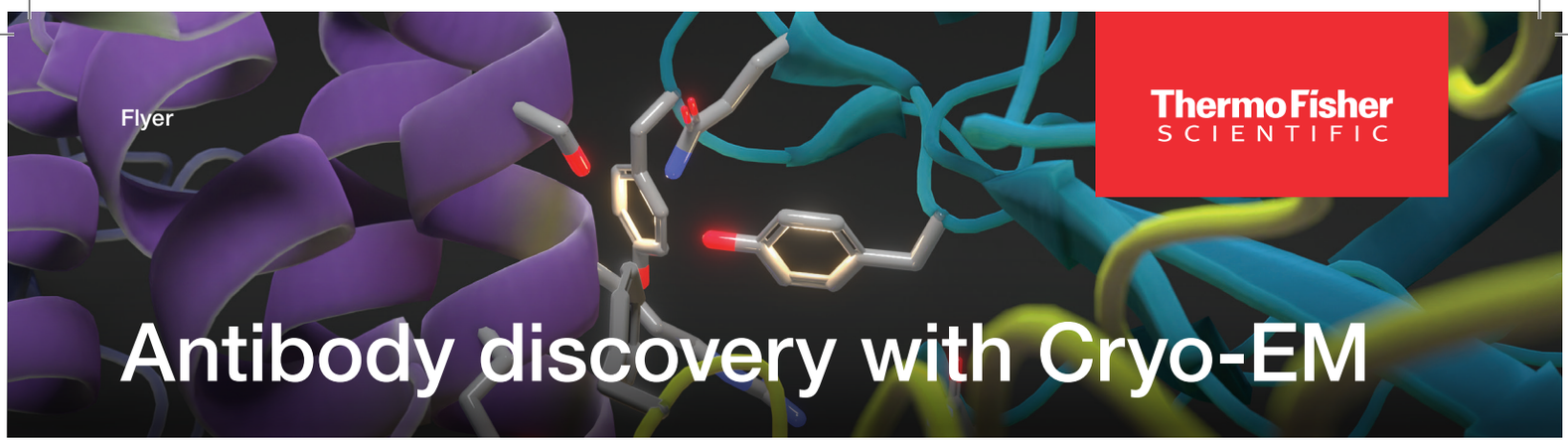
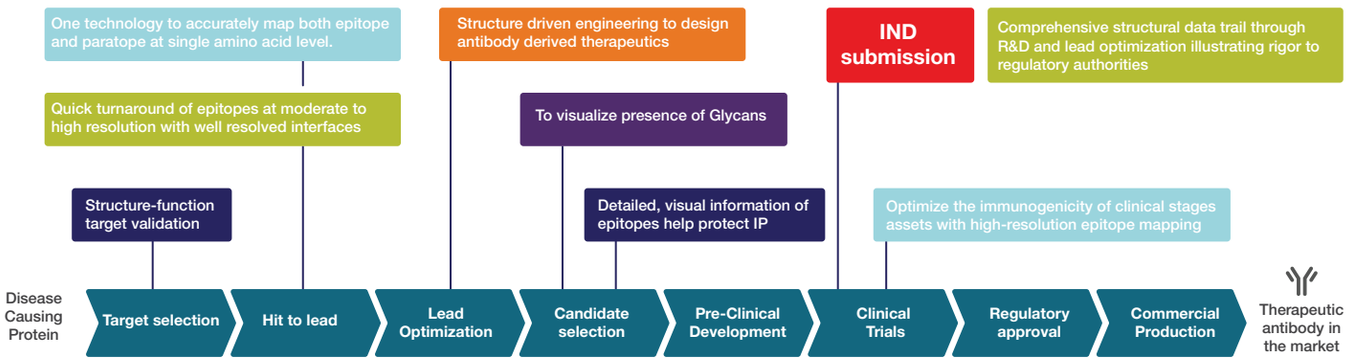


Flyer

Antibody discovery with Cryo-EM

Accelerate discovery and rational engineering of antibody modalities

Considering the growing need for novel therapeutic modalities, researchers are seeking innovative strategies and transformative technologies to overcome the limitations of traditional antibody discovery methods. Cryo-electron microscopy (cryo-EM) has emerged as a powerful technique that significantly accelerates antibody drug discovery processes. Despite only being recently adopted in drug discovery efforts, several cryo-EM supported therapies have already made their way into clinical settings. By providing high-resolution information, cryo-EM structural biology not only facilitates structure-based antibody engineering to enhance therapeutic efficacy and safety in the pre-clinical stage, but also enables the study and optimization of assets that are already in the clinical stage.



The rapid adoption of cryo-EM in antibody drug discovery pipelines is a result of its broad applicability, particularly in the field of single particle analysis. This groundbreaking technique is revolutionizing the development of various antibody modalities, ranging from monoclonal antibodies to bi- and multi-specific formats. By capturing high-resolution images of individual particles, cryo-EM offers unparalleled insights into the structural intricacies of antibodies and their interactions. This level of structural detail and understanding of antibody-antigen interactions is crucial for designing and optimizing therapeutic strategies. Compared to currently used techniques, cryo-EM offers several advantages:

- Accurate mapping of the epitope and paratope at the single amino-acid level, even for challenging and/or flexible targets
- Determination of the spatial arrangement of discrete and overlapping epitopes (both linear and conformational) on the large antigen surface
- Does not require labeling or immobilization, and there are no crystallization artifacts
- Provides early structural insights that can guide faster decision-making, saving both cost and time

As a result, this transformative technology is driving the development of novel therapeutic strategies at an unprecedented pace, offering great potential for advancements in the field.

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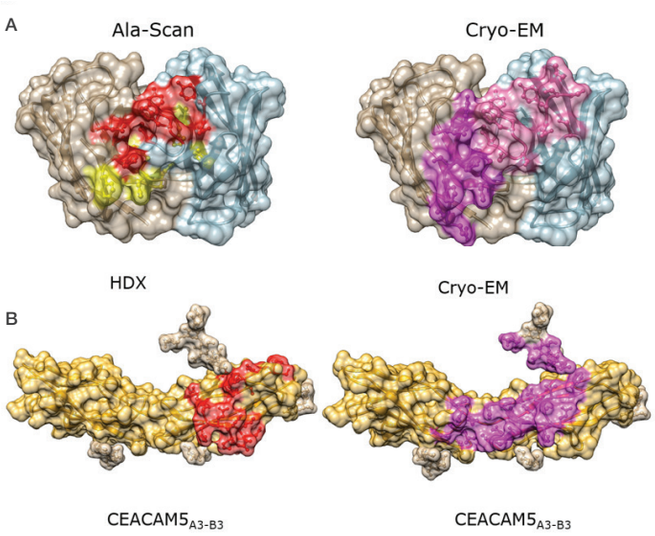


Comparison of the epitope-paratope interface between hCEACAM5A3-B3 and tusa Fab, identified using three different structural analysis techniques. Cryo-EM provided single-amino-acid resolution for both the epitope and paratope. Figure reproduced under CC BY 4.0.

Epitope/Paratope Mapping

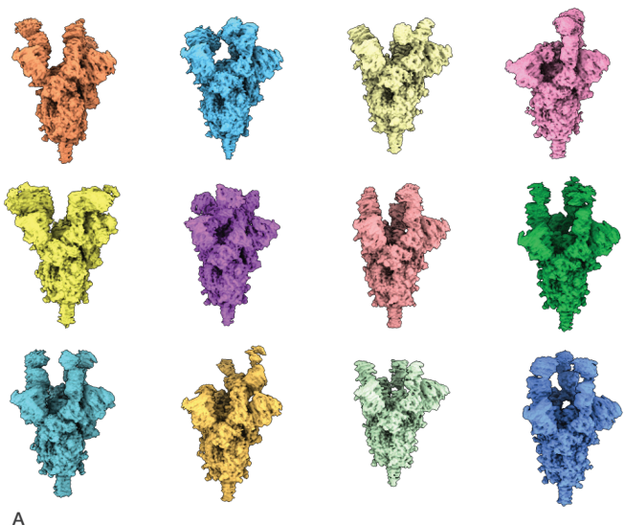
Tusamitamab ravtansine (tusa) is an antibody-drug conjugate that binds a specific region of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM); overproduction of CEACAM is associated with the growth of a number of cancers. Understanding this specificity could lead to enhanced antibody 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.

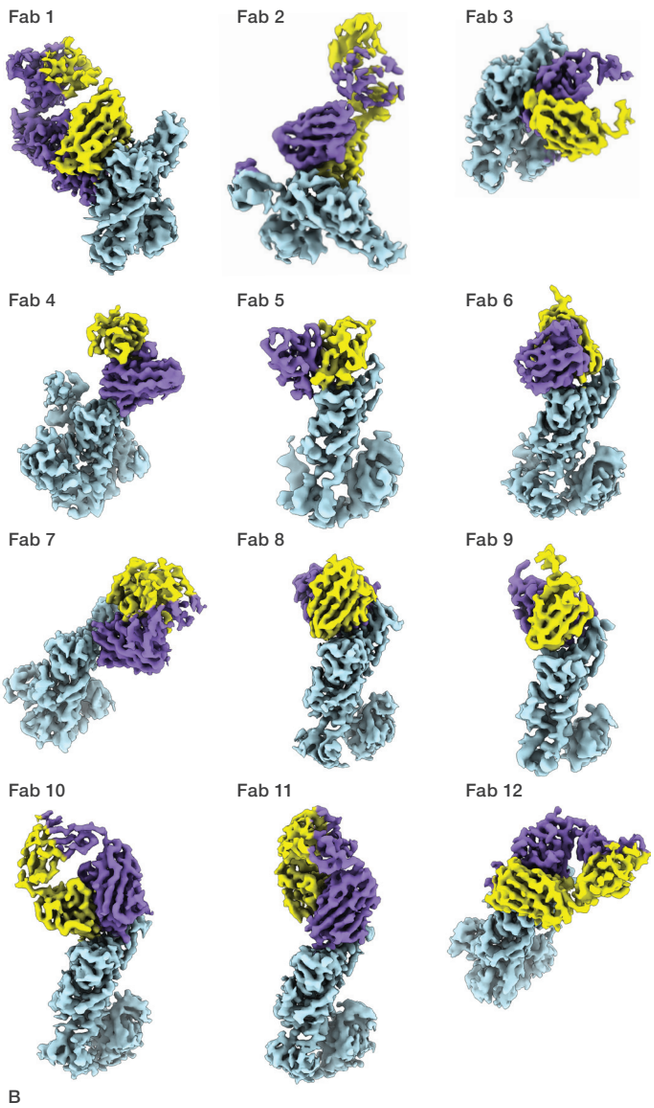
Rak, A., *et al.* **Structural insights into epitope-paratope interactions of monoclonal antibody targeting CEACAM5-expressing tumors.** Preprint (Version 1) available at Research Square (2023). doi: 10.21203/rs.3.rs-3235785/v1h

High-throughput epitope mapping

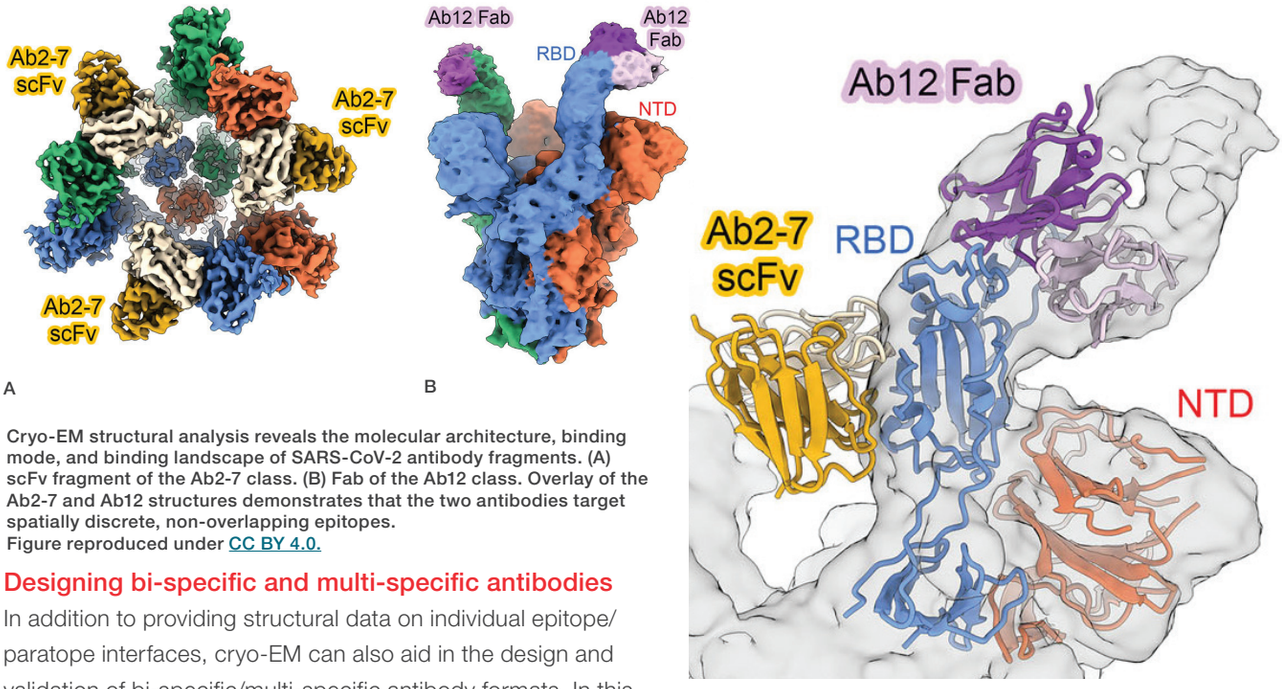
In this collaborative project, a rapid cryo-EM epitope-mapping work-flow was used to understand how 12 Covid-specific antibodies recog-nize and bind to the “Omicron” B.1.1529 strain at a structural level. This strain possesses an unusually high number of mutations in the receptor-binding domain (RBD). Cryo-EM single particle analysis ob-tained sub-3 Å structures of the SARS-CoV-2 spike:Fab complex for all 12 antibodies, with each complex’s epitope-paratope interface re-solved at high-resolution. With this information, the specific amino acids involved in the epitope/paratope interface could be identified and Fabs could be accurately assigned to their respective epitope classes.

Collaboration with Takeda Pharmaceuticals and Utrecht University (the Daniel Hurdiss and Berend-Jan Bosch groups). Manuscript in preparation.





High-throughput epitope mapping and structure analysis of 12 pre-clinical-stage SARS-CoV-2 antibodies, derived from Covid-19 patients and immunizations of transgenic animals. A) Cryo-EM structures of these antibody Fab fragments bound to spike proteins. B) Close inspection of each of the 12 Fab-antigen interfaces.



A B C

Cryo-EM structural analysis reveals the molecular architecture, binding mode, and binding landscape of SARS-CoV-2 antibody fragments. (A) scFv fragment of the Ab2-7 class. (B) Fab of the Ab12 class. Overlay of the Ab2-7 and Ab12 structures demonstrates that the two antibodies target spatially discrete, non-overlapping epitopes.
Figure reproduced under CC BY 4.0.

Designing bi-specific and multi-specific antibodies

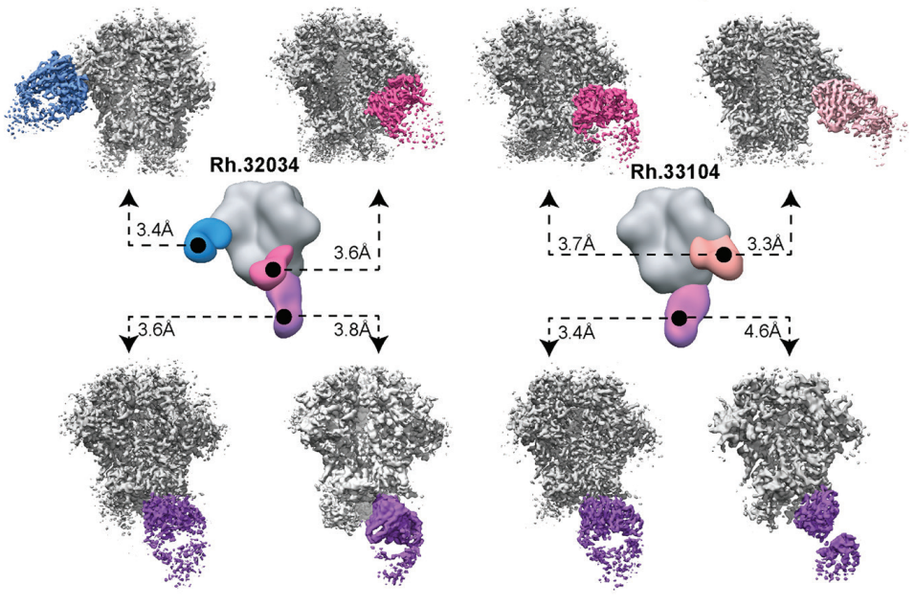
In addition to providing structural data on individual epitope/paratope interfaces, cryo-EM can also aid in the design and validation of bi-specific/multi-specific antibody formats. In this study, scientists from the Dana-Farber Cancer Institute and Harvard Medical School used structural insights to design a potent bi-specific antibody format against SARS-CoV-2.

Initially, the researchers obtained cryo-EM structures of different antibody classes bound to the receptor-binding domain, revealing their binding mode and binding landscape. Based on this structural information, they combined the Fab from one antibody class with the scFv fragment from another to design a bi-specific antibody. This new antibody has a potent and synergistic neutralization effect against the Wuhan strain of SARS-CoV-2 and numerous other variants of concern.

Chang, M.R., *et al.* **IgG-like bispecific antibodies with potent and synergistic neutralization against circulating SARS-CoV-2 variants of concernt**. *Nat Commun* 13, 5814 (2022). doi: 10.1038/s41467-022-33030-4

Determining the molecular basis of immunogenicity with cryo-EMPEM

Cryo-electron-microscopy-based polyclonal epitope mapping (cryo-EMPEM) is a novel workflow used to understand the structural basis of antigen immunogenicity, including therapeutic antibody or vaccine response. Cryo-EMPEM maps the immunogenic sites of an antigen and can be used to track an individual's immune response over time. Once these sites are identified, their antigenic properties can be enhanced with rational engineering, either to produce a vaccine or reduce their antigenicity for therapeutic antibodies. Furthermore, Cryo-EMPEM data allows high-resolution analysis of polyclonal antibody responses without the need for monoclonal antibody isolation.



Antanasijevic, A., *et al.* **Polyclonal antibody responses to HIV Env immunogens resolved using cryoEM.** *Nat Commun* 12, 4817 (2021). doi: 10.1038/s41467-021-25087-4

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 structurally distinct classes of antibodies to be resolved that bind overlapping sites. Figure reproduced under CC BY 4.0.

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Learn more

Cryo-electron microscopy is revolutionizing rational drug discovery pipelines

Rational design that leverages routine, high-resolution protein-structure determination is driving the discovery and development of diverse biologic and small molecule therapies. Cryo-EM delivers rapid epitope mapping on the atomic scale for antibody therapeutics and immune response profiling, supports the elucidation of mechanisms of action, and is also enabling more therapeutic targets than ever before for structure-based drug design. Whether you are investigating the modulation of binding affinities or optimizing drug stability, all of these questions can be answered in just one day of data collection.

In this webinar, experts from Sanofi discuss:

- How to quickly go from protein to structure
- Key examples of multi-specific drugs and structural insights of a CEACAM5-targeting antibody drug conjugate (ADC)
- How cryo-EM has significantly increased the number of the targets that can be identified for clinical trials in both biopharma and biotech

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