Antibody Research

Accelerating discovery and rational engineering of antibody modalities using Cryo-EM

Tilak Gupta, & **Surajit Banerjee***,
Materials and Structural Analysis Division, Thermo Fisher Scientific, Portland, Oregon.
Email: surajit.banerjee@thermofisher.com

Abstract

As the need for novel therapeutic modalities is growing, researchers require innovative strategies and transformative technologies to overcome the limitations of traditional antibody discovery methodologies. **Cryo-electron microscopy** (Cryo-EM) has been successfully established as a powerful technique to **accelerate antibody drug discovery** processes. Despite the recent adoption of cryo-EM in drug discovery efforts, multiple cryo-EM supported therapies have already entered the clinics. The broad applicability of single particle imaging underpins the rapidly increasing implementation of cryo-EM in **antibody drug discovery** pipelines including **monoclonal antibodies, design of bi- and multi-specific formats**, and design of **CAR binders for CAR-T cell therapy**. Here, we will provide specific and diversified applications of cryo-EM that will contribute to reshape the future of antibody research. We will share how **cryo-EM based epitope mapping** provide accurate information at the single amino acids. We will explain ways one can leverage cryo-EM structures to **design of next-generation antibody therapeutics**. We will discuss our view on how cryo-EM can provide experimental **complementation to Artificial Intelligence/Machine Learning** (AI/ML) tools. Finally, we will touch base to cryo-EM Polyclonal Epitope Mapping (Cryo-EMPEM), a workflow that allowed to **structurally characterize immunogenicity** of antibody therapeutics and vaccines.

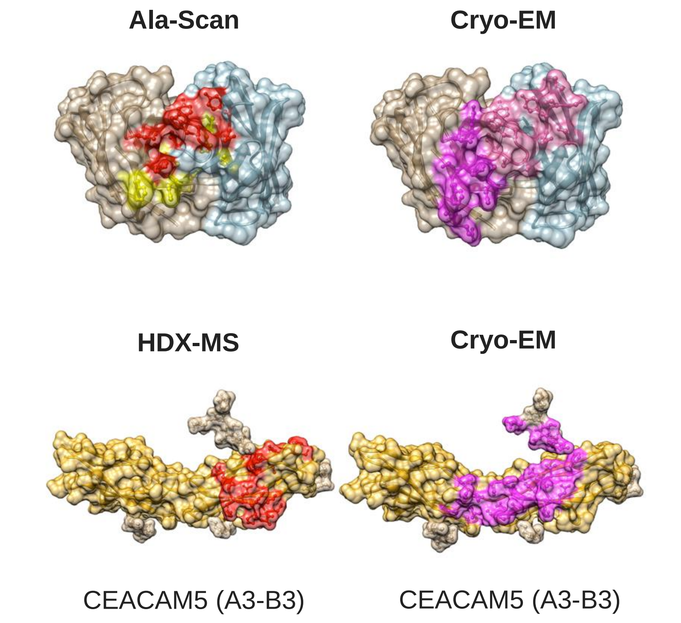

Figure 1. Comparison of the epitope-paratope interface between hCEACAM5A3-B3 and tusa Fab identified using three different structural analysis techniques. Cryo-EM provided single-amino-acid resolution for both the epitope and paratope. Figure from Rak et al. (2023).

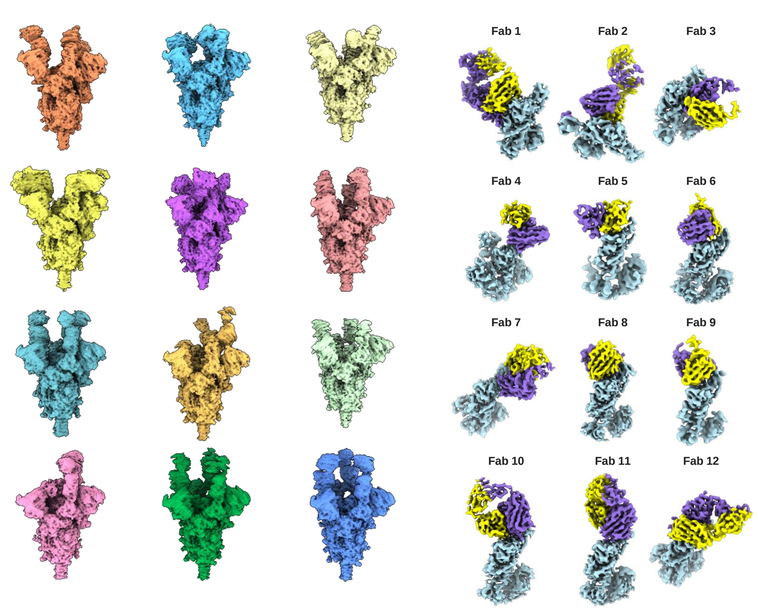

Figure 2. High-throughput epitope mapping and structure analysis of twelve pre-clinical stage SARS-CoV-2 antibodies, derived from COVID-19 patients and immunizations of transgenic animals. Cryo-EM structures of these antibody Fab fragments bound to spike proteins were solved (left panel). Close inspection of each of the 12 Fab-antigen interface were performed (right panel). Collaboration with Takeda Pharmaceuticals and Utrecht University. Manuscript in preparation.

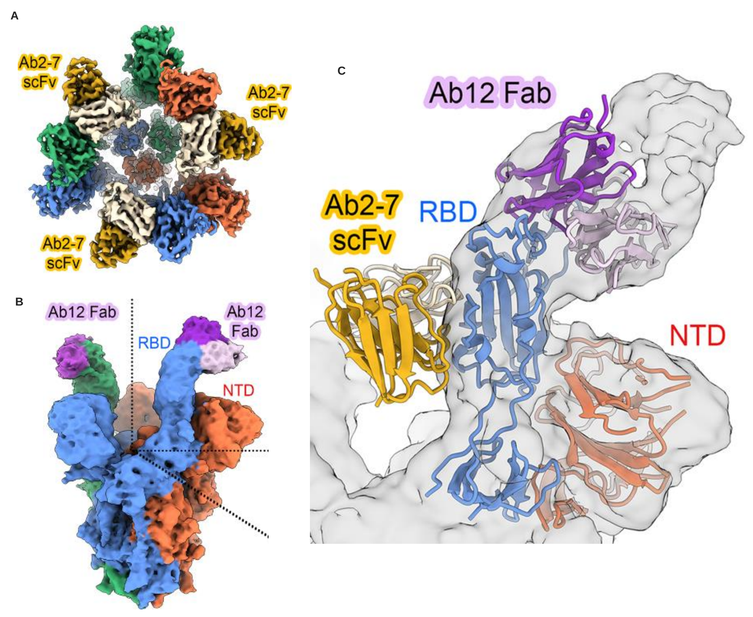

Figure 3. Cryo-EM structural analysis of scFv of Ab2-7 class (A) and Fab of Ab12 class (B) bound to SARS-CoV-2 RBD reveals the molecular architecture, binding mode, and binding landscape. Overlay of the Ab2-7 and Ab12 structures demonstrates that the two antibodies target spatially discrete, non-overlapping epitopes. Figure from Chang et al. (2022).

1. Epitope/Paratope Mapping

Tusamitamab ravtansine (tusa) is an antibody-drug conjugate that binds a specific region of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM); overproduction of CEACAM is associated with the growth of a number of cancers. Understanding this specificity could lead to enhanced antibody design. Scientists at Sanofi used cryo-EM to map the paratope of tusa (tusa Fab) and its association with the epitope, the A3-B3 domain of human CEACAM. Through the use of single particle analysis and 3D reconstruction, they found that hCEACAM exhibited a discontinuous epitope involving multiple residues throughout the A3-B3 domain. They also observed the presence of N-linked glycans in this region, which likely have a substantial impact on the conformation of the A3-B3 domain.

2. High-throughput epitope mapping

In this collaborative work, we used the Cryo-EM rapid epitope mapping workflow to gain a structural understanding of how 12 Covid-specific antibodies recognize and bind to the "Omicron" B.1.1529 strain, which contains an unusually high number of mutations in the receptor-binding domain (RBD). By using Cryo-EM single particle analysis, we were able to obtain sub-3 Å structures of the SARS-CoV-2 spike:Fab complex for all 12 antibodies, with the epitope-paratope interface resolved to high-resolution in each complex (right panel). This information helped us to determine the specific amino acids involved in the epitope-paratope interface and to assign the Fabs to the appropriate epitope classes.

3. Designing bi-specific/multi-specific antibody

In addition to providing structural data on individual epitope/paratope interfaces, cryo-EM also aids in designing and validating bi-specific/multi-specific antibody formats. In this study, structural insights were used to design a potent bi-specific antibody format by Scientists from the Dana-Farber Cancer Institute and Harvard Medical School. Initially, the researchers obtained the cryo-EM structure of antibodies from different classes bound to RBD. This helped in understanding their binding mode and binding landscape. Based on this structural information, they combined Fab from one antibody class and scFv fragment from another antibody class to design a bi-specific antibody. This new antibody has a potent and synergistic neutralization effect against the Wuhan strain and numerous other variants of concern.

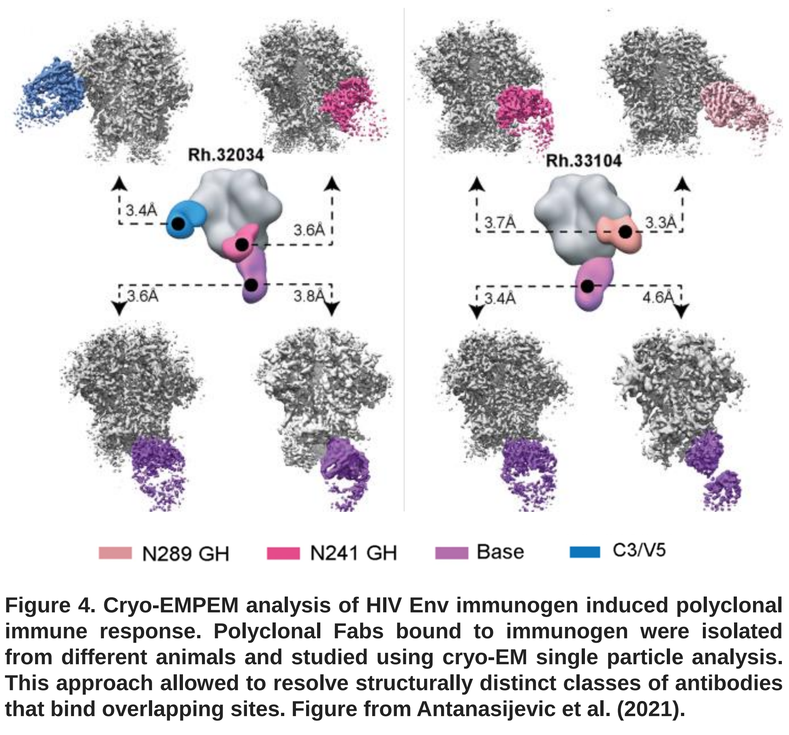

Figure 4. Cryo-EMPEM analysis of HIV Env immunogen induced polyclonal immune response. Polyclonal Fabs bound to immunogen were isolated from different animals and studied using cryo-EM single particle analysis. This approach allowed to resolve structurally distinct classes of antibodies that bind overlapping sites. Figure from Antanasijevic et al. (2021).

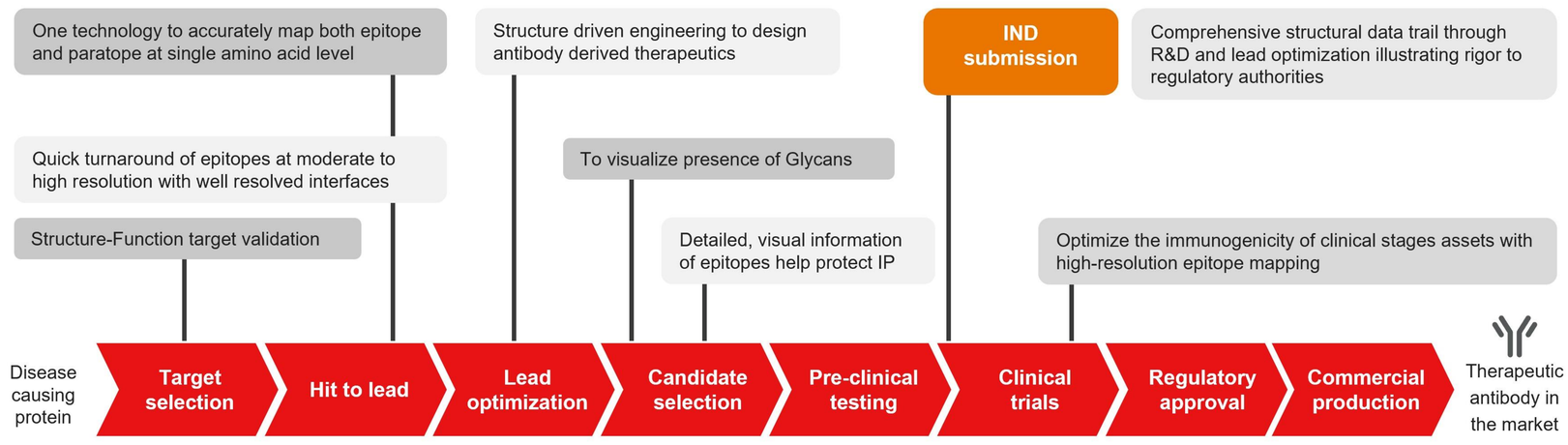

Figure 5. Antibody discovery is a long and complex process requiring multiple technologies and strategies to develop successful antibody therapeutic drug. High-resolution information obtained from cryo-EM structural biology, not only drive structure-based antibody engineering to improve their therapeutic efficacy and safety in the pre-clinical stage, but also to study and optimize assets that are already in the clinical stage.

4. Molecular basis of Immunogenicity using EMPEM

Cryo-EM Polyclonal Epitope Mapping (Cryo-EMPEM), is a specific Cryo-EM workflow to understand the structural basis of immunogenicity of an antigen including therapeutic antibody or vaccine. Cryo-EMPEM enables a comprehensive mapping of the immunogenic sites of an antigen and allows tracking of an individual's immune response over time. Once these antigenic sites are identified, it is possible to rationally engineer them to enhance their antigenic properties for a vaccine or reduce their antigenicity in the case of therapeutic antibodies. Furthermore, Cryo-EMPEM data allows high-resolution analysis of polyclonal antibody responses without requiring monoclonal antibody isolation.

5. Application of cryo-EM structural biology in antibody discovery and engineering pipeline

A versatile tool, providing structural insights early on, cryo-EM drives faster decision-making saving both cost and time associated with concept to IND application

Rich data packages that de-risk portfolios and add value to IP, contributes to higher success in securing funding in exchange for limited equity

A complementary tool providing structural data that act as a guiding map to interpret the results from multiple technologies used in the antibody discovery pipeline

High-resolution structural data obtained using cryo-EM helps train robust AI/ML algorithms and provides a mean to validate predicted antibody structures

References

Rak A, Kumar A et al., Preprint, (2023), doi: 10.21203/rs.3.rs-3235785/v1

J. Hansen et al., Science 2020, doi: 10.1126/science.abd0827

Chang MR et al., Nat Comm 2022, doi: 10.1038/s41467-022-33030-4

Antanasijevic A et al., Nat Comm 2021, doi: 10.1038/s41467-021-25087-4

Trademarks/licensing

Contact at a scan
Scan the QR code on the right with your mobile device to connect with Surajit Banerjee.

Learn more at thermofisher.com/SBDD