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Computational modeling of the effect of five mutations on the structure of the ACE2 receptor and their correlation with infectivity and virulence of some emerged variants of SARS-CoV-2 suggests mechanisms of binding affinity dysregulation

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# Computational modeling of the effect of five mutations on the structure of the ACE2 receptor and their correlation with infectivity and virulence of some emerged variants of SARS-CoV-2 suggests mechanisms of binding affinity dysregulation

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## Abstract

Interactions between the human angiotensin-converting enzyme 2 (ACE2) and the RBD region of the SARS-CoV-2 Spike protein are critical for virus entry into the host cell. The objective of this work was to identify some of the most relevant SARS-CoV-2 Spike variants that emerged during the pandemic and evaluate their binding affinity with human variants of ACE2 since some ACE2 variants can enhance or reduce the affinity of the interaction between the ACE2 and S proteins. However, no information has been sought to extrapolate to different variants of SARS-CoV-2. Therefore, to understand the impact on the affinity of the interaction between ACE2 protein variants and SARS-CoV-2 protein S variants, molecular docking was used in this study to predict the effects of five mutations of ACE2 when they interact with Alpha, Beta, Delta, Omicron variants and a hypothetical variant, which present mutations in the RBD region of the SARS-CoV-2 Spike protein. Our results suggest that these variants could alter the interaction of the Spike and the human ACE2 protein, losing or creating new inter-protein contacts, enhancing viral fitness by improving binding affinity, and leading to an increase in infectivity, virulence, and transmission.

This investigation highlighted that the ACE2 S19P mutation decreases the binding affinity between the ACE2 and Spike proteins in the presence of the Beta variant and the wild-type variant of SARS-CoV-2 isolated in Wuhan-2019. The R115Q mutation of ACE2 lowers the binding affinity of these two proteins in the presence of the Beta and Delta variants. Similarly, K26R lowers the affinity of the interaction between the ACE2 and Spike proteins in the presence of the Alpha variant. This decrease in binding affinity is probably due to the lack of interaction between some of the key residues of the interaction complex between the ACE2 protein and the RBD region of the SARS-CoV-2 Spike protein. Therefore, ACE2 mutations

appear in the presence of these variants, they could suggest an intrinsic resistance to COVID-19 disease. On the other hand, our results suggested that the K26R, M332L, and K341R mutations of ACE2 expressively showed the affinity between the ACE2 and Spike proteins in the Alpha, Beta, and Delta variants. Consequently, these ACE2 mutations in the presence of the Alpha, Beta, and delta variants of SARS-CoV-2 could be more infectious and virulent in human cells compared to the SARS-CoV-2 isolated in Wuhan-2019 and it could have a negative prognosis of the disease.

Finally, the Omicron variant in interaction with ACE2 WT, S19P, R115Q, M332L, and K341R mutations of ACE2 shows a significant decrease in binding affinity. This could be consistent with that the Omicron variant causing less severe symptoms than previous variants (Ren et al., 2022; Lauring, 2022). On the other hand, our results suggested Omicron in the complex with K26R, the binding affinity is increased between ACE2/RBD, which could indicate a negative prognosis of the disease in people with these allelic conditions.

**Keywords:** ACE2-SARS-CoV-2; Alpha; Beta; Delta; Omicron; binding affinity.

## 1. Introduction

SARS-CoV-2 genetic variants have been emerging and circulating worldwide throughout the COVID-19 pandemic. Recent research revealed that several of these variants could be resistant to neutralization by convalescent antibodies in recovered COVID-19 patients as well as recombinant neutralizing antibodies (nAbs) developed as a therapy (Fratev, 2020; Tanaka *et al.*, 2021; Weisblum *et al.*, 2020; Xianding Deng *et al.*, 2020). It is of particular concern that current treatments do not become as effective against variants preliminary studies suggest that some vaccines have low activity against some of the variants emerging (Wang *et al.*, 2021; Fratev 2020). The United States Centers for Disease Control and Prevention (CDC) have classified SARS-CoV-2 variants as variants being monitored (Alpha, Beta, Delta, Gamma, Epsilon, Eta, Iota, Kappa, Mu, and Zeta), and variants of concern (Omicron) (CDC, 2022). In our work, the variants Alpha, Beta, Delta, and Omicron were chosen (Table 1) because variants encode mutations that are related to a significant reduction in neutralization by antibodies generated during a previous infection or vaccination (Weisblum *et al.*, 2020; Riemersma *et al.*, 2021; Salleh *et al.*, 2021; Supasa *et al.*, 2021), evidence of greater transmissibility (Tegally *et al.*, 2020; Kemp *et al.*, 2021) and, evidence of greater severity of the disease (Centers for Disease Control and Prevention, 2022).

Alpha variant (B.1.1.7) was identified in the United Kingdom in 2020 and spreads approximately 50% better than the original SARS-CoV-2 (Lauring, 2021). Alpha variant presents N501Y mutation in the receptor-binding domain (RBD), and this mutation within the RBD enhances virus binding affinity to the ACE2 receptor of host cells (Starr *et al.*, 2020; Yi *et al.*, 2020; Zahradník *et al.*, 2021). Some studies reported an association with higher mortality and risk of hospitalization (Davies, 2021). The beta variant (B.1.351) with mutations K417N, E484K, and N501Y in the RBD, was reported in South Africa in 2020. This variant exhibits increased transmissibility as well as causes a reduction in T cells and other immune responses triggered against COVID-19 (Lauring, 2021). Delta variant has L452R and T478K mutations in the RBD and was originally found in India in December 2020. Based on available evidence, this variant may be associated with an increased risk of hospitalization (Sheikh *et al.*, 2021). In addition, their evidence that those people who have only received a partial scheme of vaccination would be less protected against this variant than against others, independent of the platform used (mRNA or viral vector) (Lopez Bernal et al. 2021). Finally, the Omicron variant carries an unusually high number of mutations in the RBD-Spike protein (Table 1). It was reported from South Africa in November 2021 and quickly became the dominant strain worldwide. Although the Omicron variant shows enhanced transmission and considerable humoral immune evasion and resistance to vaccines (Cao *et al.*, 2022), Currently, several studies have indicated that the Omicron variant causes less severe symptoms than previous variants (Ren *et al.*, 2022; Lauring, 2022).

| Classification | RBD- Spike protein mutations | First reported |
|---|---|---|
| Alpha (B.1.1.7) | N501Y | The United Kingdom, September 2020 |
| Beta (B.1.351) | K417N, E484K, and N501Y | South Africa, May 2020 |
| Delta (B.1.617.2) | L452R, and T478K | India, October 2020 |
| Omicron (B.1.1.529) | G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, and Y505H | South Africa, November 2021 |

**Table 1. Information on the emerging variants of SARS-CoV-2 was selected for the development of this study.** The Alpha, Beta, Delta, and Omicron variants of SARS-CoV-2 have mutations in the RBD-Spike protein and have an increase in transferability, virulence, or change in the clinical presentation of the disease, a decrease

in the efficacy of social intervention and public health, vaccine, and available treatments (Centers for Disease Control and Prevention, 2022).

Several studies have shown that the susceptibility and severity of infection by SARS-CoV-2 could be related to different external factors such as environment, socioeconomic position, and age (Guang W.F., 2020; Yang *et al.*, 2020). As well as internal factors including health status and genetic differences between people, different responses of the host immune system, the interaction between a viral protein that mediates the infection, and viral evasion mechanisms to immune response, among others (Guang W.F., 2020; Yang *et al.*, 2020). For example, it has been shown that the gene pool of populations also performs a vital role in determining susceptibility or resistance to viral infections (Debnath *et al.*, 2020). The host gene influence plays a role in the inbred population's severity, viral replication, and inflammation (Debnath *et al.*, 2020). Therefore, the identification of variation signatures at specific populations level that could be used as molecular markers for regional models, is a key aspect of disease and overall pandemic control.

During viral infection, host entry is mainly mediated by Spike (S) protein, a homotrimeric glycoprotein that includes subunits S1 and S2 in each S protein monomer. The subunit S1 contains the receptor-binding domain (RBD), which recognizes and binds to the peptidase domain (PD) of the host angiotensin-converting enzyme 2 (ACE2) (Renhong *et al.*, 2020). When protein S binds to protein ACE2, the trimer S protein cleavage is triggered by the transmembrane protease serine 2 TMPRSS2 that separates the subunits S1 and S2 (Hoffmann *et al.*, 2020). The S2 subunit exposes fusion peptides in host membranes and promotes fusion with viral membranes (Taka *et al.*, 2021). It has been suggested that the polymorphism of the ACE2 gene can modulate the interaction between ACE2 and the SARS CoV-2 Spike protein during virus entry into the host cell (Benetti *et al.*, 2020; Calcagnile *et al.*, 2020; Cao *et al.*, 2020; Debnath *et al.*, 2020; Hussain *et al.*, 2020) since some ACE2 variants can enhance or reduce the affinity of the interaction between the ACE2 and S proteins. Therefore, a predisposing genetic background may be critical for the susceptibility, symptoms, and outcome of COVID-19 infection (Benetti *et al.*, 2020; Calcagnile *et al.*, 2020). However, no information has been sought to extrapolate to different variants of SARS-CoV-2.

To understand the impact on the affinity of the interaction between ACE2 protein variants and SARS-CoV-2 protein S variants, in this study, molecular docking was used to help to understand this interaction mechanism of viral infection. In this aspect, we hypothesize that the

non-synonymous mutations reported in both the ACE2 and the S proteins could alter the affinity of the interaction between these molecules and perhaps ultimately affect the process of infection. Computationally, we examined the effect of five non-synonymous mutations of ACE2 (S19P, K26R, R115Q, M332L, K341R) binding to Alpha, Beta, Delta, and Omicron variants of SARS-CoV-2, and one hypothetical variant E484K (this mutation was selected due to it has been found in high frequency in the GISAID database), evaluating likewise their correlation with infectivity of the most important emerged variants of SARS-CoV-2. Figure. 1 depicts the general workflow used in this investigation.
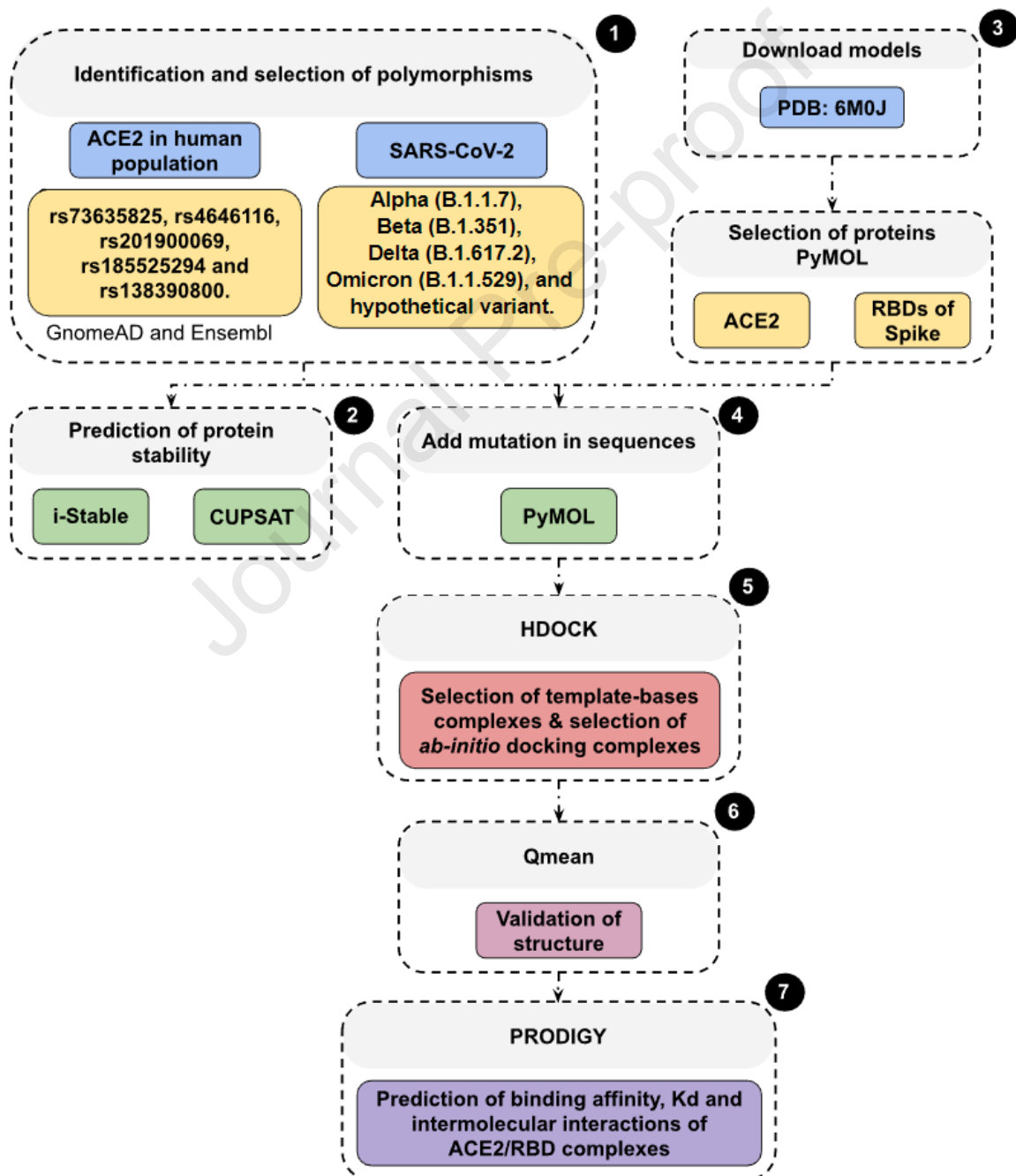
**Figure 1.** The workflow applied in this investigation. 1. Identifying and selecting polymorphisms of ACE2 receptor and Spike SARS-CoV-2. 2. Analyze the stability and structural impact of ACE2 human receptor protein upon non-synonymous mutations using bioinformatics tools. 3. The 6M0J model was downloaded from PDB. 4. The amino acid residues of the wild-type ACE2 sequence and wild-type RBD-Spike of SARS-CoV-2 were replaced with those of the missense variants selected. 5. the HDOCK server assembled ACE2/RBD complexes. 6. Qmean was used to validate HDOCK complexes. 7. PRODIGY webserver was used to predict binding affinity, the dissociation constants of ACE2/RBD complexes, and several types of intermolecular interactions. Steps 5 through 7 were repeated for the thirty-six designed complexes.

## 2. Methodology

### 2.1. Data mining for the genetic polymorphism of ACE2 gen

The genetic variability along the ACE2 gene region for the populations analyzed was obtained employing the Data Slicer tool as implemented in the Ensembl Genome Browser (https://www.ensembl.org). The dbSNP databases (https://www.ncbi.nlm.nih.gov/snp), gnomAD (https://gnomad.broadinstitute.org), and Ensembl Genome Browser were used to determine the allele frequencies of the SNPs identified in the ACE2 region. A total of 332 variants of the exonic region of the ACE2 gene were found in the world population present in the Ensembl Genome Browser and gnomAD databases. Of the 332 total variants, 239 non-synonymous or missense mutations, 88 synonymous mutations, 2 stop gained mutations, 1 splice donor mutation, and 2 frameshift mutations were found (**Supplementary Table S1**).

To evaluate the effect of mutations on the structure of the ACE2 receptor and their correlation with infectivity of some important emerged strains of SARS-CoV-2, five non-synonymous mutations from the previous databases were selected: dbSNP (rs73635825, rs4646116, rs201900069, rs185525294, and rs138390800). The selection criteria were the following; first, proximity to the site of interaction between the human ACE2 receptor and the RBD region of the SARS-CoV-2 Spike protein, considering that the region of the N-terminal peptidase domain (PD) of ACE2 is the one that interacts with the RBD of the SARS-CoV-2 Spike protein (Lan *et al.*, 2020). The domain PD has 597 amino acid residues, from serine 19 to aspartic acid residue 615 (Lan *et al.*, 2020). According to molecular dynamics studies by Jawad *et al.*, (2021), the following amino acids of the ACE2 protein have been identified as hotspots in the ACE2 / Spike interaction: R393, R357, D355, G354, K353, G352, N330, N330, T324, Y83,

M82, L79, L45, Q42, Y41, D38, E37, K35, H34, K31, D30, F28, T27, Q24, K26 and S19 (Jawad *et al.*, 2021; Low-Gan *et al.*, 2021). Second, ACE2 mutations were also selected for their high frequency in the population concerning the other mutations found for the ACE2 gene, as established from the analysis over the Ensembl databases.

It is worth noticing that even though mutations of the ACE2 gene are related to pathologies such as arterial hypertension, renal failure, cardiovascular diseases, and genetic intracranial the aneurysm have been found (Crackower *et al.*, 2002), the ACE2 mutations used in this work do not have pathological phenotypes reported to date (Karczewski *et al.*, 2020).

## 2.2. Impact analysis of non-synonymous mutations in the human ACE2 protein

The effect on protein stability of the identified amino acid substitution of ACE2 in humans was evaluated using the crystal structure of the SARS-CoV-2 S protein bound to ACE2 at 2.45 Å resolution (PDB ID: 6M0J) (Lan *et al.*, 2020). It was predicted at 37°C and pH:7 using the iStable Server (Chen *et al.*, 2013) and CUPSAT (Cologne University Protein Stability Analysis Tool) (Parthiban *et al.*, 2006).

iStable algorithm was applied to predict the stability alterations in each protein up to non-synonymous mutations. The program defines the stability of a protein as a positive (+) free energy ($\Delta\Delta G$) value $> 0$, scores higher than 0 predict increased stability, and the destabilizing data as negative (-) with a $\Delta\Delta G$ value $< 0$ predicting a decrease in the stability of the structure. Further, the iStable confidence score ranges between 0 and 1, where the higher value exposes higher confidence. Finally, iStable predicts the distribution of data based on the secondary structure and relative solvent accessibility (RSA) of the mutation site. The ranges that determine the RSA are as follows: values less than 10% as undersurface, values between 10% and 20% classified as buried, between 20%~50% as partially buried, and between 50% and 100% as exposed (Chen *et al.*, 2013).

CUPSAT algorithm uses structural environment-specific atom potentials and torsion angle potentials to predict the free energy difference ($\Delta\Delta G$) of unfolding between wild-type and mutant proteins. The output consists of information about the mutation site, its structural

features (solvent accessibility, secondary structure, and torsion angles), and comprehensive information about changes in protein stability (Parthiban *et al.*, 2006).

## 2.3. System Preparation and Molecular Docking

To predict the effect of the selected variants of SARS-CoV-2 and non-synonymous mutations of the ACE2 receptor, we generated each of the structural models using the RBD crystal structure PDB ID: 6M0J. The chloride ion, zinc ion, glycans, and water molecules in the crystal structure remained in their original positions. The energy minimization of the original protein complex was performed through YASARA (Krieger *et al.*, 2002). The resulting file, as well as the mutant complexes, were exported as PDB files and directly visualized in PyMOL Molecular Graphics System,v. 1.8 (Schrödinger, 2015).

The docked structures were refined using HDOCK (Yan *et al.*, 2020). The HDOCK server automatically predicts docked structures interaction through a hybrid algorithm of template-based modeling and ab initio template-free docking. QMEANDisCo SwissDock (Studer *et al.*, 2020) was used to validate the complexes formed by HDOCK and applied for bad bonds and angle metrics. QMEANDisCo is the default quality estimation method employed by the SWISS-MODEL homology modeling server. It is a composite score relying on a combination of knowledge-based terms and a new distance constraint (DisCo) score. DisCo assesses the agreement between observed pairwise distances in a model with an ensemble of constraints extracted from experimentally determined structures that are homologous to the model being assessed (Studer *et al.*, 2020).

## 2.4. Structure analysis of ACE2 variants and SARS-CoV-2 S Spike protein complexes

The results of the simulations performed in HDOCK were studied in PRODIGY. PRODIGY is an online tool for the prediction of binding affinity in protein-protein complexes (Xue *et al.*, 2016). The results of PRODIGY include the predicted value of the binding free energy ($\Delta G$) in kcal mol$^{-1}$ and the predicted value of the dissociation constant (Kd). The Kd is used to calculate and evaluate the order of the strength of biomolecular interactions. The smaller the Kd value, the higher the binding affinity of the ligand for its target (Vangone and Bonvin 2015). Our study selected a temperature of 37°C and the type of intermolecular contact within the 5.5Å. It is important to mention that the algorithm of PRODIGY predicts which interactions

are being formed at a distance less than 5.5Å, but it does not indicate the exact distance of those interactions detected. Nevertheless, the HDOCK server does provide the exact distances of the interactions detected. However, HDOCK only predicts interactions with distances less than 5Å. Therefore, we used both tools to build a table of interactions between the residuals of ACE2 and Spike for the developed complexes. Distances greater than 5Å were designated as (*NN*) indicating that there is an interaction, and the interaction has a distance between 5 - 5.5Å.

## 3. Results and discussion

### 3.1. Protein stability analysis of non-synonymous mutations in the human ACE2 protein

To identify the effect of the 5 non-synonymous mutations of the ACE2 protein we evaluated the stability of the ACE2 protein subjected to the selected mutations. Each mutation was evaluated individually due to amino acid residue substitutions in a protein can lead to structural changes that influence protein folding, including the persistence or elimination of non-covalent contacts (hydrophobic and van der Waals interactions; hydrogen and ionic bonds) upon mutation as well as the secondary structure and solvent accessibility of each substituted position (Masso and Vaisman, 2008). These changes affect protein function and disease occurrence. Thus, to evaluate the stability of the protein upon mutations we used iStable 2.0 (Chen *et al.*, 2013) and CUPSAT algorithms (Parthiban *et al.*, 2006).

In the S19P mutation, the proline residue (the mutant) is larger than the serine residue, being a larger residue, which could lead to bulges in the structure. Additionally, the mutation introduces a more hydrophobic residue at this position. This can result in the loss of hydrogen bonds and/or disrupt correct folding. However, our results suggest that the impact at the structural level and therefore at the functional level of the ACE2 protein with the S19P mutation is minimal since the mutation is expected to increase the stability of the structure (**Table 2**).

The stability of the ACE2 protein with the K26R and M332L mutations is reduced, however, the possible torsion angles of the structure under these mutations are favorable (**Table 2**). In the K26R mutation at the structural level, the lysine is in a region to form an α helix. The mutation converts the wild-type residue to a residue that does not form α-helices as a secondary structure. Additionally, the mutant residue is larger, which could lead to bulges. As for the M332L mutation, methionine differs in size from leucine. The methionine residue could lead

to a loss of interactions. On the other hand, the ACE2 protein with mutations R115Q and K341R is predicted to have an unstable structure (**Table 2**). In R115Q the charge of the arginine is positive while the charge of glutamine is neutral. Therefore, the charge of the wild-type residue will be lost, which can lead to a loss of interactions with other molecules or residues. There is also a difference in the size of the residues, the mutant residue is smaller, a property that could also lead to a loss of interactions. Finally, in the K341R mutation Lysine and arginine differ in size. The mutant residue is larger, this could lead to bulges.

| dbSNP | Mutation | iStable meta results | | | | CUPSAT | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ΔΔG (Kal/mol) | Prediction | RSA | Conf. Score | Overall Stability | Torsion | Predicted ΔΔG (kcal/mol) | Solvent accessibility |
| ACE2 allelic variants | | | | | | | | | |
| rs73635825 | S19P | 0,5002 | Increase | 107,5% | 0,7631 | Stabilizing | Favorable | 1,28 | 105.8% |
| rs4646116 | K26R | -0,2915 | Decrease | 43,8% | 0,6925 | Stabilizing | Favorable | 0,35 | 44.24% |
| rs201900069 | R115Q | -0,1066 | Decrease | 57,2% | 0,6756 | Destabilizing | Favorable | -1,06 | 56,77% |
| rs185525294 | M332L | -0,3772 | Decrease | 16,5% | 0,6736 | Stabilizing | Favorable | 6,21 | 15,99% |
| rs138390800 | K341R | -0,4341 | Decrease | 56,9% | 0,5713 | Destabilizing | Favorable | -0,6 | 57,37% |

**Table 2. Stability of selected mutations evaluated in ACE2.** Protein stability was predicted at 37 °C and pH: 7 using the i-Stable and CUPSAT servers. Stability is defined as a positive (+) value for a free energy difference (ΔΔG) > 0, scores higher than 0 predict increased stability and the destabilizing data as negative (-) with a ΔΔ$G$ value < 0 predicting a decrease in the stability of the structure. The confidence score ranges between 0 and 1, where the higher value exposes higher confidence. The ranges that determine the RSA are as follows: values less than 10% as undersurface, values between 10% and 20% classified as buried, between 20%~50% as partially buried, and between 50% and 100% as exposed (Chen *et al.*, 2013). CUPSAT program uses structural environment-specific atom potentials and torsion angle potentials to predict the free energy difference (ΔΔG) of unfolding between wild-type and mutant proteins (Parthiban *et al.*, 2006).

## 3.2. Types of intermolecular interactions between S-RBD-ACE2 complexes

Accordingly, we targeted the interactions of five non-synonymous mutants of the ACE2 protein with the SARS-CoV-2 variants, a total of thirty-six structural complexes were designed from

the previously optimized PDB 6M0J template. Illustratively, the position of the Wild Type (WT) residues where we locate the selected non-synonymous mutations in the RBD region of the Spike protein and the human ACE2 receptor are represented in **Figure 2**.
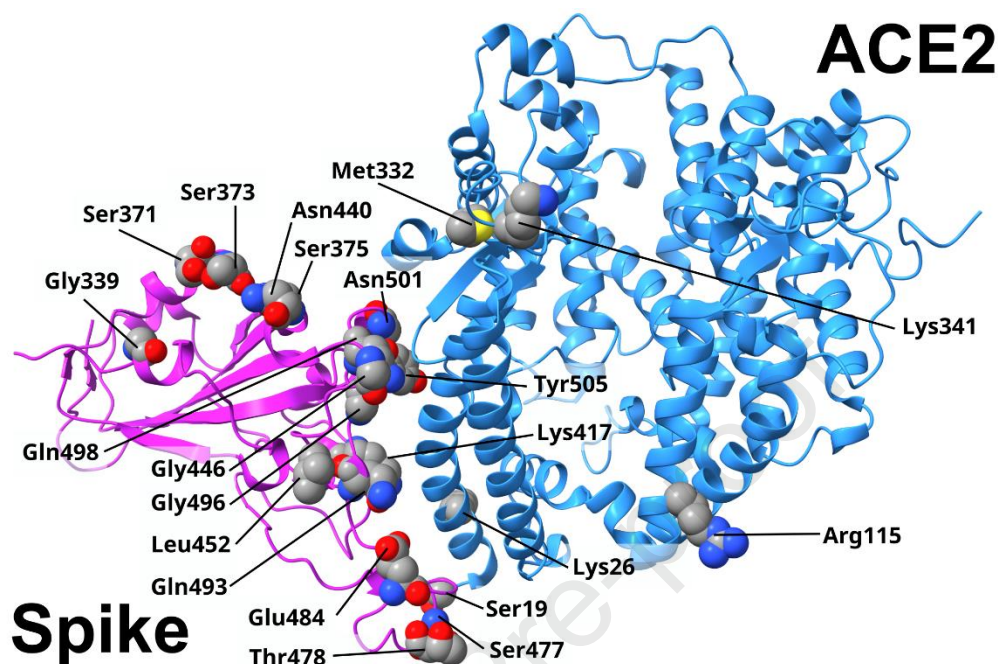


**Figure 2. Location of wild-type (WT) residues that were altered by selected variants in human ACE2 and SARS-CoV-2 Spike proteins.** In the human ACE2 protein (pink), the locations of the WT residues S19, K26, R115, M332, and K341 were highlighted, these residues were subsequently mutated to determine the impact at the structural level by the mutation's residues respectively. In the RBD-Spike protein of the Sars-CoV-2 (aquamarine), the locations of the WT residues G339, S371, S373, S375, K417, N440, G446, L452, S477, T478, E484, Q493, G496, Q498, N501, and Y505 were highlighted, these residues were subsequently mutated to determine the impact at the structural level by the mutation's residues respectively.

From now on, for greater practicality, we will name the wild-type complex WT (6M0J) and the complexes of non-synonymous mutations of the ACE2 protein (S19P, K26R, R115Q, M332L, K341R) in their interaction with the SARS-CoV-2 variants (Alpha (N501Y), Beta (417N-E484K-N501Y), Delta (L452R-T478K), Omicron (G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H) and HV as hypothetical variant (E484K)) as **ACE2 mutation / SARS-CoV-2 variant**. The quality of the models was evaluated with QMEANDisCo, which provides scores in the range of 0-1 with 1 depicting perfect quality. The QMEANDisCo analysis demonstrated the excellent quality of all the models with global scores of $0.83 \pm 0.05$, which was equal to the PDB control model (6M0J) (**Supplementary Figure S1**).

To compare and predict the diverse types of intermolecular interactions of the different ACE2 mutants and SARS-CoV-2 variants, PRODIGY and HDOCK algorithms were used. Significant differences were found in the types of interactions (charged-charged, charged-polar, charged-apolar, polar-polar, polar-apolar, and apolar-apolar) between the complex designs and the wild-type structure (WT) (**Figure 3**). The WT structure (ACE2-WT / Spike-WT) presents 82 intermolecular interactions: 4 with charge-charge, 11 charge-polar, 20 charge-apolar, 7 polar-polar, 27 polar-apolar and 13 apolar-apolar (**Figure 3**). The ACE2-WT/Beta, S19P/Delta, K26R/Delta, R115Q/Delta, K341R/Omicron, and S19P/HV complexes show the same number of intermolecular interactions as WT but show significant differences in their types.

The mutant complexes that show fewer intermolecular interactions compared to the WT complex are K26R/Spike-WT, K341R/Spike-WT, ACE2-WT/Alpha, S19P/Beta, R115Q/Beta, ACE2 WT/Omicron, S19P/Omicron, R115Q/Omicron, M332L/Omicron, R115Q/HV, and M332/HV. The mutant complexes with the highest number of intermolecular interactions compared to WT are S19P/Spike-WT, R115Q/Spike-WT, M332L/Spike-WT, S19P/Alpha, K26R/Alpha, R115Q/Alpha, K341R/Alpha, M332L/Alpha, K26R/Beta, M332L/Beta, K341R/Beta, ACE2-WT/Delta, M332L/Delta, K341R/Delta, ACE2-WT/HV, K26R/HV, and K341R/HV.  (**Figure 3**).
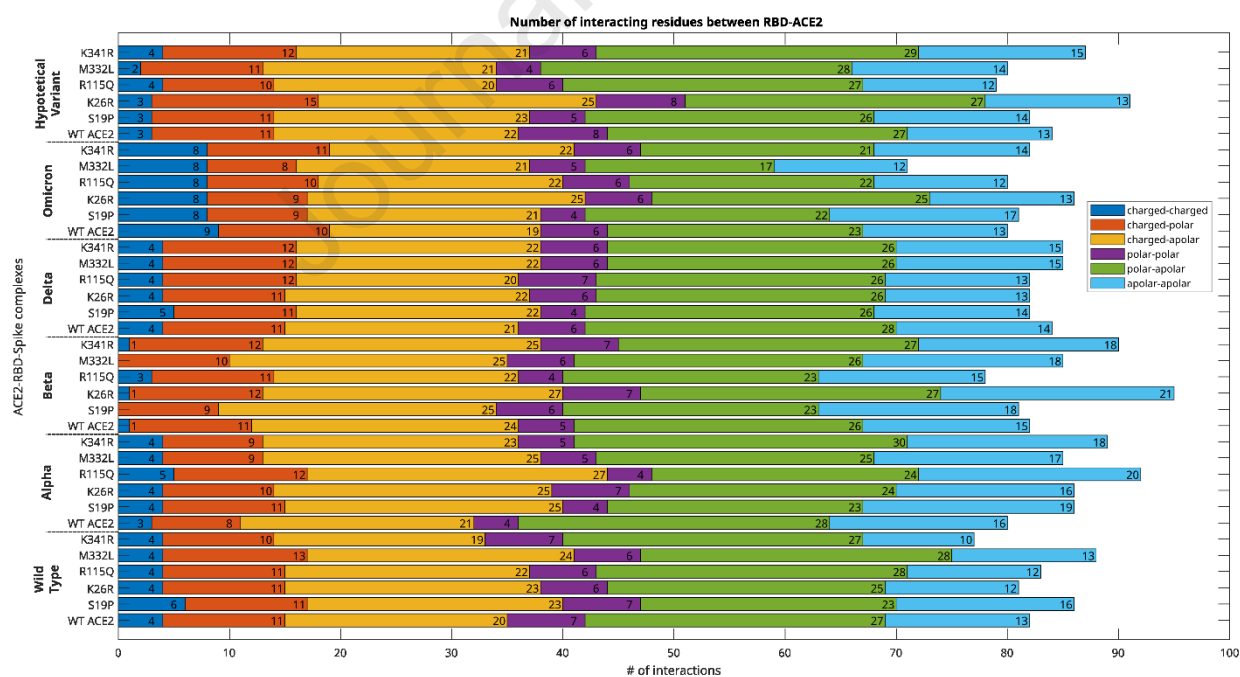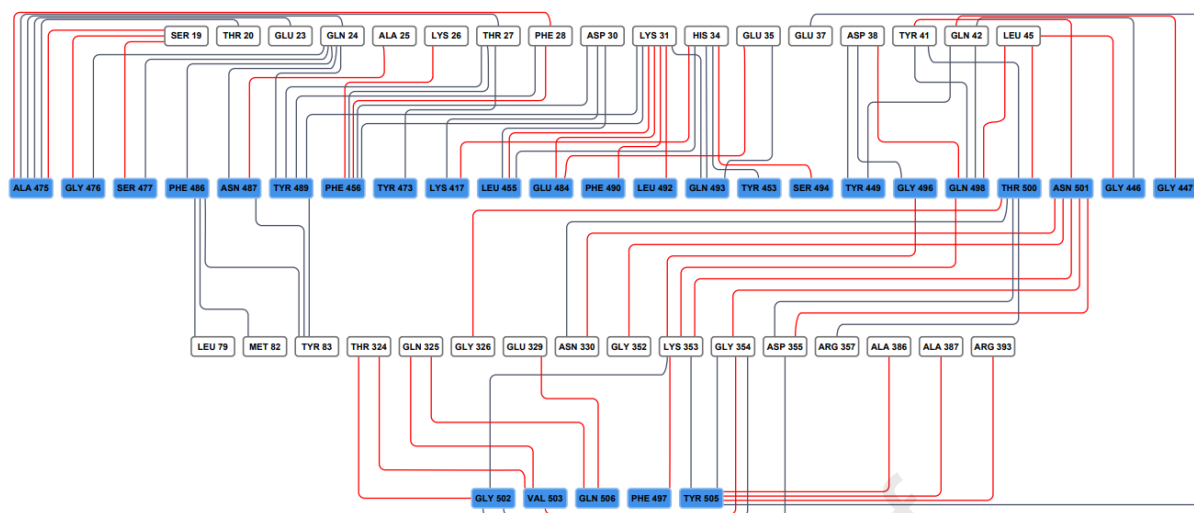


**Figure 3. Types of intermolecular interactions between ACE2 allelic variants and SARS-CoV-2 Spike variants.** The bar graph shows the number of diverse types of intermolecular contacts between ACE2 variants and SARS-CoV-2 Spike protein.

Overall, compared with the molecule WT (6M0J) the complexes that present less charged-charged, charged-polar, charged-apolar, polar-apolar, and apolar-apolar interactions could suggest that these variants in humans could present a lower binding affinity between SARS-CoV-2 and ACE2 and therefore confer some level of resistance. Here we could highlight that according to our results we observe that the complexes with the omicron variant, except for the K26R/Omicron complex, present a lower number of interactions. This could be consistent with the Omicron variant causing less severe symptoms than previous variants (Ren et al., 2022; Lauring, 2022). Likewise, the complexes that present a greater number of these interactions suggest a poor prognosis of the disease. However, considering the dynamic nature of the protein-protein interaction, it is quite difficult to draw an accurate conclusion from these observations. Therefore, to provide a compelling insight into these complex variants, below, we identify the interacting amino acid residues of both the ACE2 protein and the Spike protein and calculated the affinity binding energy and the dissociation constant (KD).

### 3.3. The different interactions between the residues of the ACE2 / RBD-S of SARS-CoV-2 are under constant selection pressure.

To identify the interacting amino acid residues in the different ACE2 mutants and SARS-CoV-2 variants, the PRODIGY and HDOCK algorithms were used. For this purpose, we constructed a matrix indicating the ACE2 residues interacting with the RBD of the SARS-CoV-2 Spike protein in each of the mutant complexes as a function of the wild-type structure PDB 6M0J (**Supplementary Table S2**). We identified eighty-two intermolecular interactions in the WT model complex (6M0J). In **figure 4** the total number of interactions between the amino acid residues of the human protein ACE2 and the Spike protein of SARS-CoV-2 are represented. Of the eighty-two interactions, forty-four remain stable in the thirty-six protein complexes proposed in this study. Not-changing interactions between these residuals are represented by black lines.

**Figure 4. The 82 intermolecular interactions between angiotensin-converting enzyme 2 (ACE2) and SARS-CoV-2 Spike protein in the WT model.** White nodes: ACE2 residues. Blue nodes: RBD residues of Spike protein. Black lines represent interactions that remained constant despite the mutations implemented in all thirty-six complexes. Red lines represent the interactions that fluctuated in the thirty-six studied complexes.

The interaction part between ACE2 and the RBD-S consists of a small section of approximately eighty amino acid residues (**Figure 4**). In the thirty-six mutant complexes studied, we were able to show that these residues are under constant selection pressure because these interactions are lost and gained due to the slight changes made. Many of these residues could be overlapping the binding epitopes of the antibodies and, therefore, could affect the effectiveness of current vaccines and therapies. If we consider that SARS-CoV-2 will continue to mutate and continue among us, we must foresee how to progressively adopt a prevention strategy against the disease, especially when the mutating virus continues to grow. Therefore, drug development must continue, and an important part of this process will be the preparation of updated vaccines tailored against all emerging variants.

## 3.4. The different interactions between the residues of the ACE2-RBD-S complexes modulate the affinity binding energy and dissociation constant (Kd) between the ACE2 and Spike-SARS-CoV-2

To connect the conformational changes of the ACE2 protein with the receptor-binding domain (RBD) variants of SARS-CoV-2, we used the PRODIGY algorithm to calculate the binding energy and the dissociation constant (Kd) of the ACE2/RBD complexes (**Figure 5 A-B**).

Binding affinity is the strength of ligand-receptor binding. The more negative the energy flow, the greater the binding force. Additionally, the affinity of interaction is described by the equilibrium dissociation constant Kd, or, in thermodynamic terms, the Gibbs free energy $\Delta G$ ($\Delta G = RT \ln Kd$). The smaller the Kd value, the higher the binding affinity of the ligand for its target (Vangone and Bonvin 2015).
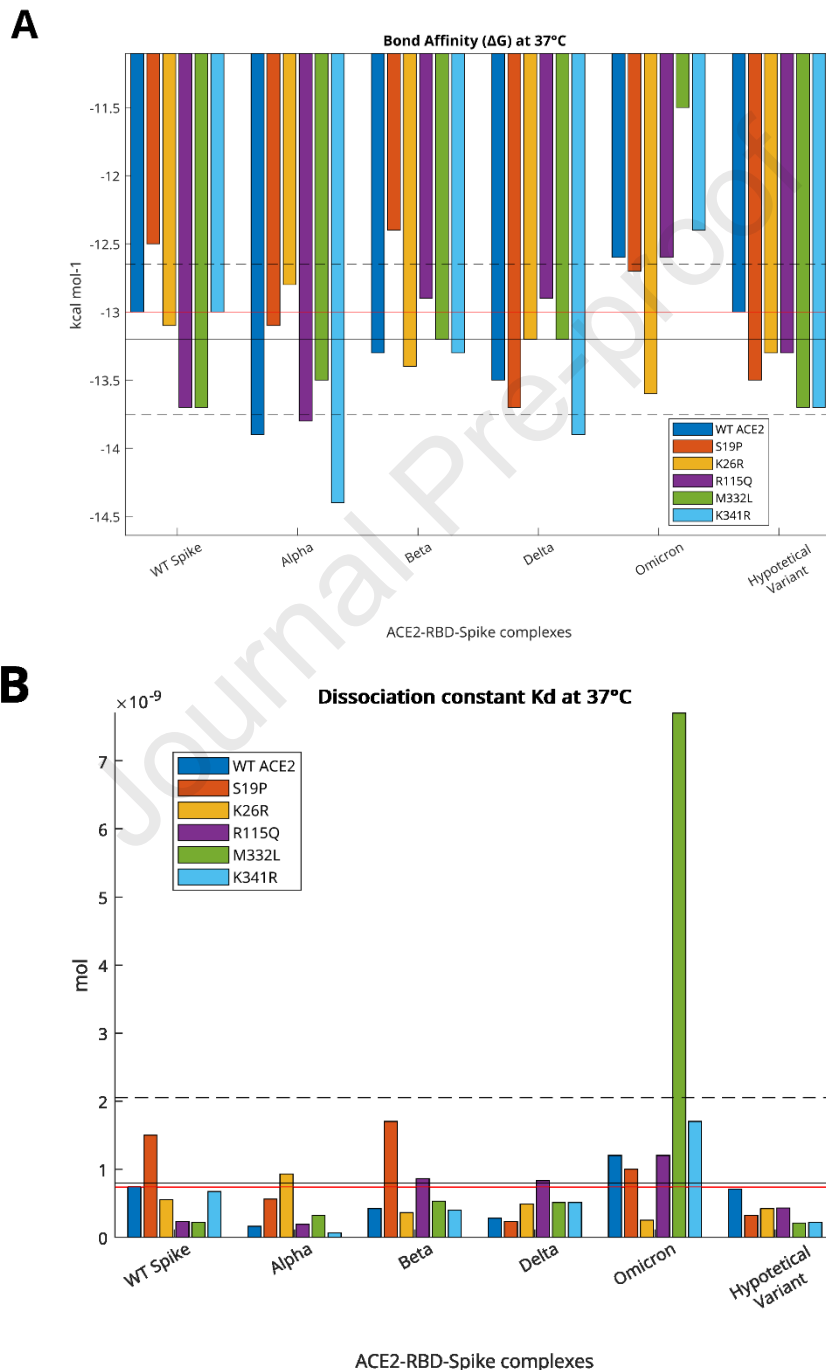


**Figure 5. Interaction between the complexes of ACE2-S.** A. Affinity comparison of the bond interaction between different ACE2 mutations and the SARS-CoV-2 variants. B. Dissociation constant (Kd) of the

ACE2/RBD-S complexes. The horizontal lines depict the reference WT value (red line), the mean (continuous black line), and the standard deviation (dotted black lines), calculated from the square root of the variance normalized with the number of samples minus one.

The Spike proteins (S) of SARS-CoV and SARS-CoV-2 interact with human angiotensin-converting enzyme 2 (ACE2) as their receptor (Li, 2015; Hoffmann *et al.*, 2020). The affinity of the Spike protein of SARS-CoV and SARS-CoV-2 for the ACE2 human receptor has been proposed to be an important determinant of the overall viral replication rate and disease severity (Li *et al.*, 2005; Chellasamy, Arumugasamy, and Govindaraju, 2020). Increased binding affinity between ACE2 and Spike proteins correlates with increased virus transmissivity and disease severity (Li *et al.*, 2005). SARS-CoV-2 has a higher affinity for human ACE2 than the SARS-CoV virus variant (Shah *et al.*, 2020; Wrapp *et al.*, 2020; Yesudhas, Srivastava, and Gromiha, 2021), indicating that the transmission rate of SARS-CoV-2 infection is higher than that of SARS-CoV infections, and this is due to alterations of a few residues in the RBD of the SARS-CoV-2 protein S leading to a ~ 10-fold increase in binding affinity between RBD and ACE2 (Chellasamy, Arumugasamy, Ienhancingndaraju, 2020; Lan *et al.*, 2020; Yesudhas, Srivastava and Gromiha, 2021). Moreover, SARS-CoV-2 being an RNA virus is highly prone to mutations during the progression of the pandemic, due to the great genetic diversity of hosts and different cellular microenvironments that can lead to diverse selection pressure in different viral strains. Consequently, adaptations within protein S are critical for the high-affinity association with the human ACE2 protein that may have contributed to the unusual severity of SARS-CoV-2. Thus, it is essential to explore the genetic diversity of the S gene around the world from the large genomic pool and evaluate their effect on human receptor binding.

Our results revealed that the RBD-S proteins of the alpha (B.1.1.7), beta (B.1.352), and delta (B.1.617.2) variants have a higher binding affinity and a lower dissociation constant (Kd) for wild-type ACE2 compared to the protein S from SARS-CoV-2 from Wuhan (S-wild-type) (**Figure 5**). The highest affinity and lowest Kd occurred in the alpha variant, followed by the delta variant, and finally the beta variant. On the other hand, our results indicated that the S protein of Omicron (B.1.1.529) has the lowest binding affinity and higher Kd for wild-type ACE2 compared to ACE2/Spike wild-type complex. These findings agree with the work of Wu L and collaborators (2022) who indicate that Omicron-RBD shows weaker binding affinity than the Delta variant and the WT to human ACE2.

The alpha variant presents one of the key contact residues in the RBD of protein S (N501Y) (Yi *et al.*, 2020). Our results agree with previous experimental studies suggesting that the N501Y mutation increases the affinity for the ACE2 receptor (Starr *et al.*, 2020; Yi *et al.*, 2020; Jiří Zahradník *et al.*, 2021). Even in experiments performed in mice, N501Y has been associated with greater infectivity and virulence, since it provides a more favorable interaction with mouse ACE2 for the coupling and entry of the virus (Gu *et al.*, 2020). This increase in affinity conferred by the N501Y mutation is due to the 501-residue lying on the right shoulder of the RBD, and the change from a relatively short asparagine side chain to the large aromatic tyrosine allowing for favorable ring stacking interactions was consistent with increased affinity (Dejnirattisai *et al.*, 2021).

Although the beta variant also includes the N501Y mutation, its binding affinity for ACE2 decreases by 4.3% compared to the binding affinity of the alpha variant (**Figure 5**). The beta variant presents two additional mutations that are key in the functional motif that forms the interface with the receptor, E484K and K417N (Yi *et al.*, 2020). The E484K mutation tested separately as a hypothetical variant, did not differ in terms of binding affinity and Kd for ACE2 wild-type compared to the S-wild-type variant from Wuhan (**Figure 5**). However, some authors mention that this mutation provides a slight increase in binding due to the formation of a transient contact ion pair with the E75 residue of ACE2 (Jawad *et al.*, 2021). This interaction between K484 and E75 is observed in our results for the ACE2-WT / HV complex (E484K), but not in the ACE2-WT / Beta complex (**Supplementary Table S2**). This result indicates that K484 in the beta variant does not participate in the increase in affinity with the receptor. The impact of the E484K mutation is focused on the role of conferring resistance to neutralizing antibodies elicited during SARS-CoV-2 infection and convalescent serum panels (Weisblum *et al.*, 2020), rather than promoting an increase in affinity-bound.

The third mutation that the beta variant presents in the RBD region is K417N. Residue K417, in our results, forms a salt bridge interaction with D30 of ACE2-WT at a molecular distance of 2.547 Å. Mutational scanning studies suggested that the K417N mutation has minimal impact on ACE2-binding affinity due to the loss of the salt bridge with the D30 residue of ACE2 (Starr *et al.*, 2020).  However, in our results there was no total loss of the interaction, N417 continues to interact with residue D30, but at a greater distance (3,528 Å). It could be inferred that, by having a greater interaction distance, the impact on the binding affinity between RBD-ACE2 is less due to this mutation. From the above, it is possible to infer that the improved binding

affinity of the beta variant relative to the WT variant points to higher transmissibility conferred by the presence of the N501Y mutation.

The Delta variant exhibits a higher binding affinity compared to the WT variant (**Figure 5**). These results are consistent with previous studies where they indicate that the binding affinity of RBD with ACE2 in the Delta variant is up to ~ 2 times higher than that of RBD WT and ACE2 (Liu H *et al.*, 201). It appears that the T478K mutation could drive this increase in affinity due to the formation of a new hydrogen bond between K478 and Q24 of ACE2, as evidenced by our results. On the other hand, we did not find molecular interactions between the L452R mutation with ACE2 residues. Hajj-Hassan et al. (2021) showed that L452 does not interact with ACE2, but the mutation R452 generates intramolecular interactions. One of the energetically allowed rotamers of the L452R mutation shows the formation of a hydrogen bond formed between R452 and S495 in the RBD (Hajj-Hassan *et al.*, 2021). This interaction helps stabilize the binding motif responsible for both recognition and binding, possibly explaining the higher affinity of the spike protein (Hajj-Hassan *et al.*, 2021). Furthermore, the L452R mutation is of particular concern, as it has been shown to provide resistance to the Delta variant against some nAbs (Qianqian Li *et al.*, 2020) since it contributes polar characteristics to the hydrophobic core of the RBD, weakening the hydrophobic interactions, which explain the complete loss of the union of RBD with some nAbs (Liu H *et al.*, 2021).

The Omicron variant carries an unusually high number of mutations, more than 60 substitutions/deletions/insertions have been identified in this sequence, and more than 30 of the total Omicron mutations identified are accumulated in the spike. Of these mutations, 15 have accumulated in the RBD (Centers for Disease Control and Prevention, 2022). Among these mutations K417N, T478K, and N501Y are found in the other variants studied in this work. These mutations as previously mentioned have indicated a higher binding affinity with ACE2, improved transmissibility, and pathogenicity (Starr *et al.*, 2020; Yi *et al.*, 2020; Jiří Zahradník *et al.*, 2021; Gu *et al.*, 2020; Liu H *et al.*, 201). However, these observations vary due to the combined effects of the following 12 mutations G339D, S371L, S373P, S375F, N440K, G446S, S477N, E484A, Q493R, G496S, Q498R, and Y505H that the RBD region of the Omicron variant presents. In our results, the mutant residues of the RBD-Omicron variant G339D, S371L, S373P, S375F, N440K, and E484A do not interact with residues of the ACE2 protein.

The residue G446 in Spike WT protein interacts with L45 and Q45 residues of ACE2 WT.  The S477 residue interacts with S19 and Q24 residues of ACE2 WT. The Q498 residue interacts with D38, K353, L45, and Q42 residues of ACE2 WT. The Y505 residue interacts with K353, G354, and E37 residues of ACE2 WT. The mutated residues S446, N477, R498, and H505 in the RBD-S Omicron variant, maintain interactions with those residues of the ACE2 protein respectively (**Supplementary Table S2**). It appears that these mutations have no impact on the binding affinity between the two proteins. Nevertheless, according to several studies, some of these residues are generating other impacts on the development of the disease. Motozono C *et al.*, (2022), showed that the G446S mutation in the Omicron variant affects antigen processing/presentation and potentiates antiviral activity by vaccine-induced T cells, leading to enhanced T cell recognition towards emerging variants and the mutation S477N appears to promote resistance to neutralization by multiple mAbs (Liu Z *et al.*, 2021).

The Q493 WT residue molecularly interacts with K31, H34, and E35 residues of the ACE2 WT protein. The mutant residue R493 maintains these interactions and additionally forms new interactions with residues D30 and D38 of ACE2 WT (**Supplementary Table S2**). These new interactions could impact the binding affinity between ACE2-RBD-S, and according to Khan A *et al.*, (2022) Q493R mutation leads to more stable interactions with the ACE2 protein. Additionally, in previous studies, it has been shown that Q493R in the RBD spike is significantly associated with Omicron disease severity in patients. Docking studies of Remdesivir with R493 mutant viral protein demonstrated a reduction in the binding strength of Remdesivir to mutant Spike proteins (Saifi S *et al.*, 2022). As for the E484 residue mutated to A484, the E484 in the spike WT of the RBD forms contact with K31 in ACE2 WT. But, in the mutated A484 residue, the side chain is too short to contact with K31 ACE2, therefore this interaction is lost, which could turn out to result in decreased binding affinity to ACE2.

Although the binding affinities of the interaction between ACE2 and RBD Spike in ACE2-RBD Omicron complexes are low, the Omicron variant has higher transmissibility and a shorter incubation period, which indicates that there are some other factors affecting viral transmission. He X *et al.*, (2020) explained this by mentioning that as Omicron has N679K and P681H mutations near the furin cleavage site, the incorporation of basic amino acids around the furin cleavage site could facilitate spike cleavage at S1 and S2, thus enhancing fusion and virus infection (He X *et al.*, 2020).

On the other hand, natural ACE2 polymorphisms have been proposed to be relevant to the pathogenesis and transmission of diseases caused by SARS-CoV-2 (Chan *et al.*, 2020). In this study, we characterize how more frequent amino acid mutations in the ACE2 protein, affect the affinity of the interaction between RBD-ACE2. We have examined human ACE2 variation data compiled from multiple data sets and identified polymorphisms that are likely to make individuals more susceptible or resistant to SARS-CoV-2 in correlation with circulating strains of the virus. Knowledge of these residues can be useful to assess the risk posed by any new SARS-CoV-2 outbreak.

One of the key residues of the RBD-ACE2 interaction is S19 of ACE2. Our docking analysis shows that the S19P mutation decreases affinity when interacting with the WT, Beta, and Omicron variants of SARS-CoV-2 (**Figure 5**). This decrease in binding affinity by the S19P mutation with the WT variant is consistent with the results of Hussain et al. (Hussain *et al.*, 2020), whose *in silico* analysis showed a binding affinity even lower than -10.3 kcal mol-1, attributing a decrease in interaction stability (Hussain *et al.*, 2020; Surya Mohan *et al.*, 2021). Structurally, the impact of the S19P mutation can differ greatly in the interaction between the RBD and the receptor. Serine (S) is a polar residue, capable of forming polar interactions such as hydrogen bonds. Proline (P), on the other hand, is an apolar residue, therefore it should establish a much weaker interaction with Spike. S19 of ACE2 forms three interactions with residues A475, G476, and S477 of the RBD of protein S. But in the S19P / Spike-WT mutation complex, the interaction of P19 with the RBD residue S477 is lost. Moreover, the results show that the K417N mutation in the S19P/Beta complex reduces the binding affinity due to the loss of the salt bridge between K417 of RBD and D30 of ACE2 (**Figure 5**). The impact of the low binding affinity and the lack of some of the key residues in the complex formation with the Spike-WT, Spike-Delta, and Spike-Omicron proteins of SARS-CoV-2 with the S19P mutation could suggest an intrinsic resistance to the interaction between ACE2 and Spike proteins and probably, in some individuals, the positive prognosis for the WT and Beta variants may be due to the existence of the S19P mutation.

In the case of the K26R mutation, a reduction in affinity is observed only when it interacts with the Alpha variant. On the other hand, when it interacts with the other variants there is an increase in the binding affinity (**Figure 5**). This increased affinity for the R26 of ACE2 with Spike-WT has been reported in previous studies (Calcagnile *et al.*, 2020; Hussain *et al.*, 2020; Stawiski *et al.*, 2020; Al-Mulla *et al.*, 2021; Suryamohan *et al.*, 2021). The K26 residue

previously establishes polar contacts with the first mannose of the glucan linked to the N90 of ACE2 and probably stabilizes the position of this glucan (Al-Mulla *et al.*, 2021). Glucan bound to N90 emerges as an important determinant of SARS-CoV-2 infectivity since it may decrease the affinity of ACE2 for RBD possibly through a steric hindrance imposed by it (Stawiski *et al.*, 2020). K26R is predicted to cancel the stabilization of polar contacts with N90, impairing glucan coordination, and leading to increased affinity of the virus for the ACE2 receptor. Therefore, the net effect of the R26 polymorphism would then be the stabilization of the central α-helices that increases the binding affinity of ACE2 to CoV-2 RBD at the cost of glucan stiffness (Stawiski *et al.*, 2020; Suryamohan *et al.*, 2021).

Although the R115, M332, and K341 residues of the ACE2 protein are not found within the site of interaction with RBD, the R115Q, M332L, and K341R mutations impact the binding affinity between ACE2 and RBD. Our results show that these three mutations increased binding affinity by interacting with the alpha variants and the hypothetical variant. Both the R115Q and M332L mutations increased the affinity in the S-WT protein complexes, while the K341R remained the same as ACE2-WT M332L and K341L increased affinity when interacting with Beta and Delta variants, while R115Q decreases it (**Figure 5**). On the other hand, the R115Q, M332L, and K341R mutations in the complex with the RBD-S Omicron variant dramatically decreased binding affinity compared to the WT complex. This increase and decrease in the affinity are probably related to the formation or loss of new interactions between the ACE2 and RBD-S residues (**Supplementary Table S2**).

## 3.5. SARS-CoV-2 variants and mutations in the ACE2 protein could not only provide resistance against the acquired immune response but could also improve viral fitness

The SARS-CoV-2 variants that we discuss in this study (Alpha, Beta, and Delta) not only offer resistance to the immune response acquired naturally or induced by vaccination, but they also improve viral fitness by enhancing the binding affinity to the ACE2 receptor compared to SARS-CoV-2 from Wuhan. Being the Alpha and Delta variants the most infectious. Similarly, our data suggest that ACE2 variants in different populations could be significantly affecting the binding affinity between SARS-CoV-2 and ACE2. We highlight that ACE2 mutations such

as S19P, K26R, R115Q, M332L, and K341R could be playing a significant role in susceptibility and/or resistance to the disease.

It is possible that, in some, if not all individuals, the positive prognosis for the WT, Beta, and Omicron variants may be due to the existence of the S19P variant of ACE2. Similarly, a positive prognosis of the disease would be expected in the presence of Beta, Delta, and Omicron variants due to the existence of R115Q; and for the Alpha variant by the presence of the ACE2 K26R mutation. On the other hand, a patient likely has a negative prognosis of the disease with the WT, Alpha, Beta, Delta, or hypothetical variant, due to the presence of the M332L, K341R , and K26R mutations (except with Alpha) in ACE2. Finally, a significative negative prognosis would be expected in the presence of K26R for the Omicron variant. These findings from this research provide valuable information for analyzing the frequencies of candidate alleles in different populations to predict the prognosis of disease caused by SARS-CoV-2.

## 3.6. Our findings can guide future attempts to design inhibitors of the virus and the human ACE2 receptor

Zahradník and collaborators (2021) proposed that the RBD domain itself can be used as a competitive inhibitor of the ACE2 receptor binding site. However, for this to work, its affinity must be significantly optimized to achieve higher affinity. Our work shows that the N501Y mutation is a good candidate to inhibit and compete against the virus for the ACE2 receptor. Soluble forms of the ACE2 protein have also been proposed for treatment in critically ill patients with COVID-19 disease (Abd El-Aziz et., 2020; Chan *et al.*, 2020). In clinical studies, they found that hrsACE2 treatment led to significant improvement in treated patients. With the data obtained in this research, we propose soluble decoys of the ACE2 protein with the S19P, K26R, R115Q, M332L, or K341R mutations as treatments since these mutations, depending on the variant, enhance the binding affinity between S-ACE2.

Consequently, the empirical data deduce the different types of binding affinity of intermolecular complexes providing important information. However, to better understand the susceptibility of the disease in human populations and their risk of infection, large-scale sequencing projects must be undertaken. Sequencing of the viral Spike gene and the human

ACE2 gene in patients with severe, moderate, and asymptomatic conditions in each population. This will provide a more reliable conclusion.

## 4. CONCLUSION

In this study, we have presented computationally the effect of five non-synonymous mutations of ACE2 (S19P, K26R, R115Q, M332L, K341R) binding to four variants of SARS-CoV-2 and one hypothetical variant E484K, with mutations in the RBD Spike protein, evaluating likewise their correlation with infectivity of the most important emerged variants of SARS-CoV-2; Alpha, Beta, Delta, and Omicron. Understanding the spectrum of the human ACE2 polymorphism not only provides information on susceptibility to assess the risk posed by any new outbreak of SARS-CoV-2 and its variants population enhancing but is also a guide for the development of possible treatments for those serious patients as are inhibitors of the virus and the human ACE2 receptor.

We highlight that the complexes with ACE2 S19P mutation have lower binding affinity for the WT variants and the Beta variant compared with the variant isolated in Wuhan-2019. The complexes with R115Q mutation lower the affinity for the Beta and Delta variants. Finally, the complex with K26R mutation lowers the affinity for the Alpha variant. The lack of some of the key residues in complex formation with the SARS-CoV-2 spike protein could suggest intrinsic resistance. Our results suggested that the complexes with K26R, M332L, and K341R mutations are expressively increasing the affinity in Alpha, Beta, and Delta complexes increased significatively the affinity between RBD and ACE2, consequently, these variants could be more infectious in people with these allelic conditions, compared to the SARS-CoV-2 isolated in Wuhan-2019.

Finally, the Omicron variant in interaction with ACE2 WT, S19P, R115Q, M332L, and K341R mutations of ACE2 shows a significant decrease in binding affinity. This could be consistent with that the Omicron variant causing less severe symptoms than previous variants (Ren et al., 2022; Lauring, 2022). On the other hand, our results suggested Omicron in the complex with K26R, the binding affinity is increased between ACE2/RBD, which could indicate a negative prognosis of the disease in people with these allelic conditions. Nevertheless, it is important to mention that the Omicron variant has higher transmissibility and a shorter incubation period compared with other variants and according to our results, it could be thought that there are

other factors beyond the binding affinity between ACE2/RBD-S that are affecting viral transmission.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

**Appendix A. Supplementary data**

The following is the supplementary data to this article:

Supplementary Table S1.

Supplementary Table S2.

Supplementary Figure 1.

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**Highlights**

- SARS-CoV-2-variants enhance viral fitness by improving binding affinity with hACE2.
- Some hACE2 mutations suggest an intrinsic resistance to COVID-19 disease.
- Omicron causes less severe symptoms due to a decrease in binding affinity with hACE2.
- Omicron interacting with K26R-ACE2 suggets a negative prognosis of COVID-19 disease.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**This document is signed by the main author on behalf of all the authors.**

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