Leveraging Cryo-Electron Microscopy for Innovative Vaccine Development

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Abstract

The efficacy of vaccines to reduce the burden of disease, as well as the associated reduction in mortality and morbidity, has been demonstrated multiple times since the development of the first vaccine against smallpox in 1796. However, although vaccine discovery, development and manufacturing have significantly advanced, it remains a challenging process that requires a deep understanding of each pathogen and associated protein targets. One powerful tool that is enabling vaccine development is cryo-electron microscopy (cryo-EM) as the structural knowledge provided by cryo-EM can be leveraged to design tailored vaccine solutions for each pathogen or virus. This method is being widely adopted by pharmaceutical companies to aid risk discovery and development pipelines and for manufacturing projects. Typically, cryo-EM can provide high-resolution structures of the virus or pathogens as well as its constituent proteins. Structural information can help design new vaccines, as well as develop a better understanding of the mechanism of action of host-pathogen interactions. As with many other fields in both academia and industry, where cryo-EM is making significant contributions, we are convinced that the increase in cryo-EM usage in vaccinology is here to stay. This wave will be amplified by the facilitation of single particle analysis workflows and new in situ innovations in cryo-electron tomography. We envision cryo-EM to become an integral tool that will accelerate time to market, improve success rates and reduce cost of vaccine development and manufacturing, leading to more clinical successes in the near future. In this paper we report on some of the applications of cryo-EM to the different areas of vaccine discovery, development and manufacturing highlighting the success stories and trends in the industry.

1. Pathogen structure

Cryo-EM is able to provide insight into the structure of complete pathogens. One example is found in work completed by Yao et al. (2020) where the researchers used cryo-electron tomography (cryo-ET), a specialized technique within the broader field of cryo-EM, to determine the molecular assembly of the intact SARS-CoV-2 virus showing the spike proteins in both a pre- and post-fusion conformation (Figure 1). They also revealed the configuration of the internal ribonucleoproteins (RNPs) providing insight into how the virus is able to package its 30kb single-stranded RNA genome into a viral lumen of 80-μm diameter. This research highlights the capacity of cryo-EM to provide detailed structural information on whole viruses, complementing other sources of datasets and contributing to our understanding of viral architecture and their underlying biological mechanisms.

2. Structure of glycoprotein B (gB) from HCMV

Cryo-EM enables the structural understanding of specific viral proteins that can be used in vaccine development. Liu et al. (2021) determined the structure of the human cytomegalovirus (HCMV) glycoprotein B (gB) using cryo-EM (Figure 2). Initial preparations of this antigen, involved in viral fusion and entry into host cells, showed primarily the postfusion conformation which would not produce an effective immune response to the wildtype virus. The cryo-EM structure was crucial for allowing redesign of the protein to increase the proportion in the prefusion state and hence development of an effective vaccine.

3. Improved Vaccine Design

Cryo-EM can also contribute to the design of more effective vaccines. Marcandelli et al. (2019) present a novel protein nanoparticle vaccine for Respiratory Syncytial Virus (RSV), a significant cause of severe respiratory infections in infants and elderly individuals. The vaccine is based on a designed protein nanoparticle that displays a stabilized version of the RSV fusion (F) glycoprotein in its prefusion conformation (Figure 3). When tested in mice, the vaccine elicited potent neutralizing antibody responses that were 10-fold higher than those induced by the soluble prefusion F protein alone.

4. Structural insight of infection

In addition to providing structural data on individual proteins that can be repurposed as antigens, cryo-EM is also able to provide information on potential targets for novel therapeutics. One such target was determined by Kern et al. (2021) in the SARS-CoV-2 encoded putative ion channel 3α (ORF3a), a multifunctional protein implicated in viral replication, release, replication, virulence, and modulation of host immune response. These high-resolution cryo-EM structures (Figure 4) shed light on the architecture, oligomerization state, and ion channel function of a critical viral protein in its native-like environment. These findings not only enhance our understanding of SARS-CoV-2 biology but also suggest potential avenues for the development of targeted therapeutics against ORF3a.

5. Structure of SARS-CoV-2 Spike protein

Understanding the biology as well as developing specific antigens for vaccines can be aided by determining the structure of specific proteins from target pathogens. Perhaps the most well-recognized example is the seminal work completed by Whapp et al. (2020) where cryo-EM was used to determine the high-resolution structure of the spike (S) protein of the SARS-CoV-2 virus (Figure 5). The ectodomain of the S protein was expressed and purified in the pre-fusion state and the structure determined to 3.5 Å resolution using cryo-EM. This showed that it forms a trimeric conformation with one of the three receptor-binding domains (RBDs) present in a receptor-accessible conformation. The RBD was revealed to be highly flexible, alternating between “up” and “down” conformations. This fluctuation likely plays a role in immune evasion by shielding critical neutralizing epitopes.

6. Epitope Mapping

Cryo-EM can also be used to map epitopes to better understand immune evasion. Liu et al. (2017) used cryo-EM to investigate the molecular basis of hemagglutinin (HA) single-chain Fv (scFv) complexes that had previously failed to be amenable to X-ray crystallography (Figure 6). The complex was determined at 4.5 Å, a resolution that still allowed for modeling of the antigen-antibody interface. In addition, a related antibody was successfully crystalized showing the complementarity of these structural biology methods.

References


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