Antibody Research

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

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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 clinic. 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 reshaping the future of antibody research. We will share how **Cryo-EM-based epitope mapping** provides accurate information at the single amino acid level. We will explain how to leverage Cryo-EM structures for the **design of next-generation antibody therapeutics**. We provide our perspective of how Cryo-EM can **complement AI/ML** tools experimentally. Finally, we will discuss how Cryo-EM-based structural insights enable the rational design **of antibody-drug conjugates (ADCs)**.

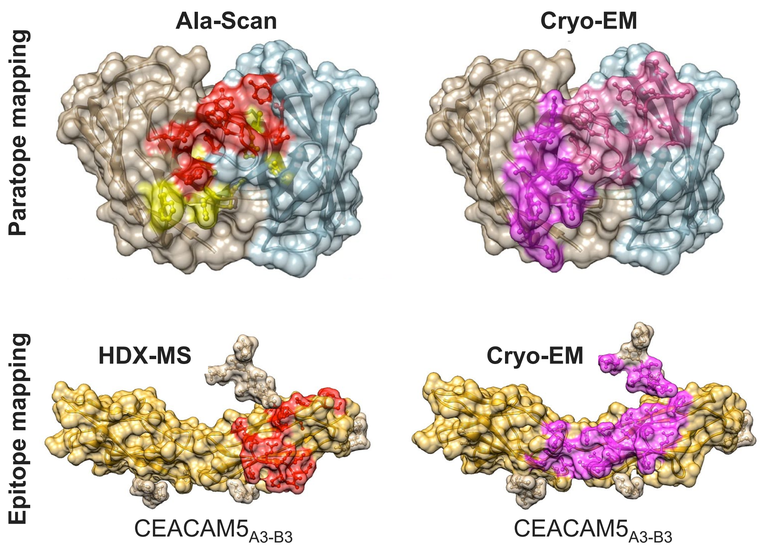

Figure 1. Comparison of the epitope-paratope interface between hCEACAM5A3-B3 and tusa Fab identified using three different structural analysis techniques. Cryo-EM provides single-amino-acid resolution for both the epitope and the paratope. Adapted from Kumar *et al.*, 2024.

1. Epitope/Paratope Mapping

Tusamitamab ravtansine, an antibody–drug conjugate, specifically binds the A3–B3 domains of human CEACAM5. Using cryo-EM, scientists at Sanofi mapped both the epitope on hCEACAM5 and the paratope of the tusa Fab at near single amino acid resolution. The structure revealed a discontinuous epitope spanning the A3–B3 domains and provided detailed insight into binding specificity. Importantly, the cryo-EM map also resolved glycan contributions, including an N-linked mannose at residue 612, along with key amino acid interactions with this glycan, highlighting the role of both protein and glycan elements in antibody recognition.

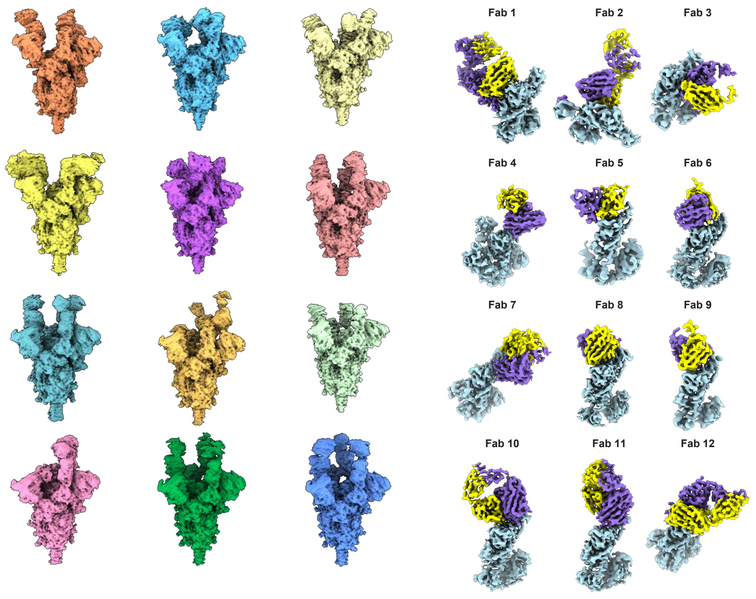

Figure 2. Cryo-EM structures of twelve preclinical-stage antibody Fab fragments in complex with the SARS-CoV-2 spike protein (left). Detailed views of the Fab–antigen interfaces highlight distinct binding modes and epitope engagement across all antibodies (right). Collaboration with Takeda Pharmaceuticals and Utrecht University.

2. High-throughput epitope mapping

In this collaborative work, we applied Cryo-EM rapid epitope mapping workflow to gain structural understanding of epitope recognition and binding of 12 COVID-specific antibodies to the “Omicron” B.1.1529 strain containing an unprecedented number of mutations in the receptor-binding domain (RBD). Cryo-EM single particle analysis resulted in 12 sub-3 Å structures of the SARS-CoV-2 spike-Fab complex, with the epitope-paratope interface resolved to high resolution in all 12 complexes (right panel). This information not only helped to obtain single amino acid-level detail of epitope-paratope interface but also to assign these Fabs to epitope classes.

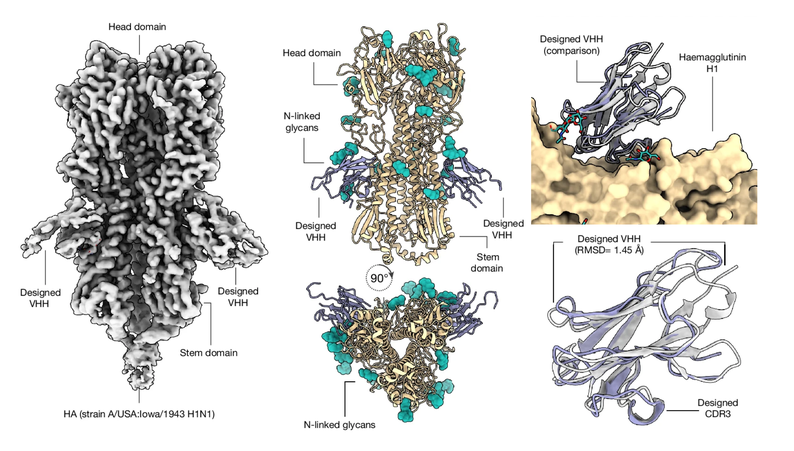

Figure 3. Cryo-EM structural characterization of VHH_flu_01 binding to influenza haemagglutinin. A 3.0 Å cryo-EM map shows VHH_flu_01 bound to the H1 haemagglutinin stem across two protomers and associated protein model. The experimentally determined cryo-EM structure closely matches the computational design. Adapted from Bennett *et al.*, 2025.

3. Integrating Cryo-EM in AI/ML-based pipelines

Cryo-EM plays a critical role in validating AI/ML-driven antibody design by providing high-resolution structural confirmation of predicted binding modes. In workflows such as RFdiffusion-based de novo antibody generation, cryo-EM enables direct visualization of how designed antibodies engage target epitopes, confirming both binding pose and atomic-level accuracy. The integration of cryo-EM with generative AI accelerates antibody discovery, improves design reliability, and supports the development of next-generation biologics with precise epitope targeting.

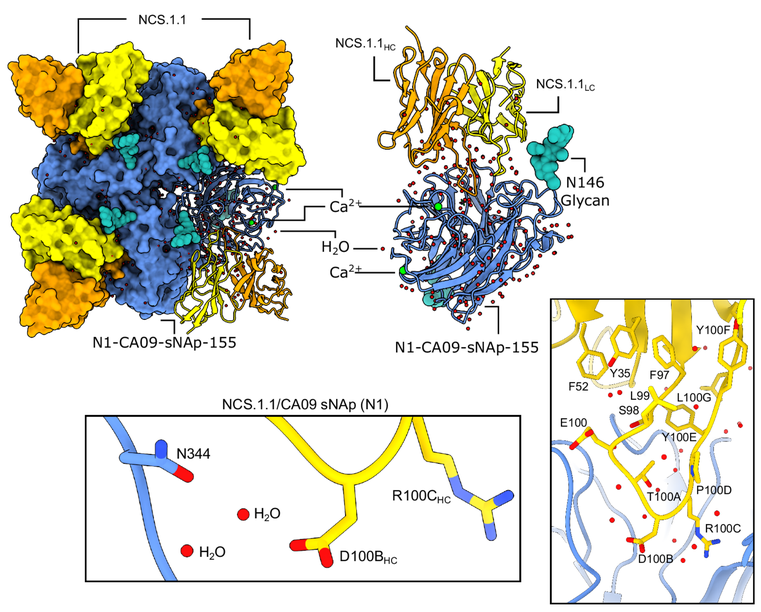

Figure 4. Cryo-EM revealed water-assisted lock-and-key binding enables mAbs to recognize and target NA variants. A 3 Å cryo-EM reconstruction showing viral neuraminidase (NA) bound by four copies of mAb NCS.1.1. Ribbon diagram of NCS.1.1 interacting with a single protomer, highlighting Ca^{2+}, glycans, and water molecules. Close-up view of the CDRH3 region with coordinated water molecules shown as red spheres. Adapted from Lederhofer *et al.*, 2025.

4. Understanding antigen-antibody binding

Cryo-EM structures at near-atomic resolution provide critical insights into the antigen–antibody interface by revealing not only the positioning of amino acid side chains but also the role of coordinated water molecules in binding. In this study, cryo-EM shows that water molecules act as essential bridges, stabilizing the interface and enabling the antibody to accommodate structural variability in the antigen. This level of structural detail enables understanding binding specificity, breadth, and inhibitory function, ultimately informing the rational design of more effective therapeutic antibodies.

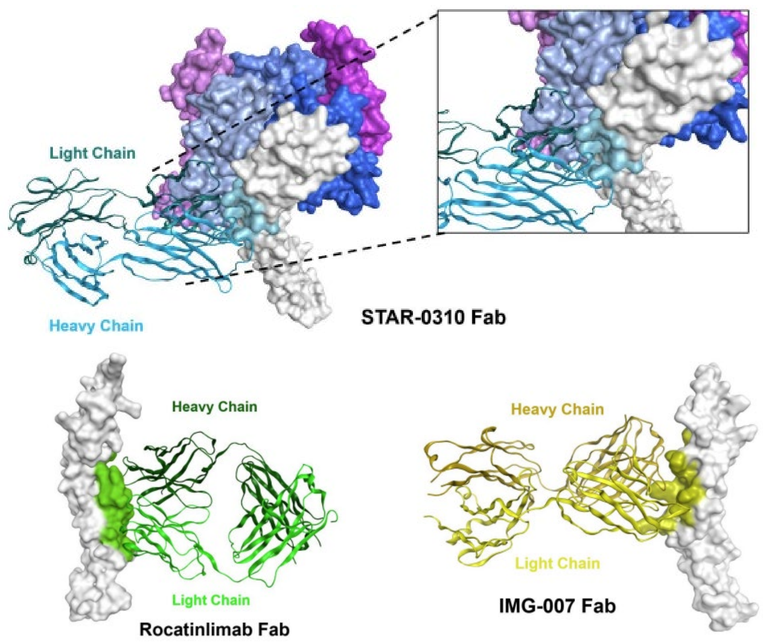

Figure 5. Rational design of a pure antagonist by revealing how antibody geometry governs signaling, safety, and differentiation. Cryo-EM structures of antibody–OX40 complexes reveal distinct binding modes underlying functional differences. STAR-0310 binds a unique epitope on CRD2 opposite the OX40L interface, explaining its pure antagonism and ability to disrupt pre-formed complexes. Adapted from Biris *et al.*, 2025.

5. Antibody Design-by-Mechanism

Cryo-EM structures can reveal how antibody geometry—such as Fab orientation and spatial positioning relative to receptors—can influence receptor clustering, signaling activation, or inhibition. In the case of OX40-targeting antibodies, cryo-EM enabled identification of a non-canonical antagonism mechanism, where steric hindrance prevents receptor trimerization without triggering agonism. Such insights go beyond traditional epitope mapping, allowing early assessment of agonism risk and enabling rational, structure-guided antibody design.

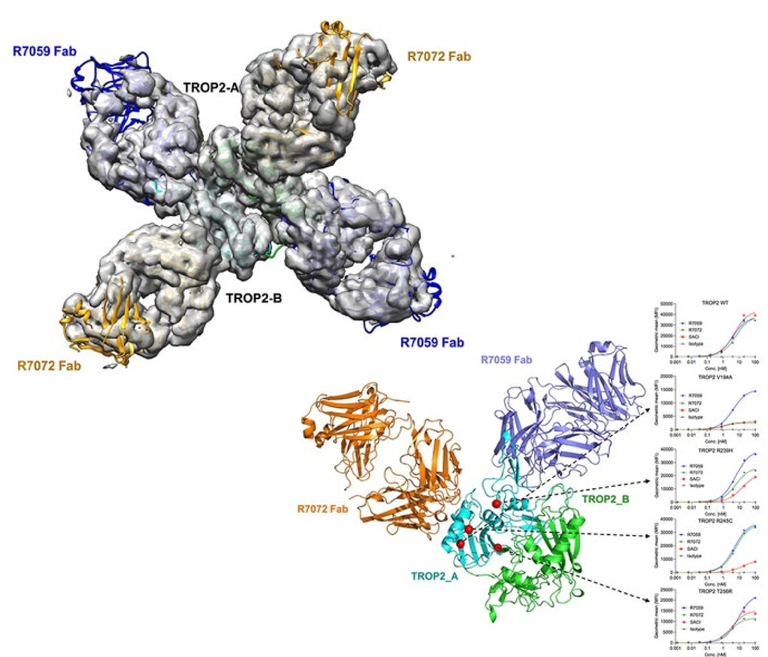

Figure 6. Cryo-EM showed that R7059 binds a unique TROP2 epitope, distinct from those recognized by antibodies used in approved TROP2-targeted ADCs, including sacituzumab and datopotamab. Structural insights helped explain the differentiated activity of R7059-DXD, including efficacy in models resistant to other TROP2-targeted ADCs. Adapted from Chen *et al.*, 2026.

6. Epitope Mapping and Designing New ADCs

While TROP2-targeted ADCs, including sacituzumab and datopotamab, have achieved clinical success, resistance mechanisms remain incompletely understood. The key question is not just whether an antibody binds TROP2, but where and how it binds across clinically relevant variants. Cryo-EM showed that R7059 engages a distinct N-terminal epitope, separate from those recognized by sacituzumab and datopotamab, providing a structural rationale for its retained binding across clinically relevant variants. Structural mapping associated reduced binding of other antibodies with mutations near the α2 helix. This positions R7059-DXD as a structure-guided, epitope-differentiated ADC.

References

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