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Structural Studies of an Anti-SARS-CoV-2 Antibody Cocktail

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Neutralizing antibodies have become an important tool in treating infectious diseases. We recently reported two separate approaches yielding successful antibody treatments for Ebola-one from genetically humanized mice and the other from a human survivor. In a similar manner, we undertook parallel efforts using both humanized mice and convalescent patients to generate antibodies against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein. These efforts yielded a large collection of fully human antibodies that were characterized for binding, neutralization, and three-dimensional structure. On the basis of these criteria, we selected pairs of highly potent individual antibodies that simultaneously bind the receptor binding domain of the spike protein, thereby providing ideal partners for a therapeutic antibody cocktail that aims to decrease the potential for virus escape mutants that might arise in response to selective pressure from a single-antibody treatment.

Single-particle cryo–electron microscopy (cryo-EM) of the complex of SARS-CoV-2 spike RBD bound to Fab fragments of REGN10933 and REGN10987 shows that the two antibodies in this cocktail can simultaneously bind to distinct regions of the RBD. A three-dimensional (3D) reconstructed map of the complex with nominal resolution of 3.9 Å shows that the two Fab fragments bind at different epitopes on the RBD, which confirms that they are noncompeting antibodies. REGN10933 binds at the top of the RBD, extensively overlapping the binding site for ACE2. On the other hand, the epitope for REGN10987 is located on the side of the RBD, away from the REGN10933 epitope, and has little to no overlap with the ACE2 binding site, although molecular modeling demonstrates that bound REGN10987 will sterically interfere with ACE2 binding to the RBD. We have additionally determined cryo-EM structures of REGN10933 and REGN10987, as well as other antibodies, bound to the complete SARS-CoV-2 spike protein, and will discuss the effects of antibody binding on RBD conformation.

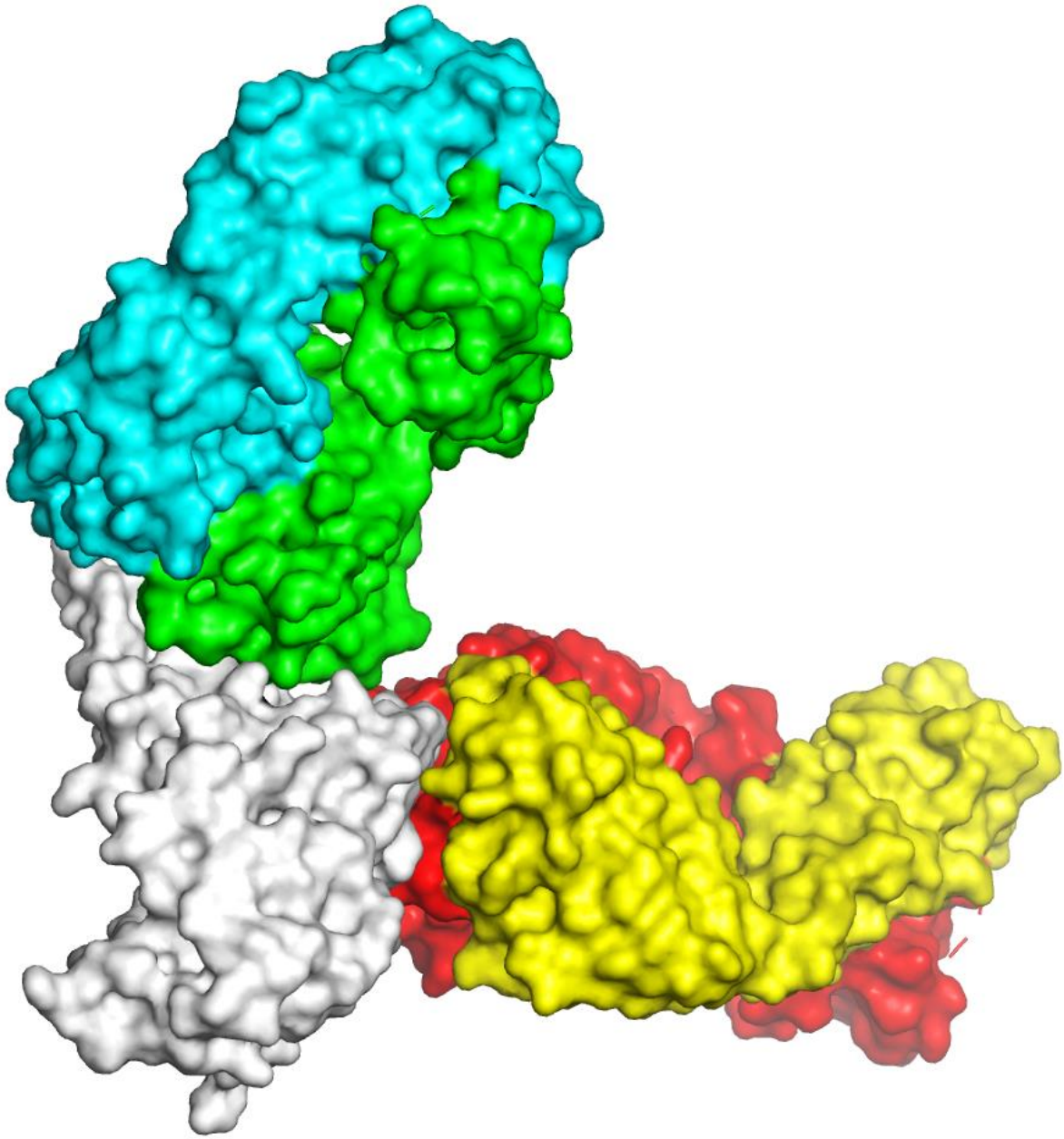

Figure 1. Complex of two inhibitory antibodies bound to SARS-CoV-2 spike RBD. The isolated SARS-CoV-2 receptor binding domain (RBD) is shown as a white molecular surface, with the Fab fragment from REGN10933 shown as a molecular surface, colored green (heavy chain) and cyan (light chain). Similarly, the Fab fragment of REGN10987 is shown in red (heavy chain) and yellow (light chain). This cryo-electron microscopy structure has been deposited in the Protein Data Bank with accession code 6XDG.

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