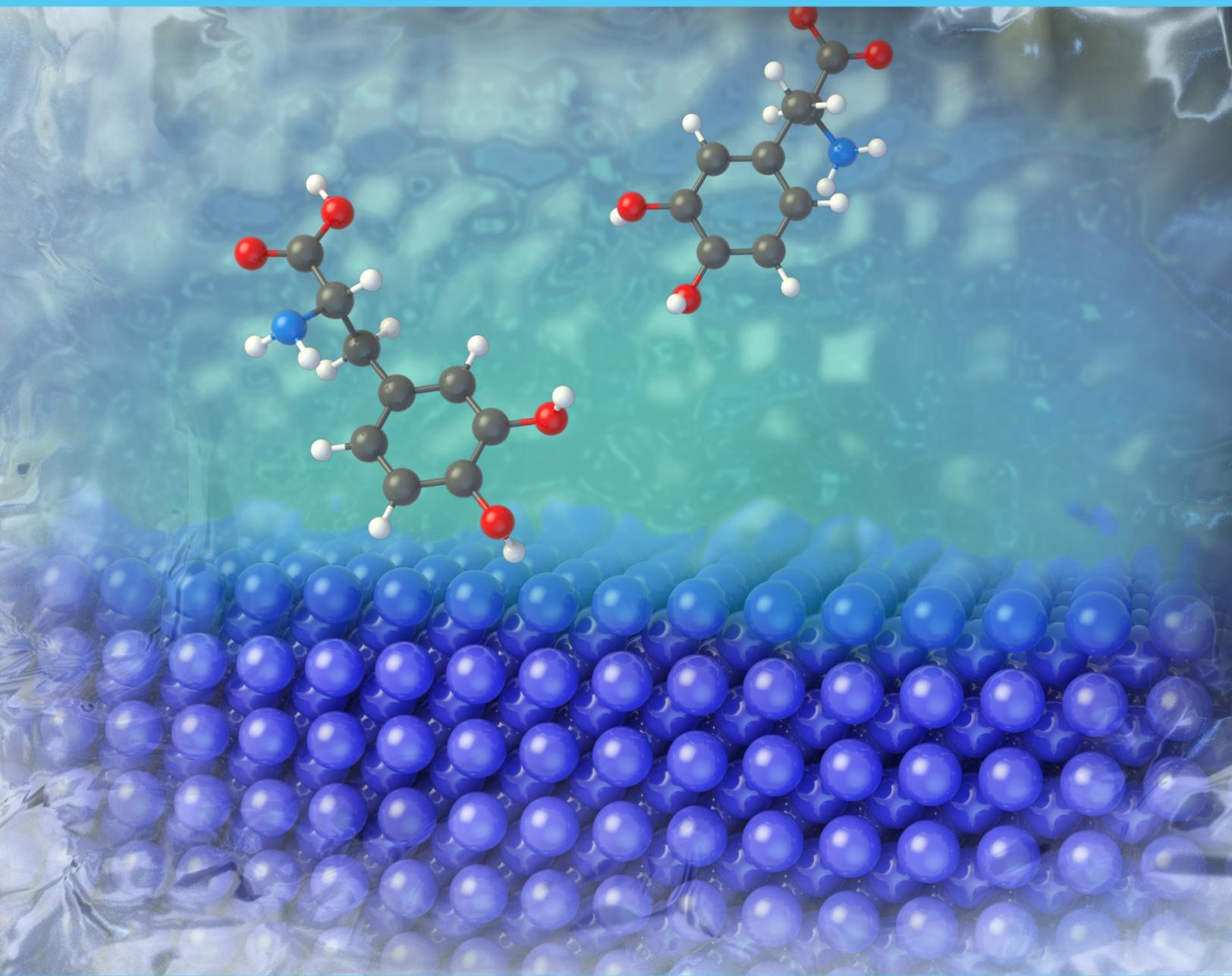


Basic Energy Sciences Roundtable

Research Opportunities in the Physical Sciences Enabled by Cryogenic Electron Microscopy



*Report of the Basic Energy Sciences Roundtable on Research Opportunities
in the Physical Sciences Enabled by Cryogenic Electron Microscopy*

May 4 – 6, 2021

The cover illustration shows a schematic of a cryoEM sample, depicting a molecule frozen in place during interaction with the surface of a crystal.

Cover illustration by Alison Mackey, Argonne National Laboratory.

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Report of the Basic Energy Sciences Roundtable on Research Opportunities in the Physical Sciences Enabled by Cryogenic Electron Microscopy

May 4–6, 2021

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Panel 2: Correlating Structure and Function in Soft Materials

Panel Lead **David Beratan** Duke University

Panel 3: Low-Temperature Phenomena in Quantum Matter

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Panel 4: Processes and Chemical Pathways in Energy Materials

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Executive Summary

The properties of materials as well as the pathways and outcomes of chemical and biological processes are controlled by microstructure, composition, and chemical and physical processes at the atomic- to nano-scale. Electron microscopy (EM) has long been used to understand the fundamental properties of materials and chemical systems because of the *local* information—as distinct from global or ensemble information—that it can provide. For numerous systems of interest, however, the powerful methods of room-temperature EM are insufficient. Challenges arise when the materials or molecules are sensitive to the incident electron beam, when fluids or solids are mobile at room temperature, or when phases or phenomena exist only at low temperatures. An emerging approach to meet these challenges is to study the samples cold, at temperatures that are below, typically far below, the freezing point of water.

Cryogenic electron microscopy (cryoEM) has proven to be a powerful method for visualizing beam-sensitive biological macromolecules and has had significant successes in material, chemical, and geochemical sciences. However, its full impact for advancing the scientific understanding of matter and chemistry has not yet been fully realized. In particular, the potential for going beyond the analysis of structure and composition to reveal the mechanisms of complex pathways and the interactions that underpin dynamic behavior has remained relatively unexplored.

To identify the most pressing research opportunities for the use of cryoEM as a decisive tool for physical sciences research, the U.S. Department of Energy, Office of Basic Energy Sciences (BES) convened a roundtable of scientists with expertise in chemistry, materials science, geoscience, bioscience and cryoEM. This group of experimentalists and theorists met remotely May 4–6, 2021, to explore research opportunities that would leverage existing cryoEM capabilities, develop forward-looking opportunities for new data acquisition and analysis approaches, and advance the broader science mission of BES. This report presents the findings of the cryoEM roundtable, summarized in the four Priority Research Opportunities (PROs) outlined below.

PRO 1: Discover emergent behavior and coupled processes at interfaces

How do complex and dynamic processes give rise to chemical functionality and physical properties at interfaces?

Chemical reactions and physical phenomena at interfaces play a vital role for energy production, conversion, and storage, as well as for information storage and communications. Meeting the increasing demands on these technologies will require the design of new types of compositionally diverse and dynamically evolving interfaces that enable new processes as well as control of those processes. CryoEM can play an essential role by elucidating the molecular-to-nanoscale, three-dimensional structure and composition of complex internal interfaces. In addition, it could capture the chemical processes and reveal the low-temperature quantum states that underpin emergent interfacial behavior and function.

PRO 2: Elucidate the role of heterogeneity in hierarchical systems

What are the dominant interactions across length and time-scales that control behavior in hierarchical systems?

Hierarchical systems are ones in which structure and composition at different length and time scales can interact to influence mesoscale and macroscale behavior. Hierarchical systems include liquids, soft and hard materials, and biosystems and have applications including batteries, bio-inspired catalysis, water-purification membranes and some magnets. These systems are intrinsically heterogeneous providing opportunities for cryoEM to elucidate how different components interact to generate hierarchical behavior and to learn to control that behavior. CryoEM can lead to a clearer understanding of important processes such as charge transport in soft materials, the formation of quantum states, and chemical separations.

PRO 3: Understand the evolution of matter in variable environments across length and time scales

How is matter assembled from its constituent units? How can we map the energy landscape that controls dynamic behavior and processes?

The dynamic behavior of matter in response to changes to its environment influences many of the properties that are harnessed for energy-related applications. Much needs to be learned about how matter is assembled from its constituents and how the dynamic behavior and processes of matter can be controlled. CryoEM has the potential for breakthroughs in understanding the assembly of matter, the emergence of quantum states, the subtleties of chemical transformations, and the dynamics of materials systems that are excited by an external stimulus. Integrating theory, experiment, and simulation, each enhanced by data science methods, will open new approaches to understanding dynamic behavior and harnessing it for new applications.

PRO 4: Harness data analytics and automation to expand the role of cryoEM in enabling scientific discoveries

What advances in artificial intelligence and machine learning can be harnessed to enable cryoEM to advance beyond what is currently possible?

Data science and data analytics will dramatically expand the capabilities and impact of cryoEM by capturing phenomena that were previously inaccessible. There are opportunities to develop machine learning (ML) and artificial intelligence (AI) approaches, to discriminate signals from noise or artifacts, and facilitate data interpretation. The ultimate goal will be autonomous discovery in which automated cryoEM experiments are guided by data science. Automated microscope tuning, high-throughput data acquisition, and “on the fly” control of cryoEM experiments can revolutionize microscope operation, data collection and mining of large-scale data sets. Data science and analytics applied to cryoEM are crucial to research efforts across the other three PROs and promise an acceleration in the rate of scientific discovery.

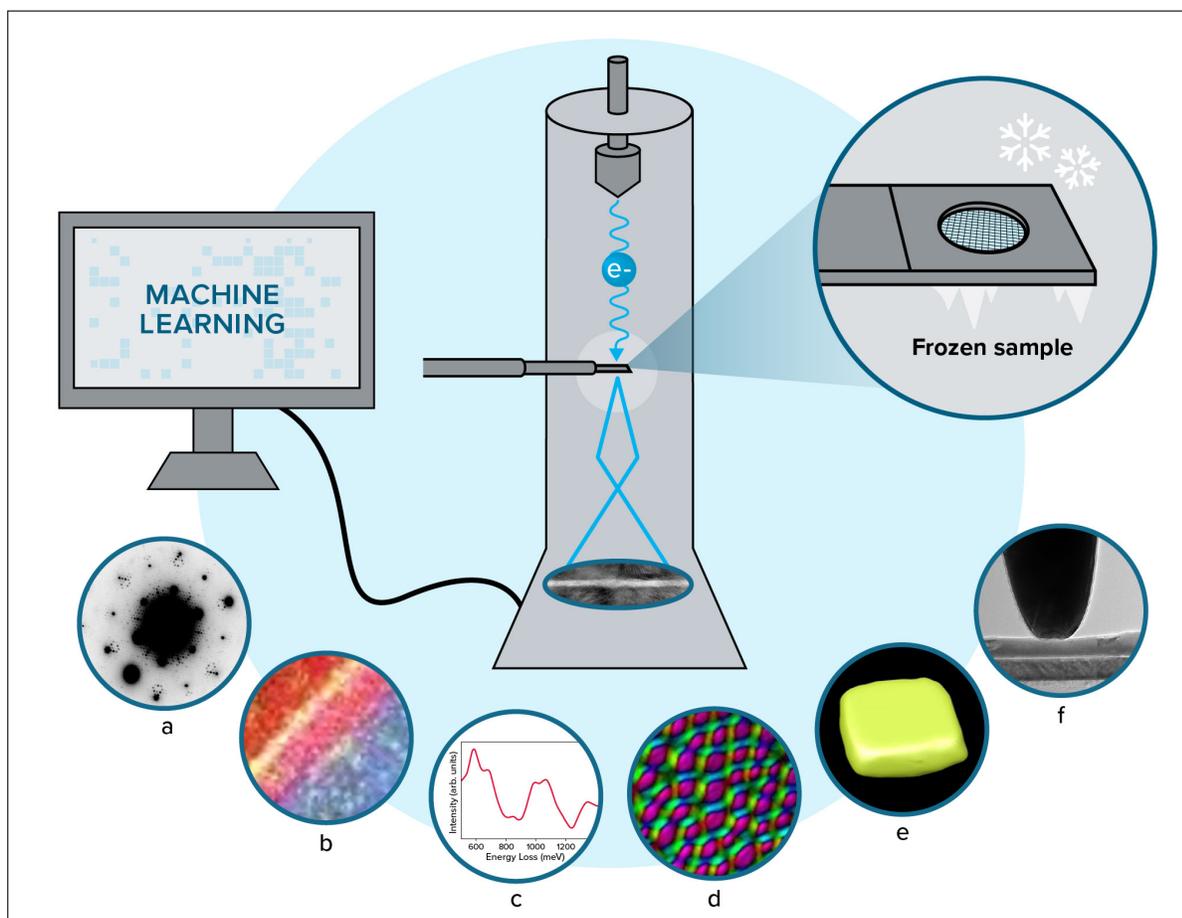
Introduction

The behavior of materials and chemical systems emerges from their distributions of atoms, electrons, and chemical species and from the interactions between these fundamental constituents over length scales that range from the atomic to the mesoscale. Many important energy-relevant systems are heterogeneous, with structures that can change over time through non-equilibrium or driven processes. Their behavior can be dominated by processes occurring at defects, interfaces, and grain or phase boundaries. Electron microscopy (EM) has long been a technique of choice for analyzing complex and heterogeneous systems because of its ability to probe *local* behavior, structure and composition, with complementary techniques such as X-ray and neutron scattering that typically characterize ensemble or global behavior. EM encompasses imaging plus electron diffraction and various types of spectroscopy, as illustrated in Figure I-1. This powerful combination of correlative methods can be used to explore microstructure down to the atomic level, reveal site-specific elemental composition, chemical bonding, and valence states, and probe functional properties such as magnetic structure and dynamics. EM can also reveal structure and behavior *in situ*, that is, subjected inside the electron microscope to an external stimulus, such as a magnetic or electric field, variable temperatures, or the composition of a gaseous or liquid environment.

The study of samples cooled to well below room temperature—termed cryogenic electron microscopy or cryoEM—has proven especially powerful in multiple fields for several reasons. First, it enables the study of a system or phenomenon that exists only at low temperatures. Second, it can capture soft, hydrated or dynamically evolving systems in a configuration that is sufficiently stabilized to permit high-resolution 2D or 3D imaging and analysis. Third, although low temperature might not prevent all electron beam damage, it can dramatically slow the consequences of electron–sample interactions, including bond breakage and atom displacements [1].

As detailed more fully in Appendix C, the first cryoEM explorations were carried out as early as the 1950s (those by Honjo et al. [2], for example) and focused on materials science research. The most impactful applications of cryoEM to date, however, have been in the field of structural biology. In 2017, the Nobel Prize in Chemistry was awarded to Jacques Dubochet, Joachim Frank, and Richard Henderson for their contributions to the development of cryoEM as a method for imaging the structures of biological macromolecules down to the atomic level. The ability to freeze hydrated proteins and combine cryoEM images from thousands of identical objects enabled a structural solution without the need for the large, macromolecular crystals required by X-ray diffraction.

Applications of cryoEM in the physical sciences have benefited in many ways from the development of biological electron microscopy, but they are almost always distinguished by the need to characterize individual and often unique features or structures rather than populations of identical objects. Consequently, the electron dose required to form an image cannot be distributed across the sample, rendering atomic-resolution imaging challenging for beam-sensitive materials. For example, cryoEM studies of the nucleation of ionic solids captured by fast-freezing aqueous solutions have provided lattice-resolution imaging and morphological analysis of non-identical, growing particles [3], but not the atomic resolution achievable for a single, beam-stable metallic particle [4].



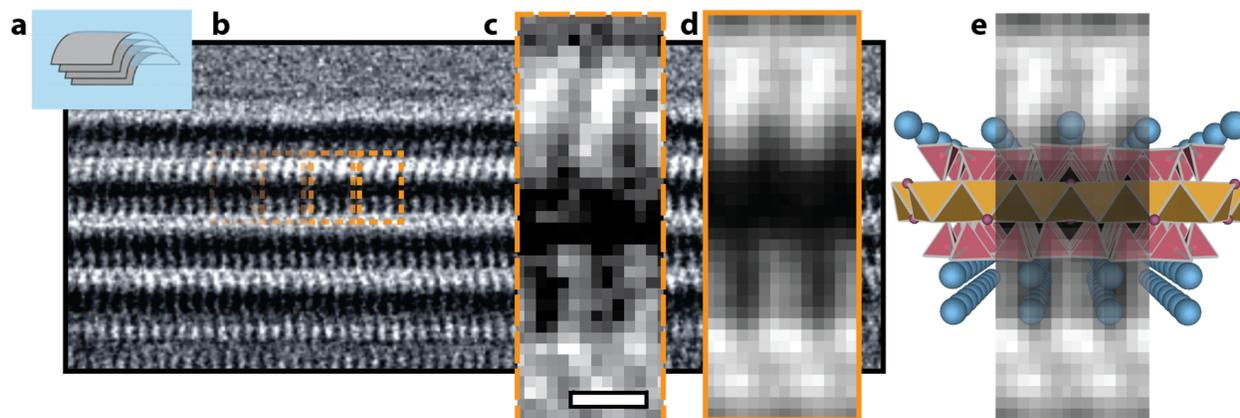
Graphic by Amanda Petford-Long and Alison Mackey (Argonne National Laboratory).

Figure I-1: Schematic illustrating the accessible information that can be obtained from cryoEM experiments on frozen samples: a) electron diffraction pattern, b) elemental map showing composition within a region, c) electron energy loss spectrum that enables access to chemical and bonding information, d) Lorentz microscopy image of low-temperature magnetic state, e) tomography data showing 3D reconstruction of a sample, and f) *in situ* biasing experiment to measure charge behavior.

Nevertheless, it has been possible to achieve important insights into the structure and function of energy materials, chemical systems, and quantum materials [5]. It is possible to access all conventional transmission electron microscopy (TEM) modes of imaging, spectroscopy and diffraction modes that are typically accessible at room temperature (Figure I-1). For example, using Lorentz microscopy, which is sensitive to magnetic field structure, it has been possible to access phenomena that exist only at low temperatures, such as vortex behavior in superconductors, or the emergence of charge- or spin-ordered phases. Cryogenic cooling can stabilize molecules in metal-oxygen framework (MOF) structures, informing, with density functional theory modeling, the principles of host-guest chemistry [6].

Advances in data acquisition, processing and quantitative image analysis have always played vital roles in the collection and interpretation of electron microscopy data, including cryoEM. These computational tools are leading the way towards low-dose high-resolution characterization of local specimen structure. Recent advances in describing electron-sample interactions and accounting for multiple scattering are enabling cryoEM study of systems composed of both

crystalline and non-crystalline materials. One example, shown in Figure I-2, is an “averaged” image of individually resolved clay nanosheets that also provides insights on the distribution of dissolved ions at the surface [7].



Source: Adapted from Whittaker 2020, figure 3.

Figure I-2: Asymmetric ion adsorption at the surface of curved swelling clay revealed by cryoEM. a) Curved-stack geometry of clay layers in aqueous solution. Each layer is ~1-nm thick and separated by variable layers of water. b) High-resolution cryoEM image. c) Detail of one unit cell indicated by orange box. Scale bar is 0.5 nm. d) Averaged image from 168 unit cells. e) Different locations of sodium ion adsorption sites above and below one clay layer [7].

Such studies illustrate the potential for high-resolution imaging of interfacial geochemical and electrochemical systems. Other exciting advances, such as ptychographic imaging [8], have the potential to further increase the resolution and information content of cryoEM data. It is clear that these techniques have not yet reached the limit of using every electron that interacts with a sample to develop composition–structure–function relationships.

CryoEM is developing rapidly and is poised to make major contributions to the fundamental understanding of material and chemical systems. To explore the potential applications for cryoEM in energy sciences, BES held the virtual roundtable *Research Opportunities in the Physical Sciences Enabled by Cryogenic Electron Microscopy*, which engaged domain science experts in many fields, from experts to non-practitioners of electron microscopy, with the goal of identifying key science drivers and research priorities that could enable physical sciences research to take better advantage of cryoEM.

The workshop panels on Day 1 focused on four scientific themes:

- Condensed phase chemical dynamics and reaction
- Correlating structure and function in soft materials
- Processes and chemical pathways in energy materials
- Low-temperature phenomena in quantum matter

Each panel explored scientific challenges in their field that could be addressed with the use of cryoEM. The reports from each panel made it clear that there were multiple high-level cross-cutting topics that emerged naturally for development into possible Priority Research Opportunities (PROs).

On Day 2, cross-cutting panels of all represented scientific areas were convened to explore the PRO topics. The PRO topics focused on the following:

- Emergent behavior and coupled processes at interfaces
- The role of heterogeneity in hierarchical systems
- The evolution of matter in variable environments across length and time scales
- Harnessing data analytics and automation to expand the role of cryoEM in enabling scientific discoveries

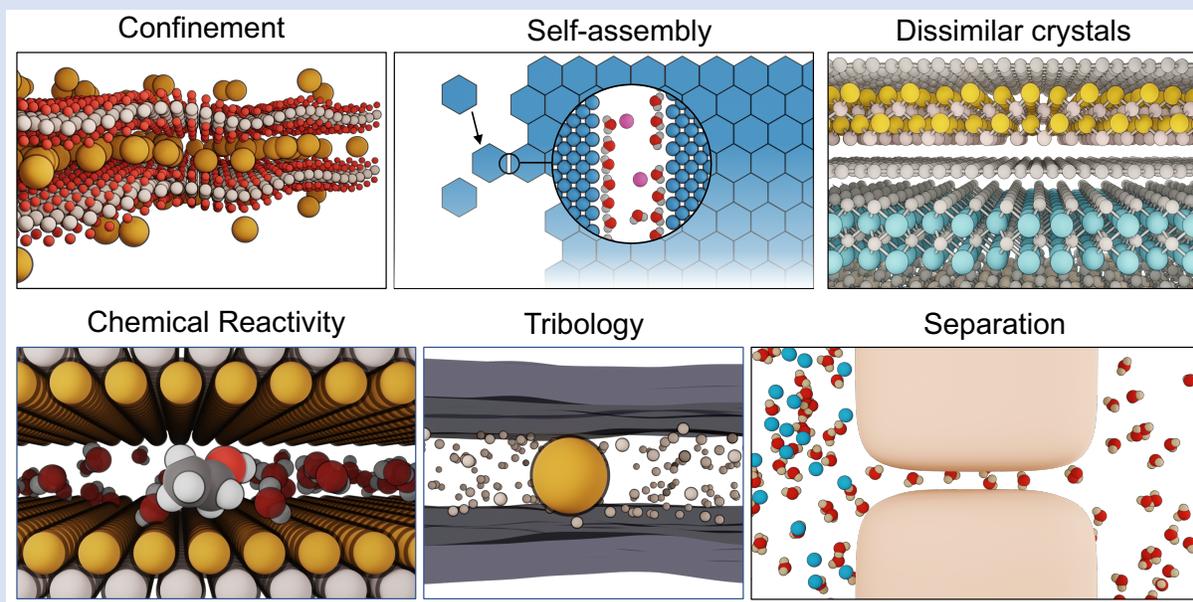
In each of these areas, the multidisciplinary panels identified exciting new opportunities for cryoEM to tackle grand-challenge problems in fundamental energy science. While some of the opportunities reflect priorities identified in prior BES workshops, the focus of this Roundtable was to explore the opportunities for cryoEM approaches to provide new perspectives that are distinctive and highly complementary.

Important themes were emphasized throughout the roundtable. In particular, all panels were excited by the opportunity to correlate local (atomic or electronic) structure to behavior and properties at the meso- and macro-scale presented by cryoEM. In addition, enhancing the role of *in situ* control of sample temperature and driving transport or transformation can lead to new insights into the behavior of matter and the pathways of chemical processes.

The following sections of the report detail the four PROs, in each case presenting an overview of the major scientific challenges that can be addressed together with specific examples and the impact that the cryoEM studies will have on the field as a whole. The report concludes with a brief outline of capability needs. The panel discussions illustrated the value of diverse scientific communities sharing goals, experiences, and perspectives, and this report aims to expand the community of researchers employing cryoEM to tackle ambitious basic science problems.

PRO 1: Discover emergent behavior and coupled processes at interfaces

Solid, liquid, and gas phase interfaces, and the chemical reactions that take place at these interfaces, play critical roles in energy production, conversion, and storage as well as information storage and communications. All of these face greater demands from today's technologies. While advances are being made in understanding the effects of composition and structure on bulk properties, the greatest unknowns, and thus the greatest challenges, lie in understanding the complex and dynamic nature of interfaces and their associated chemical, physical and functional behavior. CryoEM can play an essential role in filling fundamental knowledge gaps because it can provide both structural and chemical picoscale to nanoscale information. Emergent and coupled processes at interfaces, such as photon–electron, electron–ion, spin–electron–lattice, and mass–charge transfer, are critical to energy conversion and storage, quantum behavior, and many other properties and functions. With the *in situ* and tomographic capabilities of cryoEM, scientists can unravel the guiding principles of coupled phenomena and link them to the functionality of these interfaces.



Graphic by Qian Chen and Hyosung An (University of Illinois at Urbana-Champaign).

Figure PRO 1-1: Examples of energy-relevant processes occurring at complex condensed phase (solid–solid and solid–liquid) interfaces. The complex three-dimensional (3D) structure, heterogeneity, and close interaction with chemical species require high-resolution characterization in an environment in which the interfacial structure is frozen in, i.e., cryoEM.

Scientific Impact

Functional interfaces, due to the chemical reactions they can facilitate and the emergent behavior that they can display, have always played critical roles in energy production, conversion, and storage, as well as in information storage and communication. Interfaces can host electronic or magnetic states not found in bulk materials and attract reactive species to perform chemical transformations that are slow in homogeneous systems. In addition, coupled processes such as energy or momentum exchange through photon–electron, electron–ion or spin–electron–lattice interactions, as well as mass- and charge-transfer at these interfaces, are critical to energy conversion and storage efficiency, quantum behavior, and many other desirable properties. The breadth and complexity of functional interfaces spans all combinations of solid, liquid, and gas phases, including interfaces between highly dissimilar materials, interfaces with complex morphologies, and interfaces that confine fluids or species (Figure PRO 1-1). In many of these systems, novel behavior emerges that can be exploited in fields such as catalysis and water purification [9]. Recent years have shown that novel quantum states can emerge at complex interfaces at low temperatures that have relevance for information storage and processing and for chemical sensing [10].

The use of cryoEM will enable major advances in the understanding of heterogeneous and complex interfaces by revealing their complex morphology and chemistry and by capturing snapshots of the dynamic molecular conformations or electronic structures that emerge. For example, cryoEM observation could uncover the nanoscale interactions between dissimilar materials (e.g., soft and hard) at the complex interfaces that control the 3D conformation and temporal evolution of soft materials. By mapping out the structure, composition, and electrical polarization of complex interfacial systems, it will be possible to reveal the effects of curvature and confinement on the intermolecular interactions that generate the charge distributions that in turn control interfacial reactions and charge transport. The imaging and characterization of new low-temperature phases and behaviors that emerge at interfaces in quantum materials can enable the discovery and control of new quantum phenomena. For example, by observing emergent phenomena occurring at the interfaces of quantum materials, it will be possible to understand how heterogeneity leads to symmetry-breaking in topological and correlated materials.

Scientific Challenges

The ability to probe, understand, and control electronic states and chemical species at soft, hard, and hybrid interfaces will provide foundational knowledge that can advance innovative solutions for energy production, storage, conversion, and utilization. CryoEM can play a key role in such characterization, because low-temperature analysis that captures the evolution of coupled processes at interfaces within real environments, such as electrochemical systems, could uncover new phenomena and enable scientists to find ways to control these phenomena.

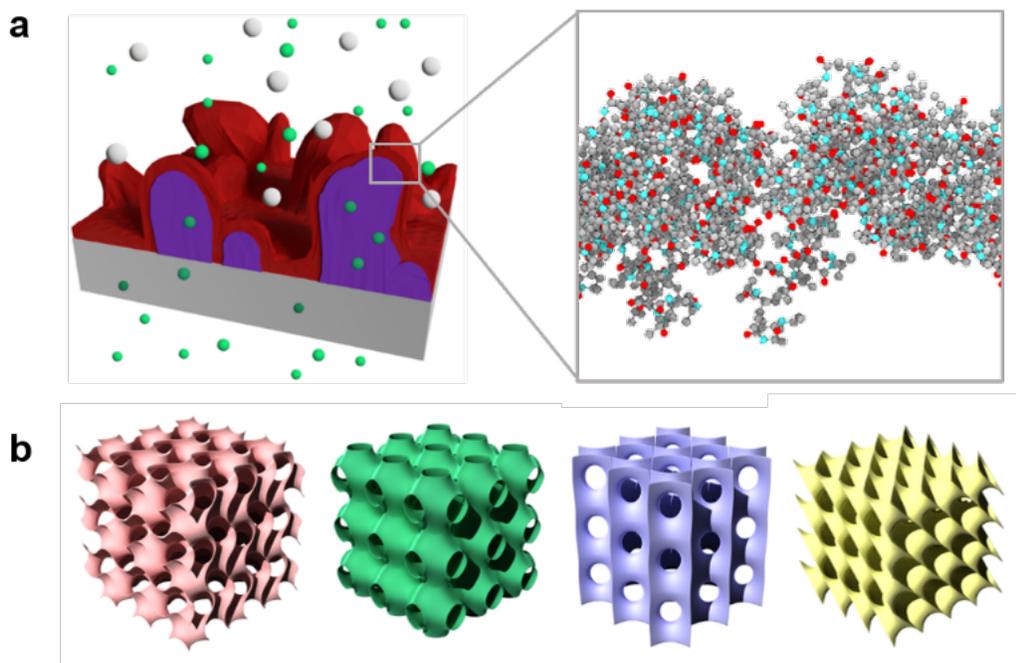
Mapping the three-dimensional conformation of complex interfaces

Complex 3D interface structures can emerge in diverse systems such as soft materials, hybrid systems, and structured liquids (Figure PRO 1-2). This conformational and interfacial complexity can originate from local gradients (e.g., concentration, temperature, or stress), from mass fluctuations during materials synthesis and processing, or from coupled multiphase dynamic processes, such as self-assembly and liquid–liquid phase separation. The 3D interface

structure can dominate bulk properties, as illustrated by polymer-based separations membranes (Figure PRO 1-2a) [10] that are formed from a covalent organic framework that spans 3D space. Voids within the 3D structure determine the permeation paths of the solvent–solute molecules, while the chemical heterogeneity and geometric attributes, such as curvature and tortuosity of the external and inner surfaces, contribute to the separation principles. Such 3D interfaces are also important for energy conversion and storage based on polymers and composites [12]. Complex interfaces are also widespread in biological systems that can provide inspiration for new materials and chemical processes. Figures PRO 1-2b shows examples of hierarchical and compartmentalized materials that have a precise 3D interfacial morphology, such as a toroidal network or hexagonally packed lamellae. These materials have applications in batteries for transport vehicles, in reaction vessels, and as structural templates [13].

CryoEM can push the boundaries of the scientific understanding of these poorly understood 3D interfacial conformations and morphologies by cooling them to temperatures at which they are stable to the electron beam and by enabling cryo-electron tomography to capture the interface morphology and composition in 3D. Atomic-scale imaging of low-atomic-number soft matter remains very challenging, so the development of new imaging modes and computational analyses will be essential. For example, it may be possible to translate recent determinations of the conformation of isolated macromolecules to establish the relationships between interface morphology and local molecular confirmation in polymeric materials [14].

3D heterogeneous interfaces from synthesis



Sources: (a) Adapted from Song 2019, figure 3. Graphics in b by Qian Chen's group (University of Illinois at Urbana-Champaign).

Figure PRO 1-2: Examples of interfaces with complex 3D conformations. (a) Polyamide filtration membrane with crumpled morphologies and voids (purple) define permeation pathways and the local interactions of the membrane with solute and solvent molecules [11]. (b) Exotic 3D bi-continuous phases can emerge from self-assembly of block copolymers, lipids, and mixtures thereof.

Understanding coupled phenomena at complex solid–liquid and liquid–liquid interfaces

Condensed-phase interfaces can exhibit complex phenomena governed by couplings between the structures and dynamics of the associated atoms, ligands, ions, and solvent molecules. These phenomena include electric double-layer formation [15], self-assembly of nanoscale colloids [16], chemical and electrochemical reactivity [17], separation of fluids [18], and tribology [19], as illustrated in Figure PRO 1-1. Although planar interfaces have been widely explored, complex condensed-phase interfaces, such as ones that include confinement or curvature effects, have remained challenging to study experimentally. As a consequence, scientific understanding of the structures of these interfaces has been limited and, to date, almost exclusively dominated by computer simulations and theory.

CryoEM presents exciting opportunities for studying complex interfacial structures in preserved states at nanometer resolution or better, so that the structural and chemical bases of energy-relevant interfacial phenomena can be discovered. In particular, cryoEM can make important contributions to understanding the roles of local electrostatic or weak molecular interactions in generating nanoscale interfacial environments that affect transport, assembly, and reaction in many settings. For example, the confinement of electrolyte ions and solvent molecules within a porous electrode leads to marked changes in the electric double layer structure, including local breakdown of electroneutrality in the confined fluid [20]. In materials synthesis, long-range electrostatic ion–solvent correlations could be observed and thus understood and harnessed to drive the oriented attachment of nanoparticles into single crystals [16]. Similarly, uneven ligand arrangements on curved nanoparticle surfaces encode heterogeneous force profiles that direct the assembly of nanoparticles into superlattices and superparticular assemblies of various dimensions. In these systems, maps of interfacial structure—measured using cryoEM—can be used as inputs for first-principle atomistic models to generate precise profiles of interionic, intermolecular, and inter-colloidal forces. Still more excitingly, cryoEM can inform the understanding of dynamic interfaces, where the interfacial structure, chemical processes, and corresponding forces can change locally as charge is stored or colloids diffuse and reorganize at different length-scales. As described in PRO 3, structural “snapshots” obtained via cryoEM at different reaction stages are crucial to revealing a comprehensive free-energy landscape of interfacial systems that control behavior and enable predictive design.

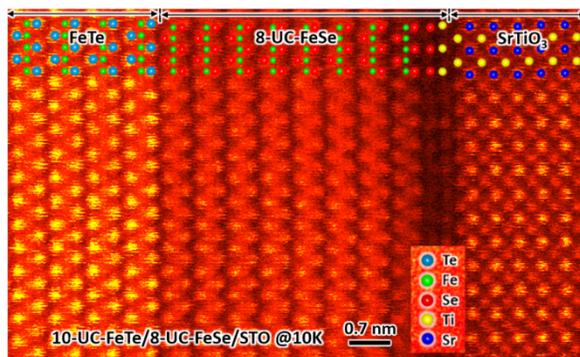
Controlling emergent quantum phenomena across interfaces

Many important solid-state systems share a common architecture in which dissimilar materials are opposed (Figure PRO 1-1), and they share similar challenges, such as chemical compatibility, interfacial reactivity (chemical or electrochemical), and mechanical and thermal stability. One excellent example is the electrode–electrolyte interface in lithium-based solid-state batteries. The cubic garnet phase $\text{Li}_{7-x}\text{Al}_x\text{La}_3\text{Zr}_2\text{O}_{12}$ (LLZO) is a lithium-ion conductor with an electrical conductivity that is higher than 10^{-4} S/cm at room temperature. However, the high impedance at the Li/LLZO interface can prevent the passage of any significant current. The origins of the high interfacial impedance have been traced to Li_2CO_3 layers, only a few nanometers thick, on the surfaces of dense LLZO samples [21].

Recent atomic imaging of a carbonate-free Li/LLZO interface, carried out using scanning transmission electron microscopy (STEM) at liquid nitrogen temperature, demonstrates that controlling the interface is the key factor allowing fast mass and charge transfer at interfaces [22]. The work has led to the optimization of carbonate contamination-free LLZO electrolytes that have made lithium metal anodes in solid-state batteries possible.

Exotic quantum phenomena, such as superconductivity, charge density waves, and magnetic spin ices, can also emerge at materials interfaces. These phenomena exist only at low temperatures and so cannot be analyzed at room temperature, making cryoEM a critical tool. One of very few examples is shown in Figure PRO 1-3, which shows an atomic-resolution image of a few-unit-cell-thick FeSe layer on SrTiO₃, recorded at 10 K. Interfacial electron transfer and its influence on superconducting electron pairing was directly investigated using atomically resolved imaging and electron energy-loss spectroscopy (EELS) at 10K. This work revealed the mechanism responsible for a significant enhancement of the superconducting transition temperature of monolayer iron selenide (FeSe) films on SrTiO₃ (STO) over that of bulk FeSe [23]; however, the experimental conditions needed to obtain this image made it a very challenging experiment to perform. Routine atomic-resolution imaging of interfaces in the range from 300 K to m K would have a profound impact on solid-state interfacial science and, by allowing materials to be studied at their operating temperatures, would have the potential to yield insights into the low-temperature-device physics critical for quantum information science.

In summary, the behavior of many functional materials and chemical systems depends critically on their nanoscale structure, their chemistry, and the electronic structure of their interfaces. These interfaces often contain complex, diverse, and dynamic chemical identities, the distribution of which is not well understood. CryoEM holds great promise to increase the fundamental understanding of coupled phenomena at complex solid–liquid and liquid–liquid interfaces and enable mapping of the chemistry, structure, and 3D conformation of complex interfaces, which in turn can ultimately enable the control of emergent phenomena across interfaces.

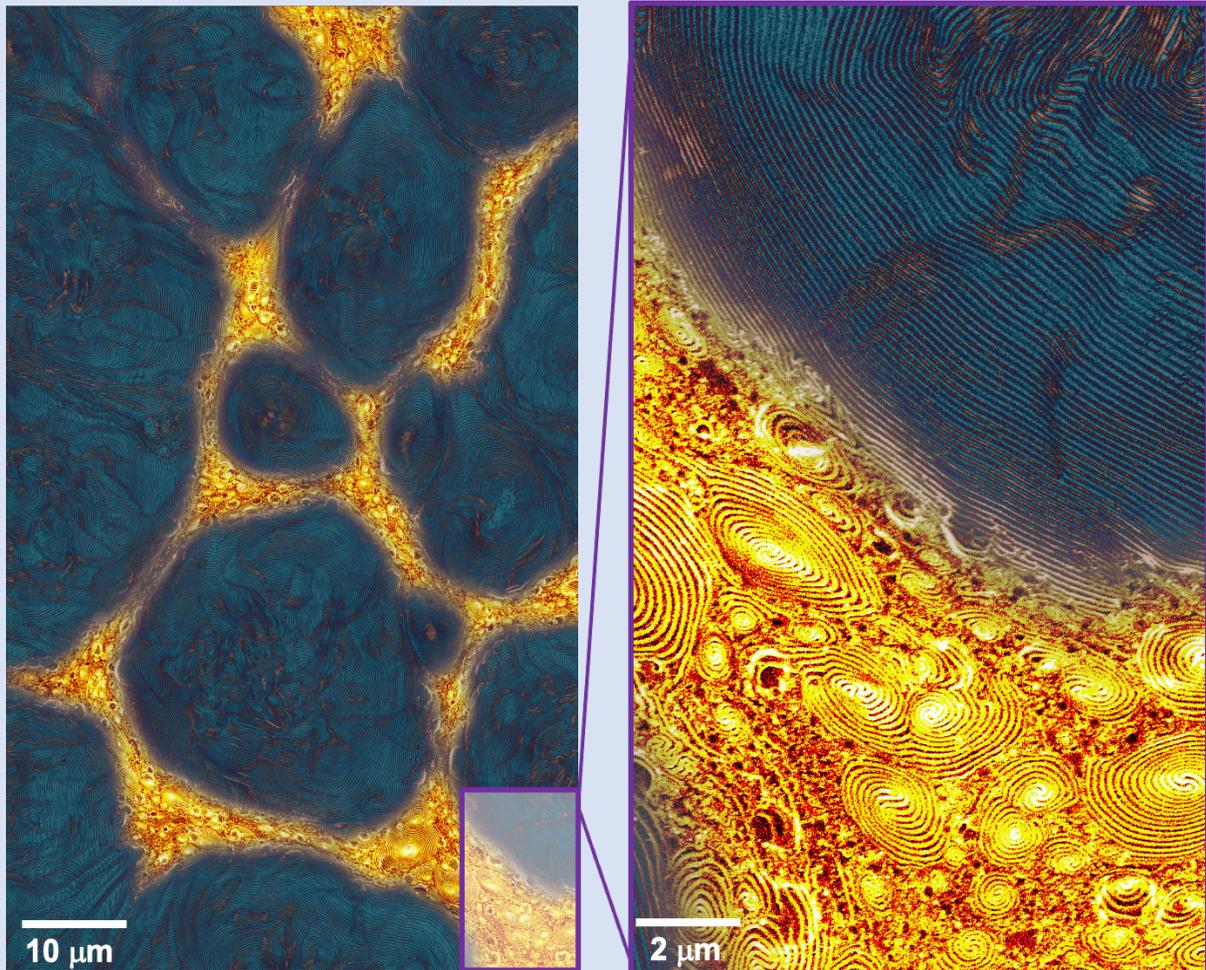


Source: Zhao, W., M. Li, C-Z.Chang, J. L. Wu, C. Liu, J.S. Moodera, Y. Zhu, M.H.W. Chan. 2018. "Direct Imaging of Electron Transfer and Its Influence on Superconducting Pairing at FeSe/SrTiO₃ Interface." *Science Advances* 4: eaao2682. <https://doi.org/10.1126/sciadv.aao2682>. Creative Commons License, <https://creativecommons.org/licenses/by-nc/4.0/>.

Figure PRO 1-3: Atomically resolved image of FeSe/SrTiO₃ (STO) recorded at 10 K. The STEM image shows a cross-section image of a 10-unit cell (UC) capping layer of FeTe on 1 UC FeSe on SrTiO₃. The image was recorded at 10 K so that the structure of the low-temperature phases could be captured [23].

PRO 2: Elucidate the role of heterogeneity in hierarchical systems

Hierarchical systems are of great importance for natural and engineered functional systems, because multiple properties and processes with different inherent length and time scales can interact to influence mesoscale and macroscale behavior. Hierarchical systems are, by their very nature, structurally and compositionally heterogeneous across a broad range of spatiotemporal scales. The interfaces in hierarchical materials exhibit properties governed by both short- and long-range interactions and by complexity across length scales. The use of cryoEM to understand the intersection of hierarchy and heterogeneity in a wide range of systems, including liquids, soft and hard materials, biosystems, and their combinations, holds promise for broad impacts on topics such as separations and nucleation, transport in soft materials, and the formation of quantum states.



Source: Adapted from Maslyn 2021, figure 8.

Figure PRO 2-1: Scanning electron microscopy images of a polystyrene-b-poly (ethylene oxide) block copolymer with an added lithium salt, which leads to the creation of distinct lamellar structures at two different length scales (colored blue and yellow, respectively). The right-hand image (b) shows a magnified view of the boxed region in (a) [24].

Scientific Impact

Hierarchical materials are characterized by combination of materials with different sizes, phases, shapes, surface chemistry, pores, and other characteristics. Their hierarchical nature can lead to properties that are substantively different from those of their constituents and can provide versatile environments for energy-relevant chemical processes. For example, hierarchical architectures can enable light harvesting and exciton capture, provide strength and fluid transport in polymeric composites or biominerals, provide mechanical and chemical resilience to novel nuclear waste forms, and host catalytic sites for chemical transformation and transport [25-32]. Hierarchical materials are by their nature heterogeneous, with properties often being dominated by heterogeneities such as defects and interfaces. CryoEM could provide key breakthroughs by directly imaging and quantifying the structure, abundance, and distribution of important heterogeneities—such as diffusion fronts, interfacial sites, and defects that trap ions or electrons—and using this information to understand the origins of observed mesoscale and macroscale properties.

The important components of hierarchical systems can range from interacting spins to molecular species (e.g., single adsorbates within an electric double layer) to clusters and nuclei (composed of tens of atoms) to nanoparticles (hundreds to thousands of atoms) to assemblies extending over many microns. CryoEM provides the ability to study heterogeneity over all these length scales and identify the critical components that control behavior. Frequently, atomic- or molecular-scale species residing within or near interfaces can be the most difficult and also the most important to interrogate. For example, imaging the active sites of heterogeneous electrocatalytic and photoelectrosynthetic assemblies would enable a better understanding of how their activity couples to reagent or product transport. Another example is understanding the larger-scale populations of subassemblies with structural or compositional heterogeneity, which are key to system properties and evolution.

CryoEM has the potential to greatly illuminate the interactions and pathways through which hierarchical systems are formed and heterogeneity emerges. For example, cryoEM has revealed the multi-step mechanisms of the nucleation and assembly of inorganic and organic structures, work that has informed contemporary ideas of materials crystallization and self-assembly [3, 32]. In other fields, variable-temperature cryoEM could enable spontaneous, temperature-dependent phase transformations in quantum matter to be imaged. These phase transformations frequently evolve in a heterogeneous manner, and understanding this heterogeneity is critical to understanding the behavior observed. Moreover, developing creative new methods to synthesize complex soft and hard matter systems will require the ability to understand and control diverse chemical interactions, including ligation or bonding as well as directional (e.g., dipole) versus non-directional (e.g., electrostatic) long-range interactions. Quantifying the size distributions and bonding interactions of crystalline or amorphous polymer assemblies could inform how they can direct larger-scale phase separation and structure formation in soft matter.

Scientific Challenges

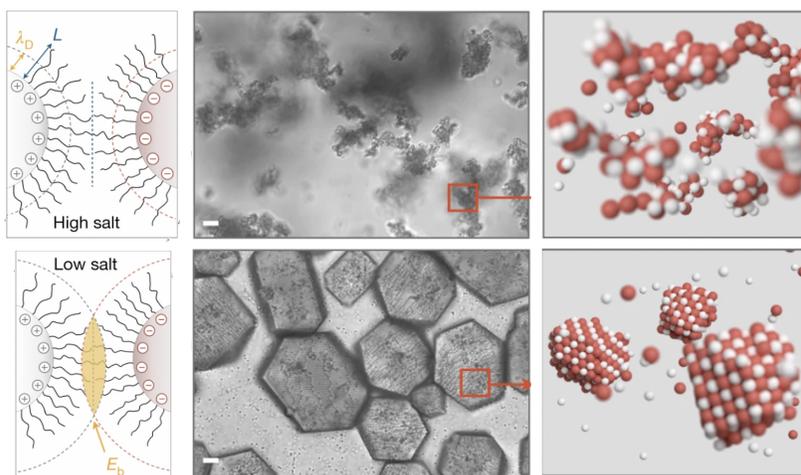
This PRO focuses on the intersection of hierarchy and heterogeneity in a wide range of materials, from liquids and soft materials to hierarchical materials composed solely of hard materials. Three examples illustrate pressing scientific problems that can be addressed with the use of cryoEM: The first is exploring how long-range interactions affect separations and nucleation through

chemical environments beyond the first coordination shell. The second is exploring mechanisms of transport in soft materials, and the third is mapping competing phases, defects, and intertwined states in quantum materials.

Bridging the gap between long-range and short-range interactions in heterogeneous systems

Strong short-range interactions, such as covalent and ionic chemical bonds, dominate local structures. However, weaker long-range interactions, such as electrostatic, dispersive, and magnetostatic forces, can critically control processes such the emergence of domains in soft matter and the self-assembly of functional materials from molecular and/or colloidal ingredients. In addition, the chemical characteristics within the *primary* coordination sphere can be influenced by the *secondary* and subsequent outlying coordination spheres. Controlling the balance of long- and short-range interactions is a long-standing goal in fundamental energy science, with applicability to the separation of solutes [33], aqueous phase transport of ions and radionuclides, catalysis in confinement, and the synthesis of novel materials [34]. For example, Figure PRO 2-3 highlights how controlling long-range interactions, through solution chemical conditions, can lead to the controlled formation of solids with markedly different morphologies but composed of the same interacting synthetic microparticles [35]. However, these images were obtained using light microscopy. Quantifying the degree and strength of these interactions over length-scales down to atomic, as well as characterizing the degree to which they impact structures and dynamics, could be aided by cryoEM and will shed light on the fundamental driving forces in play.

State-of-the-art experimental and computational methods are well suited to characterizing molecular or crystal structures that are dominated by strong short-range interactions. By contrast, studying long-range interactions remains challenging. CryoEM has the potential to show chemical and materials systems at a scale large enough to identify the roles of long-range interactions [36] while retaining information about short-range structures. Such a combination will enable increasingly powerful molecular simulations to distinguish between the changes caused by short-range chemical coordination of the solvent ions to the metal centers, by induced dipole-ion interactions [7], by hydrophobicity transformations, or by changes in long-range electrostatic interactions through modifications of the environmental dielectric constant.

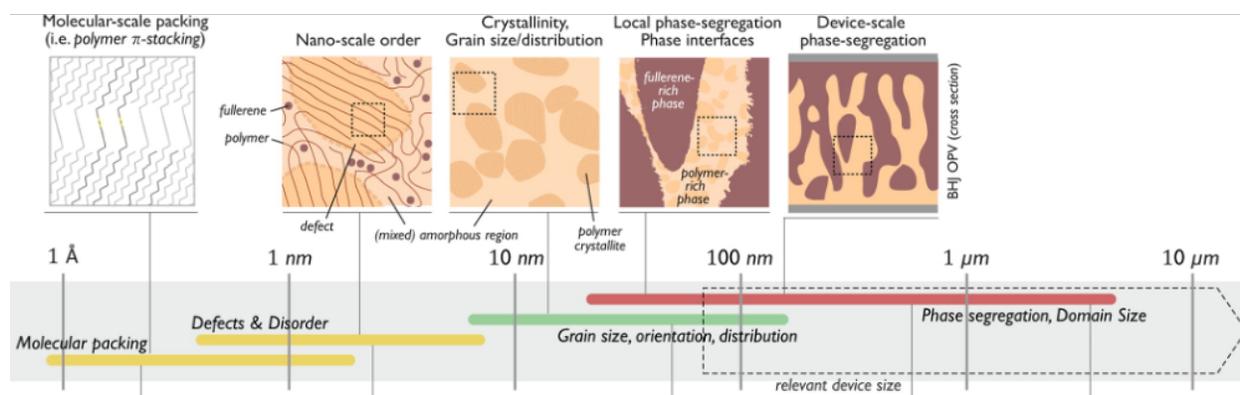


Source: Adapted from Hueckel 2020, figure 1.

Figure PRO 2-2: Polymer-attenuated Coulombic self-assembly [35]. From left to right: Schematic, bright-field optical microscope image, and computer simulations of functionalized polystyrene spheres with strong (top) and weak (bottom) Coulombic interaction strengths. Scale bars: 4 μm .

Exploring mechanisms of transport in soft materials

Recent developments in polymer design have evolved from a focus on optimization of crystalline domains to acknowledging the central roles of amorphous domains, the molecular connections between crystalline regions, and longer-range structural correlations [37, 38, 39]. For example, nanostructure has a direct effect on the functional properties of organic semiconductors used in photovoltaics, such as electrical conduction or optical absorption. Polymer electrolyte membranes, such as Nafion™, are important for a variety of technologies, such as seawater purification, hydrogen fuel cells, and electrochemical cells. These membranes comprise nanoscale hydrophilic and hydrophobic domains and display varied and often unknown structural and chemical heterogeneities at nanometer-micron length scales that can be revealed by cryoEM [40]. Correlating structural heterogeneity, with their functional properties from the atomic to system scale, is required to advance the design and optimization of soft materials. Averaging measurements, such as X-ray diffraction, can elucidate structure but cannot quantify the structure of soft heterogeneous materials, including population densities and their relative coordination with each other, and correlate structure with function. The beam-sensitive nature of these types of materials, combined with the need to characterize heterogeneities from the nano- to the micro-scale, means cryoEM can be the technique of choice.



Source: Adapted with permission from Rivnay, Jonathan, Stefan C. B. Mannsfeld, Chad E. Miller, Alberto Salleo, and Michael F. Toney. 2012. "Quantitative Determination of Organic Semiconductor Microstructure from the Molecular to Device Scale." *Chemical Reviews* 112: 5488-5519. <https://doi.org/10.1021/cr3001109>. Copyright 2012 American Chemical Society.

Figure PRO 2-3: A schematic depiction of the hierarchy of relevant morphological features in a hypothetical two-component blend film typical of a bulk heterojunction used in organic photovoltaic devices [41].

CryoEM imaging methods can enable the design and discovery of novel and high-performing macromolecular, heterogeneous, and hierarchical soft materials through quantification of structure (for example, using 4DSTEM) [42] correlated with composition (using EDX or EELS) and combined with important properties such as conductivity, stability, permeability, and mechanics. The outcome will provide insights into the fundamentals of transport in soft materials, which can identify a new paradigm of better and longer functioning electronic transfer materials.

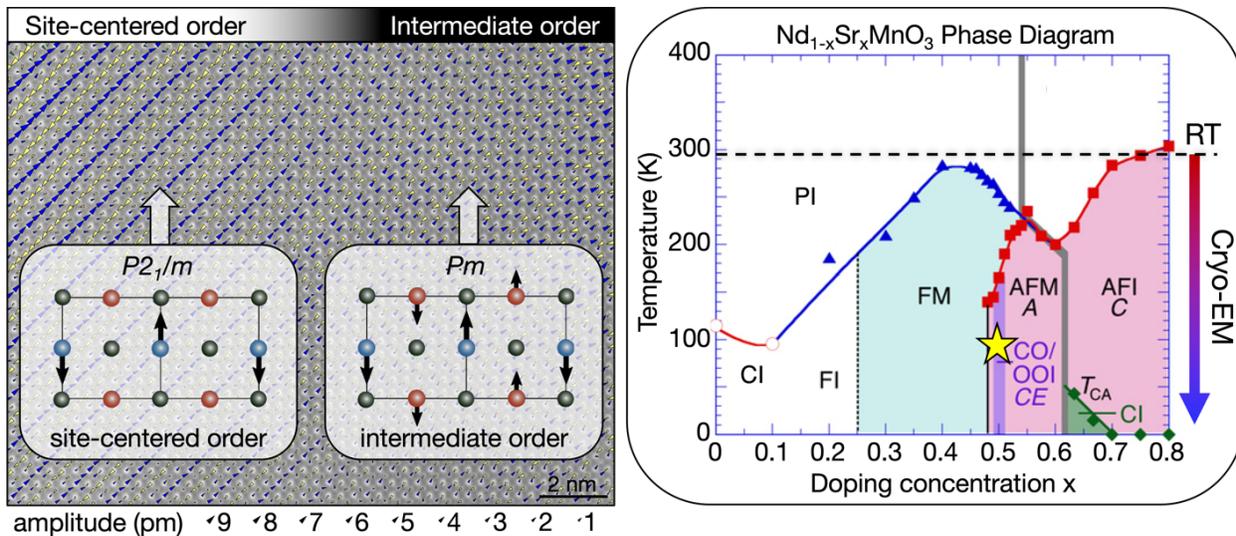
Mapping competing phases, defects, and intertwined states in quantum materials

Electrons in quantum materials can interact strongly with each other or with the atomic lattice, giving rise to novel electronic states with functionalities not achievable in conventional materials, such as multiferroicity, colossal magnetoresistance, topological protection, and high-temperature superconductivity [43]. Many of these quantum states emerge at low temperatures [44] and require cryogenic probes that enable direct mapping of charge, orbital, spin, and lattice heterogeneities inside quantum materials, as shown, for example, in Figure PRO 2-4. The hierarchy of length scales associated with the complex emergent behavior of quantum materials spans atomic to mesoscopic scales and is governed by the interplay of competing energy scales, disorder, and frustration [45]. At the atomic scale, variations in the charge or spin distributions and subtle atomic displacements define the local crystal symmetries, and they can have dramatic effects on the functional properties of the material. Over larger scales, distinct ordered states can co-exist and form intricate domain structures that are susceptible to tuning through external perturbations such as strain, temperature, fields, etc.

CryoEM is uniquely suited to probe heterogeneities of quantum phases down to the atomic scale [46]. Mapping different and often intertwined phases within the complex phase diagrams of quantum materials will advance the understanding of quantum phases and accelerate the design of entirely new materials and functionalities. Developing a microscopic picture of how quantum phase transitions occur will require tracking the way that heterogeneity evolves as a function of temperature and/or external stimuli. Atomic-resolution cryoEM at variable temperatures is required to enable such experiments. Extending the temperature range for atomic-resolution measurements of lattice, electronic, and spin structures down to tens, a few or even sub-1 Kelvin can provide access to a broader range of unconventional superconductors, Mott insulators, topological insulators, quantum magnets, spin liquids, and more.

Spatial heterogeneities that govern the mechanical, thermal, and electrical properties of crystalline materials such as domains, grain boundaries, and defects are also important for quantum phases. Electronic states such as charge density waves (CDW) or magnetic textures can form domains, defects, and boundaries with distinct properties. Domain walls in CDW systems can, for example, result in one-dimensional (1D) metallic and superconducting channels. Imaging domain walls and their functional properties using cryoEM could help devise strategies for controlling them. Heterogeneity also plays a role in stabilizing metastable quantum phases and presents new opportunities for tuning topological states. Coexistence of domains at the nanoscale or frustrated local structures can result in metastable states with remarkable long-term stability [43].

CryoEM can provide the spatial resolution needed to not only map the domain distribution but to quantify the local symmetries within each domain, at domain boundaries, and around topological defects. For quantum science, cryoEM could enable a paradigm shift in the ability to probe spatial heterogeneities of charge, spin, and lattice degrees of freedom in low-temperature quantum phases and quantum phase transitions (Figure PRO 2-4).



Source (left): Adapted from El Baggari 2021, figures 2 and 3.

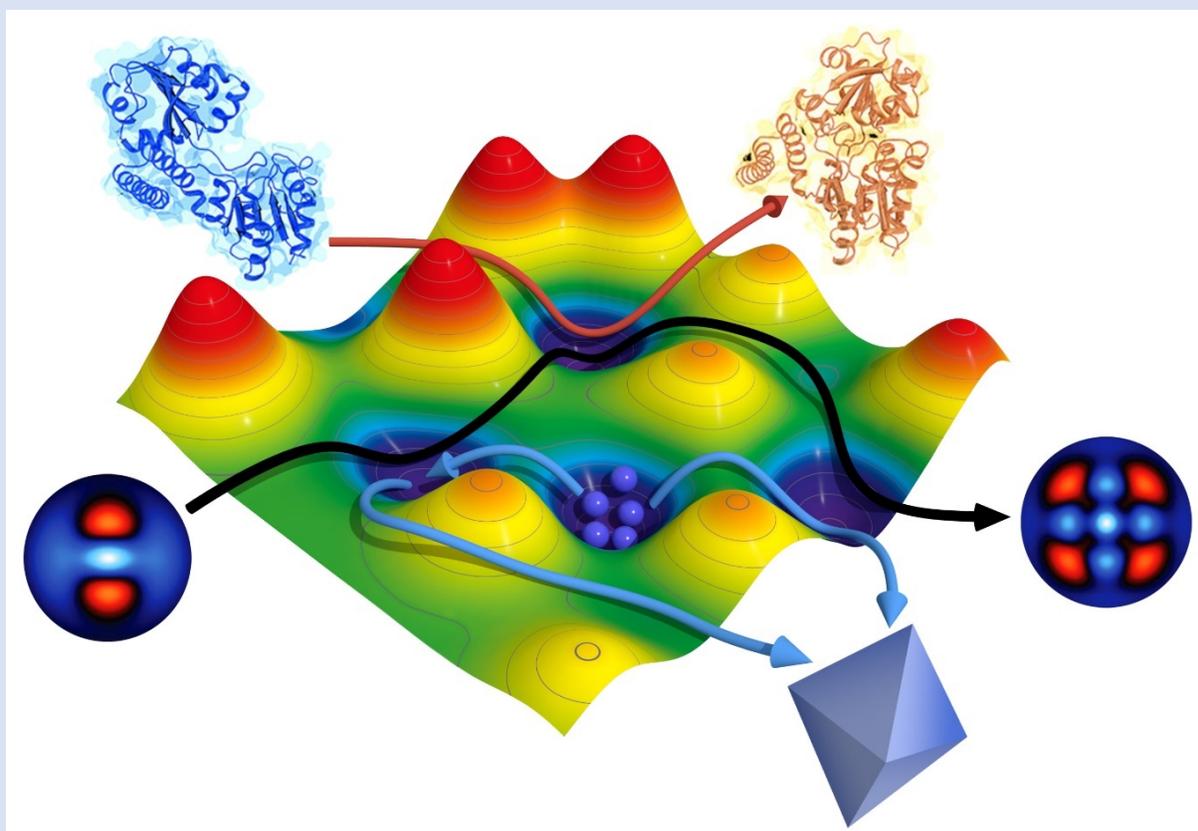
Source (right): Adapted from Tokura, Y. 2006. "Critical features of colossal magnetoresistive manganites." *Rep. Prog. Phys.* 69: 797-851. <https://doi.org/10.1088/0034-4885/69/3/R06>., Figure 5. © IOP Publishing. Reproduced with permission. All rights reserved.

Figure PRO 2-4: Heterogeneity of atomic lattice symmetry in quantum materials by cryo-STEM [46, 47]. Cryo-STEM at 93K resolves how atoms displace when a manganite crystal, $Nd_{0.5}Sr_{0.5}MnO_3$, is cooled into its charge-ordered phase. Real-space mapping of the lattice symmetries identifies two distinct ordered states that coexist within the same material. Expanding atomic-resolution cryo-STEM capabilities to lower temperatures and allowing for *in situ* tuning of the sample temperature will enable heterogeneity to be probed in a broad range of quantum phases.

In summary, heterogeneity is an important concept in many chemical and material systems, requiring them to be explored over multiple length scales. Variable-temperature cryoEM offers the opportunity to image and interrogate this heterogeneity across length-scales from the atomic to the mesoscale, enhancing understanding of the way in which heterogeneity controls chemical processes and functional behavior.

PRO 3: Understand the evolution of matter in variable environments across length and time scales

Controlling the assembly and function of materials and chemical systems requires the ability to follow structure and behavior in time, including transitions among key states, at the atomic- to much larger scales. Compelling opportunities exist for cryoEM to explore the mechanisms of dynamic processes to reveal the underlying free-energy landscape and to establish the role of such variables as gaseous or liquid environment and external fields, molecular-to-mesoscale interactions, and information transfer. CryoEM has the potential for breakthroughs in understanding the assembly of matter, the emergence of quantum states, the subtleties of chemical transformations, and the dynamics of driven materials systems. Integrating theory, experiment, and data science will open exciting approaches to understand the relevant dynamics and assist in optimizing function. For example, cryoEM could provide structural and functional constraints for simulations that can in turn predict key active states or configurations that guide cryoEM sample interrogation, data acquisition, and analysis.



Graphic by Jim DeYoreo and Michael Perkins (Pacific Northwest National Laboratory). Images of protein conformation adapted from Chu 2018 <https://pubs.acs.org/doi/10.1021/acscentsci.8b00274>, Figure appearing in abstract. Further permissions related to the material excerpted should be directed to the American Chemical Society. Images of quantum state adapted from Hofheinz 2009, Figure 3.

Figure PRO 3-1: CryoEM can observe structural and electronic states of dynamic systems evolving in a free-energy landscape. In this scheme, a colored arrow shows the path that a physical or chemical process follows as it traverses the energy landscape. Three examples are shown: a change in protein folding (top left, red arrow), the assembly of a crystal from component molecules, atoms, or nanoparticles (bottom right, blue arrows), and two different quantum states in a superconducting resonator (horizontal path, black arrow) [48, 49].

Scientific Impact

Functional materials and chemical systems evolve in time. Complex energy systems, from polymeric materials to geologic formations, evolve by coupled chemical–mechanical pathways. Catalysis and electrochemical energy storage involve cycling between molecular and electronic states. Exotic states of matter, including quantum matter, can form and undergo phase transitions as a function of temperature and develop complex dynamics when driven by external fields. CryoEM has the potential to provide unique insights into all these dynamic processes. The pathways of chemical transitions and material evolution can be studied by capturing snapshots of dynamic states through rapid freezing or by quenching competing processes through low temperatures. The dynamics of driven matter can be examined in response to system temperature and environment *in situ* in high-resolution cryoEM experiments.

Biological reaction pathways exemplify some of the fundamental questions that could be addressed through new applications of cryoEM with assistance from data science. Important biological processes such as photosynthesis or respiration involve the transient assembly of complex macromolecules, large-scale conformational change, and events when molecular chemistry occurs. Although chemical modification of proteins to stabilize certain conformations, or the use of ultrafast X-ray methods, can reveal intermediate configurations in a small subset of cases [50], most complex biological reaction pathways remain very difficult to directly determine and so are poorly understood. EM is a local structural probe and provides an opportunity for retrieving and statistically characterizing key intermediates in reaction pathways.

Opportunities for cryoEM to identify and study intermediate states and pathways in dynamic processes are illustrated in Figure PRO 3-1, which shows a schematic free-energy landscape for a biological, chemical, or materials process. For example, progress over recent decades in the field of protein folding has revealed that chemical transformations typically proceed via a finite number of metastable intermediate states. Using the data science methods described in PRO 4, cryoEM has the potential to identify the states that lie at local minima on the way to the final product. This conceptual scheme and approach are relevant to a very large range of chemically, mechanically, or field-driven processes as well as to dynamic behavior close to equilibrium. The combination of cryoEM with ML can elucidate dynamic processes in a range of systems such as biological and biomimetic systems, nanoparticle growth and assembly, quantized spin excitations, topological phases, and other dynamics in quantum matter.

Theory and simulation have played critical roles in evolving scientific understanding of reaction pathways and reaction rates as well as driven processes, and they will benefit from and inform the expanded use of cryoEM in this field. For example, when applied to chemical reactions, cryoEM determination of the structures and configurations of multicomponent reactions can provide an essential starting point for predictive simulations. Still more excitingly, simulations could predict hypothetical pathways and intermediate structures that could be used as targets for large cryoEM ML datasets and could ultimately guide autonomous data acquisition. A further exciting opportunity for cryoEM is exploring driven behavior through *in situ* manipulation of materials via control of environmental variables such as temperature, pH, illumination, strain, and magnetic and electric fields.

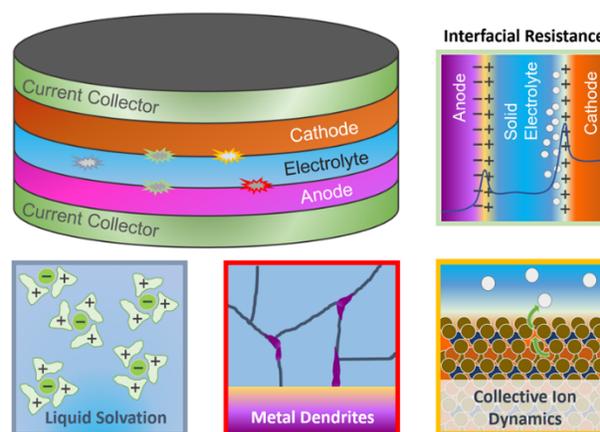
Scientific Challenges

CryoEM will enable the advancements in spatial, temporal, and energy resolution developed in conventional TEM to be fully utilized at low temperatures to study a broader range of energy-relevant materials and systems. Combining these approaches with *in situ* observation and elucidation of dynamic processes promises to be game changing in many fields, especially in addressing challenges in charge and ion transport, bioinspired energy conversion, and accessing low-temperature phases critical to mastering quantum materials design. As described in the examples below, these approaches will enable us to determine how local structure, environment, and perturbations influence transitions among functional states.

Capturing ion and electron transport mechanisms in energy systems

Charge-transport processes at complex interfaces exhibit coupled and dynamic dependencies on temperature, chemistry, strain, and charge. One scientific challenge that can be addressed by cryoEM is how the environment surrounding relevant electron- and ion-transport species defines and controls the reaction pathways and activated steps. Progress requires clarifying the correlated nuclear motions coupled to transport as well as the nature of tunneling barriers to charge transfer, which in turn requires resolving the nanoscale structure and chemistry that drive charge-transport reactions at interfaces in three dimensions. Site-specific, continuous observation by EM on nanosecond-to-second time regimes is presently impossible, however, because of electron dose limitations. CryoEM offers a quenching approach to capturing key configurations during charge-transfer processes. Freezing can fix such configurations in space and time, thereby offering snapshots with atomic resolution, and also can protect reactive or sensitive species from damage during imaging and spectroscopy.

In many electrochemical systems, the fundamental processes of concurrent electron- and ion-conduction are still poorly understood [51]. For example, battery charging and discharging involve insertion and extraction of ions into and from an electrode with a concurrent flow of electrons to compensate for charge balance (Figure PRO 3-2). Although ionic conductivity in homogeneous solid electrolytes and electronic conductivity in solid oxides are both well understood [52, 53, 54], achieving efficient, coupled ion- and electron-transfer at electrolyte–electrode interfaces remains an outstanding challenge for solid-state energy storage [55, 56, 57]. Direct observation of the interplay among charge and ion transport, lattice, and electronic structure *in situ* during cycling will be key to unlocking fundamental insights into solid electrolytes and their interfaces and thus enabling their optimal design.



Graphic by Katherine L. Jungjohann (Sandia National Laboratory).

Figure PRO 3-2: Dynamic charge transfer and ion transport processes in liquid and solid electrolyte batteries. Key mechanisms that could benefit from cryoEM studies include spatially resolved changes to interfacial resistance at electrode surfaces, liquid–electrolyte solvation structures, metal dendrite formation, and the collective ion response during charge cycling.

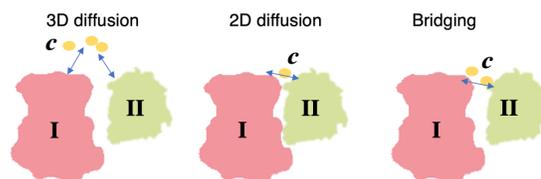
The *in situ* control of chemistry, strain, and charge along these interfaces may further identify the primary factors that promote ion transport or constrain electronic bridge pathways within solid-state electrochemical systems [58]. The challenges faced in ion and electron transport in electrochemical systems have direct parallels in biological energy transduction systems, as described below.

Controlling energy transduction and signaling processes in soft matter

High-value energy capture and conversion processes lie at the core of living systems. Photosynthesis captures the energy of the sun, and mitochondria convert chemical bond energy into electrochemical gradients that drive biosynthesis. Biology's energy transducing machines are very different from macroscopic machines. Many nanoscale machines are near-reversible and can run in forward or reverse directions: Imagine a car that is poised to either burn gasoline *or* synthesize gasoline from CO₂ and water on demand!

The multi-cofactor transmembrane proteins for energy transduction have been known for decades, yet much remains to be learned about their function. Recent studies [59], some involving cryoEM, indicate that interactions among multiple proteins are critical for machine-like function. Rather than performing electrochemical work, allosteric macromolecules in living systems convey information over long (nanometer) distances, often across membranes, and enable signaling and cooperative processes. However, the molecular mechanisms that underpin allosteric signaling over these large molecular scales are poorly understood. A tremendous opportunity exists for cryoEM to reveal key structure intermediates that define the pathways for the function of molecular machines. Understanding highly evolved near-reversible biological machine function at the nanoscale can reveal strategies for synthetic energy conversion schemes and for small molecule activation, suggesting, for example, entirely new approaches to carbon and nitrogen fixation.

Figure PRO 3-3 shows how cryoEM has been used to freeze “snapshots” of the supercomplex formed by energy-transducing mitochondrial cytochrome *bc*₁ and cytochrome oxidase, indicating that the supercomplex enables reduced-dimensionality diffusion of the small, water-soluble cytochrome *c* electron shuttle protein between the two membrane-bound proteins [59]. These studies increase understanding of the foundational electron handoff between building blocks of the respiratory chain of mitochondria. The use of cryoEM methods could be expanded beyond the structure and behavior of protein supercomplexes to biomimetic and synthetic molecules, as well as to hybrid nanoparticle-molecular systems that enable energy transduction and signaling at the molecular scale.



Source: Adapted from Moe 2021, figure 1.

Figure PRO 3-3: CryoEM showed that the pathway for electron transfer within a supercomplex of mitochondrial proteins occurs by multi-step hopping among cytochromes rather than by 3D diffusion of the cytochrome *c* electron shuttle or 2D diffusion of cytochrome *c* [59].

CryoEM also has the potential to illuminate the coordination between large-scale macromolecular docking and atomic-scale electron transfer states. Very large-scale redox-active biological nanowires have recently been identified that facilitate the exchange of electrons at biotic–abiotic interfaces [60, 61]. In contrast to conventional electrochemical systems, large-scale biological assemblies couple long-range conduction to catalytic redox reactions, offering versatile approaches to energy storage. CryoEM can provide critical insights for understanding the synthesis and behavior of these systems.

Evolution of topological states in quantum materials

Understanding emergent phenomena in quantum materials, many of which are only observed at very low temperatures, includes elucidating their phase-formation kinetics and their dependence on structural and chemical order. This order is expressed in the form of internal interfaces and surfaces, localized defects, dopants, heterogeneity, curvature, and strain. Monitoring the transient states and transitions that occur during driven and thermal nanoscale dynamics will be essential to designing correlated materials and corroborating theoretical and numerical predictions. These dynamics may span femtosecond to second timescales. One potential application for these materials is in novel electron spin-based microelectronics.

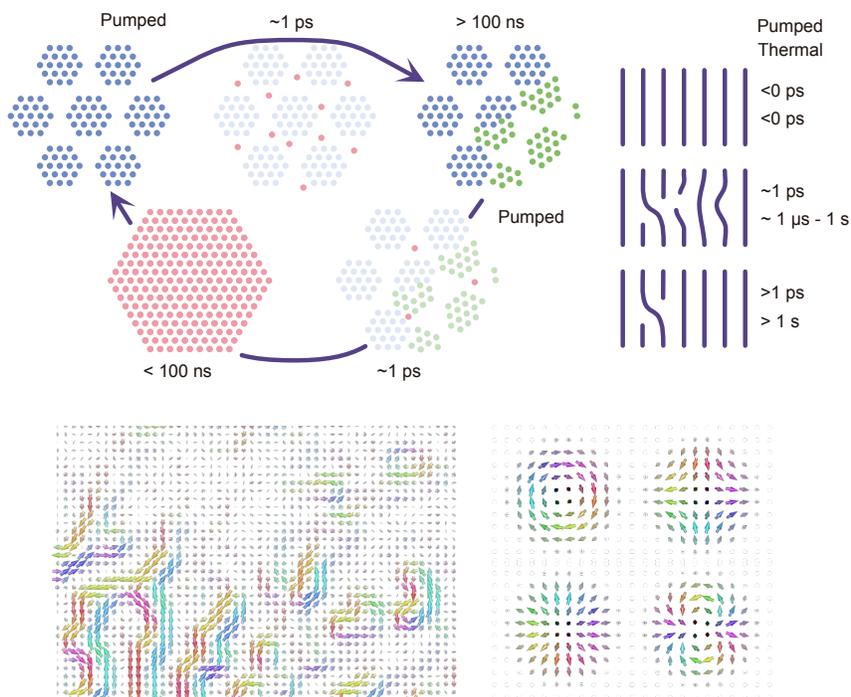
CryoEM will likely play a key role in exploring these phenomena in materials from amorphous to single-crystal: charge and spin density waves in van der Waals materials and high-temperature superconductors, spin-polarized surface states in topological insulators, and topological magnetism in exchange-coupled materials. Phase contrast imaging will provide the required sensitivity and the temporal and spatial resolution to identify spin and charge accumulation in time and space, enhanced by continuous-wave laser modulation and beam-shaping apertures to generate electron beams with orbital angular momentum or built-in reference beams [62, 63, 64]. CryoEM can enable understanding of a range of dynamic processes, such as collective excitations (breathing and rotation modes [65, 66]), transitions between localized states or extended phases with different spin chirality, symmetry, and topology [67, 68, 69], and topological defects in the charge and spin order [70, 71, 72].

Compared with leading X-ray techniques, cryoEM has the potential for providing superior spatial resolution and real-space information. Using low-dose imaging enabled by AI/ML could help answer how dynamic processes with characteristic time scales >1 ps can be correlated with order.

For example, cryoEM can be used to elucidate processes in topological magnetism, including localized and collective spin excitations in the form of quasi-static magnetization reversal processes, thermodynamic and driven spin excitations, and phase transitions. This is shown schematically in Figure PRO 3-4. The manifestation of microscopic spin textures can be used as a direct probe of the local spin-orbit coupling and its dependence on order. The overwhelming majority of experimental work to date has focused on quasi-static behavior of topological magnetic materials, including two-dimensional projections of stable configurations. However, little is known about topological protection, which could be explored through observation of processes such as unwinding and winding of skyrmions and topological knots, transient states, and the role of defects.

Similarly, collective excitations and behavior of non-rigid 1D, 2D, and 3D lattices of chiral spin textures, and of spin and charge density waves, play a critical role in phase transitions and coherence. Understanding this behavior can affect prospective applications in spintronics and magnonics. The latter may be realized using topological states such as skyrmion tubes as tunable magnonic waveguides through twisting, curving and compressing the channel or by altering topology altogether. Investigations of this kind can benefit from—perhaps will only be possible with—cryoEM supporting pump-probe and pump-free dynamics measurements.

The ability to carry out cryoEM experiments that allow a dynamic process to be followed, either by creating snapshots of the process or by driving the process *in situ*, can enable a wealth of information to be obtained that has, to date, been lacking. The resulting insights will increase the understanding of diverse dynamic behavior across a range of chemical and materials systems, including chemical reactions, structural transformations, transport and quantum phenomena, and many others.

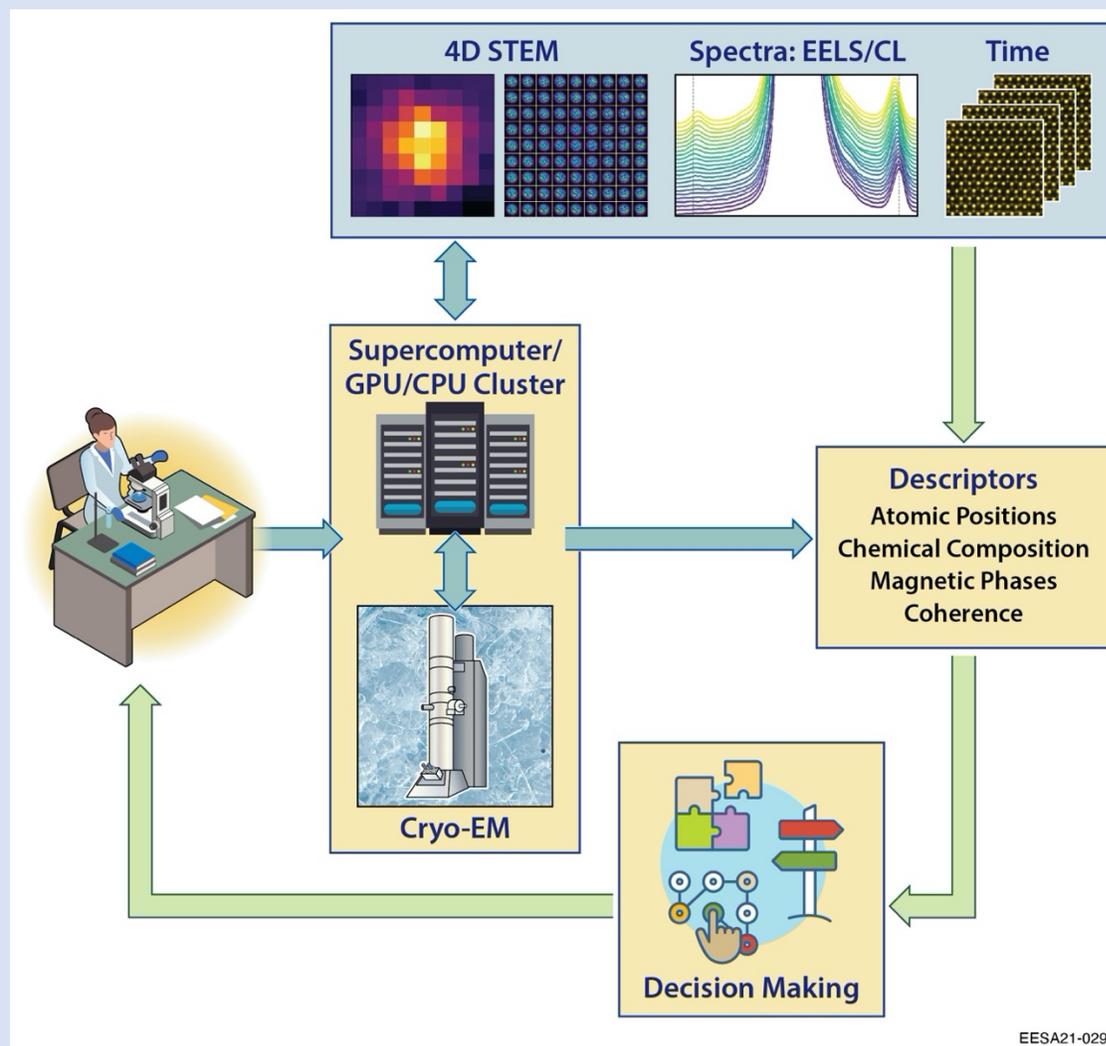


Graphic by Robert Streubel (University of Nebraska-Lincoln).

Figure PRO 3-4: Time scales for driven and thermodynamic processes in quantum materials involving phase transitions, fluctuations of charge and spin density waves, and topological magnetic states.

PRO 4. Harness data analytics and automation to expand the role of cryoEM in enabling scientific discoveries

Data science and data analytics approaches will dramatically expand the capabilities and impact of cryoEM by capturing phenomena that were previously inaccessible. Opportunities include development of machine learning (ML) and artificial intelligence (AI) approaches to discriminate signals from noise or artifacts, to detect features in sparse data, and to facilitate data interpretation, ultimately in real time to guide automated sample analysis. Automated microscope tuning, high-throughput data acquisition, and analytical protocols with workflows for “on the fly” control of cryoEM experiments can revolutionize microscope operation and the analysis and mining of large-scale data sets. Data science and analytics applied to cryoEM are crucial to research efforts across the other three PROs and promise to accelerate the rate of scientific discovery.



Graphic by Juan Carlos Idrobo (Oak Ridge National Laboratory) and Diana Swantek (Lawrence Berkeley National Laboratory).

Figure PRO 4-1: Illustration of how data analytical approaches can be used to interpret cryoEM data and to control and inform cryoEM experiments for the most effective use of every electron.

Scientific Impact

Data science and data analytics offer exciting opportunities to revolutionize the use of cryoEM in the physical sciences, expanding the range of systems and phenomena for study and accelerating discovery. Research opportunities can be considered within two broad categories: the use of automation to acquire high-quality datasets, and the enhanced analysis of cryoEM data. For example, ML models could be trained to identify low-contrast structures and to distinguish artifacts from electron beam effects while AI algorithms could employ ML methods for autonomous low-dose image acquisition. More broadly, data science methods can integrate instrument control, including the sample environment, with data acquisition protocols, theoretical modeling, and computational simulations for advanced sample analyses. These approaches also offer new ideas for the workforce training that will be required to realize these opportunities.

The application of ML and AI methods to cryoEM data can boost the extraction of information that increases understanding of chemical and materials systems. One cross-cutting challenge is the detection of “weak” features in cryoEM data, and AI/ML approaches could lead to improved discrimination of features such as transient phases, quantum states, or individual ions in solution. Applying such methods to complex systems will help capture sparse structures such as low-density defects, identify reaction pathways and dynamics, and correlate local structure to global structure in a statistically meaningful way. With these approaches, scientists have the potential to monitor complex distributions of molecules, clusters, or intermediates with shape or structure characteristics that cannot be averaged by existing single particle techniques. Given the challenges associated with training ML algorithms, new approaches will require the use of both published and unpublished datasets, using natural language processing techniques to obtain information from disparate data collections [73], and ultimately developing universal data formats and descriptors.

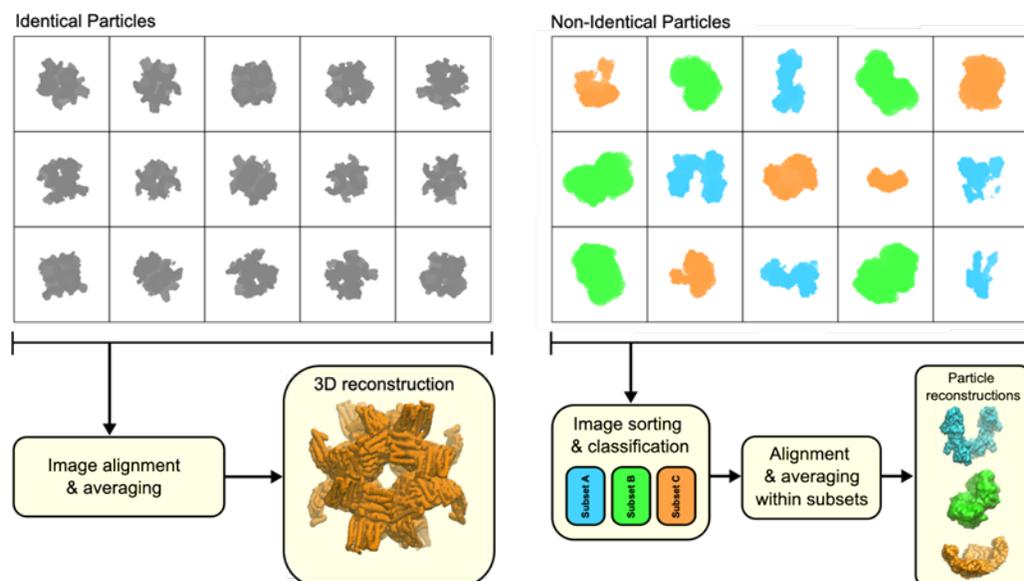
AI/ML algorithms are required to maximize the acquisition of data of sufficient quality in real-world cryoEM experiments. ML-assisted low-dose image acquisition and a data analysis workflow are urgent needs that are not limited to beam-sensitive samples. Automation of “simple” repetitive tasks can significantly increase the throughput of data acquisition and generate a large, high-quality data set for ML-assisted analysis and interpretation. New AI/ML algorithms and deep-learning solutions can create standardized data collection and analysis workflows. More excitingly, AI methods could transform the way that cryoEM experiments are designed and performed. One goal is to steer and automate instrument operation and data acquisition “on the fly” to aid decision-making associated with sample characterization and speed up scientific discovery in cryoEM experiments.

Scientific Challenges

Data science-assisted cryoEM can allow observations of matter under extreme conditions and visualization of material and chemical phenomena. It could also enable microscopists to retrieve structural information at global and local scales and establish interactions and correlations among fundamental building blocks of matter, from molecules and atoms to electrons and spins. The three examples below show where data science could solve important problems relevant to basic energy challenges. These examples range from polymers to quantum materials to single particle

Structure and dynamics in non-identical particle systems

Single-particle analysis (SPA) is a mainstay cryoEM technique that enables the 3D structure of like-shaped particles to be resolved through the alignment of a large number of 2D images. Although development has been driven largely by challenges in structural biology, the approach has a wide range of potential applications in materials systems, particularly those in which structural information is needed for species that either resist crystallization or are present in low quantities. Battery electrode particles with nanoscale conformal solid electrolyte interphases are an example of such a system. Hardware advances have gradually nudged the state of the art towards atomic resolution [81]; however, high-resolution structural reconstructions can only be obtained by averaging over a large number of particles and particle orientations [82], which limits the utility of the technique to particle systems that exhibit a high degree of conformational and compositional similarity.



Graphic by Adam F. Wallace (University of Delaware).

Figure PRO 4-3: Simplified SPA workflows for identical and non-identical particles. In each case, success depends on faithful alignment and averaging of 2D images, and particle reconstructions are generated by using a maximum-likelihood framework to optimize the agreement between the observed images and the proposed structural model(s). Non-identical particle workflows are further challenged by additional image classification requirements and the sensitivity of the optimization process to the initial structural model [83].

CryoEM holds much promise for the study of reaction dynamics, but unlocking this potential requires the ability to distinguish between populations of co-existing metastable intermediates within a single sample. In principle, SPA can be extended to non-identical particle systems if images can be successfully sorted into subsets by particle type [83] as shown in Figure PRO 4-3. Motivated by this purpose, the development of image classification, sorting and alignment algorithms is now an active and methodologically diverse research area in which ML-based approaches are gaining prominence [84]. At present, there is much interest in developing unsupervised classification methods that can identify discrete particle states within a dataset without any prior knowledge of the particle classes or their structural archetypes [85, 86].

Probing quantum states of matter by AI/ML-driven cryoEM

Quantum states of matter, such as magnetic phases, charge density order, superconductivity, two-dimensional electron gases, and topological phases, emerge across a wide range of cryogenic temperatures [5]. These quantum phenomena at cryogenic temperatures, previously probed by optical spectroscopy, are now within the reach of cryoEM [87]. However, these are extremely challenging states to create and image. Even when the temperature and position stability required for atomic-resolution imaging are achieved, key structures such as magnetic domains, domain boundaries, and coexisting magnetic phases may exhibit very low contrast. Moreover, molecular vibrations, phonons, excitons and core-loss edges in electron energy-loss spectroscopy have weak cross-sections that are about five orders of magnitude smaller than those of elastic electrons used for imaging. Consequently, spectroscopic analysis of quantum states of matter will be limited by intrinsically low signal-to-noise ratio (SNR) data.

To address these challenges, the integration of ML methods with cryoEM operation and analysis is expected to be essential for breakthroughs in exploring novel states of quantum matter. For example, suitably trained AI/ML models can enable detection of weak electronic or magnetic phases. ML tensor-based denoising approaches [88] will be invaluable in the study of magnetism at cryogenic temperatures. These methods could enable the emergence of magnetism to be probed at different length scales, ranging from single magnetic moments to micrometer-size domains and from 1D to 3D magnetic structures.

AI/ML can also reduce the time required for computationally expensive spectroscopy and vector field-imaging techniques so that they can be implemented for “on the fly” analysis, resulting in optimized experiments with higher SNR and shorter instrumentation time requirements. A combination of automated procedures that recognize defects or features could set the desired electron optical conditions for data acquisition with minimal operator input, as illustrated in Figure PRO 4-1.

In summary, data science approaches including ML and AI can enable new “Moore’s laws” for exponential discovery in materials and chemical sciences. AI-guided cryoEM capable of identifying chemical targets or novel materials in complex samples could radically increase the rate of discovering new synthesis or fabrication methods. At the same time, automation of instrument tuning, data acquisition, and analytics can revolutionize the way EM users are trained, not only in instrument operation but for data analysis and data mining. With standardized operation and analytical workflows, data science-based AI/ML could gain the advantage of democratizing scientific discovery for everyone by engaging scientists from institutions with limited resources.

Capability Needs for CryoEM

CryoEM offers the possibility of capturing, stabilizing, or creating important or novel material phases and chemical species at low temperatures for the most advanced electron-based structural and chemical analyses. All conventional and emerging EM modes can have considerable impact on fundamental energy science research when translated to studies under cryogenic temperatures. In addition to high-resolution TEM or STEM methods, complementary techniques such as nanobeam diffraction (sensitive to local structural order variation), electron energy loss spectroscopy (EELS), and energy-dispersive X-ray spectroscopy (EDS) will be needed to map structural and chemical heterogeneity (see for example Figure I-1 in the Introduction). In addition, phase-retrieval methods such as ptychographic imaging, which use the full scattered electron wavefront to obtain high-resolution, phase-sensitive images, can be harnessed. Electron tomography will be important to achieve sub-nanometer to nanometer resolution of reconstruction in materials with complex 3D structures. The highest impacts in energy science will likely be achieved with advances in the capability areas described below.

High-stability imaging and spectroscopy at cryogenic temperatures

Achieving atomic-resolution imaging at low or ultralow temperatures is a cross-cutting need that will require higher-stability dual-axis cryogenic stages. In particular, the ability to explore phenomena such as unconventional superconductivity, low-temperature metal–insulator transitions, and the behavior of topological insulators and spin liquids may require atomic-resolution cryoEM of lattice, electronic, and spin structures at variable temperatures down to tens, a few, or even sub-1 Kelvin. These opportunities face major challenges surrounding the stability of sample stages at low temperatures and the need for ultra-clean sample environments that prevent or minimize build-up of contamination. These challenges are a particular concern for experiments that require extended electron beam exposure, such as electron tomography with sub-nanometer to nanometer resolution in the reconstructed images, and ptychography analysis, which traditionally requires a higher dose than standard imaging approaches unless fast detectors are used [89].

Many chemical species and phases, such as macromolecules, metal–organic or organic–organic complexes, and structured or colloidal fluids, are of low atomic weight, leading to weak contrast, and are also sensitive to changes in chemistry induced by the incident electron beam. To map these structures reliably, dose-efficient acquisition, including new AI/ML methods described in PRO 4, will need to be developed and integrated with cryoEM to achieve minimally invasive imaging. These developments must be informed by new knowledge about the impacts of flux and dose on artifacts as well as the pathways and signatures of radiolysis phenomena in vitrified and liquid aqueous systems.

In situ and *operando* experiments to track dynamic phenomena

Exploration of dynamic chemical and materials processes using cryoEM is at a very early stage and can benefit from key methodological and instrumental developments. For example, integrating *in situ* excitations, such as magnetic fields, electric fields, laser interactions and sample environments, is critical to the exploration of pumped dynamic processes and phase transformations in quantum materials. These multimodal excitations will be needed across

temperatures ranging from sub-1 K to room temperature and potentially at frequencies up to the GHz regime, without compromising spatial resolution in cryoEM.

Some driven excitations, such as repeatable domain wall dynamics, can potentially be observed at low temperatures via stroboscopic imaging in dynamic or ultrafast instruments that synchronize pulsed electron beams and optical pulse excitations, using phase-sensitive imaging modes such as off-axis electron holography or Lorentz microscopy [90, 91]. CryoEM would have the potential to characterize qubits *in operando* (under realistic operating conditions) by integrating radiofrequency excitation with analysis at temperatures of sub-Kelvin to a few Kelvin.

Insights into a broad range of chemical reaction pathways, transport phenomena, and synthesis mechanisms could be achieved by cryoEM analyses of frozen snapshots of systems that are evolving in time under a thermodynamic gradient. Early opportunities involve pushing towards the shortest timescales for creating and cryogenically capturing reacting systems *ex situ* for sample transfer and analysis. More challenging will be the development of methods to initiate and study chemical reactions *in situ*—for example, using variable-temperature EM to manipulate reaction rates.

Sample preparation needs for cryoEM

Development of sample preparation, storage, and transfer approaches that preserve critical features such as interfaces and heterogeneity are important if cryoEM is to achieve its full potential. At the same time, factors such as crystallization damage, conformational changes, or chemical reactions that occur with cooling must be avoided. While certain samples, particularly aqueous solutions and suspensions, are readily prepared for cryoEM analysis by the traditional method of plunging the sample into a cryogen (liquid ethane in most cases), there is a pressing need to expand the repertoire of methods to make the broadest range of energy-relevant materials available for analysis. This is a particular challenge for heterogeneous systems with complex embedded interfaces or features for which advanced methods for preparing electron-transparent samples compatible with cryogenic conditions (e.g., electropolishing and focused ion beam methods) must be developed. Sample preparation challenges also arise for solid-state samples that are brittle or that undergo a large volume change as a function of temperature.

Integrating data science, theory, and correlative methods with cryoEM

For the study of complex and functional chemical and material systems, the integration of cryoEM into research workflows will be essential. In these new workflows, the experimental parameters, selection of the regions to be analyzed, the data acquisition and processing strategies, and the data interpretation are carried out through a close integration of experiment, theory, and AI/ML at all stages. Additional correlative analysis methods can be included.

The first area in which AI/ML capabilities need to be developed, as described in more detail in PRO 4, includes automated sample analysis, ultimately informed by “on-the-fly” data processing to optimize collection of the most relevant information from complex systems while maximizing throughput and minimizing beam damage.

Second, expanded collaboration between cryoEM experimentalists and practitioners of molecular and materials theory and simulation can accelerate progress in both domains. Large-scale

atomistic datasets that are made available by cryoEM can increasingly inform simulations that predict the structure and behavior of interacting systems, such as increasing understanding of complex heterogeneity by accurately accounting for the competition between long-range and short-range interactions. Computational methods could increasingly be able to predict phenomena, species, or configurations that can be considered as search targets for cryoEM analysis. Access to leadership-class computing facilities, including the upcoming DOE exascale facilities, and to new codes that integrate chemical and physical models with cryoEM data interpretation, are vital to realizing the full potential of cryoEM to address major scientific challenges in fundamental energy science.

Third, new approaches are needed to pair cryoEM data with complementary measurements of electronic or molecular structure and quantitative measures of system behavior and function, ideally on the same sample.

A final consideration is the human–microscope interface and access to advanced cryoEM facilities. The most productive approaches will borrow from the biological sciences and ensure that microscopes run 24/7, while acknowledging that cryoEM applied to the physical sciences will have more complex sample preparation and imaging requirements. In addition, as informed by experiences during the COVID-19 pandemic, new modes of operation, including remote access and potentially remote control, could enable researchers from the most diverse range of institutions and research communities to drive fundamental science using these exciting and still emerging tools.

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Appendix A: Roundtable Agenda

BES Roundtable on Research Opportunities in the Physical Sciences Enabled by Cryogenic Electron Microscopy Virtual Meeting May 4–6, 2021

All times indicated are Eastern

Day 1: May 4, 2021

10:45 – 11:00 AM	Log-In
11:00 – 11:15 AM	Welcome and Roundtable Charge Linda Horton, Associate Director of Science for Basic Energy Sciences
11:15 – 11:30 AM	Welcome, Roundtable Goals, and Logistics Amanda Petford-Long, Argonne National Laboratory Ben Gilbert, Lawrence Berkeley National Laboratory
11:30 – 12:15 PM	Invited talk 1: <i>In Aqua</i> High-resolution Imaging of Inorganic and Hybrid Earth Materials Jill Banfield, University of California Berkeley
12:15 – 1:00 PM	Invited talk 2: How Can Cryo-STEM Revolutionize Materials Science? Miaofang Chi, Oak Ridge National Laboratory
1:00 – 1:40 PM	Summary of Homework Assignments (Amanda Petford-Long & Ben Gilbert)
1:40 – 2:00 PM	Break (20 min)
2:00 – 4:00 PM	Breakout Session I – Science Focus Panel 1: Condensed Phase Chemical Dynamics and Reaction Panel 2: Correlating Structure and Function in Soft Materials Panel 3: Low-temperature Phenomena in Quantum Matter Panel 4: Processes and Chemical Pathways in Energy Materials
4:00 – 4:30 PM	Break (30 min)
4:30 – 6:00 PM	Plenary Session I: Panel report-out—discussion and identification of potential PROs
6:00 PM	Adjourn

Day 2: May 5, 2021 Revised agenda

10:45 – 11:00 AM	Log-In
11:00 AM - 11:20 AM	Introduction to New Panels Amanda Petford-Long, Argonne National Laboratory Ben Gilbert, Lawrence Berkeley National Laboratory
11:20 AM – 1:20 PM	Breakout Session II – PRO development session using quad chart approach Panels re-assigned for this session. Panel leads to prepare quad charts to bring back to Plenary
1:20 PM – 1:50 PM	Break (30 min)
1:50 – 3:20 PM	Plenary Session II: Panel Report-Out from Breakout Session II discussion to present potential PROs for Inclusion in report

3:20 – 4:00 PM **Break (40 min) Chairs/ORISE to circulate breakout materials from XLeap + quad charts for all PROs to all panels**

4:00 – 5:30 PM Panels reconvene to start writing process

5: 30PM – 6:00 PM Closing Remarks
Amanda Petford-Long, Argonne National Laboratory
Ben Gilbert, Lawrence Berkeley National Laboratory

6:00 PM **Adjourn**

Day 3: May 6, 2021 Roundtable chairs, panel leads and designated writers only

10:45 – 11:00 AM **Log-In**

11:00 – 11:30 AM Plenary III – Introduction of writing assignments/writing groups
Amanda Petford-Long, Argonne National Laboratory
Ben Gilbert, Lawrence Berkeley National Laboratory

11:30 AM – 1:00 PM **Writing/editing session 1**

1:00 – 2:00 PM **Break (60 mins)**

2:00 – 6:00 PM **Writing/editing session 2**

Day 4: May 13 Roundtable chairs, panel leads and designated writers only

10:45 – 11:00 AM **Log-In**

11:00 – 11:30 AM Plenary IV – Overview of writing progress
Ben Gilbert, Lawrence Berkeley National Laboratory
Amanda Petford-Long, Argonne National Laboratory

11:30 AM – 3:30 PM **Writing/editing session 3**

Appendix B: Workshop Participants

Basic Energy Sciences Roundtable on Research Opportunities in the Physical Sciences Enabled by Cryogenic Electron Microscopy

May 4–6, 2021

Participants

Veronica Augustyn	North Carolina State University
David Beratan	Duke University
Diana Berman	University of North Texas
Qian Chen	University of Illinois at Urbana-Champaign
Yi Cui	Stanford University
Jim De Yoreo	Pacific Northwest National Laboratory
Ben Gilbert	Lawrence Berkeley National Laboratory
Xiaodan Gu	University of Southern Mississippi
Juan Carlos Idrobo	Oak Ridge National Laboratory
Katherine Jungjohann	Center for Integrated Nanotechnologies/Sandia National Laboratories
Lena Kourkoutis	Cornell University
Andrea Liu	University of Pennsylvania
David McComb	Ohio State University
Shirley Meng	University of California, San Diego
Gary Moore	Arizona State University
Hosea Nelson	University of California, Los Angeles
Lee Penn	University of Minnesota
Amanda Petford-Long	Argonne National Laboratory
Frances Ross	Massachusetts Institute of Technology
Susanne Stemmer	University of California, Santa Barbara
Robert Streubel	University of Nebraska–Lincoln
Adam Wallace	University of Delaware
Chongmin Wang	Pacific Northwest National Laboratory
Hao Yan	Arizona State University
Ping Yang	Los Alamos National Laboratory
Yimei Zhu	Brookhaven National Laboratory

Observers

Mowafak Al-Jassim	National Renewable Energy Laboratory
Ilke Arslan	Argonne National Laboratory
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Jill Banfield	University of California, Berkeley
Chris Bradley	Department of Energy, Basic Energy Sciences
Morris Bullock	Pacific Northwest National Laboratory
Miaofang Chi	Oak Ridge National Laboratory
Matt Coleman	Lawrence Livermore National Laboratory
Bruce Garrett	Department of Energy, Basic Energy Sciences
Krzysztof Gofryk	Idaho National Laboratory
Steve Herbert	Department of Energy, Basic Energy Sciences
Linda Horton	Department of Energy, Basic Energy Sciences
Jim Horwitz	Department of Energy, Basic Energy Sciences
Helen Kerch	Department of Energy, Basic Energy Sciences
Jeff Krause	Department of Energy, Basic Energy Sciences
Margaret Lenz	Department of Energy, Advanced Scientific Computing Research
Eliane Lessner	Department of Energy, Basic Energy Sciences
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Mike Markowitz	Department of Energy, Basic Energy Sciences
Helmut Marsiske	Department of Energy, High Energy Physics
Gail McLean	Department of Energy, Basic Energy Sciences
Andy Minor	Lawrence Berkeley National Laboratory/University of California, Berkeley
Raul Miranda	Department of Energy, Basic Energy Sciences
Karren Morris	Oak Ridge National Laboratory
Brandon Rohnke	Department of Energy, Basic Energy Sciences
Tom Russell	Department of Energy, Basic Energy Sciences
Jim Rustad	Department of Energy, Basic Energy Sciences
Andrew Schwartz	Department of Energy, Basic Energy Sciences
John Vetrano	Department of Energy, Basic Energy Sciences
Liguo Wang	Brookhaven National Laboratory
John Watt	Los Alamos National Laboratory
Juliane Weber	Oak Ridge National Laboratory
Lane Wilson	Department of Energy, Basic Energy Sciences
Lin Zhou	Ames Laboratory
Jane Zhu	Department of Energy, Basic Energy Sciences

Appendix C: Factual Document

Factual Document for BES Roundtable on Research Opportunities in the Physical Sciences Enabled by Cryogenic Electron Microscopy

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Amanda Petford-Long, Argonne National Laboratory
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Table

Table 1. Examples of cryoEM studies of microphase separated or crystalline soft materials [76]. 54

1. Introduction

Motivation

There are two primary motivations for cooling a sample to cryogenic temperatures in an electron microscope. The first is to limit the negative effects of electron irradiation damage by decreasing the mobility of species produced by this damage, and the second is to study a material or phenomenon that exists only at cryogenic temperatures. While the technique of cryogenic electron microscopy (cryoEM) has varied definitions, technical approaches, and impacts for different scientific fields, in all cases the motivation for cooling a sample in an electron microscope will be one or both of these two.

The purpose of this factual document is provide workshop participants with a high-level assessment of the current status of cryoEM. This document focuses only on the existing technology/instrumentation, with a short overview of its applications in structural biology, followed by four sections that outline how cryoEM has been applied to date in each of the four fields of the workshop panels. It is meant to provide examples and describe the state of the field, but not to provide guidance on future research directions.

Technique history

Sample cooling inside an electron microscope began in the 1950s with the development of the first cryo-stages. In 1956, Honjo et al. [1] reported the design of the first liquid nitrogen cryogenic cooling stage for transmission electron microscopy (TEM) imaging and electron diffraction, capable of a temperature range from liquid nitrogen (LN₂) to 200°C. Using this stage, they were able to identify the contribution of H in diffraction patterns and observe phase transitions in mercury. They pointed out that contamination of the specimen caused by the condensation of vapors could be eliminated by improving the column vacuum and by the use of a special shield around the specimen—valuable information that guided the future design of the anti-contamination polepiece boxes in the microscope. Liquid He-based cryogenic sample stages for electron microscopy followed soon after [2–6], extending the temperature range of cryoEM [7]. However, these early cooling stages were installed in dedicated microscopes, and their operations were complicated. The use of cryoEM for materials science didn't become widespread until the introduction of commercial side-entry cooling holders by Gatan, Inc., including the option of incorporating cryo-transfer systems. In parallel, more practical liquid helium cooled systems were also introduced, making it possible to cool samples down to as low as 1.5 K in a TEM [8].

Compared to electron microscopy at room temperature, cryoEM's resolution, stability, and practical possibilities have been limited by mechanical vibrations and sample drift. Instantaneous drift velocities for specimen holders cooled below room temperature are substantially worse, as is the settling time to get to the most stable condition at low temperatures [9]. The hold times and vibrations for liquid helium cooling are even worse than for liquid nitrogen. Since one of the main strengths of electron microscopy is the ability to measure small (even sub-picometer) lattice distortions in real space, the mechanical instabilities are incredibly limiting. Practical challenges for high resolution imaging and chemical analysis at cryogenic temperatures stem from multiple factors, such as the temperature variation on the specimen tip, the mechanical instability induced by the heavy liquid dewar, bubbling and evaporation of liquid

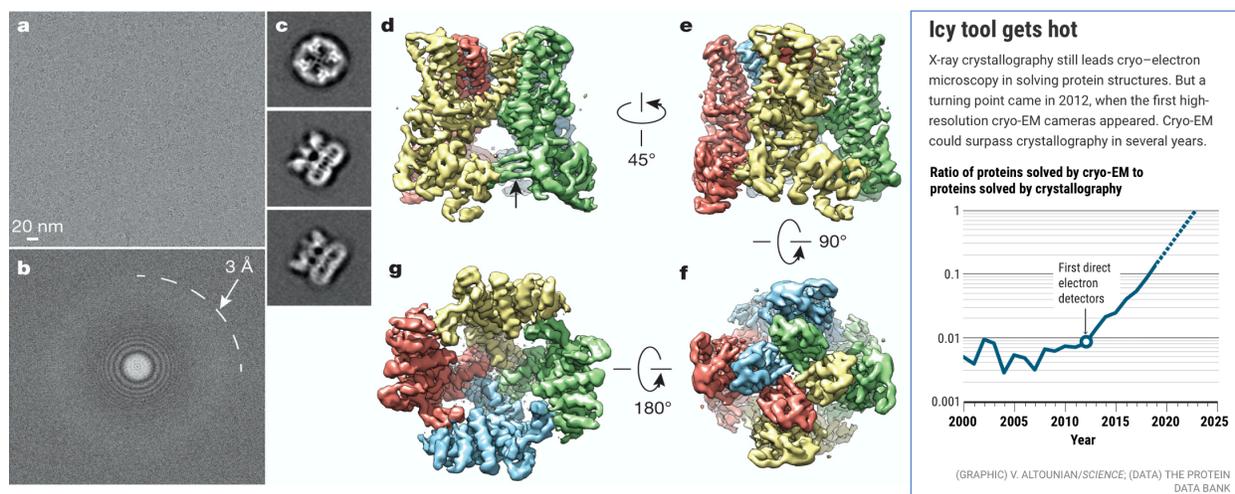
nitrogen/helium, and lack of double-tilt capabilities in modern instruments dedicated to cryoEM. Several new designs for cryoEM holders have come on the market in the past few years, including holders based on MEMS technology (from HennyZ, Inc.), combined with vacuum transfer (Mel-build, Inc.), or coupled with *in situ* biasing (Hummingbird Scientific, Inc.).

Examples of applications in structural biology

The 2017 Nobel Prize in chemistry recognized three researchers (J. Dubochet, J. Frank, and R. Henderson) who contributed key steps towards the modern use of cryoEM to image biological macromolecules in atomic detail [10]. However, the monumental expansion of the use of cryoEM in the past decade [11] for the biological sciences was based on steady progress over many years, coupled with important technological advances common to both the biological and materials sciences [12]. The success of cryoEM methods for biology has even led to the broader term “cryoEM” being commonly used to refer to the more specific technique of single-particle reconstruction of vitrified biological macromolecules, when in fact cryoEM in materials science typically refers to unhydrated studies of heterogeneous samples. Even in the biological community, there are widespread techniques, such as cryo-tomography, that are also referred to as cryoEM.

In addition to the limitations of exposing biological molecules to electron irradiation, the characterization of biological molecules in the vacuum environment of an electron microscope provides an additional constraint. Taylor and Glaeser [13] first demonstrated that high-resolution biological structural information could be retained in an electron microscope using previously frozen specimens. It was not until Dubochet et al. [14] demonstrated the technique of vitrification through plunge-freezing that cryoEM of biological structures became more common. While X-ray crystallography made use of averaging to develop atomic structures of biological macromolecules, it took advances in the merging of image data from 2D crystals by Henderson and Unwin [15], and non-crystalline samples by Frank and colleagues [16, 17], to demonstrate the basic technique of single-particle reconstruction from many images of identical samples for high-resolution biological cryoEM reconstructions. For a number of years, this technique remained a niche application for structural biology, in which X-ray crystallography was standard, until technological improvements began to enable comparable results using electrons (but without having to crystallize samples). Specifically, the development of direct electron detectors enabled the alignment of frames to overcome specimen motion [18] and had higher detector quantum efficiencies. Two breakthrough papers in 2013 [19, 20] heralded this new era of single-particle cryoEM as an established technique for the biological community [21, 22], and it is possible that cryoEM will soon surpass X-ray crystallography for solving protein structures (see Figure 1).

The single-particle method of cryoEM brings a number of unique capabilities to the visualization of biological molecules as compared to X-ray crystallography beamlines at synchrotron sources [23]. The most important is that single-particle cryoEM does not rely on crystallization, which is challenging for large molecular assemblies, membrane proteins, and heterogeneous samples. For samples that are scarce, such as samples purified from human biopsy [24], crystallization is not an option. Perhaps the most exciting capability is conformational heterogeneity, which can be addressed with imaging of particles vitrified at different states [25, 26].



Source, graph at far right: From Hand, Eric. 'We need a people's cryo-EM.' Scientists hope to bring revolutionary microscope to the masses. *Science*, January 23, 2020. <https://doi.org/10.1126/science.aba9954>. Reprinted with permission from AAAS.

Figure 1: The first cryoEM reconstruction of a small medically important molecule that could not be crystallized using a direct electron detector [20]. a.) Representative electron micrograph of TRPV1 protein embedded in a thin layer of vitreous ice recorded at a defocus of 1.7 μm . b.) Fourier transform of micrograph shown in a, with Thon rings extending to nearly 3 \AA . c.) Enlarged views of three representative 2D class averages show fine features of tetrameric channel complex. d.–g.) 3D density map of TRPV1 channel filtered to a resolution of 3.4 \AA (scaled to atomic structure) with each subunit color-coded. Four different views of the channel are shown: side (d, e), top (f) and bottom (g). The arrow in panel d indicates β -sheet structure in the cytosolic domain of TRPV1. At far right: chart showing ratio of proteins solved with cryoEM as compared to X-ray crystallography, as reported to the Protein Data Bank [27].

2. Condensed Phase Chemical Dynamics and Reaction

Chemical reactions in solution drive energy transduction, chemical transformation and material synthesis. CryoEM offers the ability to capture fully hydrated chemical and material systems either at equilibrium or as they respond to changes in composition or physical environment. Freezing also offers the opportunity for studying reagents and materials that are typically unstable under an electron beam. The spatial resolution and extent of information available from cryoEM, the ranges in sample conditions that can be studied by the plunge-freezing technique, and the accessible timescales remain to be established.

The solid–liquid interface is a distinctive nanoscale environment that can sequester solutes, stabilize species that are not found in bulk solution, and host or catalyze chemical transformations. The surfaces of reactive solids also evolve through corrosion reactions involving solvent or solute molecules. Although atomic-resolution electron imaging of solid–solid interfaces is routinely performed, and solid–gas interfaces have been imaged with molecular resolution [28], achieving high-resolution imaging of solid–liquid interfaces has been slower due to challenges with sample preparation and with high solvent scattering. Consequently, molecular models of solid-liquid interfaces have typically been developed using surface-sensitive spectroscopic or scattering methods that average over surface sites (e.g., grazing-incidence X-ray fluorescence or nonlinear vibrational spectroscopies). By contrast, cryoEM provides an approach to characterizing the structure and composition of both the solid and liquid phases that can distinguish surface heterogeneity.

Recent studies have used cryogenic cooling to capture the aqueous solution at the surface of a bulk solid, including metal oxides and metal alloys, and cryo-focused ion beam (cryo-FIB) methods to prepare thin sections for cryoEM. Lin et al. [29] used bright and dark-field cryoEM imaging and cryo-diffraction to observe the corrosion products of NiCrMo alloys corroded in an electrolyte solution containing Cl⁻. Zachman et al. [30] used elemental mapping by energy-dispersive X-ray spectroscopy in cryo-SEM to reveal the composition and structure of a hydrated device surface.

Knowledge of solute distributions at the solid–liquid interface is of great interest to many fields. In particular, many important inorganic and organic solid surfaces in an electrolyte can sorb charged solutes, forming an extended equilibrium structure called the electric double layer. An early demonstration of direct cryoEM imaging of charged species at an interface was reported by Neyman et al. [31], who showed macroions, the polyoxometalate α -AlW₁₁O₃₉⁹⁻, associated with silver particles in water (Figure 2).

The nucleation and growth of crystalline or amorphous solids from solution is a ubiquitous process in the natural world, affecting solution chemistry in atmospheric aerosols, surface waters and reservoirs and forming biominerals in many organisms, and it is a critical

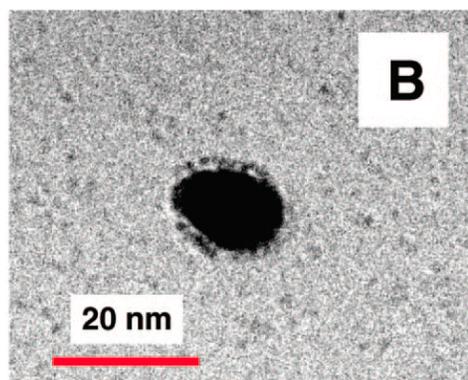


Figure 2: CryoEM observation of a single Ag(0) nanoparticle protected from aggregation by the association of polyoxometalate ions [31].

pathway for materials synthesis. Despite the countless practical consequences and the long history of study, establishing mechanistic models for nucleation and growth remains a significant challenge. Nucleation processes are rare events involving multiple species and solvent molecules, posing a challenge for direct observation and atomistic modeling [32]. Moreover, it is increasingly accepted that nucleation and growth can proceed through multi-stage processes with alternative intermediate phases and pathways for the formation of the eventual phase and morphology [33].

Both cryoEM and liquid-cell TEM have made significant contributions to understanding nucleation phenomena and continue to push boundaries in this fast-moving research field [34]. Liquid-cell studies can follow the dynamics of crystal formation, but with the solution composition significantly altered by electron-beam interactions with water (e.g., Jeon et al. [35]). CryoEM provides static snapshots of dynamically evolving systems with reduced influence from electron-beam interactions, from which intermediate structures and pathways can be inferred.

CryoEM studies, often complemented by cryo-ED, have revealed that different solids are formed through different nucleation pathways in aqueous–aqueous solution. For example, carbonate crystals can form via amorphous nanoparticulate intermediates [36], whereas magnetite crystals form via nanocrystalline intermediates [37]. CryoEM has also provided important observations that clarified the complex multistage formation process of calcium phosphate, involving ion-association complexes, amorphous calcium phosphate aggregates, and nanofilaments prior to crystalline apatite (Figure 3) [38].

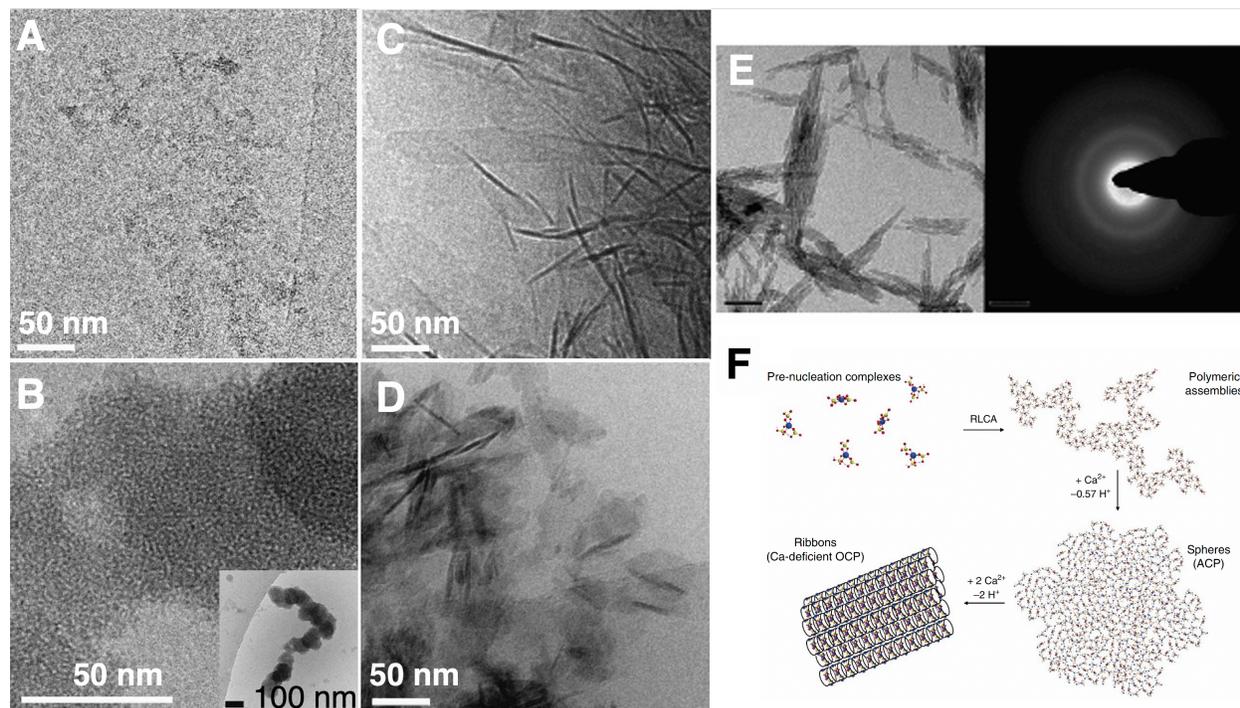


Figure 3: CryoEM observations of intermediates in the early-stage formation of calcium phosphate from aqueous solution of (A) polymeric strands from nanometer-sized units, (B) aggregated spheres, (C) ribbons, and (D) elongated plates. (E) Structural analysis using electron diffraction. (F) Mechanism of nucleation, growth and phase transformation developed from cryoEM and molecular simulation studies [38].

Colloidal suspensions of clusters, nanoparticles and clay minerals have roles in energy systems, water purification technologies and the environmental cycles of elements. CryoEM has provided direct observations of the structures adopted by colloidal particles in solution. For example, swelling clay minerals in solution form particles of ~ 1 -nm thick nanosheets separated by variable thicknesses of water (Figure 4). Cryo-tomography revealed the morphology of the particles, the interlayer spaces, and the nanoconfined pores created by regular and defective stacking structures [39]. CryoEM studies of smectite also showed that average interlayer spacing varies with the number of layers in a particle, revealing an influence of long-range attractive dispersion interactions [40].

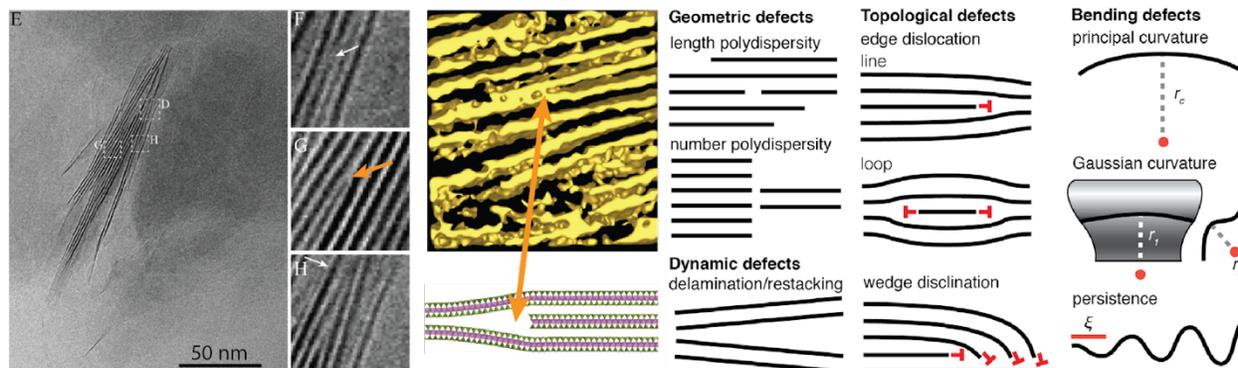


Figure 4: CryoEM and cryo-electron tomography (cryo-ET) of a clay mineral particle in aqueous solution consisting of a stack of individual clay nanosheets separated by water (left). Layers (dark linear features) are approximately 1 nm thick and have irregular shapes, leading to myriad defects, including a gap between layers within a stack (top), an edge dislocation (middle), and a wedge disclination (bottom). Rendering of three-dimensional edge dislocation created by the termination of a layer within a stack (center, top) with polygonal model of defect geometry (center, bottom). CryoEM reveals defects associated with curvature in three dimensions (right), which are not readily obtainable from 2D projection images, due to contrast resulting predominantly from phase interference when layers are face-on and absorption when layers are edge-on [39].

CryoEM has also revealed that colloidal particles can adopt anisotropic aggregation morphologies that are likely driven by crystal orientation-dependent interparticle interactions, even when the particles are separated by layers of water (Figure 5) [41]. Aggregation phenomena in solutions of metal-oxide clusters also show complexity [42].

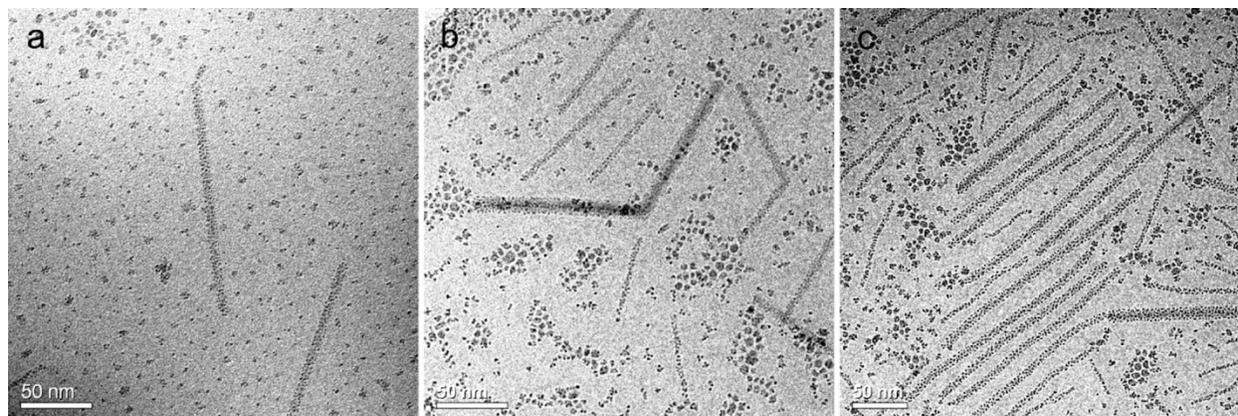


Figure 5: CryoEM images of goethite mesocrystals after (a) 5 days, (b) 10 days, (c) 24 days of aging at 80°C [41].

3. Correlating Structure and Function in Soft Materials

Hard materials like metals and ceramics are relatively stable under the electron beam and routinely survive the electron doses needed to provide atomic-scale images of their heterogeneous structure. At the other extreme, biological macromolecules are extremely unstable under electron beam irradiation, and atomic-scale images are possible only through averaging methods that reconstruct many low-dose images of homogeneous structures. Somewhere in between these extremes are soft materials, such as polymers, that are both beam sensitive and heterogeneous (meaning it is not possible to make millions of identical copies of the same structure).

A key feature of soft materials is that they have appreciable thermal motion and their effective lattice constant can exceed 1 nanometer. They are not always ordered at the atomic scale and are often found in semi-crystalline form such that there is a coexistence of both order and disorder. This combination of beam sensitivity, large lattice constants, and structural heterogeneity makes electron imaging of soft materials like polymers difficult. As a result, electron microscopy of unstained soft materials under cryogenic conditions [43], to slow down the evolution of beam damage [44–51], is critical.

CryoEM has long been used for imaging and diffraction studies of polymer samples [52]. In addition to decreasing the effect of beam damage, cryoEM also allows for the direct imaging of polymer structure in the solvated state. For example, Figure 6 shows an example comparing the structure of a polymer electrolyte membrane (PEM), both stained at room temperature and unstained but hydrated at cryogenic temperatures [53]. CryoEM of the hydrated and vitrified sample shows critical information about the structure of the PEM in conditions similar to those it would be found in during operation of a polymer electrolyte fuel cell.

CryoEM and cryo-tomography of micellar structures in solution (vitrified water) have shown hidden structural features such as planar brushes emanating from the micellar core [54]. CryoEM of organic assemblies like this and other polymeric nanostructures [55] shows the native configurations of the structures in solution. It should be noted that cryogenic sample preparation methods such as cryomicrotomy, cryoplunging and cryo-FIB [56, 57] can be as important for preserving the structure of soft materials as cryogenic imaging in the TEM.

High-resolution temporal and spatial information obtained during the formation of polymer nanostructures is crucial for understanding microphase separation mechanisms and the interactions between solvent molecules and polymer chains. CryoEM provides an avenue to obtaining time-resolved images [58]. This is enabled by freezing a thin layer of solution during the formation process on the EM grid, thereby trapping the intermediate structures as a function of time. Although this approach can achieve sub-nanometer spatial resolution, the temporal resolution is limited, due to the necessity of freezing separated samples at different stages of structure formation [59].

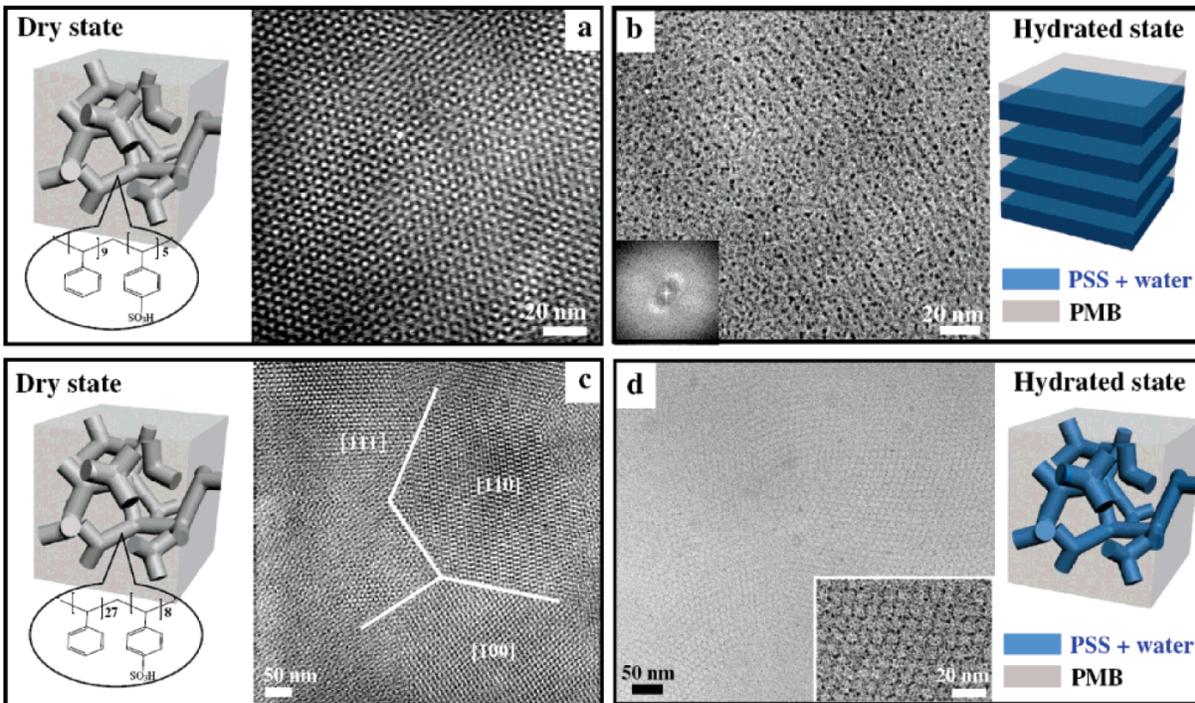


Figure 6: (a) Cross-sectional TEM image of a stained PSS-b-PMB copolymer in the dry state. (b) CryoEM image and fast Fourier transform inset of hydrated sample for unstained PSS-b-PMB copolymer. (c) TEM image of different stained PSS-b-PMB copolymer in the dry state with gyroid morphology showing three different grain orientations. (d) CryoEM image of unstained hydrated PSS-b-PMB copolymers. The inset box in (d) shows the image obtained with higher magnification. For dry samples, PSS domains appear dark due to RuO₄ staining. The images of hydrated samples are obtained without staining, thus the dark regions in (b) and (d) represent the water-rich domains. Schematics depict the change in channel geometry as a result of hydration.

Structures of crystalline polymers depend strongly on the processing conditions. Poly(3-hexylthiophene) or P3HT is a widely studied semicrystalline polymer. The π - π stacking of the conjugated backbone induces the crystallization of P3HT chains in devices obtained by printing from organic solutions, so it is important to characterize the state of P3HT crystals in solution. Wirix et al. used cryo-ET to study the nature of crystalline P3HT ribbons in organic solvents [60]. As the solvents used in this work were toxic, the vitrification process was carried out in a fume hood and the structure was preserved with cryo-transfer.

The structure of semiconducting polymers has recently been studied with cryogenic four-dimensional scanning transmission electron microscopy (4D-STEM) [61, 81]. 4D-STEM uses a focused electron beam that is rastered across an electron transparent sample, and at each scan position a diffraction pattern is acquired [62]. As their electronic properties are highly dependent on their crystallographic geometry, organic semiconductors exemplify the need for improved understanding of the link between structural and functional properties in solid thin films across a multitude of length scales ideally suited for study by electron microscopy methods. For example, Figure 7 shows the nanocrystalline structure of an organic molecular thin film using cryo-4D-STEM with a spatially localized probe (~ 2 nm). This technique revealed previously unknown structural features, such as grains overlapping through the thickness of the thin film, in information about the local crystal structure and its variations.

As a class of materials, zeolites and metal oxide frameworks (MOFs) are extremely beam sensitive, and electron microscopy studies of their structure have only begun to take advantage of cryoEM [63-65]. Some MOF families are more stable under the electron beam and can be imaged under non-cryogenic conditions [66-72]. However, understanding the complexity of MOF systems, including the host-guest interactions, requires cryoEM. A recent study by a group at Stanford used cryoEM to dramatically show the effect of a MOF framework both with and without CO₂ as the guest (see Figure 8) [64]. A ZIF-8 MOF crystal saturated with CO₂ was plunged into liquid ethane to trap the CO₂ molecules inside the crystal. As shown in Figure 8, a slight increase in the brightness at the center of the cage suggests the presence of an adsorbed CO₂ molecule within the ZIF-8 crystal. It is interesting to note that images in Figure 8 were obtained without sorting or averaging. The atomic-scale imaging of lattice structure in a MOF crystal with CO₂ shows the potential of low-dose cryoEM in characterization of well-ordered organic structures.

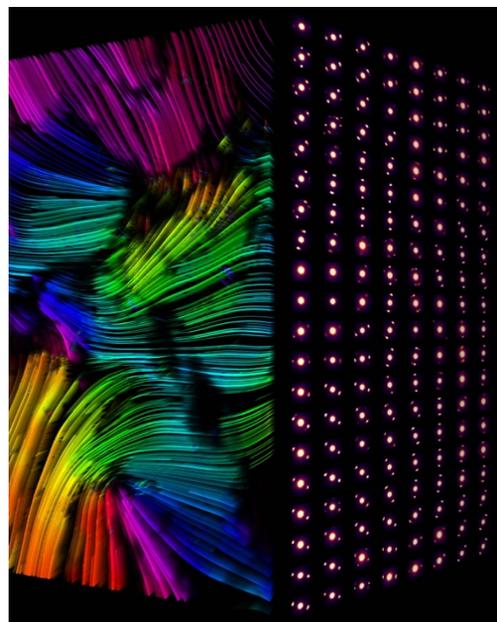


Figure 7: Cryo 4D-STEM of a dropcast p-DTS(FBTTh₂)₂ small molecule sample. At left is a flow map showing the continuity of lattice orientations in space. At right is a selection of diffraction patterns that generated the flow maps. Adapted from [81].

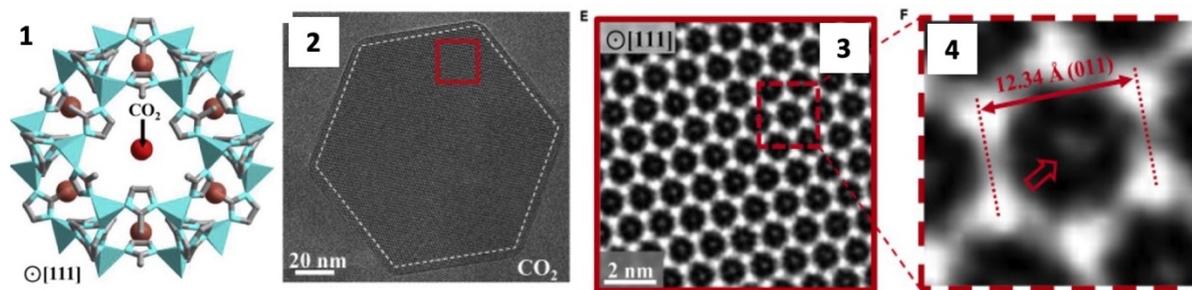


Figure 8: (1) Simulated structure of ZIF-8 with DFT-predicted binding site of CO₂ (simplified as red spheres) along the [111] projection. (2) CryoEM image of CO₂-filled ZIF-8 particle (outlined by white dashed lines) along the [111] projection. (3) CTF-corrected denoised image of the red boxed region from 2. Bright regions correspond to mass density. (4) Magnified image of a single ZIF-8 unit cell from 3. Density at the center of the unit cell (indicated by red arrow) likely corresponds to CO₂ adsorbed within ZIF-8 [64].

Recently, it has been shown that hybrid cryoEM methods to increase contrast through sorting and averaging can be successful for semi-crystalline systems. As described previously, in the biological community, cryoEM refers to an averaging method that sorts and classifies different configurations of the same molecule to be averaged together to form a higher resolution reconstructed image. Similarly, recent work by Xuan, et al. [73] used cryoEM imaging to then sort and classify different atomic configurations of peptoid molecules to increase the resolution of local motifs. Further improvement of this technique by Jiang, et al. [74,75] has demonstrated the ability to resolve the atomic structure of a polypeptoid crystal with a resolution of 1.5 Å (see Figure 9).

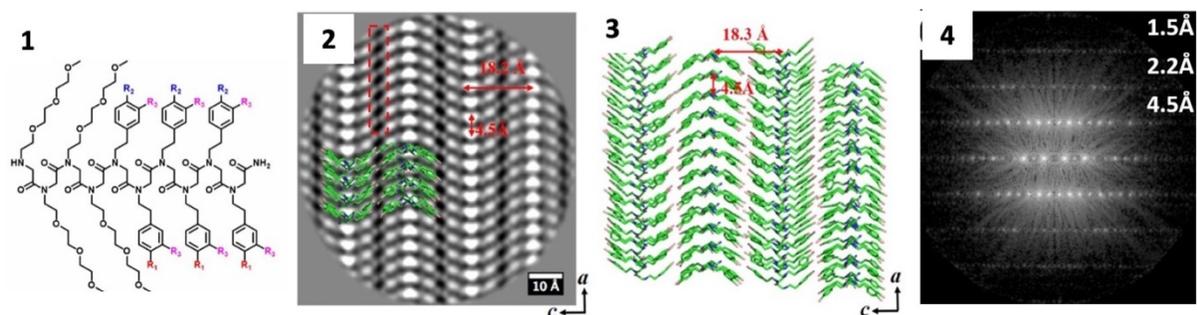


Figure 9: (1) Chemical structures of polypeptoid diblock copolymer with Br atoms at R₁ and R₂ positions. (2) Averaged cryoEM image of nanosheets from the *b* direction (top view) showing antiparallel V shapes along the *c* direction. The Br atoms show a tip-to-tip packing (red box). (3) Top view of the hydrophobic domain in 2 from the *b* direction showing antiparallel V shapes along the *c* direction. The structure is overlapped with the cryoEM image shown in 2. (4) Fourier transform of the image in 2. Reflections are visible at 1.5 Å [73].

Soft materials are beam sensitive, structurally heterogeneous, and often used in the presence of liquids and gases. For all of these reasons, the many variants of cryoEM are important for understanding the structure property relationship for soft materials. Table I is adapted from a recent review of high-resolution imaging of unstained polymers by Jiang and Balsara [76]. It lists examples of recent cryoEM studies of microphase separated or crystalline soft materials, with comparisons of imaging methods, results and details for each study.

Table 1. Examples of cryoEM studies of microphase-separated or crystalline soft materials [76].

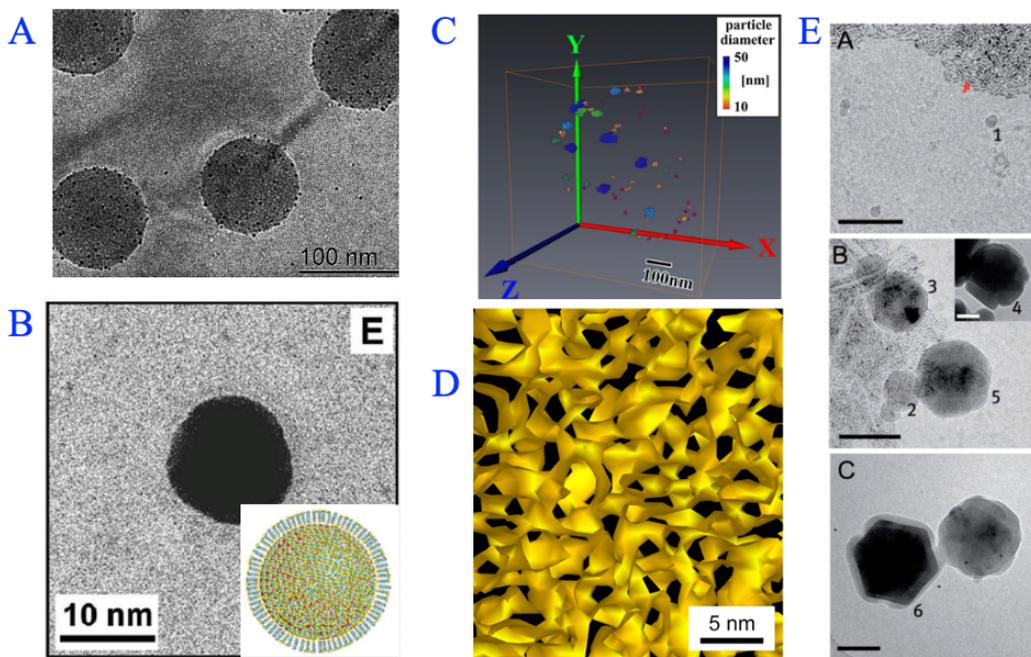
Materials	Morphology	Post-processing	Imaging method	Contrast	Accumulative electron dose (e Å ⁻²)	Resolution (Å)	Retrieved information	Reference
(1) Poly(ionic liquid)	Micelle	Tomographic 3D reconstruction	Cryo-ET	Phase	100 e Å ⁻² for tilt series	30	Morphological changes and chain conformation	[77]
(2) Conjugated polymer P3HT	Crystalline nanowire	Tomographic 3D reconstruction	Cryo-ET	Phase	2000 e Å ⁻² for tilt series ³	17	Ordered and disordered regions in nanowire	[60]
(3) Diblock copolypeptoid	Vesicle	Sorting and averaging	CryoEM	CTF corrected phase	30 e Å ⁻² per image	10	Membrane structure, chain conformation and inhomogeneity	[78]
(4) Lipid	Vesicle	Tomographic 3D reconstruction and sorting	CryoEM	CTF corrected phase	74-100 e Å ⁻² for tilt series	10	Membrane structure, chain conformation and inhomogeneity	[79]
(5) Dendronized polymers	Micelle	Single particle 3D reconstruction	Cryo-ET	CTF corrected phase	180 e Å ⁻² for tilt series	10	Backbone conformation	[80]
(6) MOF, ZIF-8	3D crystal	Direct imaging	CryoEM	CTF corrected phase	6.8 e Å ⁻² per image	1.8	Lattice structure	[64]
(7) Polypeptoid block copolymer	Crystalline nanosheet	Averaging	CryoEM	CTF corrected phase	20 e Å ⁻² per image	1.5	Lattice structure, backbone and side chain packing	[73]
(8) Conjugated polymer PBTTT	Microphase separation	Reconstructed diffractions	Cryo-4D-STEM	Diffraction	100 e Å ⁻² per diffraction	100/3.7 ²	Orientation of crystals, the degree of crystallinity and grain boundary	[81]

4. Processes and Chemical Pathways in Energy Materials

Processes and chemical pathways in energy materials inherently lie at the edge of stability, often involving metastable or transient states. CryoEM can be critical for mitigating the effects of electron beam irradiation in energy materials and devices. For example, energy materials include reactive materials for energy storage and conversion (lithium, sodium metal anodes, lithiated graphite and silicon, etc.), soft materials for membrane technologies, interphase materials after wet-electrochemical cycling, and catalysts that are extremely small (<3-5nm) or contain ligands and molecules.

CryoEM has been used to study catalysts containing surface adsorption of ligands or catalysts embedded in polymers [82, 83]. For example, cryogenic imaging revealed the correlation of Au-NPs and polyelectrolyte chains on spherical polyelectrolyte brushes (SPBs) (Figure 10-A). Cryogenic imaging of ligands on metal nanoparticles has contributed significantly to the design of new hybrid catalysts (Figure 10-B) [84, 85]. In electrocatalysis, TEM imaging and chemical analysis at LN₂ temperature has deepened understanding of the structure of Nafion™ ionomer molecules and their correlations with catalysts [86, 87]. A significant application of cryoEM is the enabling of tomographic scanning transmission electron microscopy (STEM) imaging, which requires an extended acquisition time for beam-sensitive electrocatalysis materials. Cryo-tomographic EM has visualized the positions of individual catalysts in 3D before and after cycling, thereby providing critical evidence for catalyst stability and durability. Figure 10-C shows the reconstructed 3D image of Au nanoparticles (NPs) on TiO₂ support using cryo-STEM tomography, reconstructed from 70 images with a dwell time of around 26 seconds for each image [88]. With the use of cryo electron tomography, even the hydrated as-cast Nafion nanostructure can be revealed successfully, as shown in Figure 10-D [89]. Another unique application of cryoEM for catalyst research is to elucidate the mechanism of liquid phase synthesis. By utilizing a LN₂ cryo-holder, scientists are able to track the structural and morphological evolution of materials in solution (Figure 10-E) [90, 36]. As the diffusion in such a set-up is reduced and localized, this method allows more detailed synthesis steps to be revealed at a sub-nanometer resolution and a sub-second time resolution.

While the examples above demonstrate how cryoEM enabled the imaging of beam-sensitive energy materials at a nanometer spatial resolution, atomic-resolution cryoEM is more challenging. The importance of atomic-resolution characterization for energy materials is paramount, given the fact that atomic interfaces and defects often dominate the performance of a material. For example, in solid electrolyte materials for lithium metal batteries, solid electrolytes can be considered superionic conductors that contain a large number of mobile ions, rendering them extremely sensitive to the electron beam. Recently, it was demonstrated that the atomic structure and chemistry of La₃Li₇O₁₂Zr₂ (LLZO), a typical solid electrolyte material, can be revealed with the aid of cryoEM imaging, and the interfaces of LLZO, even those containing a significantly large amount of mobile lithium ion and protons, can be imaged at an atomic resolution, as shown in Figure 11-A, providing insight into the application of LLZO in aqueous batteries [91]. Cryo-atomic-resolution TEM imaging has also been applied to the study of polymer-Li metal interfaces. Huang et al. [92] recently revealed the formation of a solid–solid interphase layer between Li metal and PEO by using atomic-resolution cryoEM imaging.



Sources: (B) Reprinted with permission from Wang, Y., et al. 2012. "Nucleation and island growth of alkanethiolate ligand domains on gold nanoparticles." *ACS Nano* 6: 629-640. <https://doi.org/10.1021/nn204078w>. Copyright 2012 American Chemical Society. (C) This article was published in *Ultramicroscopy*, Vol 110: S. Sueda, K. Yoshida, N. Tanka, "Quantification of metallic nanoparticle morphology on TiO₂ using HAADF-STEM tomography," pp. 1120-1127, Copyright Elsevier 2010. (E) From Pouget, E.M., et al. 2009. "The initial stages of template-controlled CaCO₃ formation revealed by Cryo-TEM." *Science* 323: 1455-1458. <https://doi.org/10.1126/science.1169434>. Reprinted with permission from AAAS.

Figure 10: CryoEM images of Au NP SPB composite particles. (A) The Au NPs are visible as attached black dots near the surface [82]. (B) A cryoEM image of an Au NP after complete replacement of one by 11-MU. Space-filling models of the thiolate ligands (not observed by TEM) are shown in F [84]. (C) Segregated Au particles map. The colored particles show the diameter of each particle [88]. (D) Reconstructed hydrated as-cast Nafion structure [89]. (E) CryoEM images of the early, intermediate, and mature stages of the formation of template-controlled CaCO₃ [36].

Compared to cryoEM imaging, studies of atomic-resolution cryo-STEM imaging are very limited. A recent paper has shown the imaging of lithiated MoS₂ monolayer using cryo-STEM, providing insight into the lithiation mechanism of MoS₂ being used as an anode material [93] (Figure 11-B). Liu, et al. [94] demonstrated the possibility of atomic-resolution STEM imaging of LLZO and protonated LLZO (Figure 11-C), revealing the structural and chemical stability of LLZO being used as a protection layer for lithium metal in aqueous batteries. These studies demonstrated the feasibility and the value of cryo-STEM imaging and spectroscopy at atomic resolution. However, such a limited number of related publications also reflects the challenges related to atomic-resolution cryoEM for energy materials. Atomic-resolution cryo-STEM imaging usually requires a higher dose and cleaner specimens than atomic-resolution cryoEM imaging. Nevertheless, the advantages of atomic-scale STEM imaging, e.g., simultaneous electron energy loss spectroscopy (EELS), energy-dispersive X-ray spectroscopy (EDS) analysis, and chemical information from contrast, provide a strong driver for future cryo-STEM experiments.

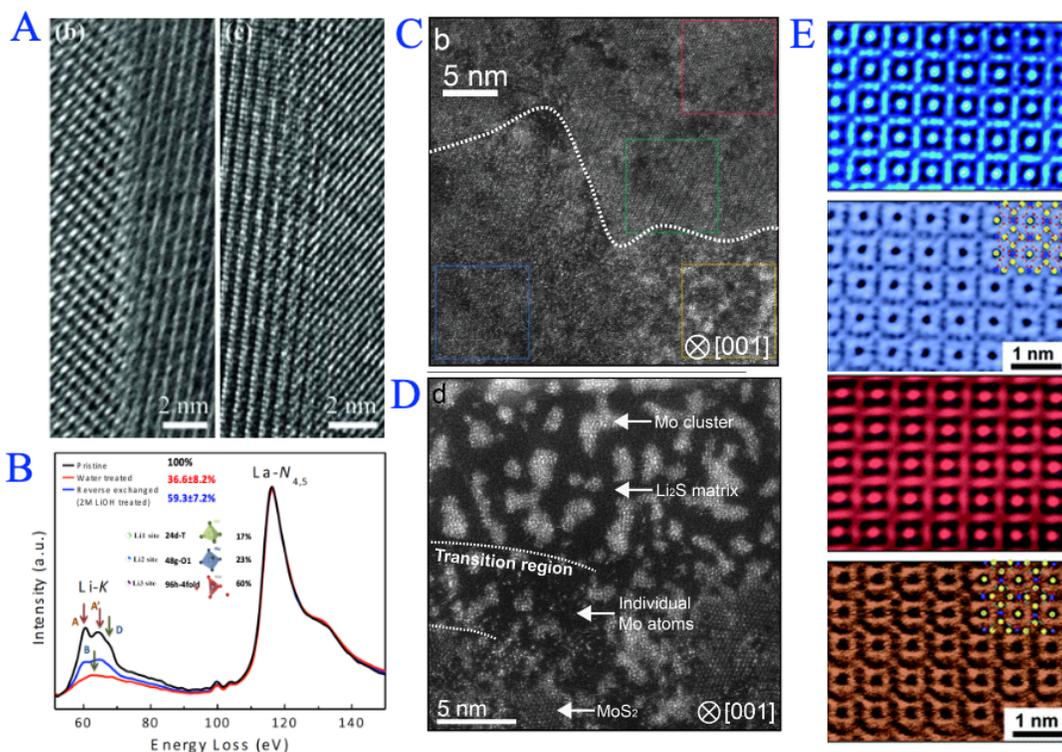


Figure 11: (A) HRTEM images of a grain boundary in a pristine LLZO specimen (left), and after Li⁺/H⁺ exchange in water (right). (B) EELS analysis of pristine, protonated, and reverse proton exchanged LLZO [91]. (C) Annular dark-field cryo-STEM image of partially lithiated bilayer MoS₂ and (D) monolayer MoS₂ [93]. (E) Atomic-resolution STEM dark-field and bright-field images of pristine LLZO (top two images) and proton exchanged LLZO (bottom two images) [94].

Perhaps the most dramatic impact of cryoEM on the research of energy materials in recent years has come from integrating cryo-FIB and cryo-transfer methods, which has paved the way for the investigation of liquid–solid interfaces in lithium metal anode in rechargeable batteries. These systems are unstable in air, potentially oxidizing or absorbing water. It has been shown that LN₂ can be used as a buffer layer to protect a sample from being exposed to air (although it is exposed to nitrogen in LN₂, the cryogenic temperature limits reactions). Thus plunge freezing and cryo-transfer techniques that have been perfected by the biological community can be used as air-free sample preparation methods for reactive materials. Use of cryo-stages in this way solves two experimental challenges at the same time: reducing sample damage during electron imaging by cooling and protecting the sample from air exposure with cryogenic transfer techniques. Several pioneering studies using these methods have been reported in recent years. In 2017, groups at Stanford University [95] (Figure 12) and the University of California–San Diego [96] (Figure 13) independently reported solid electrolyte interphase (SEI) investigations of electrochemically deposited lithium metal anode using cryo-transfer and cryoEM imaging at high resolution. More recently, nanoscale imaging and spectroscopic studies revealed the drastic differences in the morphology and chemistry of SEI following lithium deposition with different electrolytes. In 2018, a group at Cornell [97] reported for the first time the observation of LiH in SEI and revealed its impact on the microstructure of electrochemically deposited lithium. More recently, the UC–San Diego team [98] reported the discovery of glassy lithium at the very early stage of lithium electro-deposition. All these critical cryoEM observations have provided novel

high-resolution insight to guide improvements in electrolyte design and cell architecture to improve the reversibility of lithium metal anode charging and discharging.

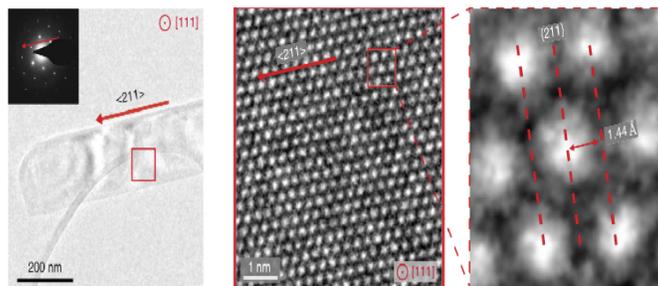


Figure 12: Atomic-resolution cryoEM of Li metal dendrites: Left, TEM images of Li metal dendrite. Middle and right, higher magnification images of the boxed region [95].

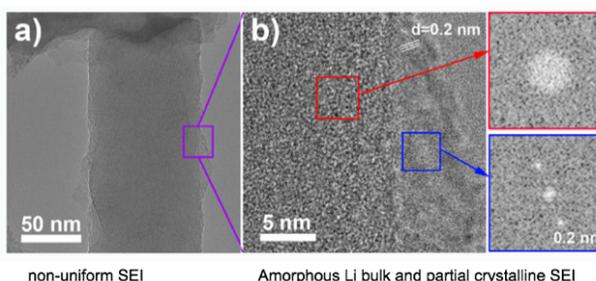


Figure 13: CryoEM (a) image and (b) regional zoomed-in image with the bulk and surface FFT result of the EDLi using conventional carbonate electrolyte [96].

One further advantage of using cryo-transfer as an air-free technique rather than a vacuum transfer holder with current holder technology is that the sample can be heated to 100°C as well as cooled to LN2 temperatures for variable-temperature studies. Another advantage is that cryo-transfer does not require a vacuum transfer holder and a glove box. For example, lead halide perovskites are being considered for energy applications due to their optoelectronic properties. Both hybrid and all-inorganic materials can be synthesized as nanoparticles, nanowires, or nanosheets. Characterization with electron beams or X-rays can result in Pb precipitating out of the crystal, which destroys the long-range order and invalidates the characterization results. Dang et al. [99] systematically examined the degradation mechanism using variable-temperature TEM (−160°C to 40°C). The process was found to proceed by desorption of the halogen species followed by reduction of the Pb^{2+} to Pb^0 . Above −40°C, the Pb^0 diffused and aggregated into metallic clusters, causing local amorphization and disruption of the perovskite structure. Cooling to −60°C suppressed the lead diffusion, minimizing structural degradation.

5. Low-Temperature Phenomena in Quantum Matter

Quantum phenomena such as superconductivity, charge density waves, magnetic phases such as skyrmion lattices, and the physics of devices for quantum information science applications are most commonly studied at cryogenic temperatures [100]. In most cases, these phenomena only exist at low temperatures, and their structure and electronic states are often spatially inhomogeneous [101]. As a result, electron microscopy is uniquely suited to exploring the defects, interfaces, phase transformations and emergent behaviors that impact novel quantum materials—key to future technologies such as quantum information science [102].

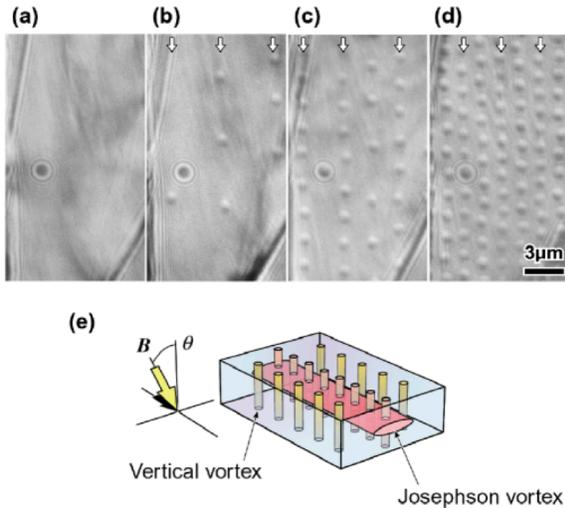


Figure 14: Vortex motion in superconductors: A series of Lorentz micrographs of vortices in a field-cooled Bi-2212 film sample obtained at 50 K with a variable magnetic field B_z perpendicular to the foil and a fixed field B_x D 5mT in-plane. B_z D 0, 0:02, 0:1, and 0:17 mT in a–d, respectively. The arrangement of the vortices in chains is clearly visible. These chains are caused by interactions with horizontal vortices produced by the in-plane field. (e) Schematic of the vortex lines consisting of vertical and Josephson vortices.

A prototypical example of low-temperature phenomena in quantum matter can be found in materials that exhibit the phenomenon of superconductivity. CryoEM using liquid nitrogen and liquid helium has been used to study the dynamics of magnetic vortices in type II superconductors. Type II superconductors do not fully expel magnetic flux, creating “vortices” of flux that create resistance as they move. Understanding and controlling the behavior of these vortices and their interaction with defects (which can “pin” the vortices and allow dissipationless current flow) is therefore of fundamental and technological importance. While other methods can enable observation of vortices, EM is critical to observing real-time vortex dynamics in order to probe interactions between vortices and vortex-defect pinning strength as a function of magnetic field strength and temperature [103, 104]. Using Lorentz microscopy, magnetic vortices were first studied in real time in niobium thin films [103]. Similar observations and confirmation of theories that govern vortex motion in undefected material have been made for MgB₂ [105], and patterning defects in a similar sample enables vortex motion to be funneled and directed [106]. Lattices of vortices can arise from thermal activation [103] or created using arrays of engineered defects [107]. By varying both temperature and magnetic field, the temperature-dependent pinning force and dynamics of pinning can also be studied in a prototypical cuprate high- T_c superconductor [107]. In Tonomura’s work, it is noted that while the interaction of the vortices with large, engineered defects can be observed, the resolution limitations of Lorentz microscopy inhibit observing atomic-scale native defects, and pinning due to these defects can only be inferred [107]. However, *in situ* experiments have revealed dramatic examples of vortices, including vortices in high T_c superconductors [107] (Figure 14). Electron holography can provide better spatial resolution [108] but greatly increases experimental complexity and post-

experiment data analysis. Extensive work on atomic-resolution microscopy by Zhu and colleagues at Brookhaven National Laboratory has been performed at 10 K over a number of years [101], including atomically resolved imaging [109] and spectroscopy. For instance, Zhao et al. [110] recently performed EELS at 10 K using a liquid He stage to understand the role of the substrate in the behavior of the superconductor FeSe (see Figure 15).

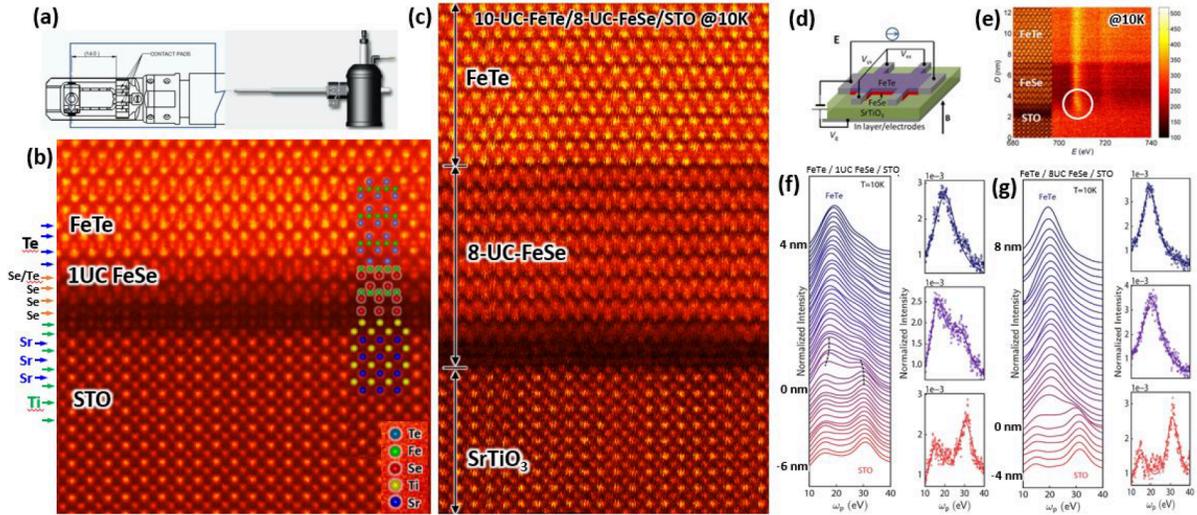


Figure 15: First atomically resolved imaging and EELS at 10 K in FeSe/SrTiO₃. (a) The custom-made liquid-He biasing holder by Gatan-UK for Brookhaven in 2014. (b) and (c) STEM images showing the cross-section heterostructure of the 10-unit-cell (UC) capping layer FeTe on 1UC-FeSe (b) and 8UC-FeSe (c) grown on SrTiO₃. The interfacial structure with an additional Se layer is identified. (d) Schematic of the same sample as a gate-tuned Hall-bar device for transport measurement to understand interface enhanced superconductivity. (e) Fe-L3 EELS map of the film showing a blue-shift within 2UC (marked by the circle) from the FeSe/STO interface. (f) and (g) Valence EELS across the interfaces from 1UC-FeSe/STO (f) and 8UC-FeSe/STO (g). Black dashed lines in (f) indicate a redshift in plasmon peaks near the interface [110].

Charge-ordered and spin-ordered phases result from the correlated interplay of electronic and vibrational excitations in complex materials. The emergence of charge- and spin-ordered phases is closely related to physical properties such as superconductivity in oxides. Cryogenic electron imaging can reveal dynamics in these quantum materials that are not readily observed in other ways. For instance, recent studies of spin-reorientation transitions [111, 112] and an unusual memory effect [113] highlight how the implementation of cryo-stages with low-energy electron microscopy (LEEM) and spin polarized low-energy electron microscopy (SPLEEM) provide a valuable capability to investigate phase formation *in situ*. The Kourkoutis group at Cornell has recently pushed the resolution of cryoEM imaging through improvements in image registration [114] that allowed for not just direct, quantitative imaging of charge order in a manganite, but also observations of their evolution [115] (Figure 16) and examination of nanoscale coexistence of site- and intermediate-order [116]. Studies such as this are key to answering important questions for low-temperature condensed matter physics, such as how new phases emerge from electron-lattice coupling.

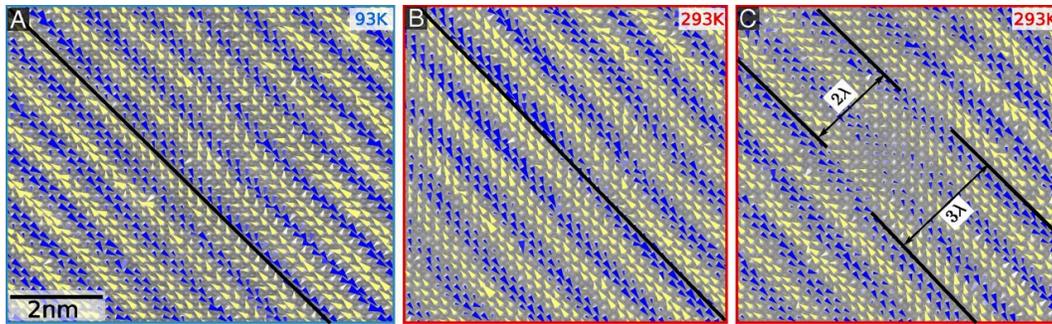
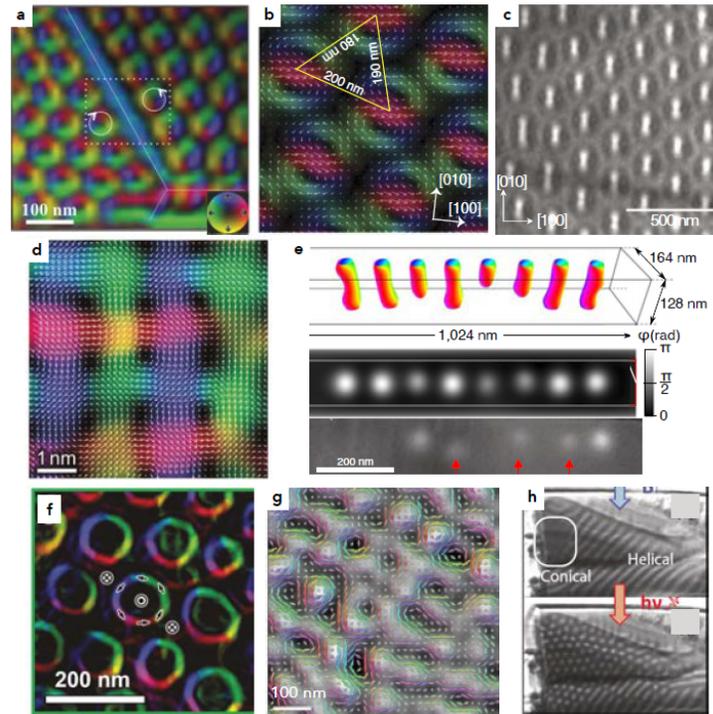


Figure 16: Atomic resolution mapping charge–lattice coupling in manganite, obtained by cryo-transmission electron microscopy, showing local variations and disorder in an incommensurately ordered charge–stripe phase. (a) and (b) Picometer-scale shear deformations of striped modulations at (a) 93 K and (b) 293 K. Shear deformation appears as a bending of the wavefronts. The black line traces the direction perpendicular to the wave vector and helps visualize the deformation of the wavefront. (c) Stripe dislocation at 293 K, in which one wavefront terminates abruptly. The color (i.e., blue or yellow) indicates the direction of modulation, and the size of the arrowheads the magnitude, with the largest distortions approximately 14 pm [115].

Understanding the low-temperature behavior of magnetic materials can help in the search for new materials operating at technologically relevant temperatures. Electron beams undergo phase shifts and deflections when passed through magnetic fields, making them well suited to sensitive, high spatial resolution imaging of the magnetic induction in and around a magnetic sample. As compared to synchrotron-based X-ray magnetic circular dichroism spectromicroscopies [117], TEM has superior spatial resolution and sensitivity to the magnetization components perpendicular to the electron beam that enable the identification of topological states, their chirality and topology. However, imaging must be carried out under well-controlled conditions of the objective lens so that the influence of the lens field is understood. At cryogenic temperatures, magnetic materials have been studied via off-axis holography [118–121], differential phase contrast [122–124], or in-line holography, where the phase is recovered from a through-focal series of Fresnel mode Lorentz images. In-field measurements of magnetization reversal can be conducted by leveraging the objective lens to generate an out-of-plane field of up to hundreds of milliTesla or by use of specialized holders to create in-plane fields. Single-tilt and double-tilt holders offer the means to retrieve the magnetization component normal to the sample plane and, if sufficiently stable, to reconstruct the 3D spatial distribution of the magnetization vector field via vector field tomography [125–127].

CryoEM has allowed for the visualization and manipulation of topological states such as skyrmions [128–130], biskyrmions [131], antiskyrmions [132], magnetic monopoles [133], and bobbars (skyrmion tubes terminated by Bloch points) [134]. Figure 17 shows some examples of these. CryoEM has been applied to complex topological states [135, 136] in metals and oxides at variable temperatures (down to 6 K) [128, 133, 137–139], magnetic fields (up to 300 mT), and at time scales ranging from milliseconds (pump-free) [140, 141] to picoseconds (pump-probe) [142, 143]. Incorporating optical lasers has allowed for laser-induced skyrmion writing and erasing [142, 143]. The development of highly sensitive direct electron detectors has provided a means to reduce the heat load and study the electron beam induced heat-driven rotational modes of skyrmion lattices [140], image distinct topological phases in B20 Fe-Mn-Ge [133, 144] including magnetic monopoles on the order of 1 nm [133], visualize skyrmions in 2D van der Waals materials [139], and monitor disordered skyrmion phases in amorphous materials [141] and domain boundary structures in skyrmion

lattices [123]. CryoEM has also been applied to understanding the nature of phase transitions in magnetic oxides [145].

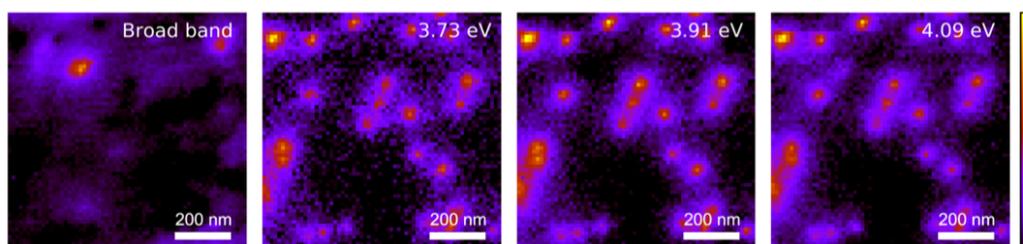


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Figure 17: CryoEM for visualizing topological magnetic states in single-crystals, 2D van der Waals materials, and amorphous materials. (a) Skymions near crystallographic grain boundaries in B20 FeGe (220 K, 100 mT) [120]. (b) Biskyrmions in La_{1.37}Sr_{1.63}Mn₂O₇ (20 K, 0.35 T) [131]. (c) Electron intensity of antiskyrmions in Mn_{1.4}Pt_{0.9}Pd_{0.1}Sn (100 K, 0.33 T)[132]. (d) Cubic skyrmion lattice with magnetic monopoles in B20 MnGe (20 K, 2.4 T) [133]. (e) Modeled bobbers (top) with simulated electron holography contrast (middle) and experimental data (bottom) in B20 FeGe (95 K, 300 mT, 200 mT field-cooled state) [134]. (f) Skymions in van der Waals Cr₂Ge₂Te₆ (17 K, 11.7 mT, 50 mT field-cooled state)[139]. (g) Electron phase and in-plane magnetization of disordered skyrmion phase in amorphous Fe_{0.52}Ge_{0.48} (155 K, 32 kA/m) [141]. (h) Laser-induced skyrmion lattice formation in B20 FeGe (233 K, 40 mT, 60 fs optical pulse with 1.55 eV and 2 mJ/cm²) [142]. (a) and (e) are visualized with electron holography, (b), (c), (d), and (f) with Lorentz microscopy and TIE, and (g) with Lorentz microscopy and GSR. The color wheel contrast corresponds to the in-plane magnetization component. The out-of-plane magnetic fields are generated by the objective lens.

Nanoscale optical measurements take advantage of local confinement and resonance [146], and thus electron microscopy is an attractive approach to identifying the defects and features of optical signals, especially the simultaneous local characterization of optical response and underlying physical and electronic structure. The lower the temperature for electron-enabled measurements such as cathodoluminescence (electron-beam excited luminescence), the less the contamination, sample damage, nonradiative recombination, and exciton ionization. Bourrellier

and colleagues [147] used low-temperature (150 K) cathodoluminescence measurements in a STEM instrument to identify a new, extremely bright UV single-photon emitter (4.1 eV) in hexagonal boron nitride. shows hyperspectral cathodoluminescence maps from this work confirming high spatial localization of the emission, indicating a point defect origin [147]. Cryogenic single-particle photoluminescence (PL) spectroscopy has recently been used [148] to directly observe the heterogeneous photophysical states present in a population of luminescent particles. In this study, Bourrellier and colleagues performed cryogenic electron tomography and correlated single-particle information directly with photoluminescence spectroscopy.



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Figure 18: Spatially resolved cathodoluminescence intensity maps of bright, ultraviolet single-photon emitters in hexagonal boron nitride measured in a scanning transmission electron microscope at 150 K. The perfect spatial correlation of the emission peaks at 3.73, 3.91, and 4.09 eV suggests the same underlying physical origin, in this case phonon replica of the 4.09 eV peak [147].

6. Summary

As this factual document and other recent reviews [150–154] on the subject have shown, cryogenic electron microscopy has had an important impact on a large and diverse number of materials and systems of direct relevance to fundamental energy sciences. Yet there are still entire fields of study to be explored with cryogenic electron microscopy. For instance, quantum information science devices (qubits) operate at cryogenic temperatures but have yet to be characterized at these temperatures with electron microscopy. The tools and techniques for cryogenic electron imaging and spectroscopy are still relatively limiting, as commercial electron microscopes are optimized for room temperature operation where they achieve their best performance and stability. Nevertheless, cryoEM is a unique and powerful method for characterizing materials that are sensitive to electron beam irradiation or that only exist at cryogenic temperatures, and the development and application of cryoEM techniques are critical for addressing the needs of fundamental energy science research.

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