Spring 2021

Spike Protein Antibody Interactions Elicited by the SARS-CoV-2 Vaccine

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Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) arose as a novel virus in Wuhan China in December 2019. Then, as it rapidly spread across the world, it was declared a global health emergency by the World Health Organization in March 2020. SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19), has dramatically disrupted both normal life and the economy. In the past year and a half, there have been over 175 million cases globally[1] (as of June 15, 2021). High death rates, disruption to education, and widespread job loss has necessitated the desperate need for a vaccine against SARS-CoV-2. A vaccine against SARS-CoV-2 will significantly reduce the spread of COVID-19 and enable a safe return to pre-pandemic social life. Across the world, money and research have been poured into the vaccine, resulting in the fastest vaccine development in history. The Pfizer-BioNtech mRNA vaccine, one of the first widely available vaccines, was approved by emergency authorization in Britain on December 2, 2020, and then by the Food and Drug Administration (FDA) in the United States on December 11, 2020. Soon after, on December 18, the Moderna vaccine, another mRNA vaccine, was also approved for emergency use[2]. There are several different vaccines in production and under development, but the focus of this paper is specifically on the Pfizer and Moderna mRNA vaccines, and the antibody responses these vaccines elicit based on the use of the spike protein as an antigen. The binding of antibodies, generated through vaccination, to the spike protein of SARS-CoV-2 effectively neutralizes the virus, and understanding the details of the interactions between the spike protein and different antibodies is essential for understanding the specifics of the immune response to SARS-CoV-2 upon which the vaccine is based and the effect novel variants may have on the effectivity of the vaccine.

Basic Biology of SARS-CoV-2

The SARS-CoV-2 genome was first published on January 10, 2020. SARS-CoV-2 is a *Betacoronavirus*, belonging to the same genus as SARS-CoV-1 and Middle-East Respiratory Syndrome Coronavirus (MERS-CoV) as well as two common cold coronaviruses, HCoV-OC43 and HCoV-HKU1[3,4,5]. The SARS-CoV-2 virus is an enveloped positive sense RNA virus. It consists of a helical ribonucleocapsid complex made of nucleocapsid proteins and the messenger RNA (mRNA) sequence which holds all of the essential genes for the virus surrounded by a membrane which protects the mRNA and allows the virus to fuse with the membrane of host cells. The membrane of the virus is populated with proteins, including the famed spike protein which allow the virus to bind to host cells. SARS-CoV-2 has four structural proteins and 16 nonstructural proteins. The four structural proteins include the membrane protein which organizes the formation of the viral membrane, the envelope protein, which like the membrane protein is incorporated in the viral membrane and helps facilitate new viral assembly, the nucleocapsid protein, and the spike protein[3] (Fig. 1). By October 2020, the structures of more than half of the SARS-CoV-2 proteins had been determined using Cryo-Electron-Microscopy (Cryo-EM) and X-ray crystallography.

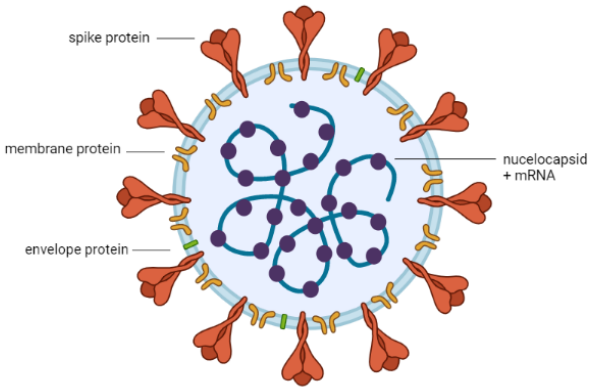


Figure 1: Schematic representation of a SARS-CoV-2 virus (created using BioRender). The four structural proteins are labeled.

Viral Life Cycle

SARS-CoV-2 invades host cells and utilizes host cell components to replicate, producing new virions which can spread to and infect other cells (Fig. 2). The SARS-CoV-2 spike protein, which is expressed on the outside of the viral membrane, binds to receptors on the host cell membrane. After the spike protein binds to the host cell, the virus either fuses with the host cell membrane and the viral mRNA is released into the host cell, or the virus is engulfed into the cell through endocytosis[5]. Once inside the host cell, the viral mRNA is released from the membrane and uncoated from the nucleocapsid proteins[5]. Ribosomes in the cell then translate the viral mRNA into proteins.

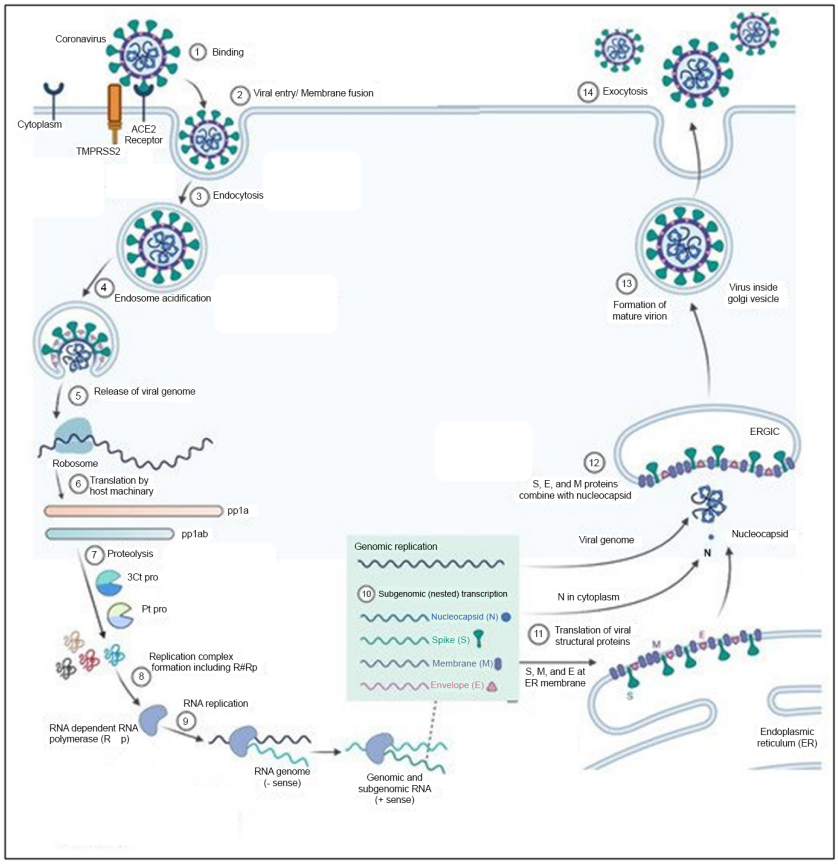


Figure 2: Complete viral life cycle of SARS-CoV-2. Entry by membrane fusion is not shown. source: Chilamakuri, R. & Argarwal, S. (2021). COVID-19: Characteristics and therapeutics. *Cells.* 10(2). https://doi.org/10.3390/cells10020206

The first open reading frame (Orf) of the viral genome to be translated is Orf1ab. Translation of Orf1ab produces polyprotein a and polyprotein ab which encode the 16 non-structural proteins (nsps)[5,6]. These polyproteins are cleaved by proteases from nsp3 and nsp5, contained in the downstream region of the genome. Cleavage of the polyproteins results in the nonstructural proteins which make up the replication and transcription complex (RTC), including the RNA-dependent RNA polymerase (RdRP)[6]. The viral RNA is then replicated to generate new mRNA which is packaged in new viruses or translated into new viral proteins. Discontinuous negative strand synthesis from the 3' end of the viral genomic mRNA produces nested mRNAs which are translated into structural and accessory proteins[5]. Membrane and envelope proteins interact with the host cell endoplasmic reticulum (ER) to initiate the formation of new viral envelopes and the engulfment of newly replicated mRNA, prompting the generation of new virions. The new virions then exit the cell through exocytosis[5].

Structure of the Spike Protein

The spike protein is a homotrimeric glycoprotein expressed on the outside of the SARS-CoV-2 viral membrane. Many studies have been done to elucidate a detailed image of the molecular structure of the spike protein through the use of Cryo-EM and X-ray crystallography. The spike protein has two distinct subunits, the S1 subunit and the S2 subunit[7,8]. The S1 subunit is the receptor binding fragment while the S2 subunit is the fusion fragment. The S1 subunit is located on the top of the spike protein and protects the prefusion S2[7]. The S1 subunit contains an N-terminal domain (NTD), the receptor binding domain (RBD), and two C-terminal domains (CTD)[7]. The S2 subunit is made of a central helix (CH), comprised of a three-stranded coiled coil, connected by the connector domain to the C-terminal heptad repeat 2 (HR2), heptad repeat

1 (HR1), the fusion peptide (FP), which becomes inserted in the host cell membrane, and a transmembrane segment (TM) which anchors the spike protein in the viral membrane[7].

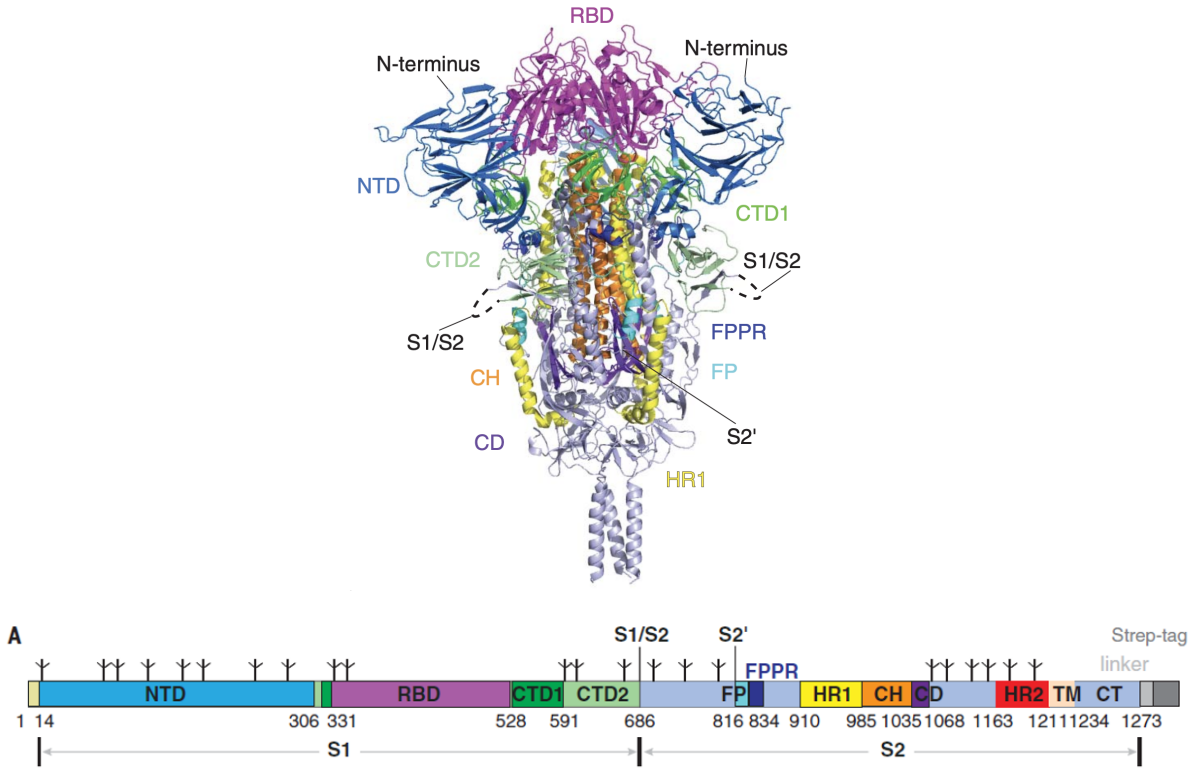


Figure 3: (A) Cartoon representation of the spike protein structure. Each domain is labeled and highlighted in a different color. (B) Schematic representation of the expression construct of the full-length SARS-CoV-2 spike protein. Glycans are represented by the tree-like symbols in black. Source: Cai, Y., Zhang, J., Xiao, T., Peng, H., & Sterling, S. (2020). Distinct conformational states of SARS-CoV-2 spike protein. *Science*, *369*(6511). https://doi.org/10.1126/science.abd4251

The spike protein has 22 glycans, or polysaccharide chains, on each trimer[3]. It has been suggested that N-linked glycans on the surface of the spike protein may have a function in inducing non-neutralizing antibodies in order to evade the immune system by shielding critical epitopes from other neutralizing anitbodies[3,7], a function that has been seen in HIV and influenza viral proteins[7]. Additionally, the presence of glycans may contribute to the conformational stability of the RBD[3].

The receptor binding domain of SARS-CoV-2 binds to the peptidase domain of angiotensin-converting enzyme (ACE2), a receptor protein expressed on the surface of host cells. The high affinity between ACE2 and the SARS-CoV-2 spike protein has been suggested to contribute to the high transfectability of the virus[7]. As described by Papageorgiou and Mohsin in a review of the SARS-CoV-2 spike protein, "The RBD is characterized by a twisted five-stranded antiparallel β-sheet with a long insertion between β4 and β7". This insertion interacts with two loops in the N-terminal peptidase domain of ACE2 in RBD binding to ACE2[3] (FIG. 4A). The RBD can be in either an up or down position (Fig. 4 B&C). The down position is receptor-inaccessible and is more stable, while the up position is receptor-accessible and less stable[7]. When the RBD binds to ACE2 and all RBDs are in the up position, the spike protein undergoes a conformational change which allows membrane fusion to occur[7].

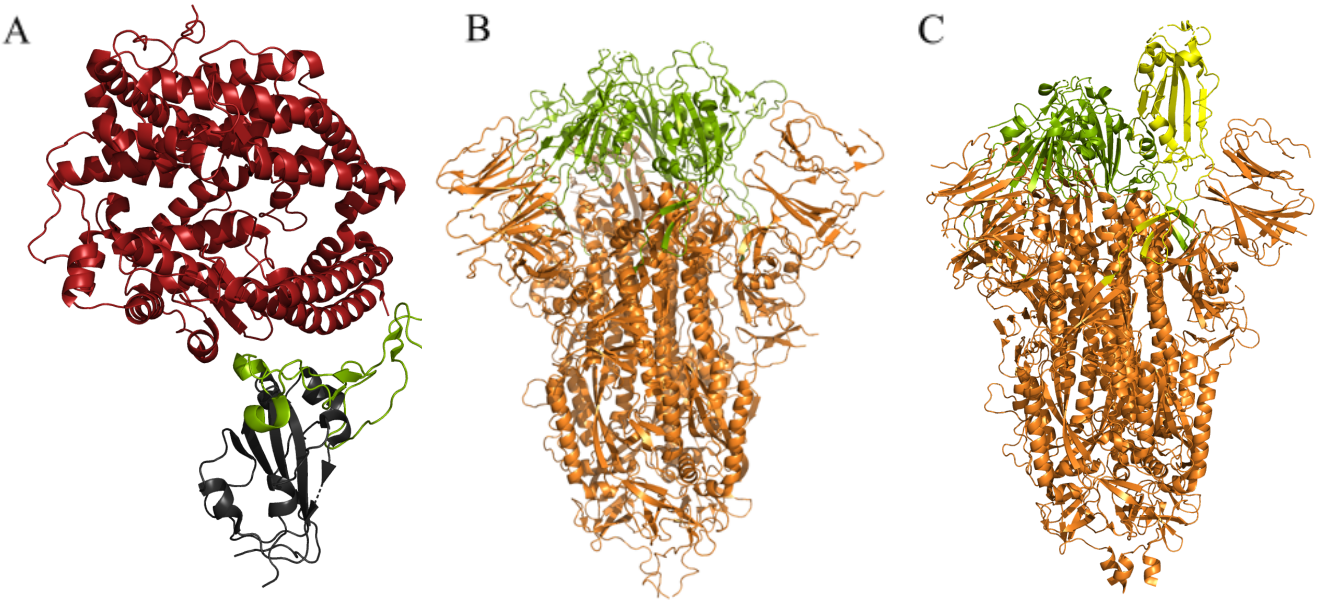


Figure 4: (A) The insertion between β4 and β7 of the RBD, shown in green, interacts with ACE2. PDB ID: 6LZG. RBDs, highlighted in green, on the spike protein in the (B) three down and (C) two down, one up conformations. The RBD in the up position is shown in yellow. PDB ID: 6XM5 and 6XKL.

Conformation Change of the Spike Protein

The spike protein has two different conformational shapes, a pre-fusion conformation and a post-fusion conformation. The pre-fusion conformational state is metastable, while the post-fusion conformation state is stable[7]. RBD binding to ACE2 leads to a 3-RBD-up conformation[7]. When all three RBDs are in the up position, the spike protein is highly unstable. This increased instability causes the S1 subunit to dissociate from the S2 subunit, subsequently causing the S2 subunit to change conformation[3,7,8]. Cleavage of the S1/S2 cleavage site also contributes to the complete dissociation of the S1 subunit[7]. When the S1 subunit dissociates, the S2' cleavage site adjacent to the fusion peptide is exposed[1] and cleaved by host cell proteases, transmembrane serine protease TMPRSS2 or cysteine proteases cathepsin B and cathepsin L[5,6,7]. This cleavage releases the fusion peptide[3]. Additionally, in the transition between pre and post-fusion states the HR1 domain of the S2 subunit flips and HR2 folds back, inserting the fusion peptide in the host cell membrane and allowing the fusion peptide and the transmembrane segment to become in close proximity and facilitate membrane bending during fusion[7]**.** The conformational change of the spike protein from a metastable pre-fusion to a stable post-fusion conformation allows viral fusion to overcome the kinetic energy barrier of membrane fusion, and proceed in a thermodynamically favorable manner[7].

Spike Protein Based mRNA Vaccines

The Food and Drug Administration has authorized the emergency use of three vaccines against COVID-19 in the United States. Two of those vaccines are the Pfizer-BioNTech (BNT163b2) and Moderna (mRNA 1273) mRNA vaccines. These mRNA vaccines are comprised of a mRNA molecule which codes for a modified spike protein encased in a lipid nanoparticle. The lipid nanoparticle protects the mRNA sequence from degradation by host

enzymes[13] and facilitates its delivery to host cells. The mRNA within the Pfizer and Moderna vaccines encodes a full-length SARS-CoV-2 spike protein with two stabilizing proline mutations in the C-terminal domain of the S2 subunit[8,9].

Once the vaccine is administered, the lipid nanoparticle is taken up by a host cell, and using host cell ribosomes, the mRNA it contains is translated into spike proteins[2]. The spike proteins and spike protein fragments made by the cell are then presented on the cell surface where they are able to be recognized by the immune system[2]. Essentially, mRNA vaccines use a mRNA transcript to produce a spike protein in order to elicit a neutralizing antibody response and file SARS-CoV-2 in the immune system's memory. Due to its role in receptor binding and fusion, the spike was identified as an antigenic target early in the pandemic[8].

The immune system works to recognize and respond to foreign materials, such as the spike proteins now expressed by cells in a vaccinated individual, through a complex pathway involving many different cell types (Fig. 5). If a cell expressing spike proteins dies, an antigen-presenting cell may take up spike proteins and fragments from the cell debris. The antigen-presenting cell then digests the spike protein and presents the spike protein fragments on its surface to be recognized by helper T cells. Helper T cells are activated by the antigens presented on the surface of the antigen-presenting cells, and activated helper T cells may function to activate either killer T cells or B cells. Activated killer T cells target and kill cells that have been infected. B cells interact with spike proteins on the surface of an infected, or vaccinated, cell, and if activated by helper T cells will either secrete soluble antibodies against the spike protein or will proliferate into memory cells. Memory B cells become a part of the innate immune system until activated by a specific antigen, such as the spike protein, which causes them to become active and secrete antibodies. Antibodies produced by the B cells will

respond to the presence of the virus, neutralizing it by marking virions for destruction, and in some cases, preventing them from binding to the ACE2 receptor. A rapid immune response to the virus prevents infection and decreases viral load, reducing transfectability.

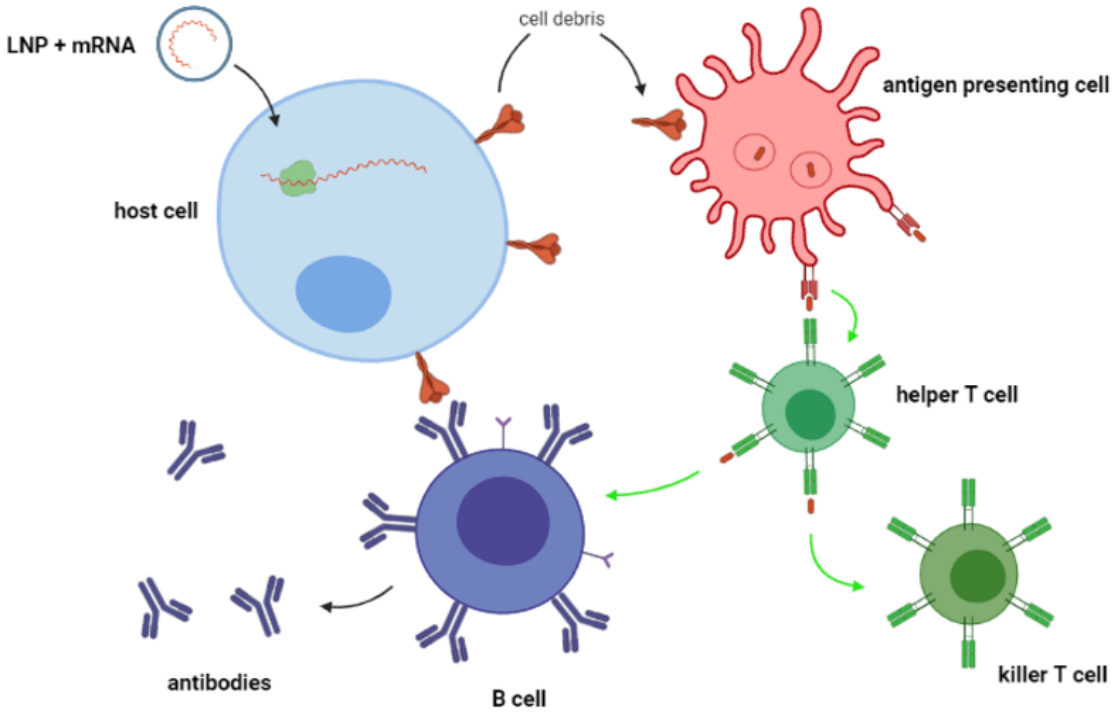


Figure 5: The immune system response elicited by the mRNA vaccine (created using BioRender).

In a study on mRNA vaccine-elicited antibodies, Zijun Wang et al. found that the mRNA vaccines provoke a very similar B cell memory immune response as natural infection by SARS-CoV-2[10]. Furthermore, the antibodies produced by individuals given either the Pfizer or Moderna vaccine were almost identical[10]. These findings allow antibodies produced by natural infection in COVID-19 patients to be considered in analysis of antibody-spike protein interactions elicited by either mRNA vaccine.

Spike-Protein Antibody Interactions

Using serum from convalescent COVID-19 patients, researchers have identified many monoclonal neutralizing antibodies against the SARS-CoV-2 spike protein. Additionally,

multiple overlapping epitopes have been identified on the spike protein, the majority of which are located on either the RBD or the NTD[13]. Antibodies that target the RBD have been found to be more potently neutralizing against SARS-CoV-2 than antibodies targeting the NTD or other regions of the spike protein, as blocking the SARS-CoV-2 RBD from binding with ACE2 prevents viral entry to the host cell[10,13]. Wu et al. expressed the SARS-CoV-2 RBD to isolate B cells from COVID-19 patients. From this experiment they found four neutralizing antibodies, B5, B38, H2, and H4 which bound specifically to the RBD of SARS-CoV-2 and not to the RBD of SARS-CoV-1. Antibody specificity for SARS-CoV-2 suggests that the two SARS viruses have different epitopes within their RBDs[11]. The spike proteins of SARS-CoV-2 and SARS-CoV-1 share 77.5 percent amino acid sequence identity, while the spike proteins of SARS-CoV-2 and MERS share only 31 percent sequence identity[4]. These differences in sequence account for the binding of unique antibodies. The B38 antibody was also identified in a proteome microarray experiment conducted by Hongye Wang et al, and further assays showed that the B38 and H4 antibodies compete with ACE2 for binding with the RBD[11,12].

The B38 antibody interacts with 36 residues in the RBD, 15 with the light chain of the antibody and 21 with the heavy chain[3,11]. Eighteen of the RBD residues that interact with B38 also are a part of the 21 residues on the RBD which have been found to interact with ACE2[11] (Fig 6), strong evidence showing binding competition between ACE 2 and B38. The interaction between the spike protein RBD and the B38 antibody is based mainly on hydrophilic interactions[3]. Using the PyMOL measurement tool, 14 potential hydrogen bonds were found between the side chains of B38 and the previously identified 36 interacting residues of the RBD. Potential hydrogen bonds were identified by measurements of 2.5-4.0Å between oxygen and nitrogen atoms of the side chains (Fig. 7).

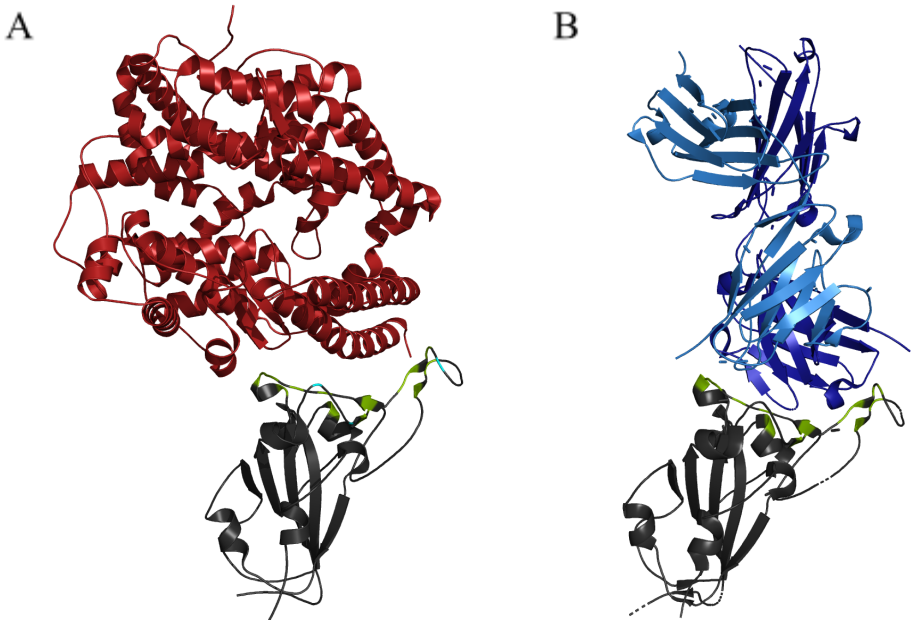


Figure 6: (A) The 21 residues on the RBD that interact with ACE2 are colored in. PDB ID: 6LZG. Cyan residues are specific to interaction with ACE2. Green colored residues interact with both ACE2 and (B) B38. PDB ID; 7BZ5.

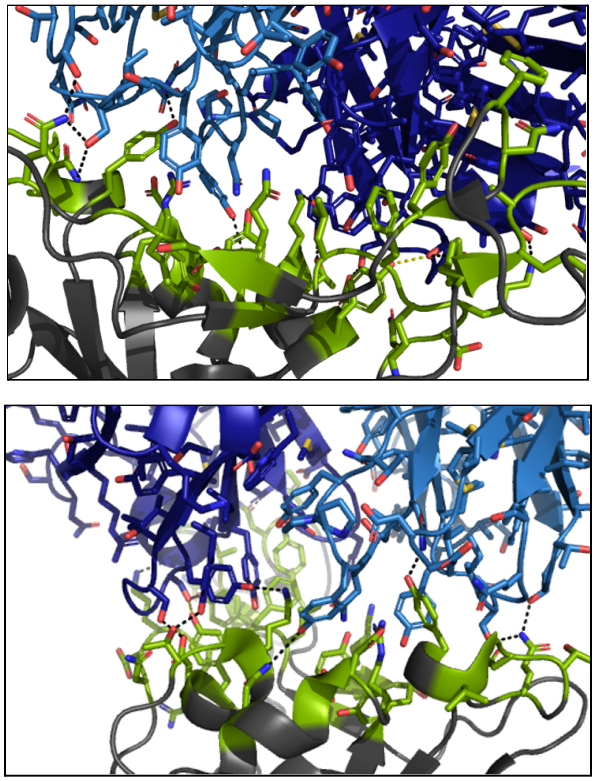


Figure 7: Representative images of the hydrogen bonds between the RBD and B38. Side chains are colored by atom and potential hydrogen bonds are shown with dashed lines.

Mice infected with COVID-19 and given either B38 or H4 had significantly lower amounts of viral RNA three days post-infection than mice in the control group that were not given B38 or H4 antibodies[11] (Fig. 8), demonstrating the neutralizing capacity of these two antibodies.

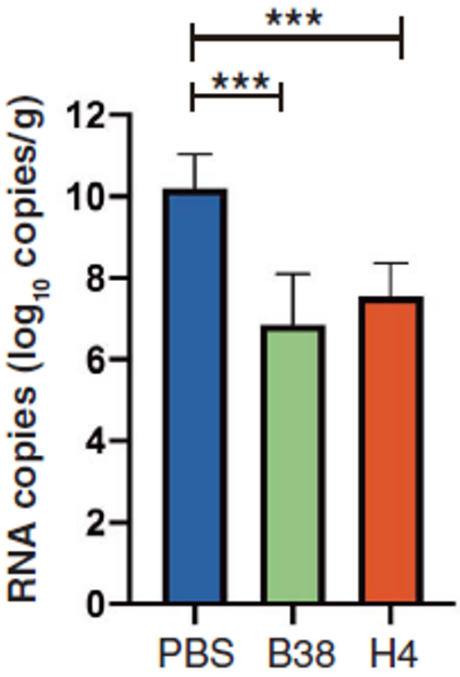
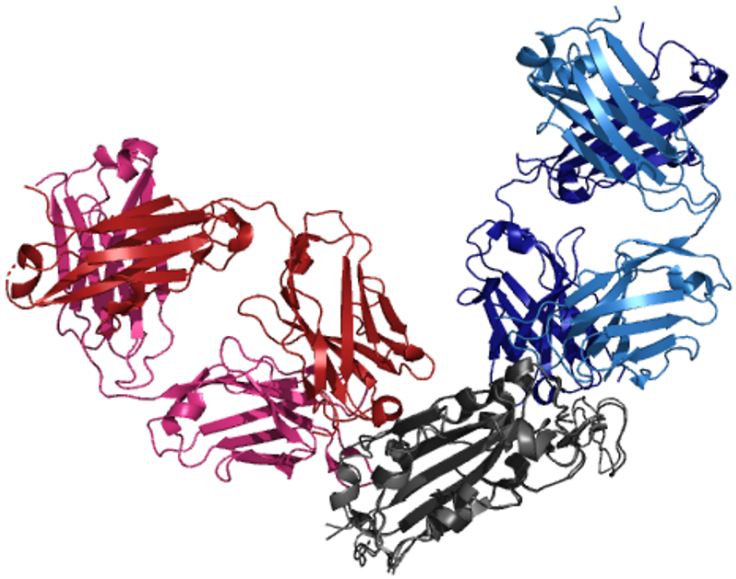


Figure 8: Viral RNA copies measured by q-RT-PCR in mice lungs treated with B38, H4, or phosphate buffered saline (PBS) 3 days post-infection with COVID-19. source: Wu, Y., Wang, F., Shen, C. *et al.* (2020). A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2.

B38 and H4 bind to different, but overlapping epitopes on the RBD[11], as can be seen in the PyMOL alignment in Figure 9A. In a competition assay for B38 and H4, Wu et al. found that after saturation with the first antibody, the second antibody still bound to the RBD, which shows that B38 and H4 are noncompetitive[11]. A BLAST alignment of the B38 and H4 Fabs shows several differences between the two antibodies in both the heavy and light chains (Fig. 9B), consistent with previous evidence for distinct epitope recognition.

A



B

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Query  3    EVQLVESGGGLVQPGGSLRLSCAASGFIVSSNYMSWVRQAPGKGLEWVSVIYSGGSTYY  61
            ++QLV+SG  + +PG SL++SC  SG+   + ++ WVRQ PGKGLEW+ +IY G S T Y
Sbjct  2    QMQLVQSGTEVKKPGESLKISCKGSGYGFITYWIGWVRQMPGKGLEWMGIIYPGDSETRY  61

Query  62   ADSVKGRFTISRHNSKNTLYLQMNSLRAEDTAVYYCAREA---YGMDVWGQGTTVTVSSA  118
            + S +G+ TIS   S NT YLQ +SL+A DTA+YYCA  +     MDVWGQGTTVTV  A
Sbjct  62   SPSFQGQVTISADKSINTAYLQWSSLKASDTAIYYCAGGSGISTPMDVWGQGTTVTV--A  119

Query  119  STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG  178
            STKGPSVFPLAPSS    GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
Sbjct  120  STKGPSVFPLAPSS----GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG  175

Query  179  LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS  219
            LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK+VEPKS
Sbjct  176  LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS  216
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C

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Query  3    DIVMTQSPSFLSASVGDRVTITCRASQGI------SSYLAWYQQKPGKAPKLLIYAASTL  56
            DI +TQSP  L+ S+G+R TI C++SQ +       +YLAWYQQKPG+ PKLLIY AST
Sbjct  1    DIQLTQSPDSLAVSLGERATINCKSSQSVLYSSINKNYLAWYQQKPGQPPKLLIYWASTR  60

Query  57   QSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPPYTFGQGTKLEIKRTVAAP  116
            +SGVP RFSGSGSGT+FTLTISSLQ ED A YYCQQ  S  PYTFGQGTK+EIKRTVAAP
Sbjct  61   ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYS-TPYTFGQGTKVEIKRTVAAP  119

Query  117  SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY  176
            SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
Sbjct  120  SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY  179

Query  177  SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE  216
            SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
Sbjct  180  SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE  219
```

Figure 9: (A) PyMOL alignment of B38 and H4. RBDs are aligned. PDB ID: 7BZ5 and 6ZH9. (B) BLAST alignment of B38 and H4 heavy chains. (C) BLAST alignment of B38 and H4 light chains. Variable residues are highlighted in the BLAST alignments..

A PyMOL alignment and BLAST alignment was also done between B38 and C105 (Fig. 10), an antibody isolated from the plasma of a COVID-19 patient via ELISA[4]. C105 shares an epitope with B38 and forms a complex with the spike with either 2 or 3 RBD in the up position, in which an antibody binds to each RBD in the up position. The heavy chains of B38 and C105 are derived from the same gene, *VH3-53*, but the light chains are derived from different genes, *KV1-9* and *LV2-8* respectively[4]. This difference can be seen in the BLAST alignment of B38 and C105. Researchers have observed an overexpression of IGHV3-53 and IGHV3-30 derived antibodies among anti-SARS-CoV-2 mAbs[4,13] produced by both natural infection and vaccination[10]. This finding suggests that the memory B cell response to the spike protein is relatively similar between natural infection and vaccination and across the Pfizer and Moderna vaccines.

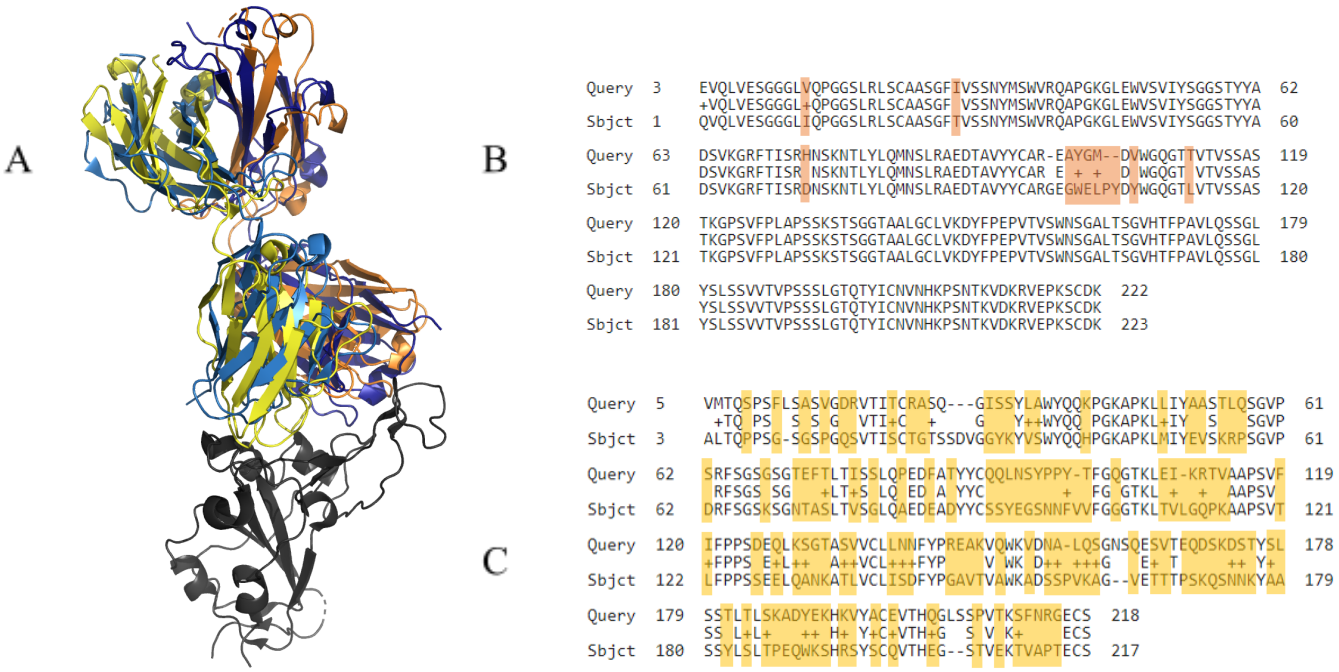

Figure 10: (A) PyMOL alignment of B38 and C105. PDB ID: 7BZ5 and 6XCA. (B) BLAST alignment of B38 and C105 heavy chains. (C) BLAST alignment of B38 and C105 light chains. Variable residues are highlighted.

The Future of mRNA Vaccines

The development of the Pfizer and Moderna mRNA vaccines, in addition to being a key to moving forward against the COVID-19 pandemic, also marks the first use of mRNA vaccines in humans. The use of mRNA vaccines has huge potential for future vaccine development in increasing the efficiency of vaccine production, the potential targets for vaccination, and the effectiveness of the vaccines produced. A significant advantage of mRNA vaccines is that they can be produced completely in vitro[9], and can be manufactured in a extremely short time relative to other live attenuated vaccines. mRNA vaccines inherently have a copy and paste design in which the mRNA in the lipid nanoparticle can easily be modified to address different variant strains and novel mutations which may arise within a virus. Such variants can already be seen in SARS-CoV-2. Knowledge of the spike protein structure in addition to identification and characterization of different neutralizing antibodies that bind SARS-CoV-2 and different epitopes on the SARS-CoV-2 spike protein is essential for further vaccine development, improvement, and modification.

References

1. *WHO Coronavirus (COVID-19) Dashboard*. World Health Organization. https://covid19.who.int/
2. Corum, J., & Zimmer, C. (2021, May 7). How the Pfizer-BioNTech vaccine works. *The New York Times*. https://www.nytimes.com/interactive/2020/health/pfizer-biontech-covid-19-vaccine.html
3. Papageorgiou, A. C. & Mohsin, I. (2020). The SARS-CoV-2 spike glycoprotein as a Drug and Vaccine Target: Structural insights into its complexes with ACE2 and antibodies. *Cells*, *9*(11). https://doi.org/10.3390/cells9112343
4. Barnes, C., *et al.* (2020). Structures of human antibodies bound to SARS-CoV-2 spike reveal common epitopes and recurrent features of antibodies. *Cell*, *182*(4). https://doi.org/10.1016/j.cell.2020.06.025
5. V'kovski, P. *et al.* Coronavirus biology and replication: implications for SARS-CoV-2. *Nat Rev Microbiol* 19, 155–170 (2021). https://doi.org/10.1038/s41579-020-00468-6
6. Chilamakuri, R. & Argarwal, S. (2021). COVID-19: Characteristics and therapeutics. *Cells.* 10(2). https://doi.org/10.3390/cells10020206
7. Cai, Y. *et al.* (2020). Distinct conformational states of SARS-CoV-2 spike protein. *Science*, *369*(6511). https://doi.org/10.1126/science.abd4251
8. Wrapp, D. *et al*. (2020). Cyro-EM structure of the spike protein prefusion conformation. *Science Magizine*, *667*(6433). https://doi.org/10.1126/science.abb2507

9. Krammer F. (2020). SARS-CoV-2 vaccines in development. *Nature.* 586. htpps://doi.org/10.1038/s41586-020-2798-3
10. Wang, Z. *et al.* (2021) mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *Nature.* 592. https://doi.org/10.1038/s41586-021-03324-6.
11. Wu, Y. *et al.* (2020). A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. *Science Magazine*, *368*(6496). https://doi.org/ 10.1126/science.abc2241
12. Wang, H. *et al.* (2020). SARS-CoV-2 proteome microarray for mapping COVID-19 antibody interactions at amino acid revolution. *ACS Central Science*, *6*(12). https://doi.org/10.1021/ acscentsci.0c00742
13. Lui, L. *et al.* (2020). Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature*. 584. https://doi.org/10.1038/s41586-020-2571-7