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Structural Analysis of Drugs by Cryo-electron Microscopy Reveals Their Mechanisms of Action

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Abstract Cryo-electron microscopy is a crucial tool for studying the mechanisms of drug action, as it provides high-resolution structural analysis of biological macromolecules, revealing the interactions between drugs and these macromolecules. This study delves into the importance of drug action mechanisms and the challenges they pose. It begins by introducing the principles, workflow, and widespread applications of cryo-electron microscopy in biological macromolecule structure analysis, particularly its unique advantages in drug mechanism studies. Through several successful cases, the study illustrates the practical applications of cryo-electron microscopy in drug mechanism analysis, explores its use in drug screening and optimization, and how it can accelerate the discovery and development of new drugs. The paper concludes by summarizing the significant role of cryo-electron microscopy in drug mechanism analysis and looking ahead to its future potential and applications in drug research and development. This research aims to provide new perspectives and methods for studying drug action mechanisms through cryo-electron microscopy, contributing to the advancement of drug discovery and development.

Keywords Cryo-electron microscopy; Drug mechanism of action; Biological macromolecule structure; Drug discovery; Interaction mechanism

The mechanism of drug action is a core issue in drug development, determining how drugs interact with biological systems to produce therapeutic effects and possible side effects. Drug interactions in the body usually involve multiple biomolecules, such as proteins and nucleic acids, which are structurally complex and functionally diverse, making these interactions highly intricate. Traditional research methods, such as biochemical analysis and genetic techniques, although informative, often fail to fully reveal the detailed mechanisms of drug interactions with biomolecules (Radostin et al., 2019).

In this context, cryo-electron microscopy (cryo-EM) has emerged as a prominent technique due to its unique advantages. Cryo-EM can capture the structures of biomolecules under near-physiological conditions and provide near-atomic resolution images, allowing researchers to directly observe the details of drug interactions with biomolecules. Moreover, cryo-EM can capture the dynamic changes of biomolecules, offering insights into the dynamic mechanisms of drug action (Nannenga and Gonen, 2018; Cheng, 2018).

This review aims to explore the application of cryo-EM in studying the mechanisms of drug action, revealing its potential and advantages in elucidating drug interactions with biomolecules. The manuscript introduces the basic principles and workflow of cryo-EM and discusses its widespread use in structural analysis of biomolecules. Through case studies, it demonstrates how cryo-EM provides robust support in deciphering drug action mechanisms, including drug binding modes and drug-induced conformational changes. The discussion extends to the application of cryo-EM in drug screening and optimization, and its role in advancing new drug discovery and development.

This review seeks to provide a new perspective and approach to the research on drug action mechanisms, aiming to advance the field of drug development. It also hopes to draw more researchers' attention to and interest in cryo-EM, encouraging the exploration of its broader applications in drug research. With ongoing technological

advancements, cryo-EM is expected to play an increasingly significant role in studying drug action mechanisms, contributing greatly to human health endeavors.

1 Fundamentals of Cryo-Electron Microscopy

1.1 Principle and workflow of cryo-electron microscopy

Cryo-electron microscopy, also known as cryo-transmission electron microscopy, is a high-resolution imaging technique used for structural analysis of biological macromolecules under near-physiological conditions. Its principles and workflow are closely linked, ensuring high-quality and accurate imaging of biological samples (Angel and Marta, 2019).

The core of cryo-electron microscopy lies in its "freezing" step. In a low-temperature environment (typically around -180 °C), biological samples are rapidly frozen to stabilize their bioactivity and structure. This freezing process minimizes radiation damage and ice crystal formation in the sample, thereby preserving its original state. The frozen samples are then placed under an electron microscope. Instead of visible light used in conventional optical microscopes, an electron microscope uses a high-energy electron beam to observe the sample. As the electron beam passes through the sample, it interacts with atoms within, causing scattering and absorption effects. These effects are captured by an electron detector and converted into visible images. Clare et al. (2017) noted that during the image formation process, the electron microscope adjusts parameters such as the electron beam focus, exposure time, and detector sensitivity to capture the fine structure of the sample. Further processing and analysis of the electron images provide three-dimensional information about the structure of biological macromolecules.

The workflow of cryo-electron microscopy requires highly precise instruments and strict operating procedures, as well as accurate sample preparation and in-depth data analysis. The advantage of this technique is its ability to perform high-resolution imaging of biological macromolecules under conditions close to physiological, thereby revealing details of the interactions between drugs and biological macromolecules, and providing important insights into the mechanisms of drug action.

1.2 Application of cryo-electron microscopy in the structural analysis of biological macromolecules

Cryo-electron microscopy plays a crucial role in the structural analysis of biological macromolecules. Because it can image biological samples at low temperatures close to physiological conditions, it has become a powerful tool for studying the structures of biological macromolecules, especially those samples that are difficult to crystallize.

In the structural analysis of biological macromolecules, cryo-electron microscopy provides high-resolution three-dimensional images, allowing for the direct observation of the fine structures and dynamic changes of biological macromolecules (Figure 1). This includes not only the structures of individual biological macromolecules such as proteins and nucleic acids but also their interactions and the formation of complexes.

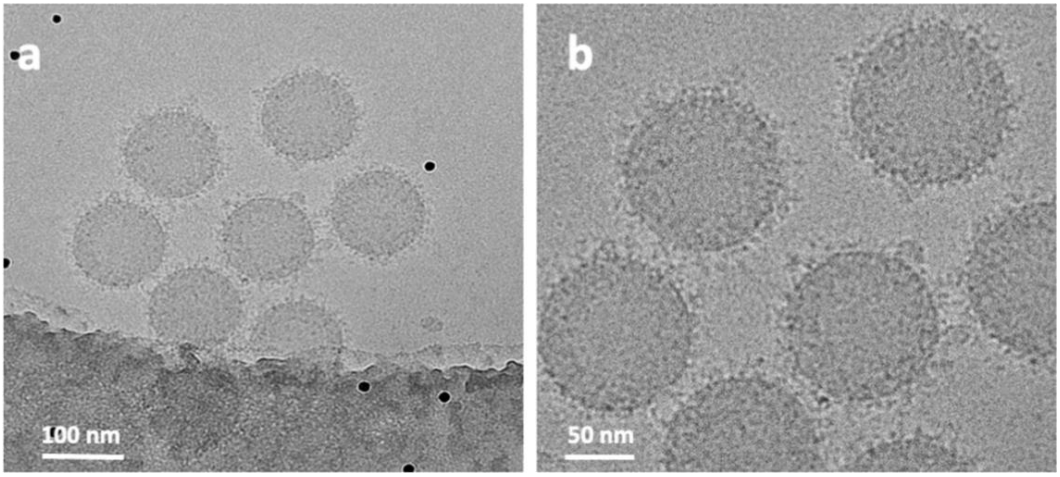

Figure 1 CryoEM technique for imaging (Sara et al., 2021)

Note: a: lower distribution of biomolecules; b: higher magnification reveals their relationship with the surface of nanoparticles

For example, in the study of drug action mechanisms, cryo-electron microscopy can reveal the binding patterns of drug molecules to biological macromolecular targets and the conformational changes in biological macromolecules caused by drugs. This information is crucial for understanding the mechanisms of drug action, optimizing drug design, and discovering new drugs. The application of cryo-electron microscopy in the structural analysis of biological macromolecules not only broadens our understanding of the structure and function of biological macromolecules but also provides significant support for the development of the pharmaceutical research field.

1.3 Applicability of cryo-EM technology in elucidating mechanisms of drug action

Cryo-electron microscopy (Cryo-EM) technology has broad applicability in elucidating the mechanisms of drug action. Whether dealing with small molecule drugs or biological macromolecules, this technology can provide key structural and dynamic information, helping to deeply understand the mechanisms of drug action. It allows for imaging of biological macromolecules under near-physiological conditions, meaning it can observe the interactions between drugs and biological macromolecules in a state close to their natural state. The structures of biological macromolecules under these conditions are usually more authentic, hence making the drug mechanism information obtained more reliable (Nannenga and Gonen, 2018).

The high-resolution images provided by Cryo-EM technology enable the capture of fine details of the interactions between drugs and biological macromolecules. These details may involve the precise binding sites of the drug molecules, modes of binding, and the consequent conformational changes in the biological macromolecules. This information is crucial for understanding the mechanisms of drug action as they reveal how drugs interact with biological macromolecules to produce therapeutic effects or side effects (Hutchings et al., 2018).

Cryo-EM technology also captures the dynamic changes of biological macromolecules under the influence of drugs. This dynamic observation provides important information about how drugs affect the functions of biological macromolecules, including drug-induced signaling pathways and conformational transitions of the macromolecules. This information is essential for understanding the comprehensive mechanisms of drug action as they reveal the complex processes of drug actions within biological systems.

2 Practical Applications of Cryo-EM in the Analysis of Drug Mechanisms of Action

2.1 Cases of successful resolution of drug mechanisms using cryo-EM

2.1.1 Analysis of the drug mechanism of G protein-coupled receptors (GPCRs)

GPCRs are a significant class of drug targets involved in various physiological and pathological processes. Using cryo-electron microscopy, Daniel and José (2020) successfully resolved the three-dimensional structures of multiple GPCRs bound with drugs, revealing how drugs bind to and either activate or inhibit their activity. These studies not only help understand the drug mechanisms of GPCRs but also provide essential guidance for the design and optimization of drugs targeting these receptors.

2.1.2 Analysis of antiviral drug mechanisms

Cryo-EM has also been widely used in the study of antiviral drug mechanisms. For example, Zhu et al. (2021) utilized this technique to analyze the binding mechanism of the influenza virus neuraminidase with the antiviral drug oseltamivir and the structure of the N501Y spike protein of the novel coronavirus complexed with ACE2 and two effective neutralizing antibodies. This analysis provided a detailed glimpse into the cryo-EM structure of the complex formed between the extracellular domain of the N501Y spike protein and the extracellular domain of the ACE2 receptor, revealing how the drug inhibits the viral replication process (Figure 2). This offers new insights and approaches for the design and development of antiviral drugs.

2.1.3 Analysis of protein degradation drug mechanisms

Protein degradation plays a crucial role within the cell, and drug development targeting protein degradation processes has always been a hot area. Cryo-EM has successfully resolved the binding patterns of various protein degradation-related complexes with drugs, such as the interactions between key proteins in the

ubiquitin-proteasome system and the autophagy pathway with drugs. These studies not only aid in understanding the drug mechanisms of protein degradation but also provide a significant basis for the design and development of drugs targeting this pathway (Kondylis et al., 2019).

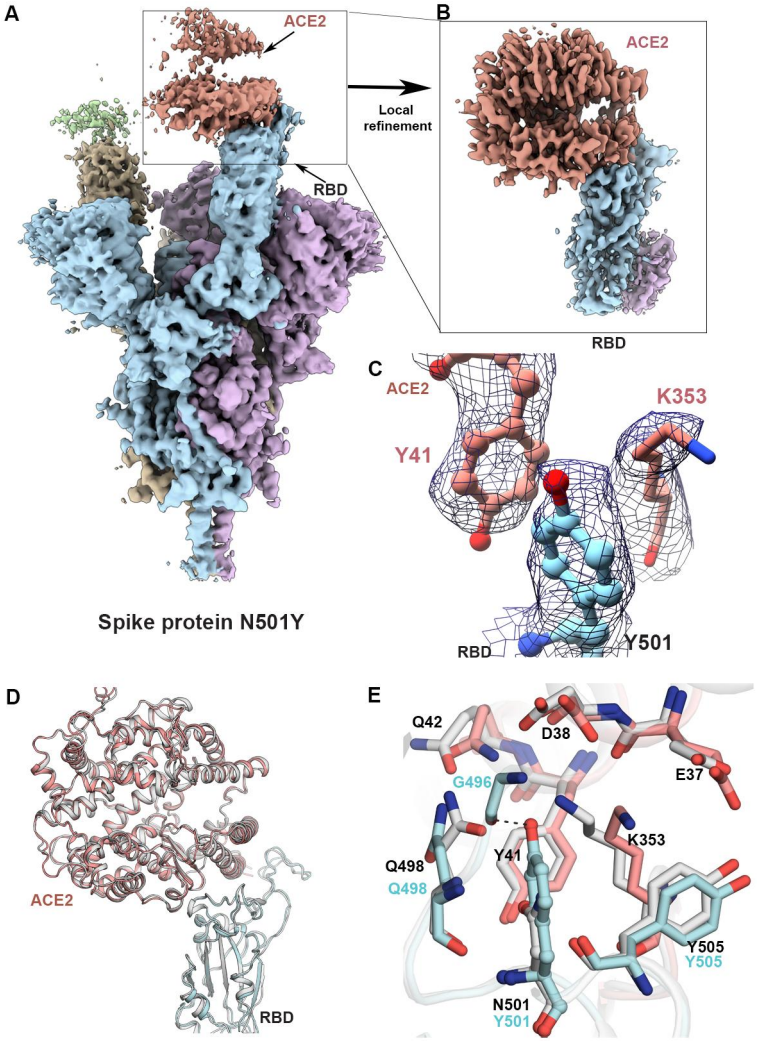

Figure 2 Structure of the SARS-CoV-2 N501Y mutant spike protein ectodomain bound to the ACE2 ectodomain (Zhu et al., 2021)

2.2 Key findings and significance in the case study

GPCR Drug Mechanism Elucidation: Utilizing cryo-electron microscopy, Liu et al. (2019) not only observed the precise binding sites of drug molecules to GPCRs but also captured the conformational changes following the drug-receptor interactions. These changes involve multiple structural domains and key amino acid residues of the receptor, thereby revealing the complete pathway of drug-induced activation or inhibition of GPCR signaling. These discoveries provide a novel perspective on understanding the complex physiological functions of GPCRs and bring significant value to drug development. By simulating and optimizing the binding patterns of drugs to GPCRs, drugs that are more selective and have fewer side effects can be designed, thus more effectively treating various diseases related to GPCRs, such as heart disease, neurological disorders, and cancer.

Antiviral Drug Mechanism Elucidation: Cryo-electron microscopy has revealed the precise binding modes of antiviral drugs to viral proteins and deeply explored how drugs interfere with the viral life cycle. These findings include the binding kinetics of drugs to viral proteins, drug-induced conformational changes in viral proteins, and how these changes affect the virus's replication and release processes. These insights are crucial for the development and optimization of antiviral drugs. They not only provide a structural basis for drug design but also offer important evidence for assessing drug efficacy and predicting drug resistance.

Protein Degradation Drug Mechanism Elucidation: Through cryo-electron microscopy, Valle (2018) meticulously observed how drugs interact with key complexes in the protein degradation pathway and how they regulate the activity of these complexes. These findings involve the interaction patterns between drugs and complexes, drug-induced conformational changes, and how these changes affect the rate and selectivity of protein degradation. These discoveries are vital for understanding the regulatory mechanisms of protein homeostasis and provide new directions for developing drugs targeted at specific diseases. For example, in neurodegenerative diseases, regulating protein degradation processes can help clear harmful protein aggregates; in cancer therapy, inhibiting the degradation of specific proteins can enhance the efficacy of anticancer drugs.

2.3 How cryo-EM provides insights into drug interactions with biomolecules

Cryo-electron microscopy (Cryo-EM) offers a powerful tool for revealing detailed information about the interactions between drugs and biomolecules through its unique imaging capabilities. Its working principle is based on rapidly freezing the sample under near-physiological conditions, thereby preserving the natural state and activity of biomolecules. Subsequently, under an electron microscope, these frozen samples are penetrated by a high-energy electron beam, which interacts with the atoms in the sample to produce scattering and absorption effects. These effects are then captured by an electron detector and converted into visible images.

These images not only possess extremely high resolution but also capture the static and dynamic details of interactions between biomolecules and drug molecules. Through in-depth analysis of these images, the binding sites of drug molecules on biomolecules can be precisely determined, providing insights into how drugs interact with specific amino acid residues on biomolecules. Cryo-EM can also reveal conformational changes in biomolecules after drug binding, which may involve protein folding, domain rearrangement, or adjustments in overall structure, further elucidating how drugs affect the function of biomolecules (Twarock et al., 2018).

In addition to providing static structural information, Cryo-EM can also capture the dynamic processes of drug interactions with biomolecules. This includes how drug molecules gradually approach and ultimately bind to biomolecules, and how binding triggers a series of biological effects. This dynamic observation provides key clues for understanding the comprehensive mechanisms of drug action, helping to more fully comprehend how drugs function at the cellular level.

3 Impact of Cryo-Electron Microscopy on Drug Discovery and Design

3.1 Application of cryo-electron microscopy in drug screening and optimization

Cryo-electron microscopy (cryo-EM) has broad applications in the drug screening and optimization process. During the drug screening phase, cryo-EM can provide high-resolution images of the interaction between candidate drugs and biological macromolecular targets. This helps researchers quickly identify potential drugs. By observing and comparing the binding modes and affinities of different drugs to the targets, it can be preliminarily determined which drugs are worth further in-depth study (Twarock and Stockley, 2019).

In the drug optimization phase, cryo-EM can reveal detailed mechanisms of interaction between drugs and biomolecules. This includes how drugs affect the conformation, function, and signaling pathways of the targets. By studying these interaction details extensively, the structure of drugs can be optimized, their efficacy improved, and potential side effects reduced. The data provided by cryo-EM offers significant theoretical support and experimental evidence for drug improvements (Michael et al., 2021).

Cryo-EM can also be used to evaluate drug resistance. The emergence of resistance is a common challenge in drug development. By using cryo-EM to observe the interactions between drugs and resistant mutants, a deeper understanding of resistance mechanisms can be gained. This knowledge allows for the design of new drug strategies to overcome resistance, providing new ideas and methods for drug development and clinical treatment.

3.2 How cryo-EM accelerates the discovery and development of new drugs

Cryo-electron microscopy (cryo-EM) significantly accelerates the process of new drug discovery and development through its unique capabilities. In the early stages of drug discovery, cryo-EM provides high-resolution structural information of biological macromolecular targets, enabling research teams to more precisely understand the three-dimensional structure and characteristics of these targets. This precise structural information is crucial for drug screening and design, as it helps identify drug candidates that can effectively bind to the target.

Cryo-EM reveals the intricate details of the interactions between drugs and biological macromolecules. By observing how drug molecules bind to targets and how this binding affects the target's function and activity, a deeper understanding of the drug's mechanism of action can be achieved. This understanding not only aids in predicting the drug's efficacy and side effects but also guides further drug optimization and improvement efforts (Sara et al., 2021).

Furthermore, cryo-EM accelerates the iterative process of drug development. In traditional drug development workflows, screening and optimizing drug candidates typically require multiple rounds of experiments and testing. However, using the high-resolution structural and interaction information provided by cryo-EM, potential drug candidates can be identified early on, and structural optimization can be rapidly conducted. This significantly reduces the time and cost of drug development, enhancing research and development efficiency.

3.3 Future trends of cryo-electron microscopy in drug discovery and design

The future trends of cryo-electron microscopy in drug discovery and design suggest that it will continue to play a significant role and will be combined with other advanced technologies to drive greater breakthroughs in the field of drug research and development. With technological advancements, the resolution and imaging speed of cryo-electron microscopy will further improve, allowing for more detailed observation of the interactions between drugs and biomolecules. This will contribute to a deeper understanding of the mechanisms of drugs, providing more precise and comprehensive information for drug discovery and design.

The integration of cryo-electron microscopy with other technologies will foster innovation in the drug development process. Twarock and Stockley (2019) found that combining it with artificial intelligence and machine learning algorithms can facilitate rapid screening and optimization of a large number of drug candidates. By integrating with multi-omics data such as genomics, proteomics, and metabolomics, cryo-electron microscopy will be able to provide more comprehensive and holistic information about the structure and function of biomolecules, offering a broader perspective for drug discovery and design.

Additionally, cryo-electron microscopy will also play an important role in other aspects of new drug development. For example, in the optimization of drug crystal forms, cryo-electron microscopy can reveal the structure and stability of different crystal forms of drug molecules, providing guidance for the development of drug formulations. In studies of drug interactions with cell membranes, cryo-electron microscopy will be able to reveal how drugs penetrate cell membranes and interact with them, providing crucial insights for the development of drugs with better bioavailability.

4 Summary and Outlook

Cryo-electron microscopy has played a crucial role in elucidating the mechanisms of drug action. It not only provides high-resolution images of the interactions between drugs and biomolecules, but also reveals in detail the dynamic processes of these interactions. Through cryo-electron microscopy, it is possible to precisely identify the binding sites of drug molecules on biomolecules, and understand how drugs exert their therapeutic effects by interfering with or regulating the functions of biomolecules (Zhu et al., 2021). This deep understanding not only enhances our knowledge of drug mechanisms but also provides important theoretical support and experimental evidence for drug research and optimization.

In the field of drug research and development, the potential of cryo-electron microscopy is enormous. With continuous advancements in technology, the resolution and imaging speed of cryo-electron microscopy will improve, allowing for more detailed observations of the interactions between drugs and biomolecules. Additionally, with the integration of multi-omics data and the application of advanced technologies such as artificial intelligence, cryo-electron microscopy will be able to provide more comprehensive and accurate information, offering more efficient and reliable tools for drug discovery and design.

More importantly, the application of cryo-electron microscopy in the field of drug research is gradually expanding. It is used not only for analyzing drug mechanisms but also plays a significant role in drug screening, optimization, and quality control. With cryo-electron microscopy, potential drug candidates can be quickly screened, their interactions with biomolecules assessed, and directions for subsequent research provided. Cryo-electron microscopy can also be used to study the distribution and metabolism of drugs within cells, providing important data for the evaluation of drug efficacy and safety.

In the future, cryo-electron microscopy is expected to continue playing a significant role in the field of drug research. On one hand, with ongoing innovations and upgrades, cryo-electron microscopy will continually enhance its imaging quality and resolution, providing more in-depth and detailed information about drug mechanisms. On the other hand, as the needs and complexities of drug development increase, cryo-electron microscopy will be integrated with other technologies to form a more complete and efficient drug development system. It is believed that in the near future, cryo-electron microscopy will bring more breakthroughs and innovations to the field of drug research and development, making a more significant contribution to human health.

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