

Cryogenic electron microscopy workflows for the characterization of electrochemical interfaces and interphases in batteries

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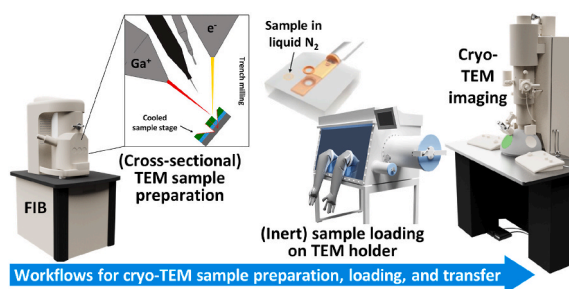
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HIGHLIGHTS

- Cryo-TEM for battery materials and interfaces sensitive towards the electron beam.
- Detailed descriptions of sample-specific preparation and transfer workflows.
- Overview of state-of-the-art cryo-TEM techniques used in battery research.
- Outlook on future developments and opportunities in the field.

GRAPHICAL ABSTRACT



ABSTRACT

Fundamental understanding of (electro-)chemical processes occurring in batteries down to an atomic level is essential to further improve the performance of commercialized battery technologies and to establish novel cell chemistries. Transmission electron microscopy techniques are well-suited for highly localized structural and chemical analysis, but many electrode materials and their corresponding electrode/electrolyte interfaces and interphases are sensitive towards ambient conditions and/or the high energy electron beam. This necessitates cryogenic cooling of the specimen during sample preparation, transfer, and/or imaging. Here, we highlight the major experimental workflows derived from sample-specific requirements, which vary in complexity and infrastructural requirements. The purpose of this Perspective is to give a comprehensive guideline on both the opportunities and requirements of cryogenic (transmission) electron microscopy to analyze materials/phenomenon-specific questions relevant to battery research.

1. Introduction

The global demand for rechargeable batteries, specifically lithium-ion batteries (LIBs), is continuously rising owing to their proven suitability for a wide range of applications from portable electronics to electric vehicles to stationary energy storage. Accordingly, tremendous research efforts are devoted towards improving the performance metrics

of LIBs regarding their energy density, power density, long-term cycling stability, as well as sustainability and cost [1,2]. Simultaneously, alternative battery chemistries are being investigated that promise further advances, including lithium-metal batteries (LMBs) and metal-batteries in general, sodium-ion batteries (SIBs), lithium-sulfur (Li-S) batteries, and organic batteries. All established and emerging battery chemistries have in common that such improvement – and in the latter cases, their

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successful commercialization - greatly rely on a fundamental understanding of the charge storage mechanism and the processes occurring inside the cell down to the atomic level. Particularly, this includes the processes occurring at the interfaces between the electrodes and the electrolyte, frequently resulting in the formation of solid interphases and the deposition of metallic phases such as lithium or sodium [3]. The latter often form extended and highly disadvantageous dendritic structures that can lead to device degradation and failure.

Transmission electron microscopy (TEM) with its related methodical portfolio (imaging, diffraction, spectroscopy) as a highly localized technique is well suited for the investigation of such microstructures and processes. However, such studies are challenging due to the high reactivity of the involved metallic species, compounds and interphases under oxidizing environmental conditions as well as the high sensitivity towards the high-energy electron beam [4]. This includes the demanding preparation of TEM-conformal (<100 nm thin) samples like extracted

cross-sectional lamellae *via* focused ion-beam (FIB) milling to reveal buried interphases and interfaces [5].

To master these issues, sample reactivity as well as the mobility of the involved atomic species have to be drastically reduced by cooling with liquid nitrogen (LN₂, best established) to prevent, or at least substantially delay, sample alterations. Moreover, cryogenic LN₂ cooling drastically minimizes ion-/electron-beam induced damage during sample preparation and microscopic investigation, respectively. Therefore, cryogenic (transmission) electron microscopy (cryo-(T)EM), which had initially been developed for bio and medical applications, was adapted for materials research [6–8]. This enables the characterization of very sensitive, but critical inorganic and organic components in batteries such as alkali metal electrodes, the solid electrolyte interphase (SEI), and the cathode electrolyte interphase (CEI) at an atomic scale. It is therefore anticipated that cryo-TEM will become increasingly relevant for battery research, particularly when it comes to the development of

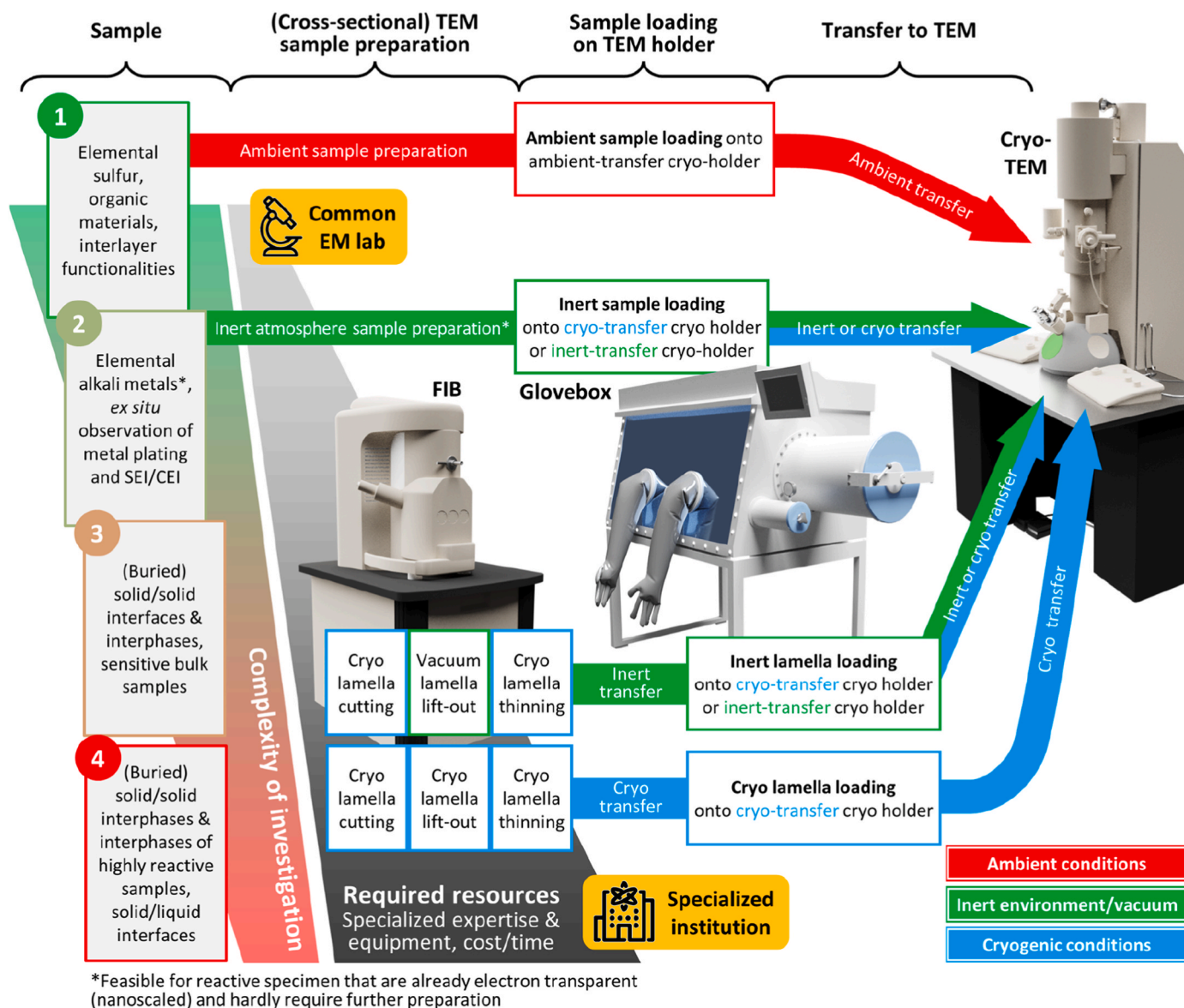


Fig. 1. Overview of different workflows (1) to (4) for cryo-TEM including examples of observable materials/phenomena, sample preparation, sample loading, and sample transfer to the TEM. Investigations/workflows increase in complexity from top to bottom with strongly different infrastructural requirements (expertise, equipment). Studies of less reactive or nanoscaled (TEM-conformal) samples may be conducted in nanoanalytical laboratories (only cryo-TEM cooling holder required), increasingly complex analyses, requiring advanced sample preparation via focused ion beam (FIB), become more suited for highly specialized institutions where cryogenic cooling and inert handling are established along the whole process chain including sample preparation, handling, and nanoanalytical characterization.

novel cell chemistries providing increased performance and sustainability.

Herein, we provide an overview of the most typical workflows for cryo-TEM for the broad audience of scientists working in the battery field, emphasizing both the workflows' specific capabilities and requirements (Fig. 1) to complement recent articles on the subject [4, 8–11]. We differentiate experimental workflows derived from the sample-specific requirements for preparation, transfer, and imaging. These workflows are characterized by different degrees of complexity and infrastructural requirements, and have already allowed for successful investigation of fundamental phenomena as discussed in the following.

2. Established cryogenic TEM workflows

Depending on the materials and phenomena to be examined, the specific sensitivity of the specimen and its dimensions, cryo-TEM users have to identify a suitable workflow for sample preparation, loading, and transfer to the microscope, as summarized in Fig. 1 and Table 1. For many TEMs, the sample dimensions are standardized (round shape: 3 mm diameter, up to 200 μm maximum thickness, with electron-transparent regions of interest of only a few 10 nm up to 200 nm). Nanoscale materials or extracted (cross-sectional) lamellae are commonly attached to so-called TEM support grids that are suited for direct holder mounting.

In the simplest case of workflow (1), the sample is not sensitive to ambient conditions but only electron beam-sensitive. Hence, routine sample preparation and handling in conjunction with a cryo-cooling TEM holder are sufficient. For air- or moisture-sensitive samples, additionally, sample preparation and handling must be conducted under inert environment, e.g., in a glovebox (Ar preferred over N_2 for highly reactive samples). In that case, a dedicated transfer holder for any respective microscope is required to facilitate the sample transfer to the TEM under cryogenic or inert/vacuum conditions (workflow (2)). Ambient- and/or electron beam-sensitive bulk materials, complex composites, or complete battery parts as well as (buried) interfaces typically require the much more demanding extraction of a thin (cross-sectional) lamella *via* cryogenic focused ion-beam (cryo-FIB) milling. Particularly challenging is the lamella lift-out procedure from the bulk sample while keeping the sample under vacuum (workflow (3)) or cryogenic conditions (workflow (4)).

In the following, successful literature examples for the different workflows are outlined, describing both the hardware requirements as well as the experimental procedure for each workflow. Concludingly, we provide a brief perspective on the potential future developments in this field.

2.1. Ambient sample preparation and loading – workflow (1)

The simplest method for cryogenic imaging of samples *via* TEM is employing a cryogenic sample cooling holder to keep the TEM sample close to LN_2 temperature during the measurement. As an example for such a cryo-holder, Fig. 2A shows a low-drift MEMS-based liquid-nitrogen double-tilt specimen holder with external copper braid (plunged into separate LN_2 reservoir (Dewar)), including a schematic

representation of the basic components. Commercial holders for standardized TEM samples typically include a small LN_2 Dewar with an inlying cooling rod directly at the end of the holder rod. The cooling rod connects the sample on the tip to the Dewar, thus maintaining the sample at low temperature after equalization. Simultaneously, a thermal isolator near the tip keeps the temperature equilibrium of the outside tube to the goniometer in order to suppress any specimen drift within a limit of less than 1 nm min^{-1} [10,12].

Such cryo-holders are mandatory for sensitive samples, which undergo significant physical, chemical, or crystallographic changes upon interaction with the electron beam. Tyukalova and Duchamp [13] succeeded in directly imaging the atomic structure of nanoparticulate $\text{LiNi}_{0.5}\text{Mn}_{1.5}\text{O}_4$ at atomic resolution even at high electron doses (Fig. 2B). At room temperature, $\text{LiNi}_{0.5}\text{Mn}_{1.5}\text{O}_4$ nanocrystals underwent a transition from the spinel to the rock salt phase when exposed to the electron beam, both at 200 keV and at 80 keV due to radiolysis. By employing cryogenic imaging of the sample, the critical electron dose threshold for radiation-induced crystallographic changes was increased by at least by a factor of 3–4, owing to reduced atomic mobility at LN_2 temperature [10,13]. The chosen example further demonstrates that the operation conditions of such a microscope including the illumination conditions (acceleration voltage, beam current/dose) as well as imaging conditions (detector/parameters) have to be carefully selected.

The use of a cryo-holder also allows for the investigation of materials with high vapor pressure, such as sulfur-containing electrode composites for Li-S batteries, for which the sulfur sublimation and redistribution within the composite pose inherent challenges for the nanoscopic characterization by TEM [14]. Sahore et al. demonstrated the successful suppression of sulfur sublimation including the observation of sulfur infusion into a porous carbon matrix via cryogenic scanning TEM (cryo-STEM) in conjunction with energy-dispersive X-ray spectroscopy (EDX) (Fig. 2C) [15]. The workflow has further proven suitable for the investigation of layered or two-dimensional materials with electron beam-sensitive interlayer species, such as hydrous layered oxides ($\text{WO}_3 \cdot 2\text{H}_2\text{O}$) [16] or surface-terminated transition metal carbides ($\text{Ti}_3\text{C}_2\text{T}_x \text{MXene}$) [17].

As illustrated by these examples, a simple cryo-holder (with no dedicated transfer functionality) is appropriate for battery research on samples which are not sensitive towards ambient conditions but the electron beam, by systematically minimizing electron beam-induced degradation. This is the easiest accessible and most established workflow of cryo-TEM as only a cryo-holder is needed in conjunction with a modern microscope. Commercial holders are available in most EM labs or may require a rather moderate investment by battery groups with access to TEM instrumentation.

However, it must be considered that, although suppressed by cryogenic cooling, electron beam-induced sample damaging occurs during most TEM investigations and limits the sample lifetime during the characterization. It is a complex process, which depends on the respective sample (atomic numbers, bonding, thickness), the microscopic illumination/imaging conditions as well as the vacuum environment [18]. Knock-on damage and radiolysis are the major primary degradation mechanisms, which strongly depend on the electron beam energy and the beam current density. Both knock-on damage (loss/displacement of individual ions/atoms from/within the sample by

Table 1

Summary of the different cryo-TEM workflows and their corresponding protections towards potentially damaging influences for specimens of certain dimensions.

| Workflow | Specimen dimensions | e - beam | Air/O ₂ , moisture | Ion beam | Thermal energy | Comments |
|----------|---------------------|----------|-------------------------------|----------|----------------|---|
| 1 | any | X | – | – | – | any TEM preparation method applicable (conventional, FIB, grid application) |
| 2 | nano-scaled | X | X | – | – | only simple TEM preparation in glovebox (application onto TEM grid, cleaving) |
| 3 | bulk | X | X | X | – | FIB: cryo-stage required |
| 4 | bulk | X | X | X | X | FIB: cryogenic nanomanipulator & sample loader additionally required |

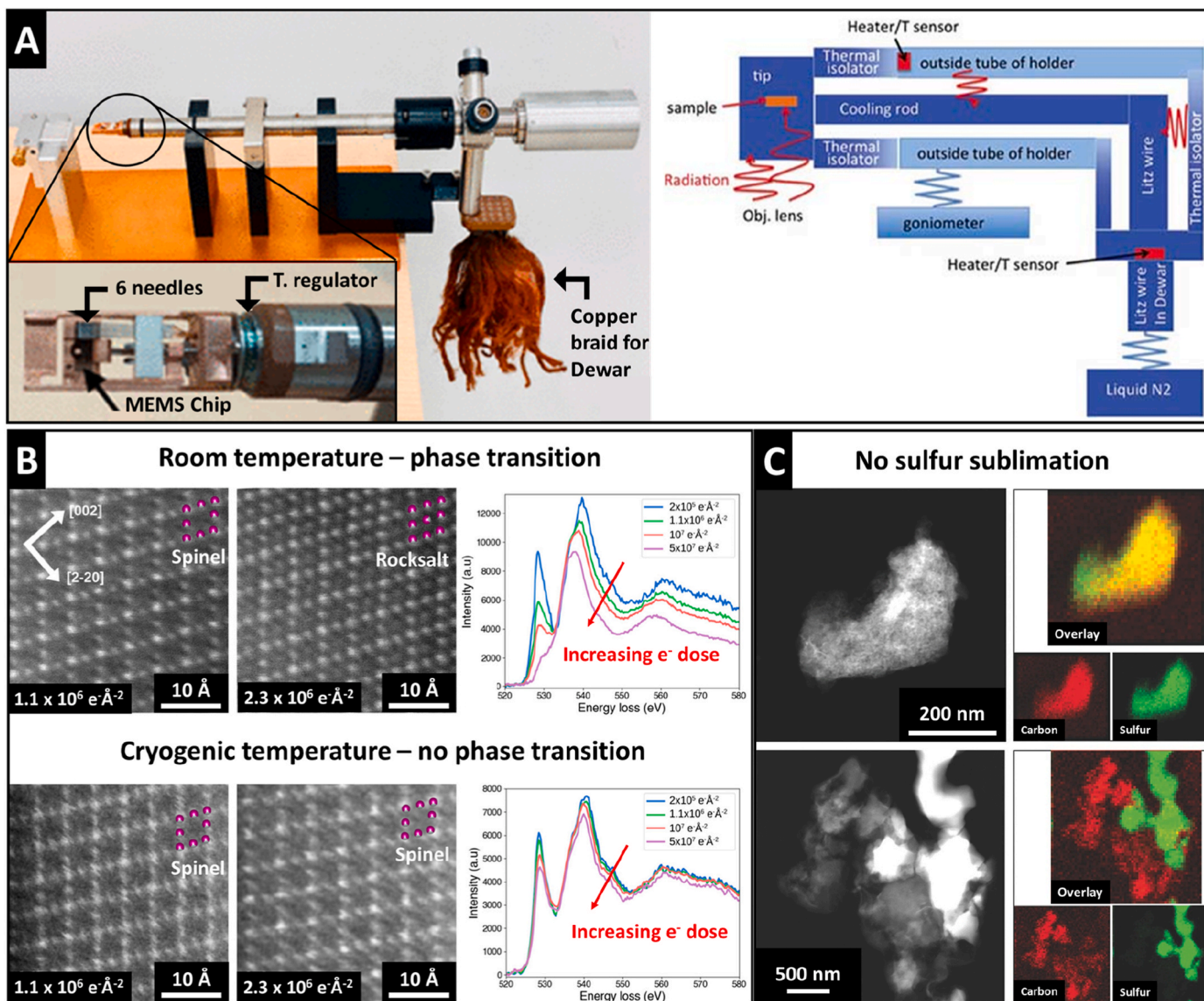


Fig. 2. (A) Photographs of a LN₂ double-tilt specimen holder (left) and a schematic illustration with a labeling of the different parts (right); adapted with permission from Ref. [10], Copyright 2021 American Chemical Society, and [12], Copyright 2016 John Wiley & Sons. (B) Atomic-resolution high-angle annular dark-field STEM (HAADF-STEM) images and a series of electron energy loss spectroscopy (EELS) datasets obtained from LiNi_{0.5}Mn_{1.5}O₄ analyzed at room temperature (top) and cryogenic temperature (bottom), revealing the preservation of the original crystal structure only under LN₂ cooling; adapted with permission from Ref. [10], Copyright 2021 American Chemical Society. (C) STEM images of two different carbon-sulfur composites and corresponding EDX mappings of carbon and sulfur, illustrating the successful determination of the sulfur distribution without sulfur sublimation when employing cryo-STEM; adapted with permission from Ref. [15], Copyright 2016 John Wiley & Sons.

sufficient energy/momentum transfer from the impacting electron) as well as radiolysis (ionization/radical formation/loss of bond electrons by electron-electron interaction) may cause the fatal disintegration of the TEM sample. While low acceleration voltages may impede knock-on damage due to insufficient electron energy, radiolysis is more pronounced under those conditions, rendering the optimum primary beam energy always a compromise. In that regard, the minimization of the beam current/electron dose is advised to extend the sample lifetime and thus to allow for a detailed characterization by complementary techniques. Measures include the application of state-of-the-art dose-efficient imaging/spectroscopy methods like low-dose imaging or 4D-STEM in conjunction with highly sensitive detectors (e.g., direct electron-detection cameras, large solid-angle X-ray spectrometer, modern electron energy-loss spectrometer). Moreover, the best achievable vacuum level in the utilized microscope is expedient, as it minimizes residual concentrations of potential reactants for undesired degradation

reactions as well as the formation of ice on the sample surfaces during cryo-TEM analysis.

2.2. Inert sample preparation and loading – workflow (2)

The preparation and handling of samples sensitive to atmosphere (and/or moisture) require inert conditions and, in the best case, cryogenic cooling throughout the whole process. Accordingly, sample preparation and loading to the cryo-holder must be conducted in an inert environment like a glovebox. In addition, the cryo-holder requires a transfer unit/functionality that allows for the transfer of the loaded sample from the glovebox to the microscope under cryogenic conditions (“cryo-transfer”) and/or under inert conditions (“inert-transfer”), as schematically depicted in Fig. 3A.

Depending on the available glovebox, the cryo-holder has to be either completely loaded into the glovebox or may be introduced from

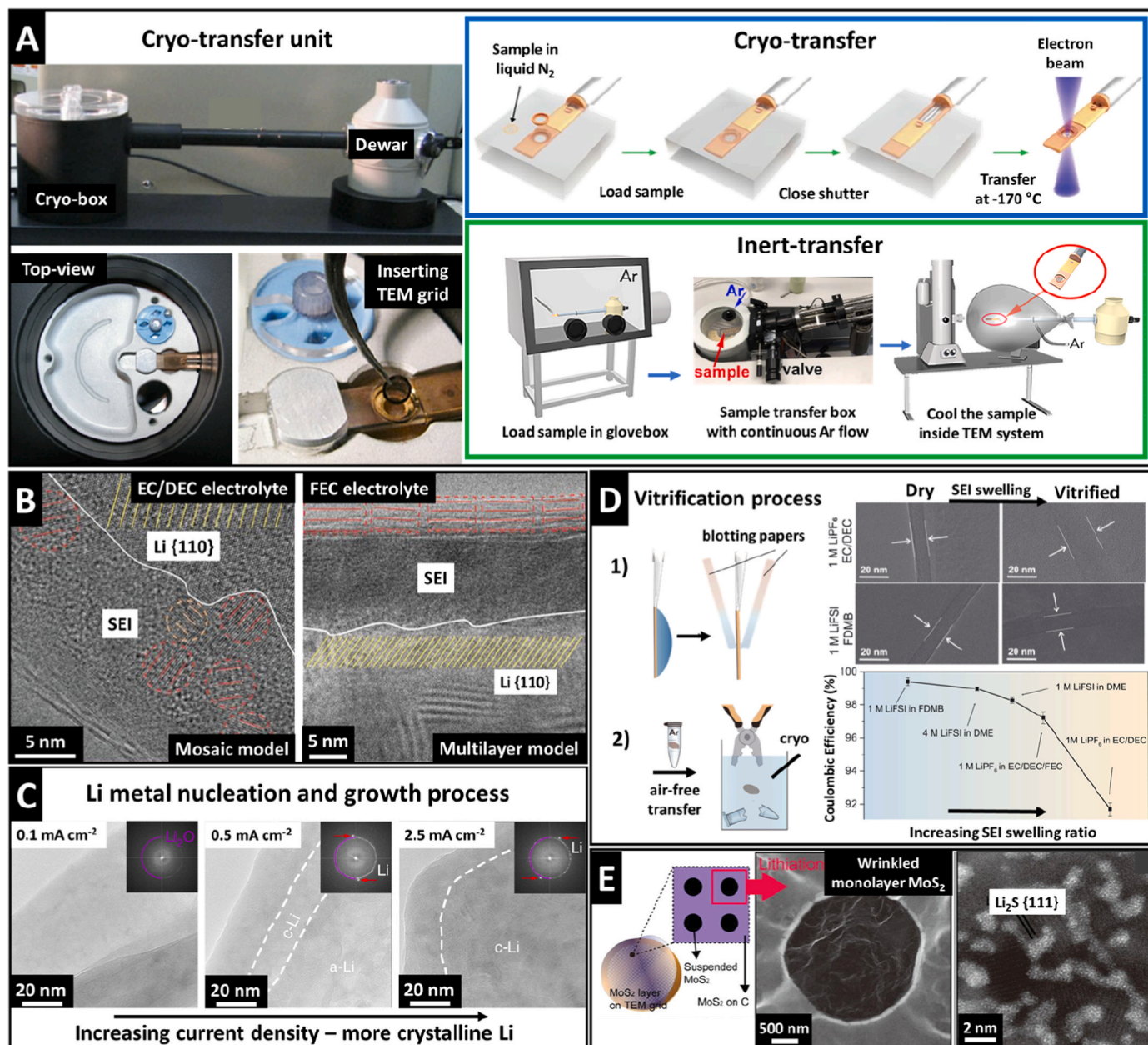


Fig. 3. (A) Photographs of a double-tilt liquid nitrogen cryo-holder in a cryo-transfer workstation and schematic illustrations of inserting a TEM grid into the holder for a cryo-transfer and inert-transfer process; adapted from Ref. [4] with permission from Elsevier, from Ref. [7] with permission from AAAS, with permission from Ref. [19], Copyright 2017 American Chemical Society, by permission from Springer Nature: [20] Copyright 2014. (B) TEM images of Li metal and the SEI grown in an ethylene carbonate/diethyl carbonate (EC/DEC)-based electrolyte vs. a fluoroethylene carbonate (FEC)-based electrolyte for comparing the different resulting SEI structures; adapted from Ref. [7] with permission from AAAS. (C) TEM images of deposited Li metal at different current densities with the corresponding diffraction patterns as insets, highlighting the (110) plane of Li metal to reveal different microstructures of the deposited Li metal depending on plating current; adapted by permission from Springer Nature: [21] Copyright 2020. (D) Schematic illustration of Li dendrite directly grown on the TEM grid that was used as the current collector in a coin cell setup, using a thin-film vitrification method to capture the “wet” state of the SEI. Different swelling effects in various electrolytes were shown and correlated to the Coulombic efficiency; adapted from Ref. [22] with permission from AAAS. (E) TEM images of MoS₂ monolayers after lithiation, showing a wrinkled structure and molybdenum metal and Li₂S nanoparticles to study and visualize the lithiation mechanism at the atomic scale; adapted with permission from Ref. [23], Copyright 2019 John Wiley & Sons.

the outside utilizing a specialized side-entry port. Most commercial cryo-transfer TEM holders have not yet provided complete gas-tightness, as the LN₂-cooled samples are only shielded for transfer (retractable metallic cryo-shield with narrow gaps to the sample holder) to minimize ice formation on the sample surfaces during transfer. Hence, slight gas exchange may occur which can significantly alter highly reactive TEM samples. In that regard, the poor vacuum in common TEM load locks during the insertion process has to be taken into account as well. A

promising development was the recent release of a commercial cryo-transfer holder with true vacuum/inert-gas transfer capability. If, in addition, cryogenic conditions are maintained during sample transfer, the cryo-holder is cooled (LN₂-filled Dewar) and may be kept in its LN₂-cooled sample mounting stand with gaseous nitrogen covering the sample tip to minimize gas exchange. Thereby, the TEM sample is kept at cryogenic temperature throughout the transfer, while the shutter on the cryo-holder tip facilitates transfer, potentially with minimal

exposure to air depending on its technical realization.

Workflow (2) is particularly dedicated to nanoscaled samples or those necessitating only minor sample preparation. In the context of battery research, these are often alkali metal structures with their corresponding SEI that were directly (electrochemically) deposited onto TEM support grids utilized as current collector in the electrochemical cell. Both the cryo-transfer and the inert-transfer methods have been successfully utilized, e.g., to analyze lithium dendrite structures and growth mechanisms to elucidate the mechanisms behind the observed capacity decrease and cell failure [24,25]. Moreover, SEI nanostructures were analyzed by cryo-TEM for better understanding of the relationship between the SEI structure and the electrochemical properties.

Using the cryo-transfer method, Cui and co-workers [7] have first reported the successful application of cryo-TEM characterization of sensitive lithium metal and SEI nanostructures. As shown in Fig. 3B, the crystalline structure of lithium dendrites was observed at atomic resolution along with two different types of SEI structures when using different electrolyte compositions. In another seminal study using the

inert-transfer method, Wang et al. [21] have shown different nucleation and growth processes of lithium metal depending on current density and deposition time as well as their correlation to the local structure (Fig. 3C). Zhang et al. [22] have successfully captured the solid-liquid interface between the (vitrified) electrolyte and the SEI on a lithium-metal anode by using the thin-film vitrification method shown in Fig. 3D. The lithium-metal with SEI was directly electrochemically deposited on a copper-based TEM support grid inside a coin cell, before being extracted and blotted with paper to form self-supporting thin films of organic electrolyte in the holes of the TEM grid, which was then vitrified in LN₂. The swelling behavior of the SEI in different electrolyte systems was analyzed, as shown in Fig. 3D, revealing that increased SEI swelling leads to decreased Coulombic efficiency of the lithium-metal anode.

Moreover, several studies on lithiated electrode materials have been reported. For example, the lithiation mechanism of MoS₂ nanosheets was studied (Fig. 3E) [23]. The lithiated states of monolayer and bilayer MoS₂ were well preserved during the cryo-transfer. The study revealed

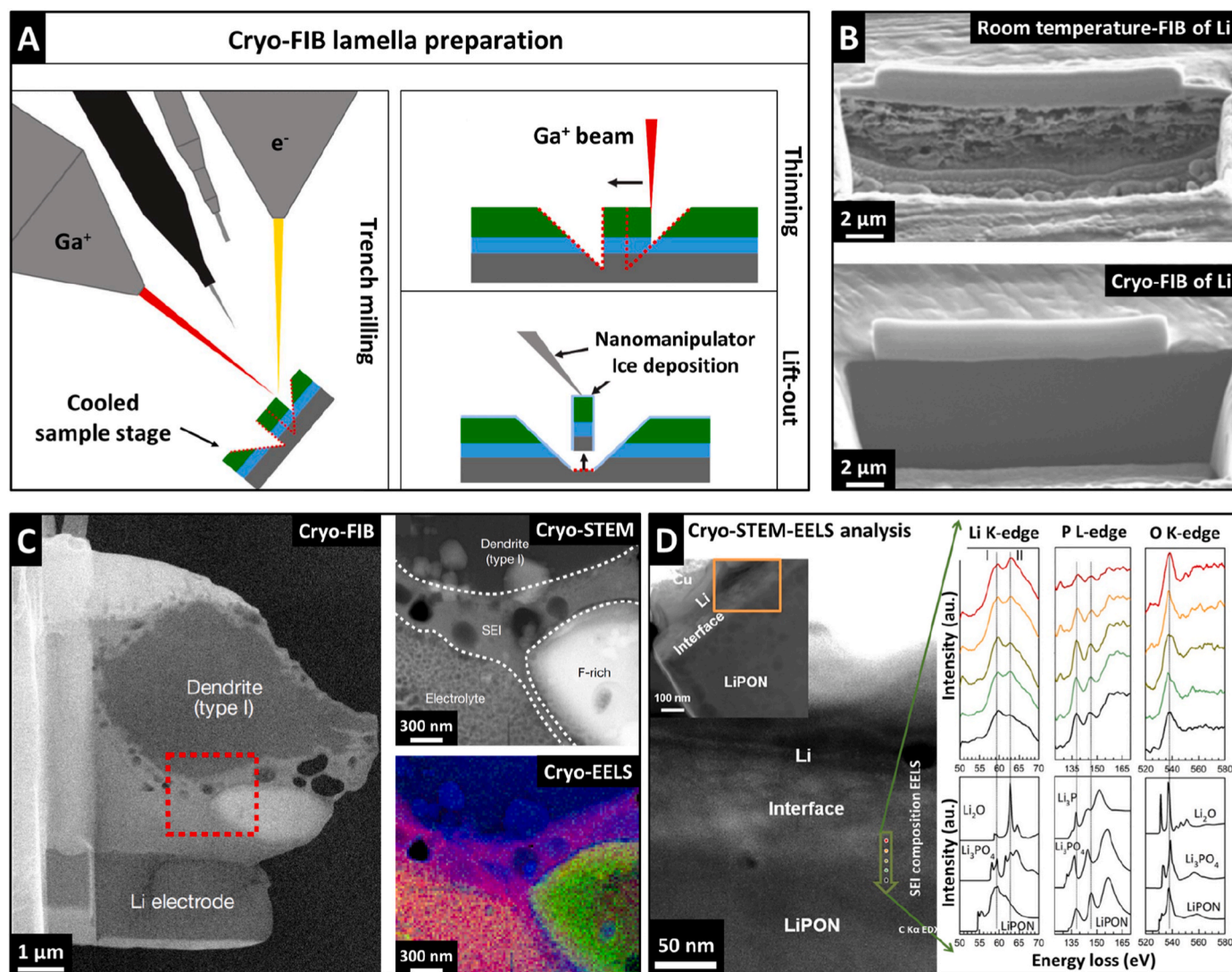


Fig. 4. (A) Schematic illustration of the lamella preparation with a cryogenic SEM/FIB, adapted with permission from Ref. [28]. (B) SEM images of metallic lithium cross-sectioned and cleaned by FIB at room temperature (top) and cryogenic temperature (bottom); adapted with permission from Ref. [29], Copyright 2019 American Chemical Society. (C) SEM micrograph of the preparation of a liquid-solid interface lamella of lithium metal and the vitrified liquid electrolyte (left) and the corresponding cryo-STEM image (right top) and cryo-EELS elemental mapping (right bottom, carbon: red, oxygen: blue, fluorine: green); adapted by permission from Springer Nature: [5] Copyright 2018. (D) Cryo-STEM image of the Li/LiPON interphase with a series of electron energy loss spectra throughout the interface, showing the changes in the composition of the SEI at the Li K-edge, P L-edge, and O-K edge; adapted from Ref. [30] with permission from Elsevier. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

highly strained (“wrinkled”) MoS₂ films after lithiation and the presence of metallic molybdenum adjacent to Li₂S nanoparticles, confirming the occurrence of a conversion-type reaction during the electrochemical lithiation of MoS₂.

It has been proven by several studies that the use of such transfer equipment in conjunction with an inert-gas glovebox is capable to conserve the native state of ambient-sensitive materials and components such as lithium metal, the SEI, and other materials in their lithiated state. This has even been successfully demonstrated for materials relevant to SIBs [26,27], where the properties of the electrode/electrolyte interfaces and interphases are considered even more crucial for the electrochemical performance compared to LIBs [3].

2.3. Cryogenic focused ion beam lamella preparation – workflow (3) & (4)

As described before, only nanoscaled and thus electron-transparent samples may be directly investigated according to workflows (1) and (2) without further complex sample preparation. For studies on bulk samples or devices, where buried interfaces and interphases are of interest, site-specific micrometer-scaled and thus TEM-conformal lamellae have to be prepared and extracted from the macroscopic specimen and attached to a TEM support grid. This procedure typically occurs by FIB under cryogenic conditions (cryo-FIB), as schematically illustrated in Fig. 4A, most often within a scanning electron microscope (SEM) [28].

Cryo-FIB is suitable to minimize ion-beam damages in the case of sensitive materials with relatively low melting points, as demonstrated by a comparison of room temperature- and cryo-FIB cross sections of metallic lithium in Fig. 4B [29]. Additionally, fragile structures like thin dendrites may be protected by the electron- or ion-beam induced deposition of a protection layer (e.g., platinum or carbon) by decomposition of a locally gas-injected organic precursor compound [24].

The cryogenic TEM lamella preparation routine (Fig. 4A) only differs slightly from the standard room temperature FIB lamella extraction procedure. The procedure starts by coarse site-specific trench milling from both sides of a respective region of interest using the ion beam. Subsequently, the coarse lamella is partially cut free and is attached to a micromanipulator either by local electron-/ion-beam induced dissociation and deposition of a (metal)organic precursor (room-temperature lamella extraction) or by the condensation of water vapor through a gas-injection system forming amorphous ice on the cold surfaces. After fixation, the complete lamella is cut free, lifted out and attached to a suitable TEM support grid. Finally, the lamella is ion-beam polished down to electron transparency and transferred to the TEM.

The transfer routines into the TEM, however, strongly depend on whether the sample must be kept under cryogenic conditions throughout the transfer. Ion-/electron-beam sensitive samples, which require cryogenic conditions only to reduce/minimize beam-induced damage, may be warmed up during lift-out and sample transfer (workflow (3)). Dedicated vacuum transfer shuttles from FIB to glovebox are commercially available and allow for a seamless transfer and loading of the TEM grid onto the TEM cryo-holder within the inert-gas glovebox. Samples like electrochemical solid/liquid interfaces with liquid components (e.g., liquid electrolyte) may require cryogenic conditions throughout the whole preparation process (workflow (4)), making the transfer routine more complex. The lamella lift-out requires a cryo-cooled nanomanipulator and continuous cooling during the complete transfer from the FIB/SEM to the TEM has to be ensured. This additionally requires a FIB cryo-load lock and a table-top cryo-transfer workstation (transfer from FIB holder to TEM cryo-holder) or a glovebox, as the TEM cryo-holder cannot directly be inserted into the FIB/SEM.

Zachman et al. were the first to publish cryo-TEM measurements of locally extracted electrochemical solid-liquid interfaces of metallic lithium and organic electrolyte [5]. The samples were vitrified by plunge-freezing in liquid nitrogen and a lamella was cut out by cryo-FIB

(Fig. 4C), enabling the distinction of different types of lithium dendrites and their corresponding extended SEI structures, including determination of local elemental composition.

Cheng et al. [30] employed cryo-FIB preparation to examine the interface between a thin lithium phosphorous oxynitride (LiPON) solid electrolyte film in contact with lithium metal, which provided detailed insights into the formed SEI. Cryo-STEM in conjunction with electron energy loss spectroscopy (cryo-STEM-EELS) revealed a nanostructured SEI consisting of Li₂O, Li₃N and Li₃PO₄ domains (Fig. 4D). Concentration gradients of N and P indicated the decomposition of the LiPON electrolyte and the elemental diffusion towards the lithium metal.

Cryo-FIB possesses not only the opportunity to prepare site-specific samples for cryo-TEM measurements, but enables the direct cross-sectional or 3D investigation of bulk samples via SEM. The morphology and elemental composition of sensitive samples can be investigated via sequential cryo-FIB milling/SEM imaging (in conjunction with EDX mapping) and subsequent reconstruction of the 3D morphology, e.g., of lithium dendrites [5] to reveal the morphology of lithium after stripping and plating cycles [24], or to investigate the homogeneity of protective coatings [31] via cryo-FIB-tomography.

Although numerous successful examples have been published utilizing the different cryo-FIB workflows, such studies are generally demanding. Sample resilience even under cryogenic conditions is limited by both the material’s reactivity and the mobility of the constituting species, limiting sample lifetime during transfer and TEM investigation. In addition, the inert glovebox environment and the high vacuum within typical microscopes are not perfect. Undesired reaction partners (e.g., oxygen, water vapor), although present at very low concentrations, may cause the corrosion of nanoscale TEM samples. Depending on the microscope conditions, residual water vapor may additionally cause the unwanted formation of ice on the during extensive TEM sessions.

3. Conclusive summary and outlook

Electron microscopy at cryogenic temperatures has been proven as a powerful method for the nanoscale characterization of samples that are highly sensitive towards detrimental environmental conditions and/or the electron/ion beam. Systematic cooling allows for suppression or at least substantial delay of unwanted sample degradation or alteration. This includes sample preparation at such temperatures via FIB and complementary characterization.

Depending on the sensitivity of the materials under investigation and the resulting complexity of the sample preparation, one can choose between four major workflows: The simplest and easiest to implement workflow only requires a TEM cryo-holder (not necessarily suited for transfer) as sample preparation and transfer are conducted under ambient conditions. Thus, it is anticipated that such studies with broadly available cooling holders may rapidly gain importance and become increasingly relevant to the field. This includes next-generation electrode materials like Ni-rich layered oxides, two-dimensional materials with surface functionalities like MXenes, or organic materials, which become more and more sensitive towards electron beam-induced changes.

For meaningful *ex situ* studies, the utilization of inert-/cryo-transfer is essential. For nanoscale and thus TEM-conformal specimens, preparation in an inert-gas glovebox in conjunction with subsequent inert-gas/cryo-transfer is suited to investigate materials as reactive as pure alkali metal. Specifically, the investigation of the interface between the electrodes and the electrolyte, including the various interphases formed, is essential for the successful development of high-energy LMBs and beyond-Li battery chemistries such as sodium, magnesium, or calcium, as well as organic batteries.

If these interfaces and interphases are not easily accessible, such as in bulk samples, the use of advanced and highly developed sample preparation procedures like cryo-FIB is mandatory for fully exploiting the

potential of cryo-TEM. Site-specific cryo-FIB preparation in conjunction with sophisticated transfer routines are highly demanding in terms of expertise, investment, time and thus cost. Consequently, such studies are limited – at least in the near future – to only a few groups and highly specialized institutions worldwide.

Currently available transfer equipment between FIB/SEM and TEM (load locks, stages, sample holders) is proprietary and consequently only compatible with the respective tools. Extension of studies by involving other complementary characterization methodology is currently hard to realize, if not impossible. To address more complex tasks on batteries and other sensitive materials in the future, standardized load locks, transfer shuttles as well as sample holders are highly required. The eventual insights will be of great relevance for an in-depth understanding of the reactions and processes occurring in batteries. Thus, further optimization of cryo-EM workflows, specifically transfer procedures, will play an important role in overcoming the present challenges and allow for further optimization of batteries.

Besides the use of cryogenic sample preparation, transfer, and imaging, complementary strategies should be considered to further minimize sample alterations. This includes the precise tuning of the imaging conditions and parameters, such as employing reduced accelerating voltages and low electron doses. Furthermore, the use of advanced plasma-based FIB methods, such as xenon ion plasma FIB (Xe⁺ PFIB) instead of gallium ion FIB, can further contribute to a decreased degradation of battery materials during the milling process [32].

Apart from cryo-TEM, *in situ* liquid cell TEM has emerged as a promising technique for studying structural and chemical properties of battery materials at the nanoscale. Electrochemical liquid cells for the TEM enable the time-resolved investigation of electrochemical processes such as the dynamic growth of metallic lithium or the formation of interfaces and interphases, i.e., the SEI [33]. The biggest advantage compared to *ex situ* cryo-TEM is the possibility to observe the dynamics of these processes. In addition, transfer steps, which can result in specimen damaging, are reduced. However, *in situ* liquid cell TEM does not avoid electron beam damages to the specimen as effectively as cryo-TEM, with radiolysis and degradation of the liquid medium (i.e., the electrolyte for *in situ* battery studies) severely limiting the technique [34]. Furthermore, limited resolution and the need for electron-transparent specimens are potential disadvantages compared to cryo-TEM. Overall, both techniques should be regarded as complementary, where the appropriate choice depends on the processes/phenomena of interest.

CRediT authorship contribution statement

Yuyoung Shin: Writing – original draft. **Dominik Stepien:** Writing – original draft. **Marco Hepp:** Writing – review & editing. **Benjamin Butz:** Writing – review & editing. **Dominic Bresser:** Writing – review & editing. **Simon Fleischmann:** Writing – review & editing, Conceptualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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