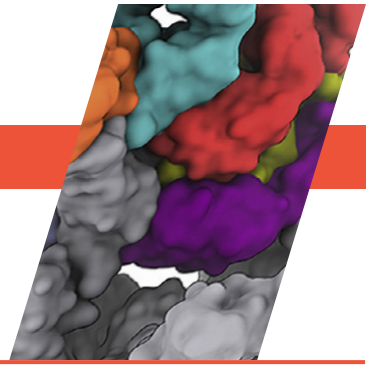
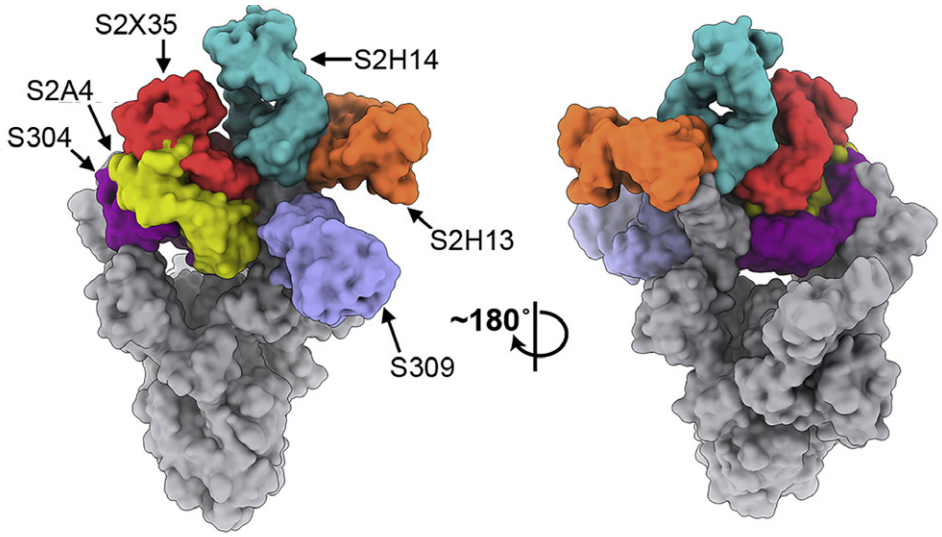
ADVANCED LIGHT SOURCE

BIOLOGICAL SCIENCES

Guiding Target Selection for COVID-19 Antibody Therapeutics





Two views of a composite model of the SARS-CoV-2 spike protein trimer (gray) with six monoclonal antibodies shown bound to one receptor binding domain (Piccoli et al.)

Scientific Achievement

Protein-structure studies at the Advanced Light Source (ALS) helped demonstrate that the primary target of antibody-based COVID-19 immunity is the part of the virus's spike protein that can most easily mutate.

Significance and Impact

The work anticipated the rise of SARS-CoV-2 variants and guides the selection of antibody therapeutics that are likely to be more resistant to immune escape.

A better understanding of immunity

To better predict the course of the COVID-19 pandemic and to develop the best new therapeutics, researchers need to understand what regions of the SARS-CoV-2 virus are most critical to the immune response and how likely these regions are to mutate and evade immunity.

Two recent papers, relying in part on protein-structure studies at the ALS, have provided detailed information about the SARS-CoV-2 virus that causes COVID-19 and the human immune response to it. The results reveal where the virus surface protein is most likely to mutate, what the consequences of those mutations may be, and which types of antibodies may be the most effective therapeutics.

Strategy for successful crystallography

For the determination of protein structures, the researchers relied on easily accessible, well-supported x-ray crystallography at ALS Beamlines 4.2.2 (part of the Molecular Biology Consortium) and 5.0.2 (part of the Berkeley Center for Structural Biology), in addition to cryogenic electron microscopy (cryo-EM). The studies focused on the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, the human ACE2 receptor through which the virus gains entry to host cells, and a panel of antibodies that neutralize the virus.

The RBD presented a challenging crystallography target. One likely reason is the presence of two glycans (sugar-molecule chains) that contribute heterogeneity and flexibility to the protein and that are required for protein expression. The researchers found that a successful strategy for each protein complex was to bind multiple Fab fragments (antibody fragments) and/or the ACE2 receptor simultaneously to the RBD. In the early stages of this work, they identified multiple non-competing antibodies that bind to different surfaces of the RBD, which made this approach feasible.

Results guide therapeutic development

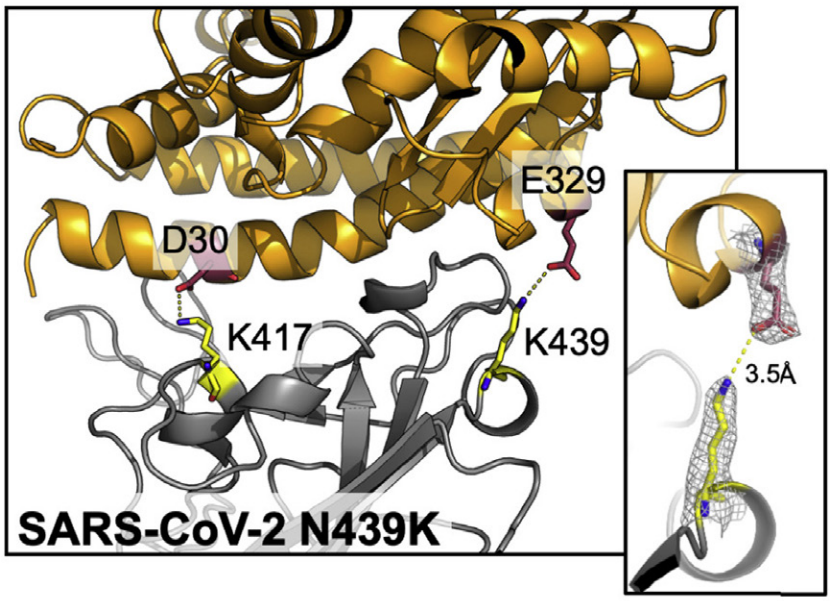
In the first paper (Piccoli et al.), the ALS data provided high-resolution information about the specific parts of the virus (epitopes) to which the antibodies in their panel attach, including the parent antibody (S309) of VIR-7831 (recently

renamed sotrovimab), a monoclonal antibody (mAb) that has demonstrated high efficacy in humans for early COVID-19 treatment. Knowing the detailed epitopes of each antibody is important for understanding their mechanisms of action and for predicting which mutations in the virus will cause a loss of antibody binding.

In the second paper (Thomson et al.), the ALS data provided the binding interface between an RBD variant (N439K) and ACE2, revealing a predicted new interaction that helps explain the higher ACE2 affinity measured for this variant.

Together, these papers demonstrate that the same region of the RBD that is the primary target of antibody-based immunity is also the most likely to mutate, and that these mutations can retain viral fitness (ability to replicate and cause illness) while resulting in immune evasion, with consequences for the long-term efficacy of vaccines and some antibody therapeutics. Indeed, this has already posed a problem for some antibody therapeutics that were approved under an Emergency Use Authorization by the FDA.

Results from these papers guide the further development of SARS-CoV-2 therapeutic antibodies and support the original selection of sotrovimab as a SARS-CoV-2 therapeutic. Sotrovimab targets a conserved epitope that is less prone to mutation, as demonstrated by its ability to neutralize all current SARS-CoV-2 variants of concern, and therefore is predicted to maintain efficacy as the virus continues its evolution. Overall, these results highlight the importance of epitope selection when developing therapeutic mAbs and provide a guide for which epitopes on the SARS-CoV-2 virus may give the best chance at avoiding future viral escape.



The first RBD–ACE2 complex structure where the RBD is a variant, in this case N439K; the figure highlights a new interaction between the N439K residue and ACE2 (Thomson et al.).

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Publication: L. Piccoli et al., "Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology," *Cell* **183**, 1024 (2020), doi: 10.1016/j.cell.2020.09.037; and E.C. Thomson et al., "Circulating SARS-CoV-2 spike N439K variants maintain fitness while evading antibody-mediated immunity," *Cell* **184**, 1171 (2021), doi: 10.1016/j.cell.2021.01.037.

Researchers: For full list of researchers and their affiliations, go to *https://als.lbl.gov/guiding-target-selection-for-covid-19-antibody-therapies/*

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