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Binding Affinity in Theoretical SARS-CoV-2 S Protein Variants

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ABSTRACT: SARS-CoV-2 first emerged in December 2019 and caused a worldwide pandemic that led to the infection of 776 million people with a reported mortality rate of 7.1. Over time, this virus has constantly mutated, leading to much more transmissible and immune-resistant strains. The appearance of these variants leads to the question of how a hypothetical variant containing all known mutations would look and function. This study analyzed all of the mutations of the Spike (S) protein data found in UniProt and selected all that had no known effect or increased transmissibility, among other things. Based on this, two experimental variants' genomes were created in SnapGene. Their 3D structure was predicted and modeled through Alpha Fold. Finally, their binding affinity to the hACE2 receptor was calculated using PRODIGY. It was found that a variant containing only mutations that are known to be beneficial to the virus resulted in a higher binding affinity to the hACE2 receptor compared to the original Wuhan strain and a theoretical strain with all mutations included. These findings suggest that some mutations counteract each other and limit the overall binding affinity of the S protein.

KEYWORDS: Microbiology, Virology, SARS-Cov-2, Spike Protein, Variants.

Introduction

In December 2019, the first human case of Covid-19 was reported, marking the start of the years-long Covid-19 pandemic. This highly infectious disease is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which belongs to the beta coronavirus group. Other notable members of this group are severe acute respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome (MERS-CoV), with each of them having caused their epidemic. However, unlike its fellow family members, SARS-CoV-2 has been proven to cause milder infections and is much more easily transmitted.[1,2] According to the World Health Organization (WHO), as of July 2024, there have been approximately 776 million reported cases of infection and 7.1 million deaths worldwide since the virus was first discovered.[3] Like all coronavirus, SARS-CoV-2 is an enveloped virus with a spherical shape and a crown-like appearance; it can be divided into two central regions: capsid and inner core.[4]

The inner core possesses the RNA viral genome, a single-stranded positive-sense RNA that is packaged by nucleocapsid proteins (N); these, along with membrane (M) proteins, spike (S) proteins, and envelope (E) proteins, are the four structural proteins present in the virus. The capsid is composed of M, S, and E proteins. There are also 16 non-structural proteins (nsp1-16) that mediate anything from RNA processing and replication to protein trafficking within the virus.[5,6] All of this is encoded by open reading frames found in the genome. In terms of replication upon cell entry, the genome possesses a significant advantage as the genomic RNA can be directly translated to proteins, acting as mRNA, shortening the overall process.[2,4] However, when it comes to cell infection, the S proteins determine initial success as they are responsible for virus-cell contact.

Spike (S) proteins are homotrimers, glycoprotein peplomers that are vital in early transmission.[7] They are composed of two domains: S1 and S2. The S1 domain is located in the N-terminus - at the start of the amino acid chain, protruding outwards of the membrane - of the protein and is responsible for the recognition and binding to the host's cell's angiotensin-converting enzyme 2 (hACE2) receptor through its receptor-binding domains (RBDs). The receptor-binding motifs (RBM) found in the RBD are behind this interaction as they possess the genomic information to recognize the hACE2 receptor. The S2 subunit is located in the C-terminus - at the end of the amino acid chain, connected to the membrane - of the protein, connected to the virus's envelope, and oversees the fusion of the virus membrane into the cell's membrane, effectively infecting the host.[1,8,9]

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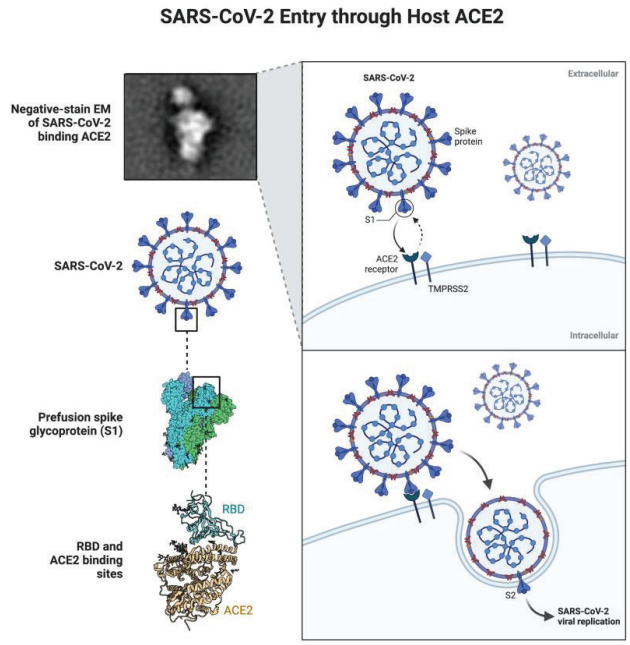

Figure 1: Negative-stain EM depicting the binding process between SARS-CoV-2 and the hACE2 receptor. The diagram expands to cover a graphical depiction of the S protein in a perfusion state and that of the hACE2 receptor and its RBD. Once the RBD detects the receptor, it attaches to it, allowing the S2 subunit to anchor itself to the cell membrane, initiating cellular infection. *Reprints with permission from (MacDaragh Ryan, Caplice)*

For binding between the virus and the receptor to be successful, the cleavage site at the meeting point between S1 and S2 must be cut by Furin, a protease enzyme, once contact with the receptor occurs.[10] Similarly, there is also another cleavage site within S2, S2', which is most often cut by TMPRSS2. This, in turn, allows S2 to unfold and anchor itself into the cell membrane by rearranging itself into a stabler, longer trimer. A condensed version of this process is illustrated in Figure 1. Without these conformational changes, the fusion between the virus and the cell would likely not be possible, and thus, the viral RNA would be unable to replicate itself, stopping the infection process altogether. Thus, the proper functioning of the S protein is crucial for successful transmission.[5,11,12] Consequently, variants of sars-cov-2 with higher recorded transmission rates present multiple mutations in the S protein.[13]

Like all viruses, SARS-CoV-2 has mutated and evolved multiple times since the start of the pandemic. WHO categorizes these variants as either a Variant of Interest (VOI) or a Variant of Concern (VOC). A VOI is a variant with changes in its genome that alter its behaviors - transmissibility, ability to cause a more disastrous disease, detectability, etc. A VOC is a VOI that has proven to cause an increase in the severity of symptoms, may overwhelm the health sector, or is resistant to current vaccines; they are named after Greek letters.[14] Some notable VOC are Alpha (B.1.1.7), Beta (1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529). All these variants presented at least eight mutations (some spanning over 30) in the S protein, and each was responsible for a peak or wave of infections when they emerged. They all have a higher transmissibility rate and have a reduced neutralization rate by antibodies.[13,15]

Mutations in Variants of Concern:

The alpha variant (B.1.1.7) was the first to be discovered in late 2020 and contains eight mutations in the S protein: N501Y, D614G, Δ H69/ΔV70, Δ144, A570D, P681H, T716I, S982A. These changes allowed the variant to attach more easily to cells expressing human hACE2, which may affect Furin processing and are associated with increased viral infection.[16,17] This variant was quickly followed by Beta (B.1.351/501Y.V2), which, at the time, decreased sensitivity to natural or vaccine-acquired immunity, as well as increased binding affinity to the hACE2 receptor. It possesses three mutations in the RBD: K417N, E484K, and N501Y, which are responsible for resistance to neutralization antibodies. Other mutations present include L18F, D80A, D215G, R264I, and A701V.[17] Compared to the original Wuhan strain, infected hosts exhibited a partial reduction in antibody response in both the Alpha and Beta variants.[18]

The gamma variant (501Y.V3/P.1) was first detected in January 2021 and has been reported to increase the virus's transmissibility by 1.4-2.2, regarding the Wuhan strain. Like the beta variant, it also possesses K417N, E484k, and N501Y mutations in the RBD. However, P.1 has a unique amino acid change in the S protein in the following mutations: L18F, T20N, P26S, D138Y, R190S, H655Y, and T1027I.[17] It also contains other mutations like the infamous D614G, which it shares with all other variants such as Delta.[19]

The Delta variant (B.1.617.2) was first detected in late 2020 and contains multiple mutations such as T19R, L452R, T478K, D614G, P681R, and D950N, to name a few. It possesses a greater transmission rate compared to the Alpha variant, and it enhances binding to the hACE2 receptor; mutations E484Q, L452R, and P614R have been shown to simplify binding.[20] Similarly, the Omicron variant (B.1.617.2) also enhances binding to the receptor and increases both transmissibility and resistance to antibody neutralization.[21] It has over 30 mutations in the S protein, overlapping with some changes present in Alpha, Beta, Gamma, and Delta. An example of these mutations are Δ H69/ΔV70, N501Y, D614G, P681H, among others.[22,23] This variant quickly gained prominence and became the dominant strain in circulation. By June 2024, most variants in circulation are sub-strains of Omicron, such as KP.3 and LB.1, giving significant importance to their mutations for studying their effects on viral advantages compared to the previously mentioned strains.[24]

Mutations in the Spike protein with known effect:

Multiple studies have shed light on hundreds of mutations present in distinct SARS-CoV-2 variants, especially in the S protein. The first mutation discovered was D614G in the Alpha variant, which quickly gained notoriety by becoming a dominant trait of nearly all strains in circulation, including all VOCs and most, if not all, VOIs. It is a single nucleotide mutation (A>G) at position 23,403 in the original genome of the Wuhan strain and position 614 of the S protein's genome.[25] It was first detected in February 2020, and by May

2020, 80% of all virus particles had this mutation.[26] D614G increases flexibility in the RBD, creates an outward rotation in S1, and has a slightly more open conformation.[27–29] Research conducted in vitro suggests that it enhances entry to hACE2-expressing cells, particularly those found in the upper respiratory tract.[30–32] This mutation has been shown to increase the virus's transmissibility but does not increase the severity of the disease on infected hosts.[25,32,33]

Another mutation that promotes the contact between the S protein and the hACE2 receptor is the deletion of amino acids (a.a.) 69 and 70. It partially compensates for losses in infectivity due to other mutations, such as N439K and Y453F, by increasing spike incorporation; it plays a role in antibody evasion.[34] Additionally, it has been recorded to have caused zoonotic transmission - from animals to humans.[35] Although mutations N439K and Y453F, found in the RBM, might have a slight adverse effect on infectivity, they have been proven to enhance binding affinity to the receptor and partake in neutralization antibody resistance.[36,37] Similarly, L452R, found in the RBD, reduces susceptibility to neutralizing antibodies, increases infectivity, increases spike stability, and promotes viral replication.[36,38] Mutations A475V, L452R, V483A, F490L, H519P, and N234Q have also been found to promote resistance to neutralizing antibodies.[39]

Three mutations found in the RBD, N501Y, E484K, and K417N, are responsible for increased transmissibility and binding affinity to receptor hACE2. Research suggests that while these advantages are present in each change, combining the three creates a significantly stronger binding affinity than the original strain.[35,40–42] Lastly, K1269A is responsible for a complete loss of the COPI binding, yet it also increases cell fusion.[43] It is important to note that there are dozens more mutations currently cataloged in UniProt; however, their effects on viral functions are still largely unknown.

Vaccines and the study's objective:

As the pandemic progressed and more variants appeared, the need for vaccines and treatment only increased. The first vaccines were introduced in late 2020 and early 2021, all using mRNA to immunize its host. To prevent infection, vaccines seek to cut off contact between the hACE2 receptor and the SARS-CoV-2 S protein; thus, the study of binding affinity between the S protein and the hACE2 receptor is of vital importance for this effort. Pfizer-BioNTech, Moderna, and Johnson and Johnson produced some of the most notable examples, all showing efficacy rates between 72%-95%.[44] However, variants of SARS-CoV-2 have been observed to surpass, to a certain degree, immunization brought forth by vaccines. For example, the Pfizer-BioNTech-Comirnaty vaccine was found to have 25% effectiveness in the Omicron variants compared to its 87% effectiveness in the Alpha variant.[45]

Mutations will continue to occur, with all types of effects on SARS-CoV-2, such as in its virality, transmissibility, disease severity, etc. Most known variants have a unique mix of mutations, with some changes overlapping between the different strains. This raises the question of what a variant expressing all these mutations in the S protein, especially those proven to increase transmissibility, would look like. Would infection rates increase exponentially, or would the combination of all mutations weaken virus transmissibility? This study aims to provide insight into this scenario, hypothesizing that a variant with a combination of mutations with known effects, will have a significantly higher binding affinity than that of a variant with random mutations and the Wuhan strain.

Method

For this study, the S protein's genetic sequence of the original Wuhan strand and subsequent variants were obtained via UniProt.[46] This data was then uploaded to SnapGene where two distinct S protein theoretical variants were created: V1 and V2, as shown in Table 1. To do this, the Wuhan S protein genome was added to two separate documents. Then, each document was manually edited to include the mutations desired in each variant. The V1 variant contains all the mutations listed in UniProt that belong to at least one VOC, regardless of whether their effect is known or not. In contrast, the V2 variant was made to only possess mutations with a known and beneficial impact on viral transmissibility; they did not need to belong to a VOC. The mutations used for the V2 variant are listed below in Table 2.

Some mutations listed on UniProt were excluded if they did not fit the criteria. This includes mutations known to diminish the efficacy of infection, those with unknown effects that are not found in any VOC, and those that require the addition of multiple amino acids into the place of one (ex. A → AAA). There are two notable exceptions in the V1 variant to what was previously mentioned. Both S13L and W152C mutations were added to V1 even though they do not belong to VOC because they represent possible advantages in terms of infection but are not significant enough to be added to V2.

Table 1: All the mutations used in this study are listed and categorized according to which experimental variant they were added to. In V1, all mutations listed in UniProt were used, excluding the ones known to hinder infection. In total, 72 mutations were used for V1. In V2, only mutations with known boosting effects from the V1 group were selected, adding up to 13 in total.

Mutations used in V1	Mutations used in V2
S13L, L18F, T19R, T20N, Q52H, A67V, △H69/△V70, D80A, V83, T95I, D138Y, G142D, △144, H146Q, K147E, W152C, F157I, Q183E, I210V, V213G, D215G, N234Q, G252V, G257S, G229D, R346T, L368I, S371F, S373P, S375F, T376A, R408S, K417N, N440K, K444T, V445P, G446S, L452R, Y453F, F456L, N460K, A475V, S477N, T478K, V483A, E484K, F486V, F490L, G496S, Q498R, N501Y, Y505H, H519P, T547K, A570D, D614G, H655Y, A701V, S704L, T716I, N764K, D796Y, N866K, D950N, Q954H, N969K, L981F, S982A, T1027I, D118H, V1176F, K1269A.	△H69/△V70, N234Q, K417N, L452R, Y453F, A475V, V483A, E484K, F490L, N501Y, H519P, D614G, K1269A

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Table 2: Listing of mutations used for theoretical V2 variant. Each mutation is matched with their known phenotype and the SARS-CoV-2 variants they are associated with. All of the information in the table was recovered from UniProt's database.

Mutations	Variants	Phenotype
△H69/△V70	Alpha/B.1.1.7, Eta/B.1.525, 19B/501T, Omicron/BA.1, Omicron/BA.4, Omicron/BA.5, Omicron/BQ.1.1	Increases incorporation of the spike into the virion.[34]
N234Q	Experimental mutation	Increases resistance to neutralizing antibodies.[39]
K417N	Beta/B.1.351, Omicron/BA.1, Omicron/BA.2, Omicron/BA.2.12.1, Omicron/BA.2.75, Omicron/BA.4, Omicron/BA.5, Omicron/BQ.1.1, Omicron/XBB.1.5, Omicron/EG.5.1	Increases binding affinity.[42]
L452R	Delta/B.1.617.2, Epsilon/B.1.427/B.1.429, Kappa/B.1.617.1, Lambda/C.37, 19B/501Y, Omicron/BA.4, Omicron/BA.5,	Leads to increased infectivity and evasion of the cellular immune response.[36]
	Omicron/BQ.1.1	
Y453F	B.1.1.298	Increases binding affinity.[36]
A475V	Experimental mutation	Increases resistance to neutralizing antibodies.[39]
V483A	Experimental mutation	Increases resistance to neutralizing antibodies.[39]
E484K	Beta/B.1.351, Eta/B.1.525, Theta/P.3, Iota/B.1.526, B.1.1.318, Gamma/P.1, Zeta/P.2 and Mu/B.1.621	Increases binding affinity.[39]
F490L	Experimental mutation	Increases resistance to neutralizing antibodies.[39]
N501Y	Alpha/B.1.1.7, Beta/B.1.351, Gamma/P.1, Theta/P.3, Mu/B.1.621, 19B/501Y, Omicron/BA.1, Omicron/BA.2, Omicron/BA.2.12.1, Omicron/BA.2.75, Omicron/BA.4, Omicron/BA.5, Omicron/BQ.1.1, Omicron/XBB.1.5, Omicron/EG.5.1	Increases binding affinity.[47]
H519P	Experimental mutation	Increases resistance to human convalescent sera neutralization.[39]
D614G	Alpha/B.1.1.7, Beta/B.1.351, Gamma/P.1, Delta/B.1.617.2, Epsilon/B.1.427/B.1.429, Eta/B.1.525, Theta/P.3, Iota/B.1.526, Kappa/B.1.617.1, Lambda/C.37, B.1.1.318, Zeta/P.2, Mu/B.1.621, 20A.EU1, 20A.EU2, Omicron/BA.1, Omicron/BA.2, Omicron/BA.2.12.1, Omicron/BA.2.75, Omicron/BA.4, Omicron/BA.5, Omicron/BQ.1.1, Omicron/XBB.1.5, Omicron/EG.5.1	Increases binding efficiency.[33]
K1269A	Experimental mutation	Loss of COPI binding and increase in cell fusion.[43]

These theoretical sequences along with the hACE2 receptor sequence (obtained from UniProt) were then uploaded to Alpha Fold, where their predicted 3D structure was modeled. Three CIF files were obtained at this stage: Wuhan Strand S protein with the hACE2 receptor, V1 with the hACE2 receptor, and V2 with the hACE2 receptor. These files were then downloaded and uploaded to Pymol to create a labeled digital rendering of the 3D structure; each structure was colored differently, and a red dot was placed over the location of each mutation. Three PDBs were generated at this stage: V1, V2, and the Wuhan S protein, and an hACE2 receptor accompanied them all. All three files were separately uploaded to the PRODIGY website to calculate the binding energy. The software analyzed the structures and calculated the values of the Gibbs free energy (ΔG), equilibrium dissociation constant (K_d), and the charge of both the inter-residue contacts (ICs) and non-interacting surfaces (NISs). Only ΔG and K_d were used in this study to determine the theoretical binding affinity for V1 and V2.

Results and Discussion

V1 and V2 Results:

Binding affinity refers to the strength at which a ligand binds to its receptor and indicates the possible success of the bonding process. The higher the affinity, the stronger and more stable the reaction is.[48] This can be calculated through the Gibbs free energy (ΔG) and equilibrium dissociation constant (K_d) parameters. Gibbs free energy is a measurement of the energy available for work to be done and what is needed for the ligand to bind to the receptor. The smaller the value, the more likely both bind spontaneously, producing an energetically favorable stable outcome. The higher the value, the more energy is needed to maintain that interaction, resulting in a weak bind. A $-\Delta G$ value indicates that a reaction is spontaneous and can occur without adding additional energy. The opposite is valid for a $+\Delta G$; more energy than available is needed, so the reaction cannot occur independently.[49,50] The equilibrium dissociation constant measures the tendency for separation or dissociation to occur in a bonded pair: the likelihood of bonding to fail.[51]

Table 3: PRODIGY results for each S protein variant tested are displayed in the table. The software measured the Gibbs free energy (ΔG) and the equilibrium dissociation constant (K_d) values of the variants. The V2 variant obtained the smallest ΔG and K_d values, followed by the V1 variant. The Wuhan strain obtained the highest values in each category.

Variant S protein	△G (kcal/mol)	K_d (M) at 25°C
Wuhan strain	-11.5	$3.9e \times 10^{-9}$
V1	-13.4	$1.4e \times 10^{-10}$
V2	-30.6	$3.9e \times 10^{-23}$

Table 3 presents the results obtained from PRODIGY.[52,53] Both theoretical variants proposed scored lower levels than the original Wuhan strain. This means both present a higher binding affinity than the original S protein. However, their efficiencies when binding are not equal. V2 scored the lowest in both ΔG and K_d, with -30.6 kcal/mol and 3.9×10^{-23} M, respectively, meaning that it has more than enough energy to bind spontaneously and is the least likely subject for dissociation. This represents an increase in ΔG of 266% relative to the original Wuhan strain. This proves this study's hypothesis correct, as the hypothetical variant containing all mutations with known positive effects did indeed possess a higher binding affinity than that of the V1 variant and the Wuhan strand.

Meanwhile, V1 scored -13.4 kcal/mol and $1.4e \times 10^{-10}$, signifying an increase of 117% in ΔG compared to the original S protein. All mutations present in V2 are also included in V1, which suggests that these mutations plus V1's other mutations, whose effects are still unknown, could counteract each other, resulting in a midpoint binding affinity. This represents an area of study for future research, as there is currently no information about these mutations' effects on the S protein and how they

interact with other mutations in the same chain. More studies are needed to determine which mutations negatively impact binding affinity and transmissibility.

Results in the context of similar studies:

There are similar studies that, through various methods, seek to find links between mutations in the S-protein and binding affinity with the hACE2 receptor. Researchers from NYU found that mutation complexes leading to high affinity are rare, representing 4% of mutation complexes. All while mutations leading to low affinity are common, accounting for approximately 60% of complexes.[54] This correlates with V1 and V2 findings, as not all mutations will increase affinity, and some may even lower it.

They also found that certain mutations in the hACE2 receptor increase affinity to the S protein, especially when paired with mutations in the S protein. However, these mutations are in the minority, as most tend to lower binding affinity. This suggests that mutations in the hACE2 receptor play a significant role in human transmissibility.[54] Additionally, it was found that the mutations with the highest binding affinity tend to concentrate near the RBD, especially those that are located at points of interaction with the hACE2 receptor. This can be observed in the V2 variant, where the great majority of its mutations are located in this area, as observed in Figure 2.

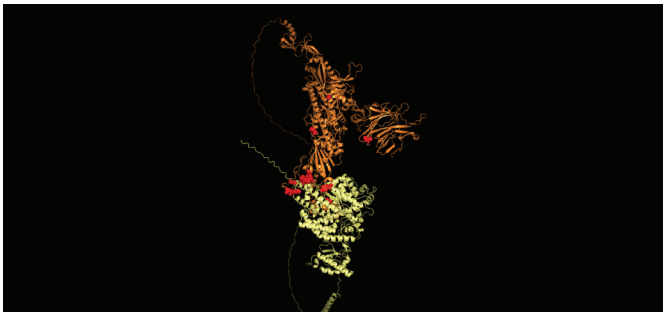

Figure 2: 3D structure of the hACE2 receptor (yellow) and the theoretical V2 spike variant (orange). Both proteins' sequences were obtained from UniProt; however, the V2 sequence was altered to contain all desired mutations (Table 2). They were modeled using Alpha fold and uploaded to Pymol to create this figure. The red spheres represent all of the mutations in the genome sequence, placed in their respective locations in the S protein's structure, with most conglomerating in the RBD region.

While studies done *in silico*, such as this one, can offer advantageous insight, they do possess limitations that may or may not interfere with a study's results. As found by Shivani Thakur *et al.*, while studying binding affinity between the hCE2 receptor and SARS-CoV-2 S protein, modeling programs may carry biases obtained during training. They observed that their results varied depending on the structure they used.[55] This is to say that for the same mutation, structure A and structure B yielded different binding affinities. In this study, this problem was not observed, as there was only one structure per variant uploaded to a single program. However, it does present a limitation in this study, as values could be inflated due to the method used.

Current Treatments and Future Variants:

Vaccines were the first available preventive treatment for COVID-19, and according to WHO, 13.64 billion doses were administered worldwide.[56] However, this is not the only option, with many drugs and therapies in clinical trials or already in use in specific demographics. This includes drugs such as polymerase inhibitors, protease inhibitors, and other antivirals.[57] Though it is important to point out that most therapies focus on RNA replication and other aspects of infection that do not include the S protein as it is less susceptible to small molecule drugs. Treatments focusing on the S protein primarily deal with neutralizing antibodies (nABs), with some potent nABs being identified in and currently in clinical trials. Although a promising avenue, nABs are highly affected by the evolution of the virus as more variants gain a level of resistance against them and vaccines. Because of this, it is essential to continue to study the mutations of SARS-CoV-2, as current treatment depends on this.[58]

■ Conclusion

SARS-CoV-2 has dramatically affected society, producing a months-long lockdown with everlasting effects in all sectors of life. With over 700 million recorded infections, the health sector has been paralyzed by its various lineages. Prominent mutations that affect transmissibility are in the S protein, a vital part of virus transmission. There are dozens of known mutations, but not all their effects are understood. Compared to the original Wuhan strain, this study determined that combining all the mutations augments the binding affinity between the protein and the hACE2 receptor. However, not all changes increase the affinity. The combination of known positive changes gives a higher affinity than the mix of all mutations without a filter. This leads to the conclusion that some mutations counteract each other, but further research is needed to determine the individual effects of each. Understanding and calculating a virus's binding affinity offers multiple benefits as vaccines, whose job is to prevent the union between the cell and virus, could be developed with higher efficacy and immunization rates. Additionally, information could also help scientists and doctors stay ahead of a virus's variants, as they will be able to study the effects of mutations before they are present in the circulating variants. This would allow for preventive treatment and prevent an oversaturation of the healthcare system. Similarly, binding affinity would also be an asset in drug development for treatment post-infection, such as in the case of SARS-CoV-2 where medication focuses on treating the systems instead of fighting the actual virus. Binding affinity represents a field of study that has the potential to lessen and even prevent the effects of pandemics if only more time and research are allotted to it.

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