Leveraging Cryo-Electron Microscopy for Innovative Vaccine Development

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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. Cryo-EM is a powerful tool in vaccine development to enable better antigen design, map epitopes through analysis of antibody–epitope complexes, and understand the molecular basis of vaccine efficacy. This method is being widely adopted by pharmaceutical companies to de-risk discovery and development pipelines and for manufacturing projects. We envision cryo-EM to become an integral tool that will accelerate time to market, improve success rates, and reduce the cost of vaccine development, leading to more clinical 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.

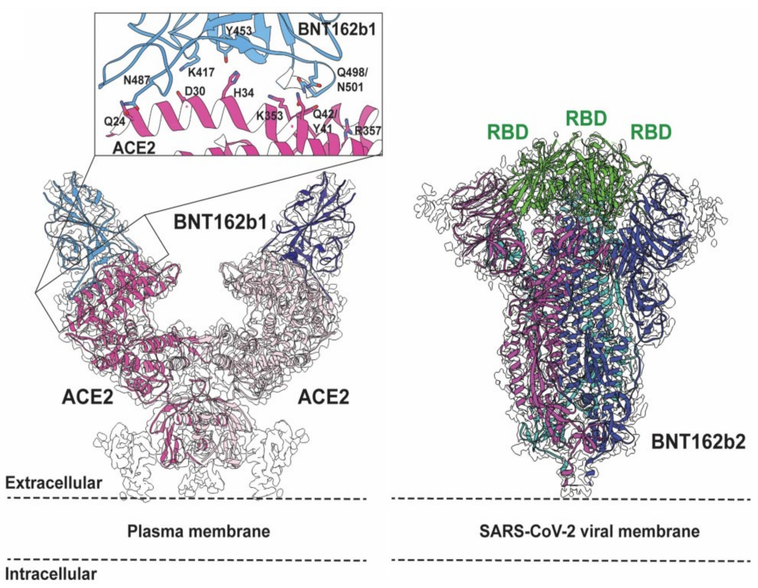

Figure 1. Cryo-EM structures of SARS-CoV-2 vaccine antigens BNT162b1 and BNT162b2. Left side, dimeric ACE2 (pink) bound to two copies of BNT162b1-encoded SARS-CoV-2 spike protein RBD (blue). Right side, Cryo-EM structure of trimeric pre-fusion-stabilized SARS-CoV-2 spike protein encoded by BNT162b2, superimposed with its cryo-EM density. Figure from Vogel A et al., Nature (2021)

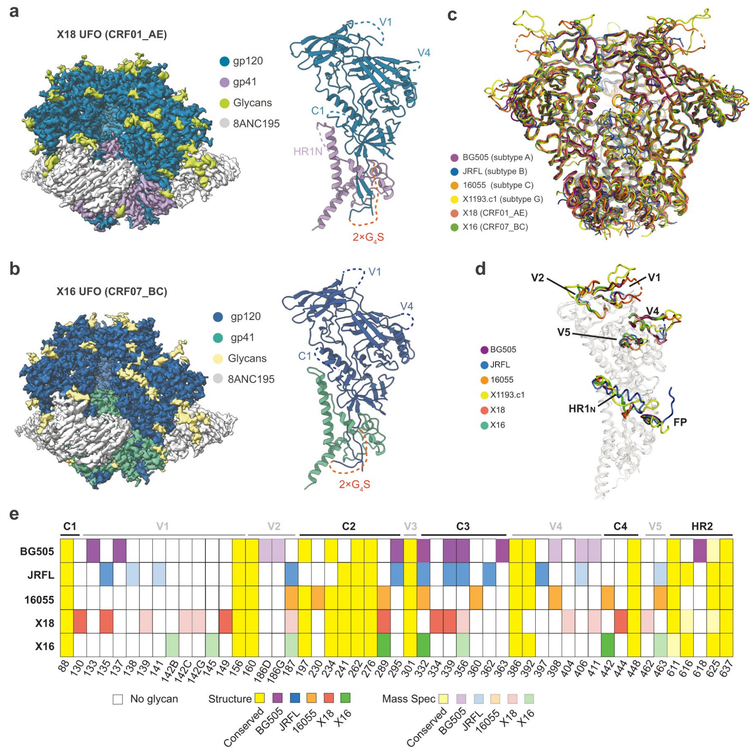

Figure 2. Cryo-EM structural analysis of Env antigen of two HIV-1 sub-types isolated from infected individuals. a) and b) Cryo-EM maps of HIV-1 subtypes (left) and reconstituted models of protomers (right). c) These atomic models are superimposed onto the atomic models of other known Env trimers and conformational differences in variable loops are highlighted in d). e) Out of 30 potential glycans, 20 of them were visualized in the Cryo-EM structures. Figure from Niu J et al., Nat Commun (2023)

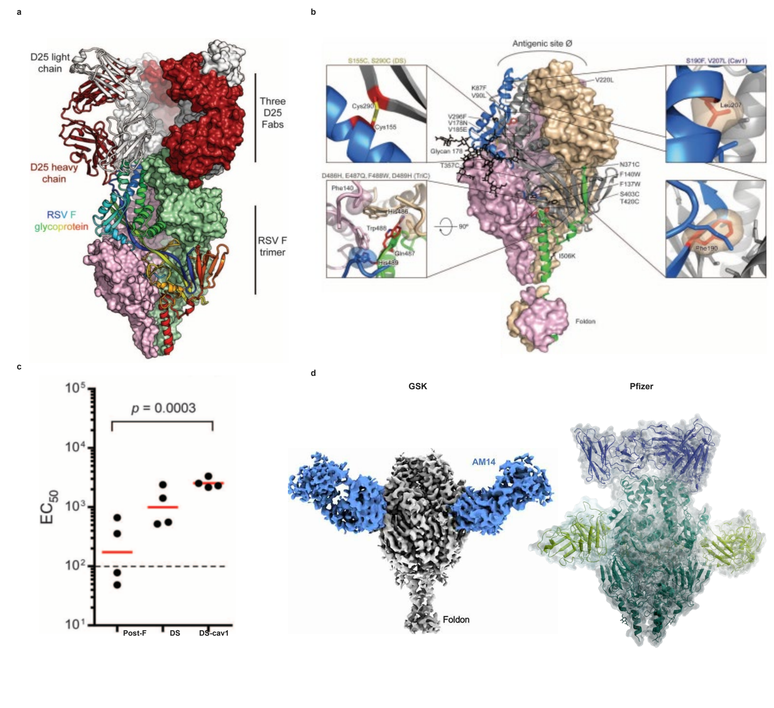

Figure 3. Cryo-EM structural analysis of RSV F protein. a) Prefusion structure of RSV F protein in complex with D25 Fab. b). Structure-guided antigen engineering was performed to obtain pre-fusion-stabilized RSV F named DS-cav1. c) Pre-fusion stabilized antigen elicited potent neutralizing antibodies. d) Cryo-EM structure of two FDA-approved vaccines against RSV. Figure from McLellan J et al., Science (2013), Harshbarger W et al., mAbs (2021), Ye C et al., Sci Transl Med (2023)

1. Antigen structure

Cryo-EM enables the structural understanding of specific viral proteins that can then be used in vaccine development. In response to the COVID-19 pandemic, Pfizer and BioNTech developed novel mRNA-based vaccines. Vaccine candidate BNT162b1 encoded an artificially trimerized version of the spike protein receptor-binding domain (RBD), in contrast with a pre-fusion-stabilized version of the full-length spike protein (BNT162b2). Given the novelty of mRNA-based vaccines, structural characterization was critical to ensure the correct epitopes were presented to patients' immune systems.

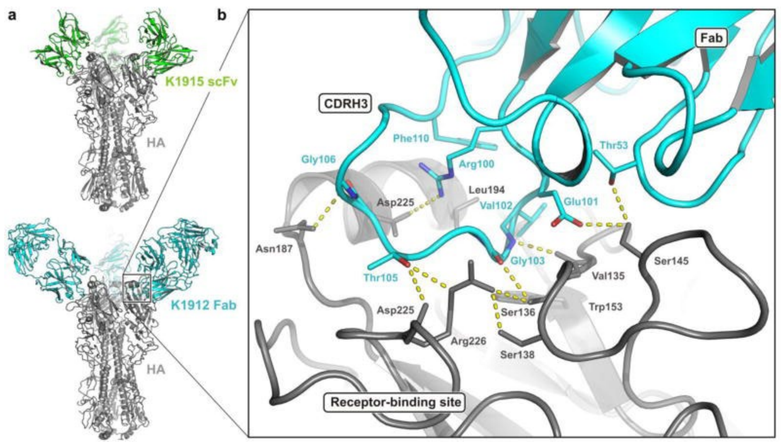

Figure 4. Cryo-EM analysis of hemagglutinin (HA):single-chain Fv (scFv) complexes at 4.5 Å resolution reveals the molecular basis of the antigen:antibody interface in a system previously intractable to crystallography. The study highlights the complementarity of cryo-EM and X-ray crystallography in addressing challenging structural biology problems. Figure from Liu Y et al., J Mol Biol (2017).

4. Epitope Mapping

Cryo-EM can also be used to map epitopes and better understand the immune evasion process. 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 crystallography. The complex was determined at 4.5 **Å,** 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.

2. Glycan visualization and immunogen design

Structure-guided HIV-1 vaccine design entails a comprehensive understanding of Envs from diverse HIV-1 subtypes. Here, scientists compared the structures and glycosylation patterns of Envs from different subtypes and performed cross-clade analyses to reveal that V1 loop in CRF01_AE is significantly longer and glycosylated more heavily than other subtypes. Latest advancements in Cryo-EM technology enable to locate the position of glycans in the Cryo-EM structure.

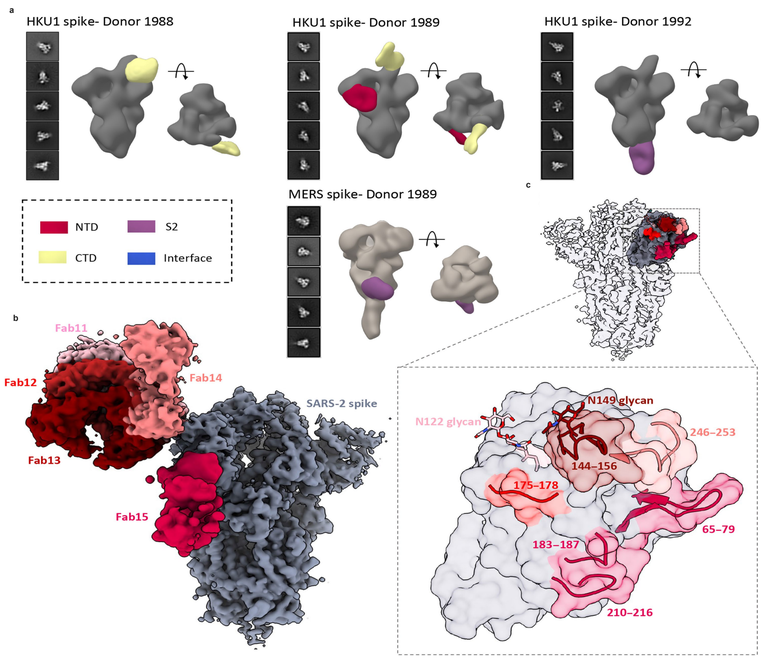

Figure 5. ns- and cryo-EMPEM analysis of antibody Fabs from SARS-CoV-2 infected donor sera. a) ns-EMPEM analysis of polyclonal Fabs. b) Cryo-EMPEM analysis showing five unique antibody classes, against SARS-CoV-2 spike NTD providing high-resolution details on the epitope-paratope interface. c) A zoomed-in view displaying the loop residues comprising each epitope. Figure from Bangaru S et al., Sci Adv (2022).

5. Molecular basis of immunogenicity using EMPEM

Cryo-EM Polyclonal Epitope Mapping (Cryo-EMPEM), is a specific cryo-EM workflow to study the structural basis of immunogenicity of vaccine antigen candidates. Cryo-EMPEM enables a relatively complete mapping of the immunogenic landscape of an antigen as well as allowing the tracking of an individual's immune response over time. Once these antigenic sites are identified, rational engineering can aid in improving the immunogenic properties of vaccine candidates.

3. Structure-guided stabilization of vaccine antigen

The RSV fusion (F) glycoprotein is a key antigen for vaccine development against RSV. RSV F protein exists in two conformations Pre-Fusion and Post-Fusion. Prefusion F is metastable and can spontaneously rearrange to the highly stable Postfusion conformation. Structure-guided antigen engineering strategies enable to identification of specific mutations to help stabilize immunogenic conformations, mask unwanted non-neutralizing antibody epitopes, optimize antigen thermostability and introduce additional epitopes on the antigen.

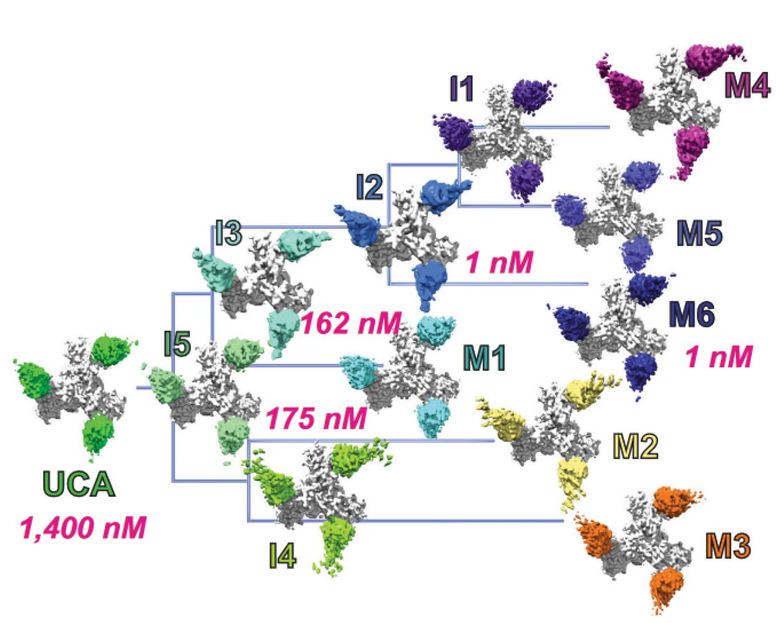

Figure 6. Cryo-EM structural analysis of broadly neutralizing HIV-1 V3-glycan targeting DH270 antibody clonal B cell lineage. These structures track the development of neutralization breadth from the unmutated common ancestor (UCA). By elucidating contacts mediated by key mutations at different stages of antibody development, scientists identified key sites for affinity optimization. Figure from Henderson R et al., Nat Commun (2023).

6. Structural basis for breadth development

Defining antibody maturation pathways to strategically inform immunogen design and enable the acceleration of this process through vaccination is a primary goal of current HIV-1 vaccine efforts. Antibody maturation involved staged, site-specific optimization of epitope-paratope contacts. Cryo-EM helps to visualize and study the interactions of a complete DH270 clonal tree with the co-evolving virus Env.

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