Leveraging Cryo-Electron Microscopy for Innovative Vaccine Development

Zuben P. Brown* & Anass Jawhari*,
Email: Zuben.brown@thermofisher.com; Anass.Jawhari@thermofisher.com
Materials and Structural Analysis Division, Thermo Fisher Scientific, Portland, Oregon

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 de-risk discovery and development pipelines and for manufacturing projects. Typically, cryo-EM can provide high-resolution structures of the virus or pathogen 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 clinic successes in the near future. In this poster 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.

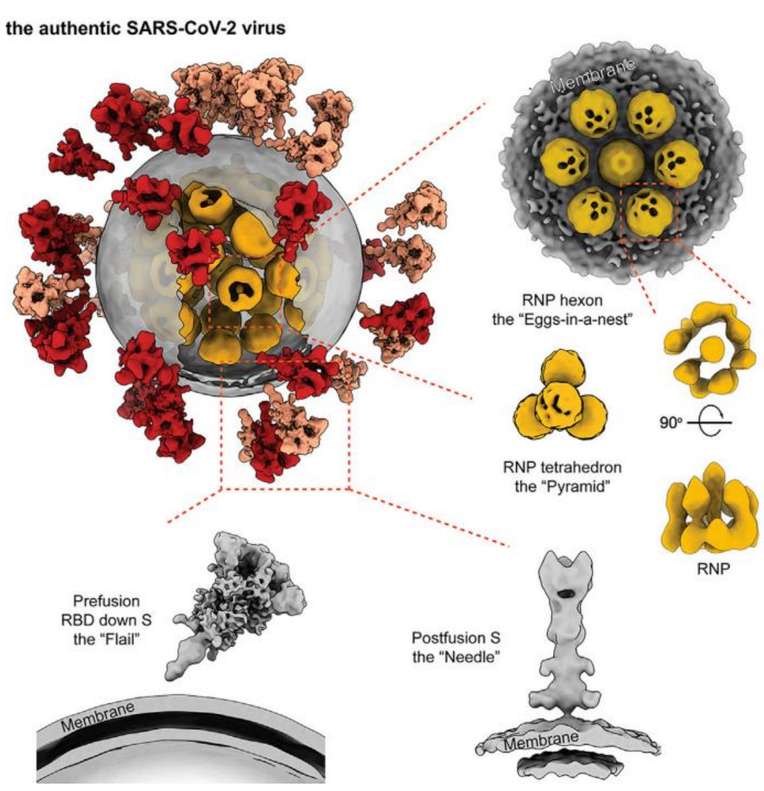

Figure 1. Cryo-ET analysis of intact SARS-CoV-2 virus architecture, revealing spike protein conformations and RNP packaging within the viral capsid. Resolution for these components was between 8.7-11 Å. Figure from Yao et al., (2020).

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 determined 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 it's 30-kb single-segmented RNA genome into a viral lumen of 80-nm-diameter. This research highlights the capacity of cryo-EM to provide detailed structural information on whole viruses, complementing other sources of structural data and contributing to our understanding of viral architecture and their underlying biological mechanisms.

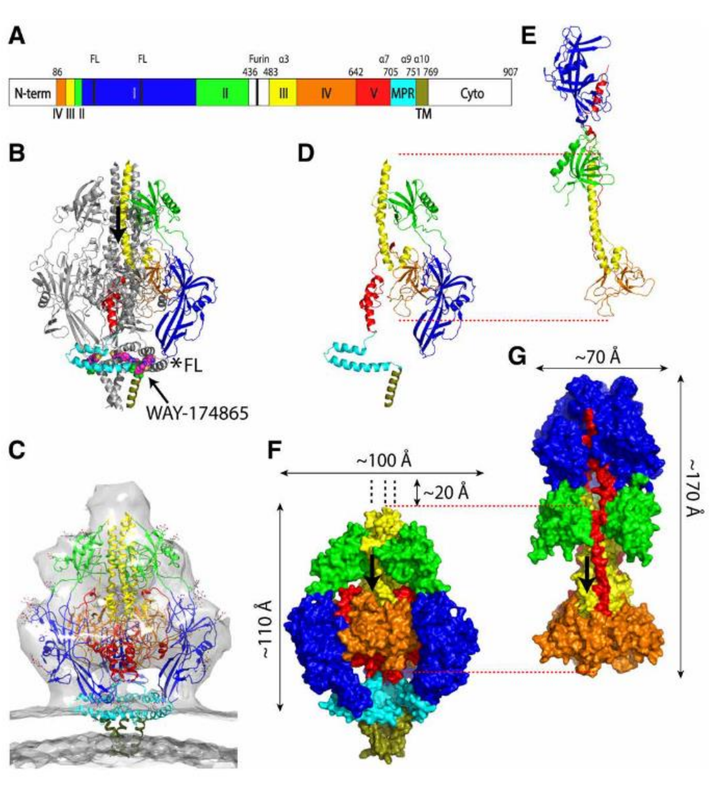

Figure 2. Cryo-EM analysis of the postfusion conformation of HCMV gB reveals trimeric architecture, domain organization, and fusion loop arrangement, elucidating the molecular basis of HCMV entry and enabling the design of effective gB HCMV antigens for vaccine development. Figure from Liu et al., (2021).

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 this protein to increase the proportion in the prefusion state and hence development of an effective vaccine.

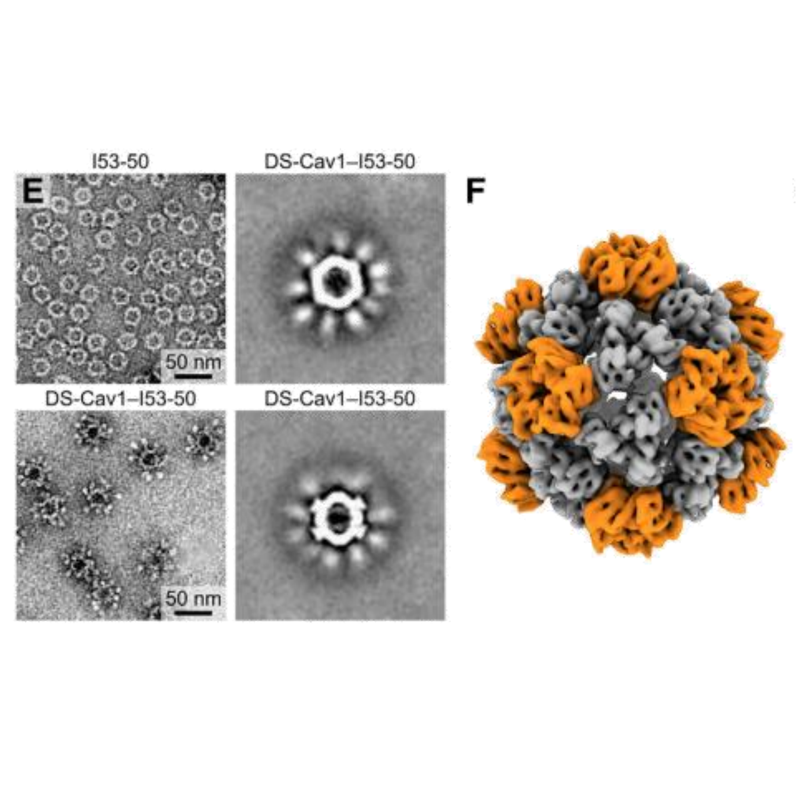

Figure 3. Protein nanoparticle vaccine for Respiratory Syncytial Virus (RSV), displaying a stabilized version of the RSV fusion (F) glycoprotein in its prefusion conformation. The vaccine induced potent neutralizing antibody responses in mice, 10-fold higher than those induced by the soluble prefusion F protein alone, and with broad reactivity to diverse RSV strains. Negative stain images of the vaccine (left) and the cryo-EM structure (right) at 6.3 Å. Figure from Marcandalli et al., (2019).

3. Improved Vaccine Design

Cryo-EM can also contribute to the design of more effective vaccines. Marcandalli 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.

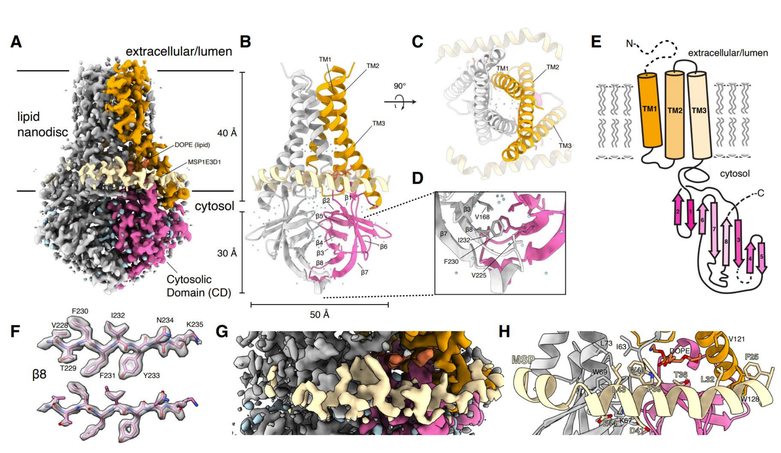

Figure 4: Cryo-EM structural analysis of SARS-CoV-2 ORF3a reveals the molecular architecture, oligomerization state, and elucidate ion channel function of this critical viral protein. The 2.1 Å resolution structure highlights a unique fold with a large polar cavity that spans halfway across the membrane, providing potential therapeutic targets. Figure from Kern et al., (2021).

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 3a (ORF3a), a multifunctional protein implicated in viral 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.

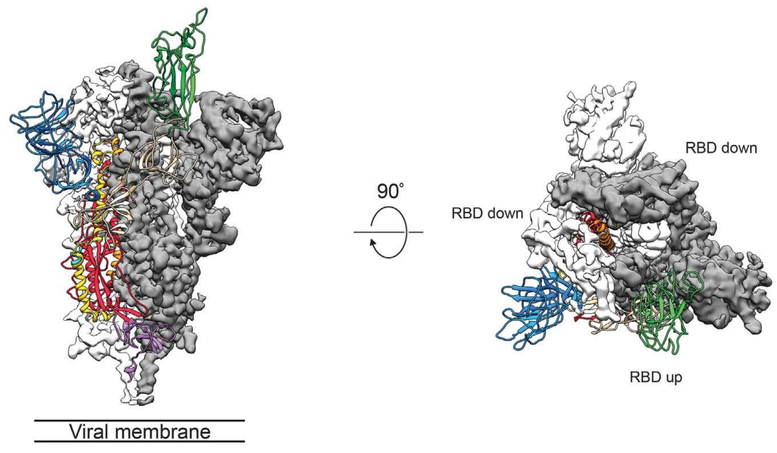

Figure 5. Wrapp et al. (2020) determined the high-resolution structure of the SARS-CoV-2 spike (S) protein using cryo-EM. The trimeric conformation, with one of three RBDs in a receptor-accessible configuration, revealed a highly flexible RBD that may contribute to immune evasion. This structure facilitated the identification of potential antigenic sites, informing rational vaccine design. Figure from Wrapp et al., (2020).

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 Wrapp 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.

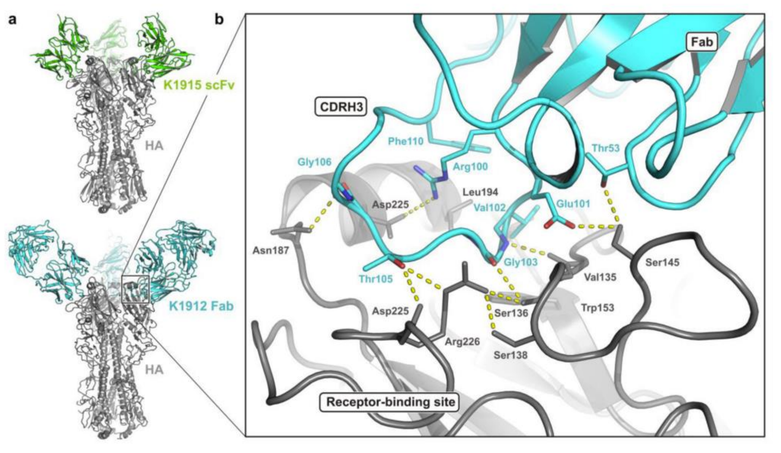

Figure 6. Cryo-EM analysis of hemagglutinin (HA):single-chain Fv (scFv) complexes by Liu et al. (2017) at 4.5 Å resolution reveals the molecular basis of the antigen:antibody interface in a system previously intractable to crystallography. The study highlights the complementarity of cryo-EM and X-ray crystallography in addressing challenging structural biology problems. From Liu et al., (2017).

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 A, a resolution that still allowed for modelling of the antigen:antibody interface. In addition, a related antibody was successfully crystallized showing the complementarity of these structural biology methods.

References

Kern, D. M. et al. (2021). Nature Structural & Molecular Biology, 28:573–582.

Liu, Y. (2017). Journal of Molecular Biology, 429:1829–1839.

Liu, Y. et al. (2021). Science Advances, 7:eabf3178.

Marcandalli, J. et al. (2019). Cell, 177:1420-1431.

Wrapp, D. et al. (2020). Science, 367:1260-1263.

Yao, H. et al. (2020). Cell, 183:730-738.

Reach us via email: