

How do Spike Proteins of SARS-CoV-2 and ACE2 Make Covid-19 Pandemic Inevitable?

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Abstract. People's lives throughout the world have been impacted by the Covid-19 epidemic. There are numerous viruses that can cause human disease, so what exactly made SARS-CoV-2 different from others and caused a quite severe impact? Unlike other common viruses, SARS-CoV-2 is highly infectious and virulent. The most crucial surface protein of coronaviruses is the spike (S) protein, and it is the structure that is responsible for binding human cells and infecting them. And the mutation of the S protein that gives the virus higher affinity with the host receptor might be a possible explanation for its strong infectiousness. Angiotensin-converting enzyme 2 (ACE2) receptors are the sites on cell surfaces where SARS-CoV-2 binds, it is an important enzyme in the human body that has various regulatory roles. This paper will also discuss their structure and functions, as well as the possibility of treating ACE2 proteins as a target to diminish the infection of SARS-CoV-2.

Keywords: SARS-CoV-2, Spike Protein, ACE2, Angiotensin.

1. Introduction

SARS-CoV-2 is a newly discovered form of coronavirus which is the pathogen for the Covid-19 pandemic. It spread unexpectedly rapidly and had a severe impact on people's lives. Understanding the mechanism of infection and recognizing the key particles involved would be the premise of coming up with solutions for Covid-19. The first step for an enveloped virus to infect cells is to bind its glycoproteins on the envelope to receptors on the cell surface, which leads to membrane fusion and genome delivery in the cytoplasm. S protein which is the glycoprotein on the SARS-CoV-2 envelope and ACE2 receptors on human cell membranes are the two particles that are responsible for the infection of the virus in human cells. Consequently, S protein and ACE2 might be the most relevant factors determining the infectiousness of SARS-CoV-2. The potential treatment targeting these two particles should be emphasized.

2. Structure of SARS-CoV-2

Coronavirus is a common form of the virus; it can cause a variety of diseases including from the common cold to SARS, MERS, and HPV. Coronavirus is large, roughly spherical particles with unique surface projections. And the S protein is the outermost projection on the viral envelope, and it is responsible for binding with the host cell.

S proteins are made of subunits with different compartments. The outer most structure is the signal peptide, the receptor binding domain (RBD) in subunit1, and the transmembrane domain on subunit 2 is connected to the viral envelope. Therefore, S protein has a transmembrane (TM) domain anchored in the viral membrane, an extracellular N-terminus, and a brief intracellular C-terminal portion (Figure 1).



Figure 1. The schematic representation of S protein [1].

The viral S proteins are highly glycosylated which means that they have decorating sugar moieties that help defend the immune system. The basic structure of S proteins is a collection of alpha-helix

and beta-sheeted polypeptides. They fold into three intertwined chains with identical amino acid sequences which are called protomers. However, the, protomers have non-identical 3-dimensional structures forming two subunits. They are S1 and S2 subunits respectively shown in Figure 2. S1 binds to the human cell receptors ACE2 and S2 mediates the fusion of viral and cellular membranes.

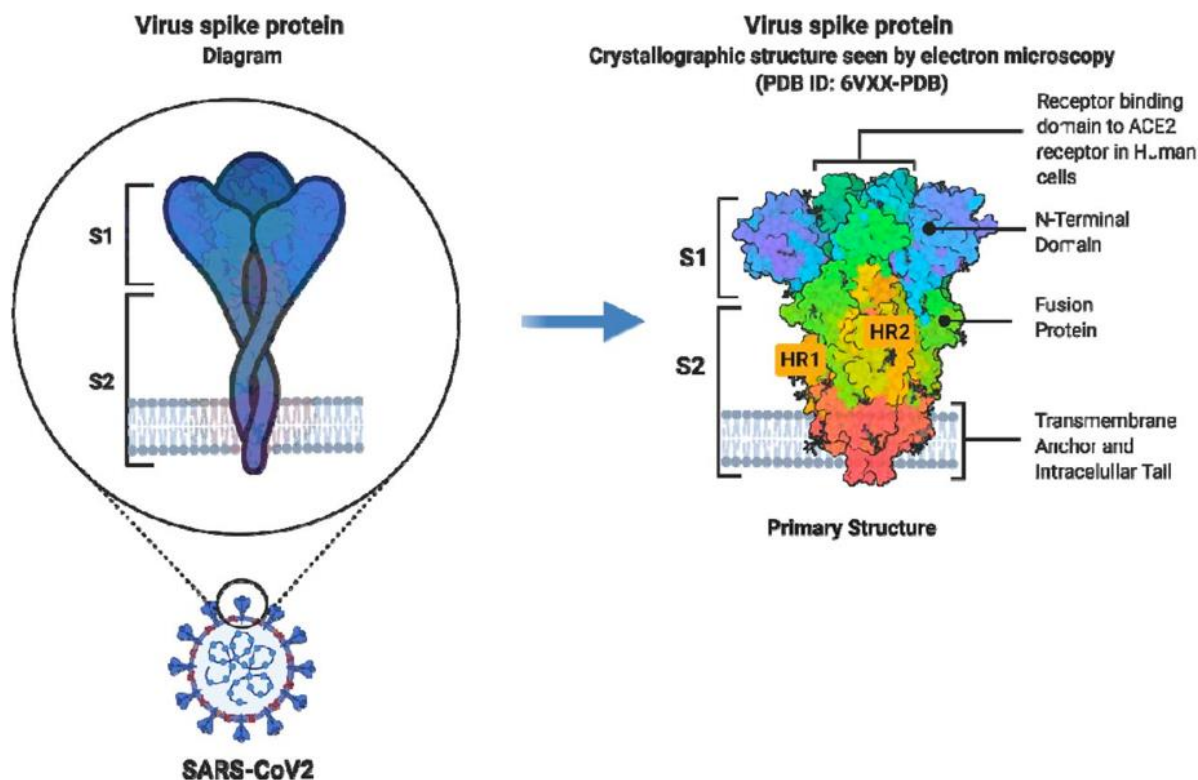


Figure 2. The structure of S protein [2].

3. Binding of S protein to the host cell

The beginning of virus infection occurs when the virus particle binds to cell receptors on the surface of the host cell. Because of this, receptor recognition is a key factor in viral entry and a crucial target for therapeutic development.

The RBD binds to the ACE2 receptors on human cells, they are expressed in the lungs, heart, kidneys, and intestines. The wide range of symptoms, ranging from no symptoms to death, could be attributed to human-to-human variability in either the configuration or number of ACE2. Extensive H-bonding occurs between them, resulting in a 20-fold increase in affinity over SARS-CoV-1. Our own protease, which are peptide-breaking enzymes, can cut the peptide at specific sites, and the conformational changes in the S protein allow fusion to occur. The virus will release its genetic material into the host cell after the viral and cellular membranes fuse (Figure 3).

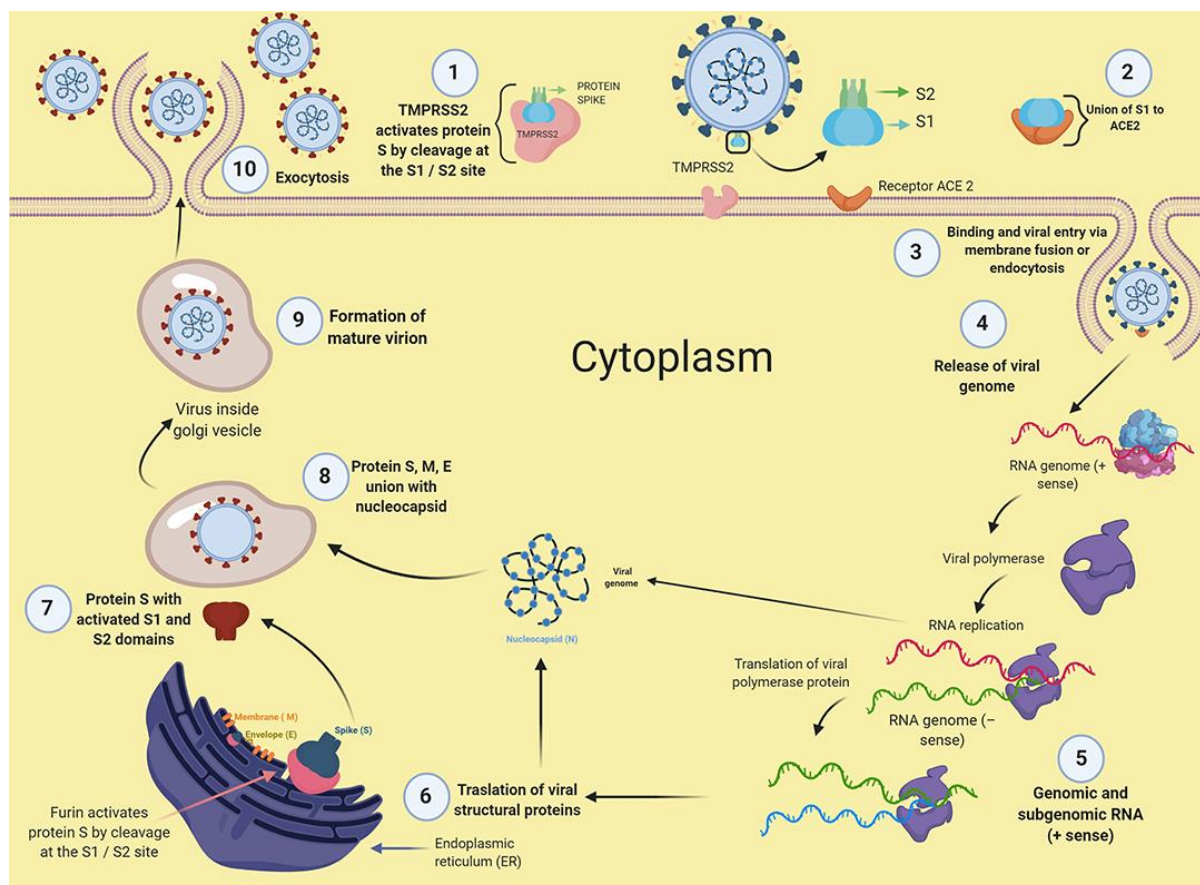


Figure 3. The binding mechanism of SARS-CoV-2 infection and the reproductive process [7].

4. Mutants' variability

SARS-CoV-2 is constantly mutating creating a variety of mutants with different levels of infectiousness and virulence, omicron is the one that is most rapidly spread with a low case fatality rate. Omicron is more infectious but less virulent than the wild type, and that is possibly due to mutations in the S protein which could allow it to bind tighter with ACE2 receptor of human cells. Figure 4A depicts the interaction of omicron BA.2 with the human ACE2 receptor at hotspot 353. Figure 4B depicts the interaction of omicron BA.2 with the human ACE2 receptor at hotspot 31 and the hotspot ridge. The Omicron Receptor Binding Domain is shown in cyan, while the ACE2 receptor is shown in grey. The hydrogen bonds are represented by yellow dotted lines. The same holds true for Figure 4C and D, except that instead of Omicron BA.2, the wild-type SARS-CoV-2 is now binding to the ACE2 receptor. The Wild Type SARS-CoV-2 is in orange.

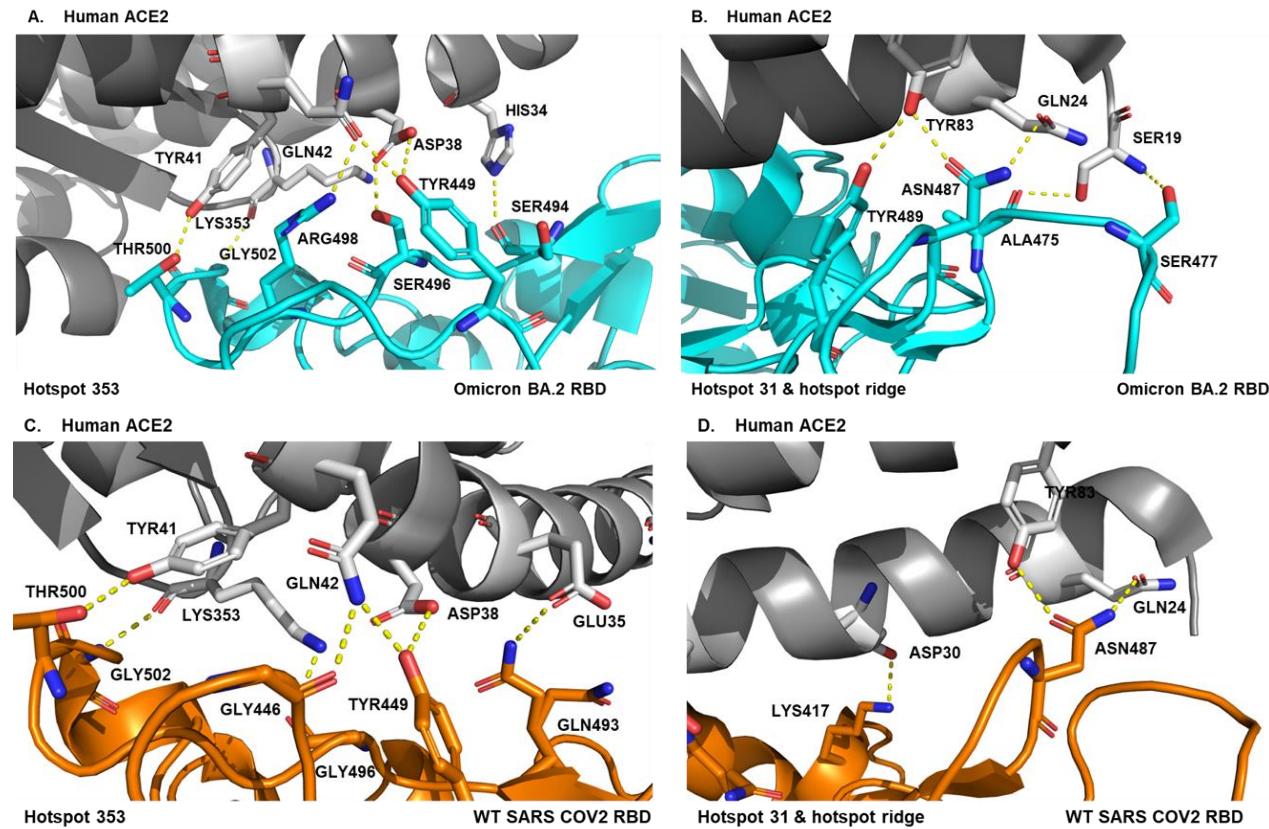


Figure 4. The number of hydrogen bonding between the ACE2 receptor and the S protein in the wild type and Omicron mutant. Figure 4 was created using the software PyMOL to generate crystal structures (PyMOL, 2019). Protein Data Bank was used to obtain the crystal structures used to create the diagrams (RCSB, 2010). SARS COV2 wild type and omicron variant accession codes are 6m0j and 7u0n, respectively [8].

Although the numbers of hydrogen bonds at hotspot 353 are the same, two more hydrogen bonds in the omicron mutant at hotspot 31 are shown in Table 1.

Table 1. Summary of the number of hydrogen bonds in hotspots 353 and 31 and the total number of hydrogen bonds of the omicron mutant and the wild type respectively.

	H-bonds in hotspot 353	H- bonds in hotspot 31	Total number of H-bonds
Omicron	7	5	12
WT	7	3	10

However, as previously stated, H-bonds have varying bond energies. A mutation that introduced two weak H-bonds after replacing a strong H-bond may not improve binding. As a result, simply counting the number of H-bonds does not address the claim that mutations on the S resulted in the virus becoming more effective at infecting people. The total number of interaction contacts (ICs) between ACE2 and the two SARS-COV-2 were obtained using Z-dock, and Omicron has a higher total IC of 78, implying that it interacts with ACE2 more frequently. And a lower HADDOCK score for Omicron indicates that the binding is therefore more favorable [9].

Despite the various mutations of SARS-CoV-2 leading to high virulence and infectiousness, they all rely on binding with the ACE2 receptors in our cells to infect humans. Understanding the functioning of ACE2 provides a possibility for us to consider treating Covid-19 via blocking or interrupting ACE2 receptors.

5. ACE2

ACE 2 receptors share a quite similar structure with the testis-specific form of ACE (tACE) and the *Drosophila* homolog of ACE (AnCE). The ACE2 model's architecture and the tACE crystal structure are quite similar (Figure 5A). the ACE2 model structure is superimposed over the tACE and AnCE template structures (Figure 5B). The catalytic site is located in a deep channel on top of the molecule, which is a key component of the ACE2 structure [12].

As previously mentioned, ACE2 expression occurs in the human airway epithelial, heart, kidney, and intestines. The presence of ACE2 in the airway epithelial is previously proved by previous studies [13]. Jia, H. P. et al firstly used lysates from the human airway and distal lung tissue and performed Western-blotting. The presence of immunoreactive bands indicates the presence of glycosylated form of ACE2. However, they cannot tell which specific tissue expression ACE2 the most from the first experiment. They then further evaluated ACE2 expression using more differentiated cell culture. The most abundant signal was detected on the apical rather than the basolateral surface and the ciliated cells showed the strongest intensity. Furthermore, they also found that ACE2 expression might depend on the state of cellular differentiation. They compared the morphology of the apical surface. Submersion of apical surfaces of primary cells caused cilia loss probably due to the reduction in the ACE2 mRNA and protein expression. Additionally, the coordinated expression of a transcription factor and ACE2 also provides evidence for their statement. Foxj1, a transcription factor required for well-differentiated epithelial cilia phenotype, is found to have a correlated expression with ACE2. However, there is no evidence to prove a causal relationship which means that foxj1 alone does not regulate ACE2 expression.

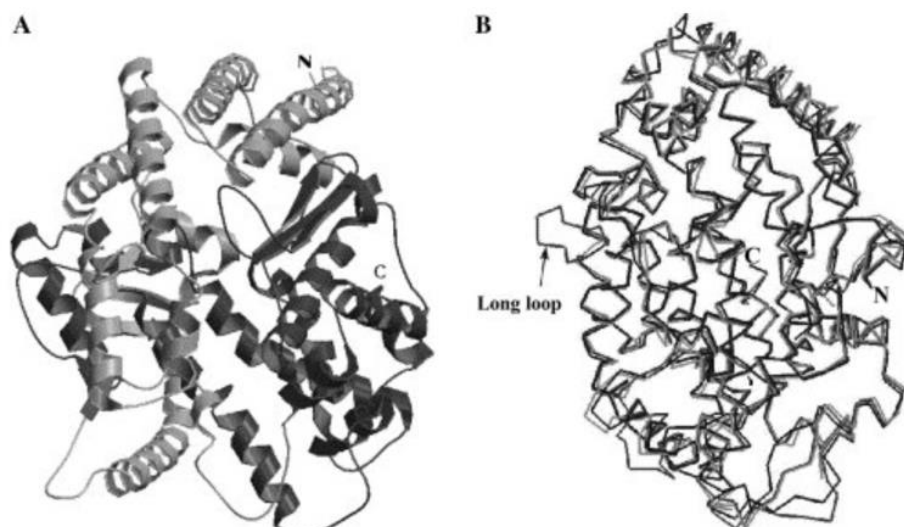


Figure 5. The ACE2 structure's model [12].

6. Crucial Regulatory Roles of ACE2 in the Human

ACE2 is a homologue of ACE, it produces a homeostatic regulation of angiotensin II (Ang II) circulation. ACE and ACE2 are both ectoenzymes, transmembrane proteins, on the cell surfaces, and ACE2 functions as a zinc metalloenzyme and carboxypeptidase [13]. Apart from regulating Ang II, ACE2 also hydrolyzes many other physiological substrates. Additionally, it also carries non-catalytic roles like regulating intestinal neural amino acid transport and being a receptor of SARS-CoV-2. There are two differentially spliced forms of ACE2, N- and C-domain somatic ACE, single domain testicular form is essential for male fertility. Moreover, ACE2 plays crucial roles in multiple aspects, apart from regulating fertility which is just mentioned, ACE2 is also responsible for regulating cardiovascular functions.

The Renin-angiotensin system (RAS) is the basic system that regulates the cardiovascular system. Angiotensin II (Ang II) is the main regulatory hormone involved. This system had evolved to now

become complex networking. Angiotensinogen is the precursor protein of Ang I and II. The aspartic protease, another enzyme involved in the system apart from ACE, will cleave Ang I off from the precursor. Afterward, ACE will convert Ang I to Ang II. Although ACE and ACE2 are quite similar, they have distinct substrate specificities. ACE works mainly as a carboxypeptidase which removes a dipeptide on C-terminal producing Ang II from Ang I. ACE2 functions exclusively as a carboxypeptidase which removes a single amino acid from the C-terminal. It generates Ang-(1-7) from Ang II and Ang-(1-9) from Ang I. The production and hydrolysis of Ang II of ACE and ACE2 counterbalance each other. Thus, ACE2 has its own unique and important role in regulating cardiovascular function which is different from the function of ACE. The Cardiac function of ACE2 is also proved by previous experiments. Hamming and colleagues produced ACE2 knockout mice which showed cardiac dysfunction [16].

ACE2 is also crucial in maintaining kidney and lung function. A deficiency in ACE2 will lead to a disrupted balance between renal ACE and ACE2 which causes a high level of Ang II. Excessive Ang II might contribute to progressive renal damage. The expression of RAS elements, including ACE and ACE2, is highly concentrated in the lung. The development of lung damage may be influenced by intrapulmonary RAS activation. Acute respiratory distress syndrome, sarcoidosis, pulmonary fibrosis, and pulmonary hypertension have all been linked to higher levels of ACE. ACE2 may be involved in mother-fetus interactions as well, as the placenta has both Ang-(1-7) and ACE2 expression [17].

7. Conclusion

In conclusion, by analyzing the binding mechanism of S protein and ACE2 receptor, this article pointed out the decisive role of S protein in viral infectivity and proposed that the infectivity changes of SARS-CoV-2 variants are closely related to the variation of S protein, and the structural changes of spike lead to the difference in the amount of hydrogen bond formed between spike and ACE2 receptor, thus leading to the rate of binding with host cells. Through analyzing in silico structures of Omicron mutant and wild type, the paper proved that there is potentially more hydrogen bonding, and they have higher strength when omicron binds to the host cell. However, the paper is not stating a causal relationship, from the data produced, it can only be proved a possible correlation between infectiousness and spike mutations. There are also many more factors that need to be considered.

Besides, the paper also evaluated the functioning of ACE2 in human bodies and stated its importance in various aspects including lung, heart, kidney functioning, and fertility. Although it is principally possible to diminish the expression of ACE2 receptors to prevent SARS-CoV-2 from binding to and infecting human cells. Upregulation of ACE2 expression and function is increasingly being regarded as a possible therapeutic strategy that can be used to treat cardiovascular disease and hypertension, as well as type I and II diabetes, lung damage, and fibrotic disorders. However, it is shown that upregulation of pulmonary ACE2 is identified in ever-smokers compared to nonsmokers, and an increased risk of binding with SARs-CoV-2 [18]. Researchers could work on proper approaches to repress ACE2 expression or function to a controlled level like using artificial substrates to block a small portion of ACE2 receptor, or could reduce the number of ACE2 by downregulating ACE2 gene expression. However, it should be very careful with the level of downregulation and make sure not to affect the normal regulatory functions of ACE2 too much.

References

- [1] Recombinant Proteins for COVID-19 / SARS-CoV-2 Research [EB/OL]. www.biocat.com. <https://www.biocat.com/corona-proteins>.
- [2] Grobbelaar L M, Venter C, Vlok M, et al. SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19 [J]. Bioscience Reports, 2021, 41 (8): BSR20210611.

- [3] Mansbach R A, Chakraborty S, Nguyen K, et al. The SARS-CoV-2 Spike Variant D614G Favors an Open Conformational State [J]. *Biophysical Journal*, 2021, 120 (3): 298a.
- [4] Bergmann C C, Silverman R H. COVID-19: Coronavirus replication, pathogenesis, and therapeutic strategies [J]. *Cleveland Clinic Journal of Medicine*, 2020, 87 (6): 321 - 327.
- [5] Shang J, Ye G, Shi K, et al. Structural basis of receptor recognition by SARS-CoV-2 [J]. *Nature*, 2020, 581 (7807): 221 - 224.
- [6] Li L, Liao H, Meng Y, et al. Structural basis of human ACE2 higher binding affinity to currently circulating Omicron SARS-CoV-2 sub-variants BA.2 and BA.1.1 [J]. *Cell*, 2022, 185 (16): 2952-2960.e10.
- [7] Guadarrama-Ortiz P, Choreño-Parra J A, Sánchez-Martínez C M, et al. Neurological Aspects of SARS-CoV-2 Infection: Mechanisms and Manifestations [J]. *Frontiers in Neurology*, 2020, 11: 1039.
- [8] PyMOL | pymol.org [EB/OL]. pymol.org. /2022-10-10. <https://pymol.org/2/>.
- [9] Ortega J T, Jastrzebska B, Rangel H R. Omicron SARS-CoV-2 Variant Spike Protein Shows an Increased Affinity to the Human ACE2 Receptor: An in-Silico Analysis [J]. *Pathogens*, 2021, 11 (1): 45.
- [10] Geng Q, Shi K, Ye G, et al. Structural Basis for Human Receptor Recognition by SARS-CoV-2 Omicron Variant BA.1 [J]. T. Gallagher. *Journal of Virology*, 2022.
- [11] RCSB PDB: Homepage [EB/OL]. Rcsb.org. 2010. <https://www.rcsb.org/>. [Accessed 1st September, 2022]
- [12] Prabakaran P, Xiao X, Dimitrov D S. A model of the ACE2 structure and function as a SARS-CoV receptor [J]. *Biochemical and Biophysical Research Communications*, 2004, 314 (1): 235 – 241.
- [13] Jia H P, Look D C, Shi L, et al. ACE2 Receptor Expression and Severe Acute Respiratory Syndrome Coronavirus Infection Depend on Differentiation of Human Airway Epithelia [J]. *Journal of Virology*, 2005, 79 (23): 14614 – 14621.
- [14] Turner A J. ACE2 Cell Biology, Regulation, and Physiological Functions [J]. *The Protective Arm of the Renin Angiotensin System (RAS)*, 2015: 185 – 189.
- [15] Gonzalez-Villalobos R A, Shen X Z, Bernstein E A, et al. Rediscovering ACE: novel insights into the many roles of the angiotensin-converting enzyme [J]. *Journal of Molecular Medicine*, 2013, 91 (10): 1143 – 1154.
- [16] Crackower M A, Sarao R, Oudit G Y, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function [J]. *Nature*, 2002, 417 (6891): 822 – 828.
- [17] Hamming I, Cooper M, Haagmans B, et al. The emerging role of ACE2 in physiology and disease [J]. *The Journal of Pathology*, 2007, 212 (1): 1 – 11.
- [18] Cai G, Bossé Y, Xiao F, et al. Tobacco Smoking Increases the Lung Gene Expression of ACE2, the Receptor of SARS-CoV-2 [J]. *American Journal of Respiratory and Critical Care Medicine*, 2020.