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Key Target for Vaccine and Drugs – The Spike Protein

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Abstract

The single standard RNA virus that causes the fatal respiratory tract infection is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The way this virus works is by attaching itself to the angiotensin-converting enzyme 2 (ACE2) receptors on host cells. The virus then enters the receptor through endocytosis. The cell that hosts machinery is then used to replicate further copies of itself and the many virions are released by exocytosis. The SARS-CoV-2, Spike protein (S) consists of two subunits: the S1 subunit has a receptor-binding domain that interacts with the ACE2 host cell receptor, and the S2 subunit facilitates the interaction between the membranes of the virus and the host cell. The spike (S) glycoprotein of SARS-CoV-2 is a homo-trimeric class 1 fusion protein divided by host cell proteases furin and TMPRSS2, resulting in the structural rearrangement. This allows the binding of ACE2 as the host cell receptor followed by the viral invasion by membrane fusion. So, this review summarizes the lifespan of the global coronavirus and the structural and functional properties regarding SARS-CoV-2, S protein. Furthermore, several recent studies have focused on medications that target or disrupt the spike protein−ACE2 interaction. The spike protein that can be utilized as a druggable target, convalescent sera from people who received three doses of the Pfizer or Moderna vaccines revealed lower neutralization of the omicron variant relative to ancestral SARS-CoV-2. Following two doses of the Pfizer vaccine, sera from people naturally infected with ancestral SARS-CoV-2 produced noticeably higher levels of neutralizing antibodies against both the ancestral virus and all volatile organic compounds.

Key Words*:* ***N-terminal domain, cytoplasm terminal domain, Receptor binding domain, Angiotensin-converting enzyme 2, 1, and 2 Heptad repeat, Fusion peptide, Transmembrane domain, Spike protein***

Introduction

As we know SARS-CoV-2 is a serious acute respiratory disease, that first occurred in 2003. Second, the closely related Coronavirus, MERS-CoV, or the Middle East Respiratory Syndrome Coronavirus, in 2012. As of November 30, 2019, the World Health Organization (WHO) registered 2494 MERS-CoV cases worldwide. The first report of SARS-CoV-2 appeared in China in December 2019. The covid -19 is characterized by a range of symptoms, primarily including fever, cough, dyspnea (shortness of breath), and myalgia (muscle aches and pain), (Poduriet al, 2020). The primary cause of mortality from COVID-19 is a compromised immune

system, leading to respiratory failure. The spread of infection depends on the pattern of ACE2 expression and the immune system's response. The spike glycoprotein of beta-coronavirus SARS-CoV-2 is a homotrimeric class 1 fusion protein that is presented in the metastable conformation for cleavage by host cell proteases furin and TMPRSS2, undergoing consideration of structural rearrangement for ACE2 host cell receptor binding and then the viral entry by membrane fusion. TMPRSS2–mediated cleavage and priming of the S protein of SARS-COV-2 is required for binding to ACE2, membrane fusion, and cell entry that requires the action of a virus and the host cell machinery comprising S protein, TMPRSS2, and ACE2. S1 and S2boundaries of SARS-CoV-2 (S) have multiple residues of arginine that are not found in SARS-CoV and SARS-CoV-2. The host cell protease furin, which mimics subtilisin and is widely expressed in humans, cleaves at a site on the subunits S1 and S2. The spike (s) glycoprotein interacts with the host's angiotensin-converting enzyme 2 (ACE2) receptor to initiate the SARS-CoV-2 infection process. The host membrane serine protease 2 (TMPRSS2) then cleaves the (s) protein before its fusion with the host cell membrane (Sternberg et al, 2020). The RBDs within the S1 subunit are more exposed on the viral surface than the S2 fusion machinery and are probably will be subject to selection pressure from immune close observation, the S2 fusion machinery is densely decorated with heterogeneous N-linked glycans present above the S2 surface that could obstruct the elicitation of humoral immune responses and accessibility to neutralizing antibodies. In this review paper, we discuss the structural and functional properties of the SARS-CoV-2 (S) protein (Gill et al, 2020).

History of SARS-CoV-2

In December 2019 Investigations into a cluster of pneumonia cases that occurred in Wuhan, China, revealed that the illness was caused by a recently identified coronavirus, which was given the moniker covid-19. Early victims of the illness had mentioned traveling to or working with seafood and live animals. As the outbreak expanded throughout China, the World Health Organization on January 30, 2020, deemed it a public health emergency of international concern. Remember that this is a novel illness and that current understanding is developing quickly and subject to change, so what exactly is a coronavirus? This is a broad class of viruses that are made up of a core genetic material encased in a lipid envelope and adorned with protein spikes, giving it the shape of a crown and the name Corona in Latin. This is the origin of the word coronavirus. A variety of coronaviruses can infect humans and animals, causing respiratory infections ranging from the common cold to more serious illnesses like SARS in humans. The severe acute respiratory syndrome coronavirus (SARS-CoV) was first identified in China in 2003; the Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in Saudi Arabia in 2012; and the virus that causes COVID-19 is known as SARS-CoV-2, which was first reported in December 2019. So where did the novel COVID-19 come from? It is well known that the coronavirus spreads among a variety of animals. Occasionally, these viruses can spread to humans, a phenomenon known as "spillover," and this can happen for several reasons, including changes in the virus itself or an increase in the number of animals and humans nearby-Although it is known that (MERS-CoV) is spread by camels and (SARS-CoV) by civet cats, the animal reservoir for the novel coronavirus remains unknown at this time. (www.who.org)

Structure of SARS-CoV-2

Nucleocapsid (N), Membrane (M), Spike (S), Envelop (E), and four structural proteins are encoded by it, along with a few non-structural proteins (nsp). The nuclear capsid, also known as N-protein, is attached to the virus's single positive strand RNA inside the capsid, which is the protein shell that the virus uses to enter human cells and transform them into virus factories. The viral RNA genome, which is coated by the N protein, aids in transcription and replication. The N protein's N-terminal binds to genomic and subgenomic RNAs in IBV and MHV virions and facilitates transcription and viral replication. RNA binding to the N terminal domain of the coronavirus N protein may be inhibited by drugs. The M-protein is thought to be the primary organizer for the coronavirus assembly since it is more prevalent on the viral surface Coordinated across the virus's surface, the S-protein aids in the virus's attachment to host cell surface receptors and membrane fusion with the host cell, which allows the virus to enter the host cell. The E-protein, a minor component of the virus particle and a tiny membrane protein of around 76–109 amino acids is crucial for virus assembly, host cell membrane permeability, and virus–host cell contact. A lipid sheath holding the genetic material. The viral surface contains the Hemagglutinin-Esterase dimer (HE) (Urquiza et al., 2020). The HE protein is present and crucial for the infection of the natural host cell, but it is not necessary for virus replication and may be involved in virus entrance. The whole structure of the Spike (S) protein in both its closed and open (perfusion) forms has been made available by cutting-edge cryo-EM research. Such glycoprotein is built of three identical chains with 1273 amino acids each and it is constituted of two well-defined protein domain regions: S1 and S2 subunits which are related to cell recognition and the fusing of viral and cellular membranes respectively. Later on, various proteins undergo alterations that are still unclear and contribute to the process. Many human cells, especially those in the lungs, have (ACE2) receptors on their surface, which the coronavirus spike (S) protein adheres to and facilitates virus entrance through. Two locations on the coronavirus S protein, close to the junction of the S1 and S2 subunits, are known to undergo proteolytic cleavages by host proteases. The fusion peptide is later released by the S2 domain's cleavage. (Huang et al, 2020).

Nucleocapsid (N) protein: The multifunctional coronavirus N protein binds to RNA and carries out its essential role in the transcription and replication of viral RNA. The central ser/Arg-rich linker connects the two highly conserved domains of the primary CoV protein—the C-terminal dimerization domain and the N-terminal RNA-binding domain. These significant structural discoveries will aid in the search for ligands that specifically target the target of the coronavirus to prevent transcription and replication.

Non-structural proteins (nsps): The 16 nsps of the coronavirus are highly conserved and have various roles. The likelihood of infection is increased by the existence of the crystal structure, the ligand that has been characterized, and an important function in viral infections. The two targeted proteases, RNA-dependent RNA polymerase (kdRP) and helicase (nsp13) are 3CLpro and PLpro.

Spike (S) protein: Per monomer, it has between 1200 and 1400 amino acid residues and is found on the virion's outer membrane. The method of recognizing a host-guest is specific to a virus,

and its selectivity and specificity dictate the virus's tropism as well as its pathogenicity. (Gill et al, 2020).

The spike protein structure

Cryo-electron microscopy has established the atomic SARS-CoV-2's structure trimeric S protein, revealing the difference within the S RBD domain. The protein of SARS-CoV-2) is 1273 as long overall. Other human pathogenic coronaviruses including MERS-CoV and SARS-CoV have class 1 trimeric fusion proteins with a transmembrane homotrimeric glycoprotein of about 180 kDa, known as the S protein. The receptor binding domain, or RBD, of S, comprises two subunits: the apical V-shaped S1 subunit, which can bind with the ACE2-recognition motif at every monomer, furthermore the S2 subunit, which is necessary for the fusing with the viral and cellular membranes (Sternberg et al, 2020).

Polysaccharide molecules are placed regarding the spike protein to mask the immune system of the host when it enters. The N-terminal domain, the domain that binds to receptors (RBD), the fusion peptide (FP), the heptapeptide repeat sequence 1 (HR1), the HR2, and the cytoplasm domain comprise the S1 subunit. The Spike protein surrounds the virus molecule in the shape of a crown. The S protein of SARS-CoV-2 continues to function in its original condition. Target cell proteases cleave the Spike protein into S1 and S2 subunits during the S protein's interaction with host cell ACE2. The serine protease TMPRSS2 is employed as a protein primer (C. Jiang et al, 2020).

S1 subunit's structure

This receptor recognition is an essential feature of the virus entrance since infection starts when virus particles attach to cell receptors on the exterior of the cell membrane. RBD connected with the NTD and CTD of the ACE2 cell receptor's aminopeptidase N region found in the S1 subunit. More residues on the SARS-CoV-2 (S) CTD directly interact with ACE2, and if it shares a complex with ACE2 instead of the SARS-CoV-2-RBD, a greater surface area is buried.F486 in

SARS-CoV-2 makes a powerful aromatic-aromatic interaction with ACE2 Y83 in place of I472 in SARS RBD, while E484 in SARS-CoV-2-CTD results from an ionic bond with K31, as opposed to P470 in SARS RBD, which leads to a higher intensity for receptor binding than SARS-CoV RBD. In the CoV RBD, four of the nine ACE2-contacting residues are partially preserved. The majority of the SARS-CoV residues required for ACE2 binding are intact in the SARS-CoV-2 (S) protein, according to the examination of the structures of SARS-CoV-2 and SARS-CoV. Studies demonstrated that SARS-CoV-2 (S) protein-specific murine monoclonal antibodies (mAbs) and polyclonal antibodies exhibited changes in antigenicity between SARS-CoV-2 and SARS-CoV. In 2020, (Wang Q et al).

S2 subunit's structure

It is composed of a fusion of the FP, HR1, HR2, TM, and cytoplasmic domains (CT), which is in charge of viral entrance and fusion. The viral family's brief section of 15-20 conserved amino acids known as FP, supports the target membrane when the (S) protein adopts the hairpin conformation by mostly consisting of hydrophobic residues like glycine (G) or alanine (A).

Considering the study, the host cell membrane's lipid bilayer is disrupted and connected by the FP, which is essential in effecting fusion. The repeating heptapeptides HPPHCPC, which are made up of the H- hydrophobic, HR1, and HR2 are composed of dense residue, P- polar or hydrophilic residue, and C- charged residue. The 6- 6-helical bundle (HB), made up of HR1 and HR2, is necessary for viral fusion and the entrance of an S2 subunit. HR1 is situated at the C-terminus of a hydrophobic FP, whereas HR2 is situated at the N-terminus of the TM domain. The downstream TM domain links the S protein to the viral membrane after the S2 subunit terminates at the CT tail. S2 changes its conformation by inserting FP into the target cell membrane after the connection of RBD to ACE2, bringing the cell membrane and envelope together by releasing the HR1 domain's hairpin spiral and causing the HK2 domain and HK1 trimer to engage to produce 6-HB, closer for the viral fusion and entry. Then to communicate with the HR1 domain, the HR2 domain creates a flexible loop additionally a stiff helix. And for the "fusion core area" is where the HR1 and HR2 domains interact most strongly in the post-fusion hairpin conformation of CoVs (HR1 core and HR2 core regions).

While the S1 RBD domain is a section found in highly changeable regions and is not an excellent target location for the creation of broad-spectrum antiviral inhibitors, the S protein is an essential target protein for the development of certain medicines. However, the S2 subunit's HR region, which functions as the manner of contact between HR1 and HR2, is conserved among HCoVs and has a major part in CoV infections. (Huang et al, 2020).

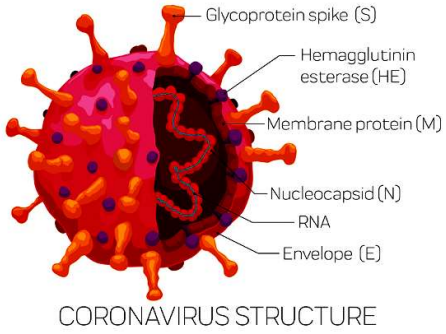

Figure 1. Structure of SARS-CoV-2. The spike protein is showing S1&S2 subunit, proteins for the membrane (M), the envelope (E), nucleocapsid (N), and single-stranded RNA (ssRNA) (www.jmu.edu.)

The role of spike (S) proteins

The virus's surface Protein is an essential component in the spread of infection. It's a class 1 TM (trimeric glycoprotein) that is the factor that allows entrance through viral. All varieties of (HCoV) human coronaviruses Moreover, other viruses have Hit like HIV, influenza virus, paramyxovirus, and Ebola. Similar to other coronaviruses, S proteins of SARS-CoV-2 also have the receptor recognition factor which helps in the attachment and fusion when the infection first started.

The S protein has a trimer that is situated on the viral envelope's surface and facilitates the interaction between the S protein and the receptor. The RBD (receptor binding domain), which is responsible for the virus's binding to the receptor, is located in the S1 domain, while the HR

(heated-repeat) domain is mostly situated in the S2 domain, consisting of HR1 and HR2 which is closely related, fusion with the virus (Urquiza et al, 2020).

Binding of a receptor

The SARS-CoV-2 S protein recognizes the ACE2 receptor to attach to the host cell (angiotensin-converting enzyme 2). ACE2 is homologous to ACE and ACE2 is widely distributed in the lung, colon, heart, kidney, and alveolar epithelial type 2 cells. Angiotensin 1 to 9 are also present and they are major expressing cells. ACE2 is additionally recognized as the receptor of SARS-CoV. To encourage the evolution of endosomes, the S1 component of the SARS-CoV S protein binds with ACE2 and initiates the fusion activity at low PH. There are binding of ACE2 and SARS-CoV-2 between species 2 analyzed the binding affinities and found that only the ACE2 proteins from the Bovidae and Cricetidae families successfully interacted with SARS-CoV-2 Sprotein (RBD). With a dissociation constant (KD) of 14.7 nm, SARS-CoV-2 binds to human ACE2, while SARS-CoV S protein binds to ACE2 of humans with a (KD) of 325.8nM, this difference shows that the SARS-CoV-2 possesses greater sensitivity than SARS-CoV. So, researchers calculated the difference between SARS-CoV and SARS-CoV-2 is about ~24% and the difference between RBD is about ~23%.

Viral fusion

A virus fuses with the host cell, which means there is the cell of the host with the viral DNA. SARS-S1 CoV-2's and subunits that are S2 are being cleaved, which is causing the viral fusion. Host proteases, cleave the subunits S1 and S2 into two parts and remain in the form of non-covalently present till the viral fusion. Researchers found that there's a specific furin cleavage site but it is not present on other SARS-like CoV. The S protein remains uncleaned during the splitting of the SARS-CoV-2, S1, and S2 subunits. SARS-CoV-2 has multiple furin-like cleavage sites and that's why it has more affinity for infection. Host cells have TMPRSS2 (transmembrane serine protease2), which is a cell surface protein that is located on the endothelial cells of the respiratory and digestive tract. This holds significance for S protein priming and it is activated during the entry of SARS-CoV. Trypsin is also known to cleave the S protein.

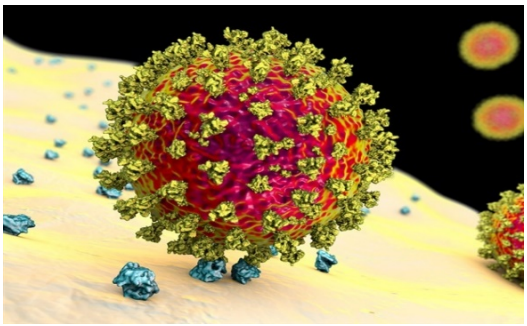

Figure 2. The binding spike protein in combination with the host receptor, ACE2, was (www.news-medical.net.)

The establishment of 6-HB (6-helical bundle) is helpful for viral fusion. FP (fusion peptide) for viral fusion is the 2HR domains on S2 and the N-terminus of SARS-CoV-2. FP cleaves the

Spike protein before starting the viral fusion. The protein modifies its shape after fusing and reaches the membrane of the host cell.

When the HR1 Spike protein of the domain is enclosed in the membrane of the host cell and HR2 is closer to the viral membrane side, the viral membrane and the host cell membrane are reduced. The viral membrane is subsequently forcefully stretched towards the membrane of the host cell as HR2 folds back to HR1, creating the 6-helix anti-parallel shape of the fusion core and they bind together, and finally, 2 membranes fuse (Zhu N et al, 2019).

Potential medication that targets the spike protein

The primary role of the S protein in viral infection suggests that it is a possible target for the creation of vaccines, antibody-blocking treatments, and small molecule inhibitors. The host immune response is triggered by the functional protein of SARS-CoV-2 and nAbs that target the S protein can trigger protective immunity against viral infection. A strong association between these two viruses are demonstrated by the sequence and striking structural resemblance of the SARS-CoV-2 and SARS-CoV proteins, which opens the door to the potential of treating COVID-19 with antibodies that target the SARS-CoV S protein. In contrast to SARS-CoV-2 RBD, SARS-CoV-2-CTD interacts with ACE2 via the c-terminal domain, demonstrating a greater capacity for receptor binding. So, the RBD shows high potency nAb responses and it shows the potency towards an efficient and secure subunit vaccination against SARS-CoV-2 was created. The rate of entry of SARS-CoV-2-MLV was decreased to 10% by SARS-CoV polyclonal antibodies produced by mice that have received vaccinations, which entirely prevented the arrival of SARS-CoV S-MLV (murine leukemia virus). The anti-SARS S1 polyclonal antibody T62 can't inhibit the introduction of the SARS-CoV-2 S pseudovirus particles. The S230, m396, and 80R, SARS RBD-directed mAbs, are unable to attach to SARS-CoV-2RBD. In humans, CR3022mAb has the potency to show the property of the prevention and controlling of SARS-CoV-2 infection, therapeutic either by itself or in concert with other nAbs—an S1-targeting mAb made derived from transgenic mice that have been vaccinated. In human IG variables, both light and heavy chains neutralize the infection whatever the case may be a blocked connection between RBD-hACE2 and SARS-CoV-2. several human blocking mAbs (311mab-31B5, 311mab32D4, 47D11, n3130, n3088, s309, p2c-1F11, p2B-2F6, B38, H4) are successfully cloned from single memory B cell from the recovered covid-19 patient (Ramirez et al, 2020).

Fusion inhibitors to prevent infection

The SARS-CoV-2 S protein is less persistent than the SARS-CoV S protein. They have the same HR2 sequence alignments between SARS-CoV-2 and the SARS virus. To stop SARS-CoV-2 from merging and entering a designated cell and prevent SARS-CoV-2 S and SARS-CoV-2 pseudovirus, the SARS-CoV-2 HR 2p (1168–1203 residues) was developed. Its LC50 values are 0.18 and 0.98 micrometers, respectively. There is perhaps a connection between several mutant leftovers throughout the HR1 area and improved binding in the HR2 region. Therefore, it was created and established that EK1 C4, a lipopeptide obtained from EK1, inhibits SARS-CoV-2 S-mediated cell-cell fusion. EK1 C4, which was 149-fold more powerful than the original EK1 peptide and had a 1C50 of 15.8 nm, also prevented the entry of the SARS-CoV-2 S pseudovirus.

Additionally, pseudovirus infection and SARS-CoV-2 S protein-mediated cell-cell fusion may be inhibited by the sequence-based lipopeptide fusion inhibitors, IPB02.

Peptide fusion inhibitors, such as nelfinavir mesylate Stop SARS-CoV-2 S and SARS-CoV S from generating cell-cell fusion by employing an anti-HIV protease inhibitor. Simultaneously inhibiting the TMPRSS2 that activates the S protein. Early on is mostly when it is effective.

Furin, a member of the pc family and proprotein convertase subtilisin kexin3 (pcsk3), catalyzes the hydrolysis of paired basic residues in peptide and protein substrate. The existence of an S furin Cleavage site (682–685 residues) in SARS-CoV-2 S may make SARS-CoV-2 transmission more likely. As a result, SARS-CoV-2 is treated with drugs called furin inhibitors. Alpha-1 PDX, D6K, pI8, and peptidomimetic furin inhibitors are furin inhibitors (Huang et al, 2020).

Evolution of spike protein

Evolutionary change inside the framework of the SARS CoV family from alpha to gamma so, S1-CTD and host galectins may share the same evolutionary ancestor. One scenario is that S1-CTD was produced by gene duplication of S1-NTD after S1-NTD was produced through gene capture (Figure 2). The particular tertiary architectures of alpha- and beta-coronavirus S1-CTDs suggest that S1-CTDs evolve at an accelerated rate. They may be situated in the prefusion trimeric spike's most exposed and projecting region

(Figure 2), which is connected to this. As a result, there is strong selective pressure on S1-CTDs to avoid host immune surveillance. Gamma-coronavirus has a unique reading frame and delta-coronavirus has a unique transcription regulatory sequence**.** The primary role of coronavirus spikes is membrane fusion since coronaviruses need to enter cells to replicate. Consequently, S2 may have been the only component existing inside the original form of coronavirus spikes (Figure 2). Because the ancestral virus would need to spread non-specifically to access the vicinity of target cells to fuse the membrane, such a primordial spike might not operate effectively. Later, through gene capture, the spike would develop an S1-NTD that was similar to a galectin, improving its effectiveness in facilitating virus entrance. The spike would then develop an S1-CTD through gene duplication of its S1-NTD or other means, improving means, which would improve its ability to recognize receptors (Fang Li, 2016).

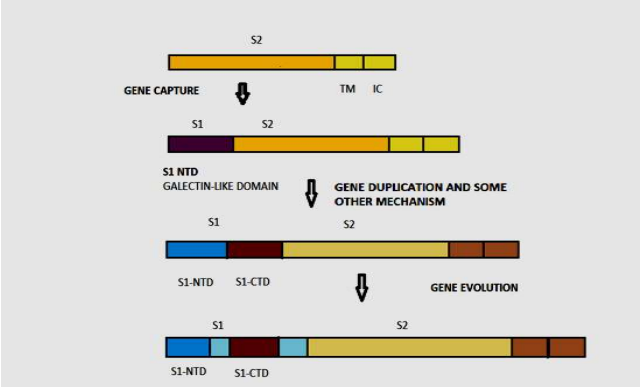

Figure 3. Hypothesized evolution of coronavirus spike proteins. Abbreviations: HCoV-NL63, human coronavirus NL63; IC, intracellular tail; S1-CTD, S1 C-terminal domain; S1-NTD, S1 N-terminal domain; TM, transmembrane anchor

Conclusion

The SARS-CoV-2 cycle is crucial for developing effective treatments to combat this coronavirus. Many host- and virus-based targets are important for pharmacology and host immune response during the examination of the virus life cycle. Our interaction with the host receptor ACE2 is significantly influenced by the structure spike S protein. Antibodies further demonstrate that the intended outcome against S protein can be achieved by structurally druggable sight and determinants. There are 16 nonstructural proteins in this coronavirus. The RNA-dependent RNA polymerase (RdRp), the helicase, and the two proteases (3CLpro and PLpro) are the unique sites for virus replication that are also good candidates for therapeutic targeting. The main cellular protein engaged in the process is the investigation of the molecular mechanisms underlying viral entrance. For example, the host receptor ACE2, proteases such as TMPRSS2, furin, cathepsin L, or kinases these are perform the part in the regulation of intracellular viral trafficking during endocytic entry. A great way to prevent viral escape by mutation is to target human proteins. Combining antiviral medications that target many targets in a multi-target approach, which has been shown to improve efficacy and generally prevent virus resistance, is another intriguing option. There has been a lot of research conducted during the COVID-19 pandemic to prevent the infection of coronavirus drugs, and a study of the coronavirus life cycle has produced many successful and beneficial results to prevent the infection. However, there is currently no effective medication for the therapeutic mechanism that can be demonstrated to stop the infection after the early stages. There are 214 structures found in the PDB for various SARS-CoV-2 S proteins as of May 2020. In addition to X-ray diffraction, other experimental methods have been employed, including as electron microscopy and nuclear magnetic resonance (NMR). The structure of the SARS-CoV-2 major protease has been the subject of the most study, with over 136 distinct crystals formed. Since possible antiviral action against SARS-CoV-2 being explored will increase dramatically. By examining the composition, role, and evolutionary background of coronavirus spikes, we can gain a deeper understanding of the genesis of viruses and the evolutionary relationship between viruses and host cells.

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