State of Art Review on

Cryo-EM Structural Determination of Low Molecular Weight Proteins and Biomolecular Complexes

BIO-467 Individual Report

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Brief summary of the field

Cryo-electron microscopy (Cryo-EM) is a revolutionary method used in structural biology that started to prevail in the 2010s. By applying cryogenic temperatures to freeze the samples, Cryo-EM preserves their original structures and reduces radiation damage, thus, achieving high-resolution imaging and allowing researchers to observe the native states of biomolecules. Its general workflow includes the following steps: first, the sample is applied to a grid and rapidly frozen in liquid nitrogen or helium to form a vitrified layer, in this way, avoid the formation of undesired ice crystals; next, electron microscopy and direct electron detection are applied to do the data acquisition; lastly, the data obtained can be put into image processing and structural analysis. Besides, compared to traditional structural imaging techniques such as X-ray and NMR, Cryo-EM is applicable for a wider range of targets. With these merits and characteristics, Cryo-EM provides a promising solution to the structural determination of low molecular weight proteins and biomolecular complexes, which has long been a tricky task, due to the weak electron scattering, low signal-to-noise ratio, and inherent dynamic properties of the low molecular weight substances.

Recent achievements and progress of Cryo-EM for small proteins and complexes lie in many aspects, from electron detection to resolution enhancement. The implementation of direct electron detectors (DEDs) [1] has significantly increased sensitivity and reduced noise, together with the invention of related software such as Relion [2] and CryoSPARC [3], the convenience and quality of Cryo-EM structural determination is greatly enhanced, especially for precise three-dimensional reconstruction. Meanwhile, new sample preparation techniques, such as graphene oxide grids [4] and gold-coated support films [5], have helped to reduce background noise and improve imaging stability, which further extends Cryo-EM's ability to investigate small-scale molecules. For instance, Cryo-EM has successfully been used to determine the high-resolution structure of sub-100 kDa complexes [6].

Current research focuses on the field of Cryo-EM for low molecular weight proteins and biomolecular complexes including the enhancing of resolution and the expansion of applications. With the fast development of AI and deep learning, image processing algorithms for Cryo-EM structural analysis are continuously being polished, thus, improving sensitivity and signal-to-noise ratio. Sample preparation procedures are also being optimized for better stability and uniformity. Time-resolved Cryo-EM imaging is another research hotspot, which can reveal dynamic molecular conformations [7]. Lastly, the integration of Cryo-EM with other techniques like X-ray [8] and NMR [9] is providing more and more possibilities in structural biology study, facilitating both fundamental research and applications like drug discovery. In general, Cryo-EM has now become a powerful tool in the structural determination of low-weight proteins and complexes, while challenges still exist in low signal-to-noise ratio and heterogeneity.

Major publications, groups working, and demonstrations

- **Direct electron detection yields cryo-EM reconstructions at resolutions beyond 3/4 Nyquist frequency, 2012 [1].** The study enhances the resolution of Cryo-EM structural analysis beyond the limit of 3/4 Nyquist frequency, by the implementation of the direct electron detectors (DEDs), which makes the future exploration of Cryo-EM on small proteins possible.
- **CryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination, 2017 [3].** The paper introduces a novel computational framework, CryoSPARC, which makes Cryo-EM more convenient and efficient, especially for determining structures of low-molecular-weight proteins.
- **Cryo-electron microscopy structure of a human PRMT5:MEP50 complex, 2018 [10].** The study uses high-resolution Cryo-EM to elucidate the 3D structure of the human PRMT5:MEP50 complex, and thus, reveals the mechanism of how it regulates protein methylation. This serves as a milestone of Cryo-EM application on low molecular weight protein complexes.
- **Cryo-EM in drug discovery: achievements, limitations and prospects, 2018 [11].** The paper digs into the Cryo-EM's usage in drug discovery, especially its function in analyzing protein-ligand complexes and identifying detailed targets. It also talks about Cryo-EM's potential in determining detailed and small structures.
- **High-resolution structure determination of sub-100 kDa complexes using conventional cryo-EM, 2019 [6].** The study serves as another typical instance of small molecular complexes structural study (under 100 kDa) using Cryo-EM, it presents many advancements, especially in SNR ratio improvement.
- **Integrating cryo-EM and NMR data, 2020 [9].** The paper discusses the combination of Cryo-EM and NMR in structural determination, Cryo-EM is good at resolving larger complexes, while NMR can look at relatively dedicated regions. The co-function of the two methods enhances the efficiency and the resolution of targeted regions.
- **Electron microscopy holdings of the Protein Data Bank: the impact of the resolution revolution, new validation tools, and implications for the future, 2022 [12].** The paper discusses the impact of Cryo-EM's resolution revolution on the protein data bank and points out that current Cryo-EM techniques have overcome the limitations of traditional methods, to be suitable for smaller, dynamic molecules.
- **High-resolution cryo-EM of a small protein complex: The structure of the human CDK-activating kinase, 2024 [13].** The study uses cryo-EM to successfully determine the structure of the human CDK-activating kinase, which is a small protein complex essential for cell cycle regulation.

Timeline and statistics on the publication volume

To analyze the timeline and statistics on the volume of publications about Cryo-EM structural determination of small proteins and complexes, the PubMed database is used. I first search the keyword "Cryo-EM" or "Cryo-electron microscopy" for an overview of the general topic. Then, I used the combination of "Cryo-EM" or "Cryo-electron microscopy" and "small biomolecular complexes," "low molecular weight," or "sub-100 kDa", to look for statistics in Cryo-EM for small proteins and complexes alone. The results of the two searches are presented in the following histogram (Figure 1). The numerical results regarding small proteins and complexes may be different from the actual number, as the keywords method could miss some papers and include irrelevant ones, but the overall trend can be concluded.

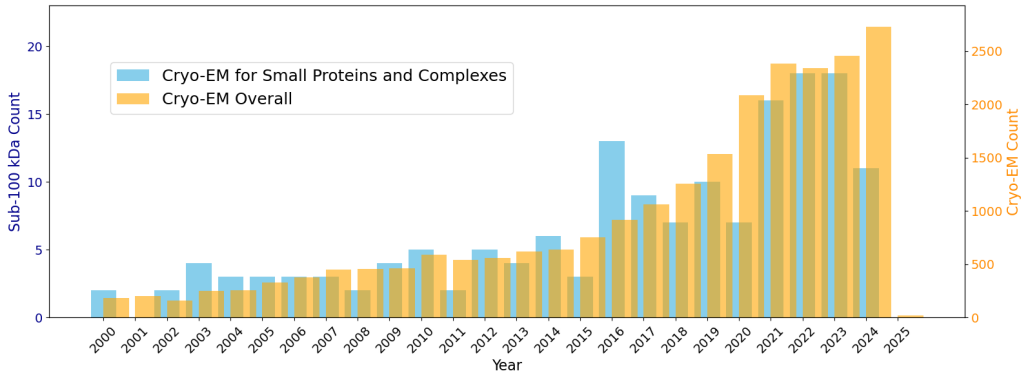

Figure 1: Statistics on the Publication Volume

As indicated by the yearly change in publication volume, there is a synchronous growth of Cryo-EM in both the overall field and its application to small proteins and complexes, and the growth can be summarized into three periods. First, from 2000 to 2010, publication numbers were relatively low, as there went the foundational development of the Cryo-EM method, we can see that early approaches of using Cryo-EM on small particles already appeared. Moving on to the 2010s, the papers' number grew significantly. Contributed to key technological breakthroughs such as the introduction of direct electron detectors (DEDs) and advanced image processing techniques, Cryo-EM showed its potential to address smaller, more dynamic biomolecules. Lastly, from 2020 to now, the field is still developing rapidly, driven by AI-based methods and wide application in structural biology and drug discovery.

Future Directions

The future directions of Cryo-EM's application on structural analysis of small biomolecules include several aspects. The first is the ongoing innovation and improvement of the existing techniques like processing algorithms, detection settings, and sample preparations. Algorithm development, such as AI-based analysis, is an essential direction in dealing with weak signal issues, it can refine or reconstruct the signal gained from small particles to make it more usable. Electron detector with higher sensitivity is another heated topic, together with low-noise electron sources, which could significantly enhance image quality. Meanwhile, the process of sample preparation is keep being polished, researchers are trying novel materials to reduce background noise and stabilize samples.

The second direction is the attempts to implement Cryo-EM on small particles in wider scenarios. For example, the analysis of dynamic or transient states of small proteins, such as ion channels and signaling particles. The analysis of small proteins by Cryo-Em is also increasingly used for drug discovery, as it can provide insights into druggable targets and small biding points. Moreover, in situ studies are largely benefiting from Cryo-EM development, as, ideally, the small biomolecules inside the cell can be observed without losing their native states.

Lastly, the interdisciplinary technique is also an essential direction of Cryo-EM's usage in analyzing low molecular weight proteins and complexes. Not only the combination with computing approaches or AI, but also the co-work of Cryo-EM and other imaging methods. As mentioned, there have been successful studies that tried combining Cryo-EM with NMR or X-ray, by more and more approaches like this, we can expect in the near future, there will be a better comprehensive Cryo-EM-based method to determine the structures of low molecular weight proteins and biomolecular complexes.

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