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# The Latest Progress of Cryo Electron Microscopy Technology in Protein Structure Analysis

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**Abstract** With the rapid development of Cryo Electron Microscopy (Cryo EM) technology, significant progress has been made in protein structure analysis, opening up new avenues for research in the fields of biology and medicine. This study aims to review the latest progress of cryo electron microscopy technology in protein structure analysis, and explore its research significance and application prospects. Through in-depth analysis of technological optimization, method innovation, and its application in the study of complex biological macromolecular structures, it was found that cryoelectron microscopy technology not only improves resolution and signal-to-noise ratio, but also successfully analyzes various complex protein structures, providing powerful tools for research in the fields of biology and medicine. The development of cryoelectron microscopy technology not only deepens the understanding of protein structure and function relationships, but also provides new ideas and methods for drug development and disease treatment. Therefore, further promoting the development and application of cryoelectron microscopy technology is of great significance for promoting progress in the field of life sciences.

**Keywords** Cryoelectron microscopy; Protein structure analysis; Advantages; Technological progress; Application prospect

As an important carrier of life activities, protein's structure determines its functional performance. Deeply analyzing the structure of proteins is of great significance for understanding the essence of life activities, revealing the pathogenesis of diseases, and developing new drugs (Walport et al., 2021). In the field of biology, protein structure analysis helps to reveal the interactions between biomolecules and elucidate the molecular mechanisms of complex biological processes such as cell signaling and metabolic pathways. In the field of medicine, protein structure analysis can provide key theoretical basis for early diagnosis, precise treatment, and new drug development of diseases. Therefore, research on protein structure analysis has always been a hot topic and frontier in the fields of biology and medicine.

Cryoelectron microscopy, also known as cryoelectron microscopy, is a technique that uses an electron microscope to perform high-resolution imaging of biological samples in a frozen state (Thompson et al., 2016). The principle is to quickly freeze biological samples, fix biological molecules in a conformation close to physiological state, and then scan the sample with an electron beam to obtain structural information of the sample. This technology not only has atomic level resolution and can reveal the fine structure of biomolecules, but also the sample preparation is relatively simple, without the need for complex crystallization or staining treatment, and is closer to the natural state of biomolecules (Mitra, 2019). More importantly, cryo electron microscopy technology can capture the dynamic conformational changes of proteins under physiological conditions, thereby more comprehensively revealing the functional mechanisms of proteins.

Freezing electron microscopy technology has shown unparalleled advantages in protein structure analysis due to its unique principles and characteristics. It not only expands the scope of protein structure research, but also provides strong technical support for scientists to have a deeper understanding of the functional mechanisms of proteins. With the continuous progress and improvement of technology, cryo electron microscopy technology will play a more important role in future protein structure research (Thompson et al., 2016).

This study aims to explore the latest advances in protein structure analysis using cryo electron microscopy technology, and to reveal the potential and value of cryo electron microscopy technology in the field of protein structure research. This study reviews the development history of cryo electron microscopy technology, outlines its advantages and applications in protein structure analysis, and looks forward to the future development trends of cryo electron microscopy technology in protein structure analysis, in order to provide useful references and insights for researchers in this field. Through this study, it is expected to promote the further development of cryoelectron microscopy technology and provide more efficient and accurate tools and methods for protein structure research in the fields of biology and medicine.

## 1 The Development History of Cryoelectron Microscopy Technology

### 1.1 Limitations of early cryoelectron microscopy techniques

The development history of cryoelectron microscopy technology has a long history, going from early exploration and experimentation to today's maturity and widespread application. In the early stages, cryoelectron microscopy technology faced many limitations and challenges. Chlanda and Locker (2017) discussed how the development of electron microscopy (EM) technology for biological samples from the 1940s to the 1950s changed people's understanding of eukaryotic cell architecture, and how in the following decades, despite the emergence of significant new methods, EM technology seemed to have entered a dormant phase. The discovery of rapidly frozen samples gave birth to the world of cryo EM, and in the past 15-20 years, cryo EM has aroused great interest due to significant technological advancements. In the 1960s, cryo electron microscopy imaging technology began to develop as a branch of scanning electron microscopy imaging technology.

However, before the 1970s, the application of cryoelectron microscopy technology was mainly concentrated in the fields of polymer chemistry and biochemistry, and its imaging effects were not ideal. This was mainly due to technical limitations at the time, such as low instrument resolution and severe radiation damage, which made it difficult to obtain high-quality cryo electron microscopy images.

In the 1970s, researchers began to explore methods to improve the resolution of cryo electron microscopy samples. The resolution of the sample has been improved to a certain extent through gas enhanced freezing technology, but there are still many limitations. At this time, cryo electron microscopy technology still faces difficulties in sample preparation and unstable imaging effects, which greatly limits its application in protein structure analysis and other fields.

In the 1980s, with the rapid development of computer technology, cryoelectron microscopy imaging technology was significantly improved. Taylor and Glaeser (2008) found that advances in computer technology have made image processing and analysis more precise and efficient, thereby improving the resolution and reliability of cryoelectron microscopy images. At the same time, researchers are constantly optimizing the freezing sample preparation technology to reduce the formation of ice crystals, allowing the samples to maintain better structural integrity in the frozen state.

Despite significant progress made in the 1980s, cryoelectron microscopy technology still faces some challenges. For example, imaging of large biomolecular complexes remains challenging, and the potential radiation damage during the imaging process has not been fully resolved.

### 1.2 Technological breakthroughs and improvements

In the development process of cryo electron microscopy technology, technological breakthroughs and improvements have played a crucial role, driving its application in protein structure analysis and other fields to continuously advance. Tsuchiya (2019) found that early cryoelectron microscopy techniques faced the problem of ice crystal formation during the freezing process of samples, which greatly affected the quality of imaging. Researchers developed rapid freezing and vitrification techniques, effectively reducing the formation of ice crystals and improving the resolution of cryoelectron microscopy imaging.

Wang et al. (2020) argue that traditional electronic detectors are prone to damage when subjected to high-energy electron beam bombardment, which reduces the signal-to-noise ratio of imaging. However, with the invention and continuous optimization of electronic detectors, it is now possible to directly detect the number of electrons. The new electronic detectors have improved the imaging signal-to-noise ratio, continuously increasing the resolution of cryo electron microscopy and supporting the analysis of fine structures of biological macromolecules such as proteins.

With the rapid development of computer technology, researchers are able to efficiently process and analyze cryoelectron microscopy images using more advanced algorithms, according to Vilas et al. (2022). These algorithms can automatically identify and correct errors in images, improving the accuracy and reliability of imaging. At the same time, the improvement of computing power also enables researchers to process larger datasets, accelerating the application process of cryo electron microscopy technology in protein structure analysis and other fields.

### 1.3 Recent technological progress

Recently, cryoelectron microscopy technology has made significant technological progress in hardware, software, sample preparation technology, and application fields. These advances not only improve the performance and efficiency of cryoelectron microscopy technology, but also provide more accurate and efficient tools for research in fields such as protein structure analysis. With the continuous progress and improvement of technology, cryo electron microscopy technology will play a more important role in the future.

Zhao et al. (2019) analyzed the structures of AMPA receptors in 10 different complexes in the brain using cryo EM, revealing the diversity of subunit composition and spatial configuration, providing new insights into the role of these receptors in rapid excitatory synaptic transmission.

Watson et al. (2020) used cryoelectron microscopy technology to determine the structure of E. coli 70S ribosomes (Figure 1), with a global resolution of 2.0 Å. This work reveals the unambiguous localization of protein and RNA residues, their detailed chemical interactions, and chemical modifications, providing in-depth insights for future structural analysis.

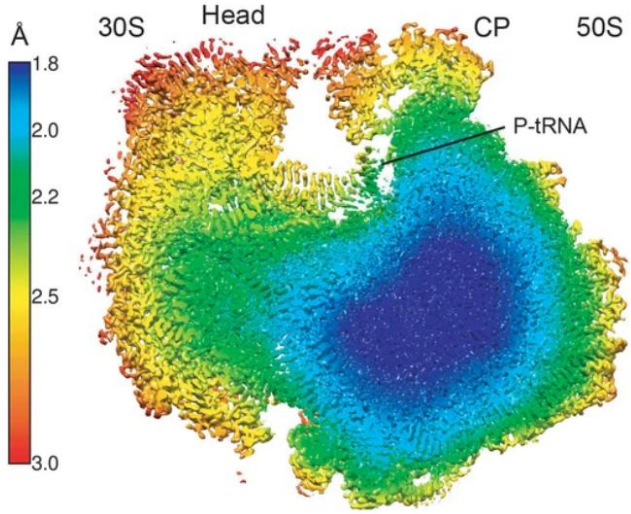


Figure 1 Section of Local Resolution Image of 70S Ribosome Reconstruction (Watson et al., 2020)

Kordyukova et al. (2023) used a freeze transfer rack equipped with JEOL JEM-2100 transmission electron microscopy to investigate influenza A and B virus strains and their efficacy β- Preliminary cryoelectron microscopy data analysis was conducted on SARS-CoV-2 inactivated with propionate. These analyses help to distinguish virus particles with and without nucleocapsids, observe the lipid bilayer of the virus envelope, recognize influenza virus surface antigens and M1 protein layers, as well as the different morphologies of SARS-CoV-2 spinous processes.

## 2 The Advantages of Cryo Electron Microscopy Technology in Protein Structure Analysis

### 2.1 High resolution imaging capability

The advantage of cryo electron microscopy technology in protein structure analysis is first reflected in its high-resolution imaging ability. Although traditional X-ray crystallography methods can also provide information on protein structure, their resolution is often limited by factors such as crystal quality and radiation damage. And cryo electron microscopy technology can image individual protein molecules or complexes under conditions close to physiological conditions, without the need for crystallization, thus avoiding the limitations of crystal formation (Figure 2).

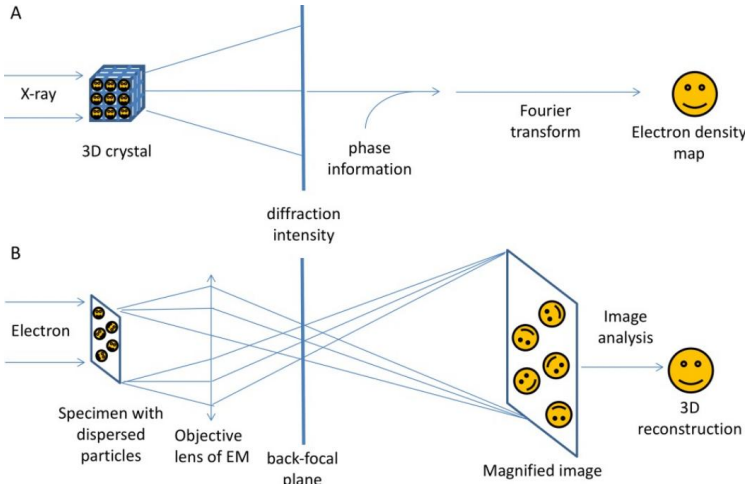


Figure 2 Technical differences between X-ray crystallography and single particle cryo electron microscopy (Wang and Wang, 2017)
Note: A: X-ray crystallography solves the physical and mathematical principles of structure; B: The physical and mathematical principles of EM in solving structures

Freezing electron microscopy utilizes low-temperature rapid freezing technology to fix protein samples in structures close to their physiological states, effectively reducing structural changes during sample preparation. Mielanczyk et al. (2014) focused on different freezing preparation methods, starting with sample size dependent vitrification methods, followed by cryo FIB as a potential alternative to cryo electron microscopy (CEMOVIS), as well as discussions on cryo substitution and resin embedding for structural analysis. This demonstrates the crucial role of cryo EM and low-temperature rapid freezing technology in sample preparation, providing electron microscopy with near native sample observations.

By combining advanced electron microscopy technology and image processing algorithms, cryo electron microscopy can achieve atomic level resolution and clearly reveal the fine structure of protein molecules. Yip et al. (2020) reported the use of a newly developed electron microscope to obtain a 1.25 Å resolution apoferrin structure. This result is almost twice the 3D information content of the current world record reconstruction (1.54 Å resolution). This study demonstrates significant progress made by cryo electron microscopy in structural biology, which now allows for direct visualization of individual atoms in proteins.

The high-resolution imaging capability enables cryo electron microscopy to capture subtle structural features within protein molecules, such as the orientation of amino acid side chains, arrangement of hydrogen bonds, and intermolecular interactions. Liu and Yeates (2019) designed protein skeletons to bind and symmetrically display 12 small 26 kDa proteins (green fluorescent protein GFP), achieving cryo EM imaging at 3.8 Å resolution. This modular protein skeleton design can bind and display multiple proteins through small amino acid sequence changes.

The high-resolution imaging ability of cryo electron microscopy technology also enables its application in a wider range of protein system studies. Whether it is prokaryotes or eukaryotes, whether it is a single protein or a large complex, cryoelectron microscopy can provide high-resolution structural information. This provides a powerful

tool for researchers to explore the structure and function of proteins in different biological systems.

### 2.2 Wide applicability

Cryo EM technology plays an important role in the field of protein structure analysis due to its unique advantages, especially its wide range of applications, highlighting the value of this technology. Shoemaker and Ando (2018) delved into the significant advantage of cooling without sample crystallization, which overcomes the main obstacles in traditional X-ray crystallography and enables the structural analysis of proteins or macromolecular complexes that are difficult to crystallize. This technology is particularly suitable for studying large molecular complexes, such as viruses, ribosomes, and membrane protein complexes, which play crucial roles in biological processes and can be observed and analyzed under conditions close to their physiological states.

By rapidly freezing samples at extremely low temperatures, cryo electron microscopy can lock molecules in their almost pristine state, preserving their true dynamic structure and morphology. Baker et al. (2017) discussed how to use electron freeze chromatography (cryoET) of frozen hydration samples to determine the structure of macromolecular complexes in their native environment, thereby avoiding possible sample damage or structural changes during crystal formation. This ability to maintain the original ecological state of biomolecules, combined with high-resolution imaging of large molecules and complex assembly structures, provides a powerful tool for a deeper understanding of biological mechanisms.

The flexibility and diversity of cryo electron microscopy technology have further expanded its applicability, enabling it to capture the dynamic processes of molecular transitions between different states and reveal how molecules perform biological functions by changing their shape. Trabuco et al. (2009) developed the Molecular Dynamics Flexible Fit (MDFF) method to combine high-resolution atomic structures with cryo EM images, representing the atomic model of the conformational states captured by cryo EM. This method has been successfully applied to ribosomes, a ribonucleoprotein complex responsible for protein synthesis.

Combined with other biophysical and biochemical technologies, cryo electron microscopy can provide comprehensive information on the structure, dynamics, and function of biomolecules, thereby promoting a comprehensive understanding of biological processes from atomic level to cellular and even tissue level. These advantages make cryo electron microscopy a powerful tool for understanding the mysteries of life sciences, and its wide applicability provides unlimited possibilities for biomedical research and drug development.

### 2.3 Ability to analyze proteins with different conformations

Freezing electron microscopy technology is particularly adept at handling sample heterogeneity, able to identify different conformational states from tens of thousands of protein molecule images. This single particle analysis (SPA) technique is crucial for understanding how proteins participate in biological processes in different conformational states. Freezing electron microscopy does not require protein crystallization and can be directly analyzed in its natural state, which is particularly important for proteins that are difficult to crystallize and have multiple conformations.

Ki et al. (2021) introduced the nanoparticle assisted cryo EM sampling (NACS) method to access the conformational distribution of protein molecules. By measuring the distance between two gold nanoparticles as a structural parameter, various protein conformations can be captured even for small or disordered proteins that are typically inaccessible through cryo EM.

Jonić Reviewed with Vénien Buryan the methods and latest developments of cryo EM for structural analysis of protein and biomolecular complexes. These complexes are either too large or too heterogeneous to be studied through traditional X-ray crystallography or nuclear magnetic resonance (NMR), and these latest developments provide exciting opportunities for determining the three-dimensional structure of macromolecular complexes.

Nakane et al. (2020) obtained human membrane proteins using a novel electronic source, energy filter, and camera β The 1.7 Å resolution cryo EM reconstruction of 3 GABA A receptors provides a true atomic resolution view,

demonstrating the powerful ability of cryo EM in capturing different protein conformations and providing atomic level resolution structures.

In addition, cryo electron microscopy can not only capture the static structure of proteins, but also reveal their dynamic transitions between different states, providing valuable information for understanding protein responses to biochemical signals or interactions with other molecules. By combining the structural data of cryo electron microscopy with data from other biochemical and biophysical technologies, scientists can comprehensively understand the function of proteins from multiple perspectives, thereby revealing their roles in complex biological processes.

## 3 Application of Cryo Electron Microscopy Technology in Protein Structure Analysis

### 3.1 High resolution protein structure analysis

The application of cryo electron microscopy (Cryo EM) in the field of protein structure analysis marks a significant advancement in biochemical and molecular biology research, especially in achieving high-resolution protein structure analysis. This technology enables scientists to reveal the three-dimensional structure of proteins with near atomic level clarity, thereby gaining a deeper understanding of their biological functions.

Freezing electron microscopy technology is also particularly suitable for analyzing the heterogeneity and dynamics of proteins, capturing the instantaneous state of protein transitions between various conformations. This is crucial for understanding how macromolecular machines and complexes perform their functions through precise assembly and dynamic changes in their components. In the study of ribosomes, membrane protein complexes, and viral particles, cryoelectron microscopy has become an irreplaceable tool. For example, Yi (2018) proposed a method that combines microfluidic single-cell extraction with electron microscopy single particle analysis to characterize protein complexes from individual Caenorhabditis elegans embryos, revealing the structure of ribosomes directly from single embryo extracts.

In addition, the contribution of cryo electron microscopy to drug discovery and design cannot be underestimated. By providing high-resolution structural images of target proteins, it provides drug designers with the basis for designing highly specific drugs that can more effectively bind to target proteins, improve efficacy, and reduce side effects. In the development process of antiviral drugs and cancer therapeutic agents, cryo electron microscopy has shown its enormous potential.

Renaud et al. (2018) found that historically, the application of cryo EM in drug discovery has been extremely limited by the minimum size of the structures it can be used to study and the resolution of the images. However, the development of direct electronic detectors and the latest advances in more effective computational image analysis techniques are overturning the practicality of cryo EM, leading to the explosion of high-resolution structures assembled with a large number of large macromolecules. These advances enhance the hope that single particle cryo EM may soon become an important tool in drug discovery, especially if they can make it possible to determine the structure of "difficult to handle" targets that currently cannot be analyzed by X-ray crystallography.

By analyzing the multi protein interactions that make up large complexes, cryo electron microscopy revealed the mechanisms of complex biological processes such as intracellular signal transduction networks, protein synthesis, decomposition, and pathogen invasion. For example, Chen et al. (2020) discussed the method of using generative adversarial networks (a form of artificial intelligence) to denoise individual particles, which effectively restores the global structural information of synthetic and real cryo EM data, providing assistance in evaluating individual particles from noisy original images. With the continuous advancement of detector technology, image processing software, and sample preparation methods, the resolution and application range of cryo electron microscopy continue to expand, making it the preferred technology for biomolecular structure analysis.

### 3.2 Structural analysis of complex protein complexes

Complex protein complexes, such as those involved in cellular signaling, gene expression regulation, and immune response, are often difficult to cope with traditional structural analysis methods due to their high complexity and

dynamic changes. The application of cryo electron microscopy technology enables scientists to reveal the assembly methods, functional mechanisms, and interactions between subunits of complexes by providing high-resolution structural details, greatly deepening their understanding of these biological processes.

Freezing electron microscopy technology is particularly suitable for capturing the structural changes of complex protein complexes during their biological function execution. These dynamic structural information provide valuable perspectives for understanding how complexes transition between different functional states. In addition, this technology can handle the heterogeneity of samples and identify different conformational states from a large number of images through single particle analysis technology, which is of great significance for studying the regulatory mechanisms and functional diversity of complexes. The unique ability of cryo electron microscopy to analyze asymmetric or extremely large protein complexes provides an unprecedented perspective to explore the secrets of these complex biological entities.

Costa et al. (2017) described the method of using single particle cryo EM for studying the structure of biological complexes, which has now become a mature technology in structural biology competing with X-ray crystallography. The latest progress in EM enables us to determine the structure of protein complexes at a resolution of 3-5 Å, with a wide range of sizes ranging from approximately 200 kDa to hundreds of megadaltons.

Schmidt and Urlaub (2017) explored a method for elucidating the structure of large protein complexes by combining cryo EM and cross-linking mass spectrometry (CX-MS). The proximity information between amino acid residues provided by CX-MS serves as a distance constraint for homology or de novo modeling, providing valuable information for addressing unclear areas in cryo EM images.

Danev et al. (2019) emphasized the rise of cryo EM as a powerful structural determinant technology. Single particle analysis (SPA) is its most prolific branch used to determine high-resolution protein structures in laboratories worldwide.

### 3.3 Analysis of membrane proteins and dynamic protein structures

Membrane proteins are the core components of biological cell functions, playing crucial roles in key processes such as signal transduction, substance transport, and intercellular interactions. However, due to the poor stability and low solubility of membrane proteins in lipid bilayers, traditional structural biology methods such as X-ray crystallography are difficult to obtain their high-resolution structures. Freezing electron microscopy technology has broken through this limitation and can directly observe the structure of membrane proteins in close proximity to their original ecological environment without the need for crystallization, providing extremely valuable information for drug design targeting these key biomolecules.

Similarly, in the study of dynamic protein structure, cryo electron microscopy has also demonstrated its unparalleled ability. The function of these proteins usually depends on their ability to transition between different conformations, and cryoelectron microscopy can capture the instantaneous and transitional structures of these proteins during their functional execution. This ability to analyze dynamic protein structures provides a deeper perspective on how proteins regulate their function through structural changes, and opens up new avenues for developing new therapeutic methods, especially drugs that can precisely regulate these dynamic processes.

Bonomi and Vendruscolo (2017) discussed how to use cryo EM to simultaneously determine the structure and dynamics of proteins, and provided an effective way to provide all this information in the form of structural sets through an integrated approach combining experimental and computational methods Nygaard et al. (2020) discussed the challenges and techniques of using single particle cryo EM structures to analyze small-sized membrane proteins, such as increasing size through the use of antibody fragments and providing markers for particle alignment, as well as some unresolved issues such as complex displacement at the air water interface. Thonghin et al. (2018) discussed the use of cryo EM for membrane protein structure in breakthrough studies, which can handle relatively small samples and tolerate the presence of detergents. These studies demonstrate the potential of cryo EM technology in revealing membrane proteins and dynamic protein structures, providing

valuable information for a deeper understanding of the functions of these biomolecules.

## 4 Latest Developments

### 4.1 Application of New Detectors

The application of new detectors in cryo electron microscopy (Cryo EM) technology marks a significant improvement in protein structure analysis ability, triggering a technological revolution in this field. These advanced direct electron detectors greatly enhance the resolution and quality of images, providing scientists with unprecedented detailed perspectives on observing the structure and function of biological macromolecules.

The high frame rate imaging capability of the new detector has opened up a new way to study the dynamic changes of proteins, enabling scientists to capture a large number of images in a very short time, thereby observing and analyzing the structural changes of proteins in different functional states. This is particularly important for understanding dynamic proteins that rely on rapid conformational changes to perform their biological functions, providing a powerful analytical tool.

With the surge in data volume, new detectors have also brought challenges in data processing and analysis. To address this challenge, it is possible to develop more efficient image processing algorithms and software that can quickly extract meaningful structural information from large datasets, accelerate the process of structural analysis, and improve its accuracy.

Cheng et al. (2015) outlined the different steps and considerations in determining the structure of single particle cryo EM, with particular emphasis on the latest advances in detector technology and software algorithms, which now allow for recording unprecedented quality images and determining the structure at near atomic resolution.

Faruqi and McMullan (2018) reviewed the electronic detectors used in electron microscopy imaging, emphasizing the advantages of direct imaging detectors in terms of improved resolution, fast readout speed, and sufficient resistance to radiation hardness.

Spilman (2020) discussed how the development of direct detection cameras has led to unprecedented improvements in the quality of recorded images. These large format (>4 million pixels) cameras can provide continuous image streams and have near ideal detection quantum efficiency (DQE), which has led to the important role of bioelectronic cryo EM in the "resolution revolution".

The introduction of new detectors not only improves the resolution and processing efficiency of images, but also greatly expands the application range of cryo electron microscopy technology, enabling scientists to study small molecule proteins and complex biomolecular complexes that were previously difficult to decipher. These advances have had profound impacts on drug discovery, virological research, and many other aspects of the biomedical field.

### 4.2 Development of automatic data collection and analysis

In the field of cryo electron microscopy (Cryo EM) technology, the development of automated data collection and analysis has become the core driving force for advancing protein structure analysis. This progress not only significantly improves the efficiency of experiments, but also improves data quality, making it possible to process large datasets and accelerating the entire process from data collection to structural analysis. Through automated systems for data collection, scientists can achieve rapid identification, localization, autofocus, and calibration of potential areas on the sample grid while reducing human intervention, thus efficiently collecting image data under preset conditions. This automated process not only improves the consistency and repeatability of image acquisition, but also greatly accelerates the collection speed of a large amount of high-quality data.

Danev et al. (2019) emphasized the latest technological and methodological developments in cryo EM, including single particle analysis (SPA) and cryo electron chromatography (cryo ET), which have made automated data

collection and analysis possible, further improving the efficiency and resolution of structural biology research.

Punjani et al. (2017) introduced cryoSPARC: a fast and unsupervised cryo EM structure determination algorithm that utilizes stochastic gradient descent (SGD) and branch and bound maximum likelihood optimization algorithms, enabling the main steps of cryo EM structure determination to be completed in a short period of time on affordable desktop computers.

With the improvement of collection efficiency, the amount of data generated has also greatly increased, making data analysis a bottleneck in research. To address this challenge, researchers have developed a series of high-throughput data analysis tools that utilize advanced algorithms to automatically complete the process from image recognition to protein 3D structure reconstruction. The application of machine learning and artificial intelligence has further optimized this process, improved the speed and accuracy of data processing, and made the analysis of complex protein structures faster and more accurate.

Kumar et al. (2021) introduced a user-friendly, high-throughput, and fully automated data acquisition software suitable for single particle cryo EM. By demonstrating the application of this software package in automatic imaging of SARS-CoV-2 spike proteins, the potential for fully automated image acquisition processes was emphasized.

In addition, the integration of experimental processes is crucial for improving overall experimental efficiency. By integrating data collection, processing, and analysis into a continuous automated process, research efficiency has been significantly improved, while also ensuring standardization of the experimental process, reducing possible errors, and improving the reliability of structural analysis. This advancement in automation and integration not only accelerates the research of basic biology, but also has extremely important value in quickly understanding disease mechanisms, discovering new drug targets, and drug development.

### 4.3 Freezing electron microscopy combined with other technologies

The combination of cryo electron microscopy (Cryo EM) technology and other scientific technologies is becoming an important trend in advancing the field of biomedical research. Through this interdisciplinary integration, scientists can analyze the structure and function of proteins from a more comprehensive and in-depth perspective, thereby deepening their understanding of the biomolecular world. For example, the combination of mass spectrometry technology not only enables researchers to accurately identify the composition and modification status of protein complexes, but also combines with spatial arrangement images provided by cryo electron microscopy to gain insight into complex biological processes at the molecular level. Engen and Komives (2020) emphasized the benefits of combining single particle cryo EM and hydrogen/deuterium exchange mass spectrometry (HDX-MS) methods, including low resolution density analysis, structural validation, analysis of individual proteins in large complexes, and research on isomers, protein quality control, and protein dynamics/motion during functional processes.

By combining cryo electron microscopy with X-ray crystallography, a more accurate three-dimensional protein structure can be obtained based on the atomic level information provided by X-ray crystallography, while analyzing the structure of macromolecules using cryo electron microscopy. The application of this dual technology greatly improves the accuracy and reliability of structural analysis. Orlov et al. (2017) emphasized the integrated role of cryo EM in molecular and cellular structural biology, and examples of its combination with other methods such as X-ray crystallography, fluorescence imaging, or focused ion beam milling, such as studying ribosomes, viruses, chromatin, and nuclear receptors.

Meanwhile, the combination of cryo electron microscopy and optical microscopy techniques, such as super-resolution microscopy and live cell imaging, provides possibilities for studying the function and behavior of proteins in a broader biological context. Li et al. (2018) introduced a new solution for non-integrated multiscale cryo related optical and electron microscopes (cryo CLEMs), enabling scientists to study biological samples at near atomic resolution while preserving their spatial localization in the cellular environment, aiming to improve

the accuracy issues currently reduced during sample transfer processes.

The combination of computational simulation techniques, especially molecular dynamics simulations, further expands the application of cryo electron microscopy. This technology can reveal the dynamic characteristics and functional mechanisms of protein structure based on experimental data from cryo electron microscopy, providing a new perspective for understanding how biomolecules respond to environmental changes. Through this interdisciplinary combination, scientists are not only able to analyze protein structure and function in a more precise way, but also accelerate the transformation process from basic research to applied research.

## 5 Challenges and Prospects

Cryo EM technology, as a powerful tool in biomedical research, has shown great potential in the field of protein and macromolecular complex structure analysis. However, in the pursuit of deeper understanding of biomolecules, Cryo EM still faces many challenges, especially in sample preparation, data collection and processing (Kühlbrandt, 2022). The difficulty of sample preparation, especially in rapidly freezing samples to preserve their structure close to the original ecology without forming ice crystals, and finding suitable preparation methods for proteins that are difficult to express or stable, is a major challenge. In addition, although the development of automated data collection systems has improved the efficiency of data collection, it has come with challenges in managing, storing, and analyzing large amounts of data, requiring continuous technological innovation and software development to optimize this process.

Despite these challenges, Cryo EM still has extremely broad prospects in future biomedical research. Especially in analyzing the structures of large-sized and complex macromolecular complexes as well as low abundance proteins, Cryo EM can provide valuable information that helps to gain a deeper understanding of biological processes. With the advancement of technology, especially innovation in sample preparation techniques and image processing software, Cryo EM's ability to capture low abundance proteins and transient complex structures will be greatly enhanced, further promoting research in areas such as cell signal transduction and protein interactions.

The combination of Cryo-EM with other structural biology techniques such as X-ray crystallography and nuclear magnetic resonance (NMR) (Tsegaye et al., 2021) will open up new research avenues. This cross technology integration not only breaks through the limitations of a single technology and provides more accurate and dynamic structural information, but also provides a more comprehensive perspective for the study of the structure and function of biological macromolecules, accelerating the transformation from basic research to applied research. With the continuous development and improvement of these technologies, the role of Cryo-EM in the field of life sciences will become more important in the future, providing strong impetus for revealing the mysteries of the biomolecular world.

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