状态. 在此, 我们回顾了cryo-EM和其他新兴冷冻技术的发展及其在材料科学、能源存储与转换等领域的应用. Cryo-EM技术能够直观地观察敏感材料和电催化反应过程, 将极大地革新学界对材料科学及其相关机制的理解.

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