Cryo-EM as a powerful tool for epitope mapping of difficult targets

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Introduction

Determining the structure of antigen-antibody complexes is crucial for understanding the mechanism of action and rational design of potential biotherapeutics. However, many clinically relevant biological targets are intractable to X-ray crystallography. As a result, characterizing epitope-paratope interfaces for such complexes can be extremely challenging using this technique.

Cryo-electron microscopy (cryo-EM) is a powerful technique to visualize proteins in a near-native state at resolutions comparable to X-ray crystallography. Advances in cryo-EM are rapidly improving accessibility, automation and structure throughput (1,2).

Epitope mapping of large antibody panels

One of the main concerns in the ongoing COVID-19 pandemic is the SARS-CoV-2 variants that may be able to evade immunity. Epitope mapping of large antibody panels allows researchers to:

- Identify vulnerable sites on the spike protein.
- Predict and interpret the effect of new mutations.
- Speed up decision-making in selecting antibody combinations with non-overlapping epitopes.
- Identify liabilities in drug discovery pipelines in response to new emerging variants.

High-throughput cryo-EM epitope mapping of SARS-CoV-2 spike protein antibodies

Solving 12 structures from a single microscope session

Twelve pre-clinical stage SARS-CoV-2 antibodies, derived from COVID-19 patients and immunizations of transgenic animals, were selected for this study.

6P-stabilized (3) SARS-CoV-2 pre-fusion spike ectodomain was complexed with fragment antigen-binding regions (Fabs) and vitrified. Twelve pre-screened grids were imaged on a Krios G4 cryo-TEM equipped with an E-CFEG and Selectris X-Falcon 4 using EPU Multigrid.

Single particle analysis of individual datasets 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 maps.

Impact

- Significant advances have been made in speed, quality, and automation of cryo-EM data collection.
- Cryo-EM can be used for FBDD or epitope mapping of large antibody panels.
- High-throughput cryo-EM can impact existing applications by improving their efficiency and reducing the cost per dataset.

Table 1. Global and local resolutions achieved for each spike:Fab complex.

#	Global (Å)	Local (Å)
Fab 1	2.8	3.7
Fab 2	2.7	4.0
Fab 3	2.9	3.7
Fab 4	2.9	3.7
Fab 5	2.8	4.2
Fab 6	2.5	3.7
Fab 7	2.9	3.4
Fab 8	2.4	3.1
Fab 9	2.8	3.9
Fab 10	2.6	3.4
Fab 11	2.5	3.4
Fab 12	2.9	3.4

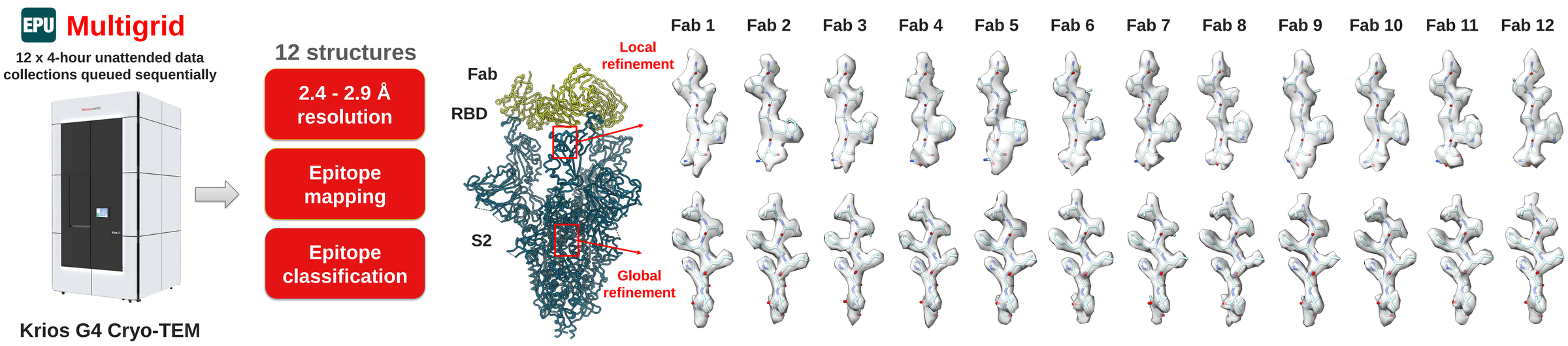

Figure 1. High-throughput cryo-EM epitope mapping of SARS-CoV-2 spike protein antibodies using EPU Multigrid. 48-hour EPU Multigrid session resulted in 12 sub 3 Å cryo-EM reconstructions, which allowed for mapping of epitope-paratope residues and assignment of Fabs to the epitope classes.

De novo inactive GPCR cryo-EM structure and epitope mapping

Cryo-EM is the go-to structural method for GPCRs

At the time of writing, there were over 450 GPCR cryo-EM in the Protein Data Bank. Active-state structures grew exponentially after the first structure depositions in 2017. A similar trend is starting to form for fiducial-assisted inactive-state cryo-EM structures. Additionally, high-resolution GPCR structures can now be routinely achieved (4).

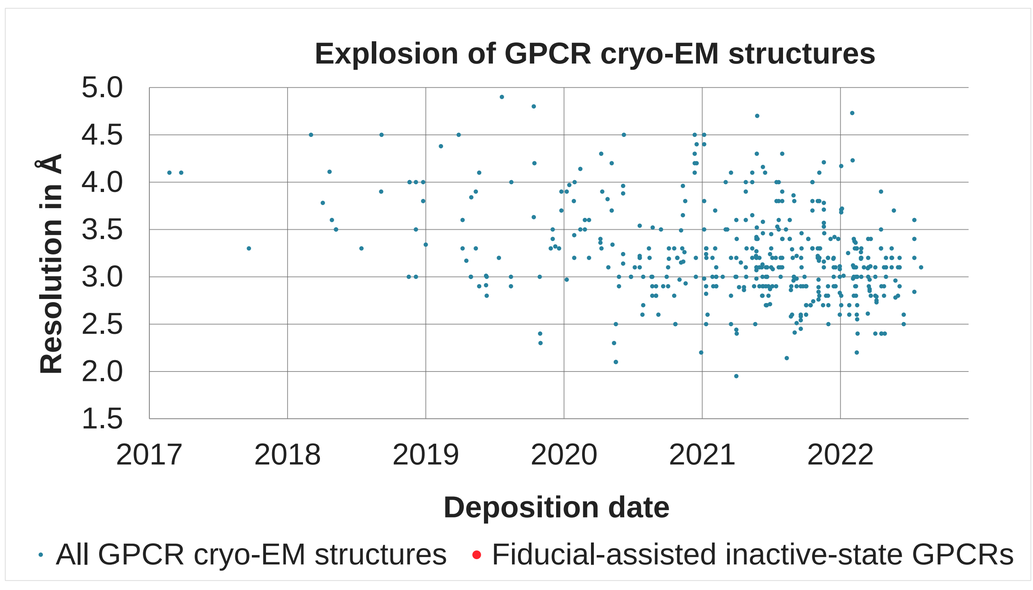

Figure 2. GPCR cryo-EM depositions in Protein Data Bank in the last 5 years.

3.5 Å structure of GPCR:Fab complex

Processing the ~4000 movie dataset resulted in a 3.5 Å *de novo* reconstruction of a GPCR target in a complex with a preclinical development antibody Fab fragment.

The analysis of the epitope-paratope interface revealed the nature of the interactions between the receptor and the Fab and facilitated the mutagenesis studies to improve antibody selectivity and specificity.

Sample optimization

1. The detergent was changed from DDM to GDN.
2. Protein concentration was increased to ~15 mg/ml.
3. A single-domain antibody (VHH) was added to rigidify the Fab and increase the mass of the complex.

Impact

- The protein to structure pipeline can be executed in less than a week, allowing cryo-EM to go hand in hand with the drug development cycles.
- Cryo-EM can be used for structure elucidation of GPCR-antibody or -small molecule antagonists within the pharmaceutical and biotechnology industry.

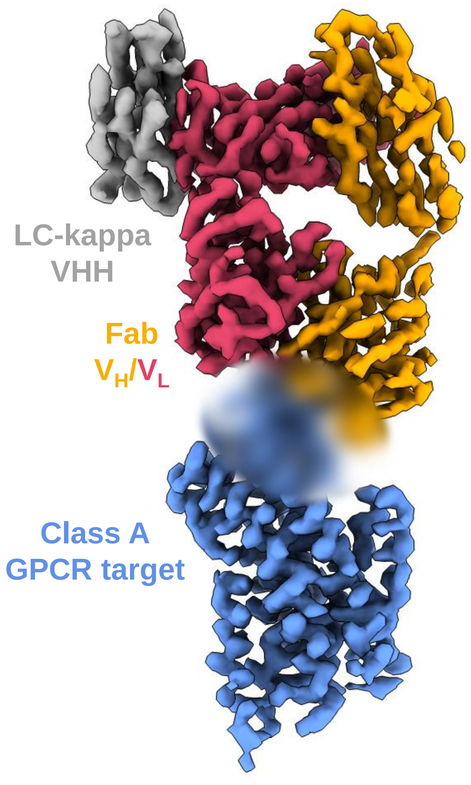

Figure 3. Cryo-EM reconstruction of the GPCR:Fab:VHH complex.

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