THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 12 Issue 9

RESEARCH ARTICLE

In silico prediction of COVID-19 vaccine efficacy based on the strain-specific structural deviations in the SARS CoV-2 spike protein receptor binding domain

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OPEN ACCESS

PUBLISHED
30 September 2024

CITATION
Vissapragada, M., Aggunna, M., et al., 2024. *In silico* prediction of COVID-19 vaccine efficacy based on the strain-specific structural deviations in the SARS CoV-2 spike protein receptor binding domain. Medical Research Archives, [online] 12(9). https://doi.org/10.18103/mra.v12i9.5718

DOI
https://doi.org/10.18103/mra.v12i9.5718

ISSN
2375-1924

ABSTRACT

Coronavirus disease-2019 (COVID-19) pandemic gave the opportunity for various vaccine design strategies and their evaluation. However, the emergence of variants severely challenged the efficacy of the COVID-19 vaccines. The changes in the amino acid sequences and corresponding changes in the epitope topology of the variants could be the primary reasons for the loss of antibody binding affinity. The structural deviations in the epitopes may not be the same for all the variants resulting in differential efficacy of the same vaccine against different variants. In this study we performed an *in silico* evaluation of the strain-specific structural deviations in 15 variants of coronavirus in order to predict the antibody binding affinity and in turn estimate the success/failure of the current vaccines. The spike protein trimer homology models of 15 variants along with the wild type were prepared in close, open and semi-open conformations. The variant models were superposed onto the wild type model to calculate the C_α root mean square deviations (RMSD) in their epitopes and ACE2 binding sites. Our results show that both the epitopes and the ACE2 binding sites of variants have RMSDs >35 Å suggesting that such large structural deviations in the epitopes are potentially responsible for the loss of binding affinities of the neutralizing antibodies that are generated in response to the current vaccine shots. Based on our analysis, we further prepared two hand-drawn clover leaf plots one for the main variants and the other for the omicron sub-variants summarizing all the structural deviations in the epitopes and ACE2 binding sites in open, closed and semi-open conformations. These plots serve as quick reference charts to predict the efficacy of current vaccines. For example, large structural deviations that are >20 Å can certainly lead to total loss of the antibody binding. Based on our findings in this study we propose alternative and improved strategies of future vaccine design for COVID-19.

Introduction

Severe-acute-respiratory syndrome coronavirus-2 (SARS CoV-2) causes the respiratory infection known as Coronavirus disease-2019 (COVID-19). It is extremely contagious and escalates quickly[1]. The World Health Organization (WHO) identified the novel coronavirus, 2019 novel coronavirus (2019-nCoV), as the cause of the pneumonia outbreak in late December of the year 2019, in Wuhan City, China[2]. COVID-19 is transmitted through aerosols and fomites as well as close contact between the source and the affected person. This virus can cause illnesses ranging from a simple cold to more serious diseases[3]. As of March 31, 2024, there are over 774 million confirmed illnesses and more than 7 million deaths worldwide[4]. Infected people in Europe and America correspond to about 40% and 30% of the total reported cases, respectively[5].

Coronaviruses are positive-strand RNA viruses[6]. Coronaviruses are classified into four genera based on genetic and serological characterization: alpha coronavirus (alpha-CoV), beta coronavirus (beta-CoV), gamma coronavirus (gamma-CoV) and delta coronavirus (delta-CoV). Alpha-CoV and beta-CoV are found principally in mammals[7]. The coronaviruses responsible for recent epidemic and pandemic outbreaks of infections in humans, such as SARS, MERS and COVID-19 are members of the *Sarbecovirus* subgroup of beta-CoV[8]. SARS-CoV2 fits in the *Orthocoronavirinae* subfamily of the *Coronaviridae* family and the *Nidovirale* order[9]. Coronaviruses endure frequent recombination. It materializes that such events occurred during SARS-CoV-2's evolutionary history resulting in the emergence of a strong strain capable of proficiently infecting human cells[7].

SARS-CoV-2, like other coronaviruses, has a genome of approximately 30 kb long and encodes four structural proteins: spike protein (S), envelope (E), membrane protein (M) and nucleocapsid (N)[10-12]. The SARS-CoV-2 genome encodes a number of non-structural open reading frames (ORFs) as well. The coronaviruses infect host cells using their spike glycoprotein (S protein)[13,14]. The transmembrane spike glycoproteins exist as homotrimers that protrude from the viral surface. The spike glycoprotein is crucial for coronavirus entry, thus rendering it as an alluring antiviral target[13-16]. S protein consists of two functional subunits, S1 and S2. The S1 subunit is made of the N-terminal domain (NTD) and the receptor binding domain (RBD). The S1 subunit binds to the receptors on the host cell. The S2 subunit contains the fusion peptide (FP), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic tail (CT). The S2 subunit's function is to cohere the spike protein with the host cells[15,17]. The cleavage site between the S1 and S2 subunits is known as the S1/S2 protease cleavage site[17,18]. For all coronaviruses, host proteases cut the spike glycoprotein at the S2' cleavage site to activate the proteins, which are required to fuse the membranes of viruses and host cells via irreversible conformational changes[17-19]. N-linked glycans are crucial for proper folding and neutralizing antibody binding. The spike protein exists in three conformations, closed, open and semi-open. In the closed state, all three RBDs are in the "down" conformation[20,21]. In the open state, all three RBDs are in the "up" conformation and is necessary for the fusion of the SARS-CoV-2 with the host cell membranes, thereby facilitating SARS-CoV-2 to enter the host cells. In semi-open state, one of the RBDs is in "up" conformation[20-22].

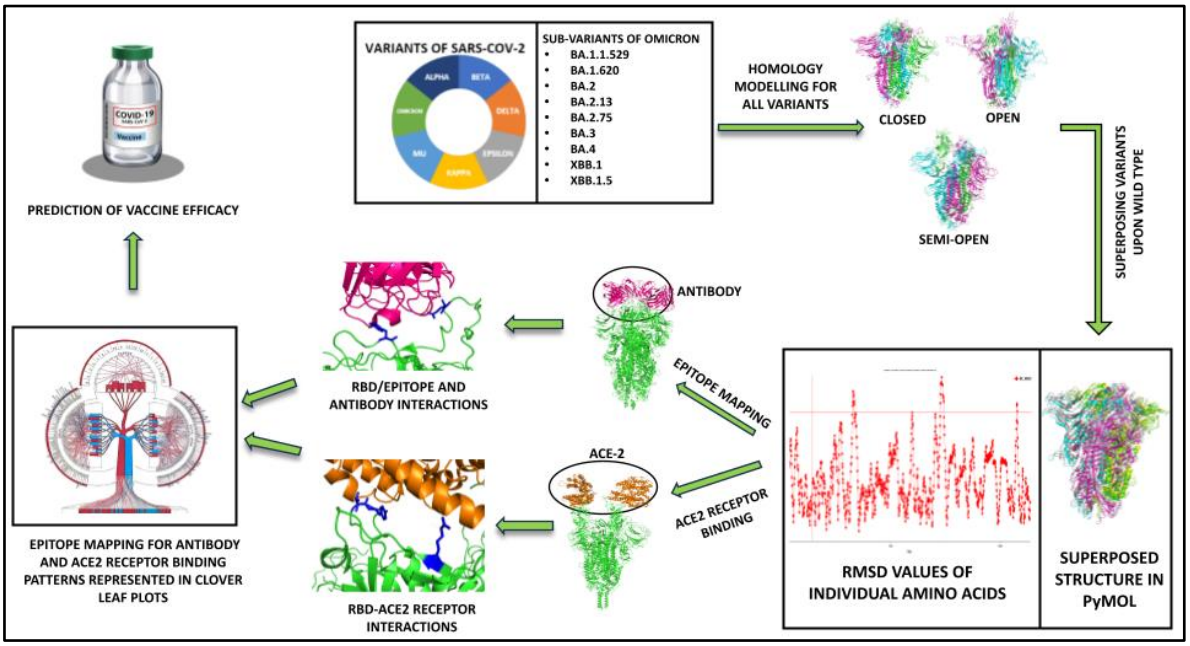
There are several variants in SARS-CoV-2 and the micron variant itself has nine sub-variants (BA.1.1.529, BA.1.620, BA.2, BA.2.13, BA.2.75, BA.3, BA.4, XBB.1 and XBB.1.5)[23-29]. Delta developed severe sickness than other variants in those who were not immunized. Delta instigated more than twice as many infections as earlier variants and it is believed that it is 80% to 90% more transmissible than the Alpha variant[30,31]. Omicron sub-variants are thought to be predominantly active disease spreaders. The initial Omicron strain was more transmissible than the Delta variant partially due to the reason that more

than 30 of omicron's mutations occur on the virus's spike protein, which attaches to human cells and some of them are thought to boost the likelihood of infection[23,24]. Beta strain was approximately 50% more infectious than the initial coronavirus strain. There was evidence suggesting that Beta can, more likely than other variants, result in hospitalization and death[32]. Some mutations in Alpha strain spike protein were suggested to increase its infection. The B.1.1.7 lineage was thought to be 30-50% more contagious than the original SARS-CoV-2 strain[33].

The human angiotensin converting enzyme-2 (ACE2) protein has been identified as a key target for the SARS-CoV-2 S-protein, enabling the virus to adhere to host epithelial cells. Recently, the binding affinity of ACE2 for SARS-CoV-2, S-protein RBD was found to be similar or 10-20 times higher than that of SARS-CoV. Structural studies on SARS-CoV-2 revealed that RBD has a greater interaction with ACE2[34-37]. A unique phenylalanine (F486) in the flexible loop may play an important role since it can penetrate ACE2's lengthy hydrophobic pocket[38]. Analysis of 3-D structures and biophysical properties of protein-protein interactions revealed a large interaction surface with high binding affinity between SARS-CoV-2 and ACE2, which was at least 15-fold more intense than between SARS-CoV-1 and ACE2. These high-affinity interactions between the S-protein and the ACE2 receptor occur due to multiple interacting residues in the viral S-ACE2 protein-protein interface, which establish the strong (multi-epitope) adhesion synapse between the viral surface and the host's epithelial layer[39].

Vaccine efficacy is defined as the guard offered by immunization in a certain group. It provides both direct (vaccine-induced) and indirect (population-related) protection[40]. Antibodies, particularly neutralizing antibodies, are thought to have a role in providing protection against viral infections. These antibodies inhibit viruses from entering the host cell. The fundamental objective of vaccination has been to generate sufficient neutralizing antibodies. Variable vaccinations elicit varying amounts of serum antibodies, which act as substitutes for protective immunity[41]. Researchers have made significant efforts to develop more effective and safer vaccines and drugs against COVID-19, such as virus-like particle vaccines, mRNA vaccines, subunit and adenovirus-based vector vaccines, neutralizing antibodies and inactive as well as live-attenuated vaccines. The conventional neutralizing approach involves blocking the receptor-binding site between the RBD and ACE2. The substantial similarity of amino acids in RBD between the two viruses can improve their capacity to neutralize antibodies[42]. However, vaccines might fail to work effectively for all variants due to alterations and structural deviations in RBD at the molecular level. In this study we performed an *in silico* evaluation of structural deviations in the RBDs of different strains and their effect on the antibody-epitope interactions as well as RBD-ACE2 interactions with the goal to predict the efficacy of vaccines against the existing strains of SARS CoV-2 (Figure 1).

Figure 1. Summary of the current study. Homology models of spike protein trimers in closed, open and semi-open conformations were built for the chosen variants of SARS CoV-2 and were superposed onto the wild type spike protein trimer. The RMSD values of each variant were used to calculate and analyze the structural deviations to understand their effects on the epitope-antibody binding and RBD-ACE2 receptor binding. The observations were plotted as a clover leaf plot to predict the vaccine efficacy.



Methods

HOMOLOGY MODEL BUILDING FOR VARIANTS: Three-dimensional structures for most of the SARS CoV-2 variant spike protein trimers are available in the protein data bank. However, some of these structures have breaks in the backbone of the protein. In order to avoid any variations caused by such backbone brakes, homology models were built for all the variants including the wild type spike protein trimers to establish uniformity in the structural analysis. For each variant, the amino acid sequence containing the strain-specific mutations was used for building the homology models. Model building was performed using the SWISS MODEL server (https://swissmodel.expasy.org/) as described previously[43]. Briefly, the amino acid sequence of each variant was copied to the SWISS MODEL server page and model building was performed on the server. Among the final models built by the server for each amino acid sequence, the one with highest sequence homology with the template was finalized. Ramachandran plots for the final models were analyzed for the stereochemical quality and displayed in Figures S1 and S2.

CALCULATION OF STRUCTURAL DEVIATIONS BY SUPERPOSITION: The homology models for each variant including the wild type spike protein trimers were built in three conformations, closed, semi-open and open. In each case, the like conformation of the variant was superposed onto the wild type spike protein trimer in order to calculate the root mean square deviations (RMSD) of the variant C_α atoms compared to the wild type C_α atoms. Structural superpositions were performed using the LSQKAB program[44] from the CCP4 (collaborative computational project number 4) suite of programs[45]. The RMSD values were taken as the structural deviations in the variant compared to the wild type spike protein trimer as described previously[46,47]. RMSD values below 2 Å were ignored and above 2 Å were considered as structural deviations.

EPITOPE MAPPING IN THE VARIANT SPIKE PROTEIN TRIMERS: We initially attempted to perform docking of antibody Fab fragment bound to the wild type spike protein trimer to each variant spike protein trimer homology model for epitope mapping. However, the docking was unsuccessful

due to structural deviations in the epitopes. Hence, epitope mapping was performed for all the variants by superposing the variant spike protein trimer homology model onto the wild type spike protein trimer bound to the Fab fragment of antibody taken from the protein data bank (PDB IDs: 8ELJ). The coordinates for wild type spike protein trimer were then removed so that the resultant model contains the variant spike protein trimer bound to the Fab fragment of antibody. Amino acid residues on the variant spike protein trimer that were at the variant and antibody interface were considered as the epitope for that variant. This process was repeated for all three conformations, close, semi-open and open, of the variants. We focused on these residues and their RMSD values compared to the wild type in order to understand the possibility of antibody Fab fragment binding to these skewed epitopes.

ACE2 RECEPTOR BINDING SITE MAPPING IN THE VARIANT SPIKE PROTEIN TIMERS: As mentioned above, the ACE2 receptor binding site mapping was performed by superposing the the variant spike protein trimer homology model onto the wild type spike protein trimer bound to the ACE2 receptor taken from Benton *et al.*[48] The coordinates for wild type spike protein trimer were then removed so that the resultant model contains the variant spike protein trimer bound to the ACE2 receptor. The interface residues on the variants were then analyzed to understand the possibility of ACE2 receptor binding to these skewed trimers.

THE CLOVER LEAF PLOT: Epitope mapping of antibody and ACE2 receptor are represented in a distinct hand-drawn plot titled clover leaf plot. We published similar hand-drawn circular plots previously[43]. Two clover leaf plots were generated, one covering the variants alpha, beta, delta, epsilon, kappa and mu and the second one covering the nine sub-variants of omicron. Each clover leaf plot has a stalk connecting correlations from the base displaying the total number of amino acids interacting with either antibody alone (specified in red color) or both antibody and ACE2 receptor (specified in cyan color) in bars and also three lobes indicating each conformation of the spike protein (closed, open and semi-open conformations). Each lobe consists of red and cyan colored bars indicating averages of RMSD values of epitopes and ACE2 receptors binding sites, respectively of the spike protein trimers. Each lobe of the clover leaf contains three rims. The innermost rim represents amino acid positions irrespective of the variant. The middle rim contains the amino acid (variable depending on the variant) that interacts with antibody alone or both antibody and ACE2 receptor. The outer rim gives a quick color-based reference whether a particular amino acid in the middle rim is interacting only with antibody (red color) or both antibody and ACE2 receptor (cyan color). The outer rim of the lobe representing closed conformation is an exception because the spike protein trimer does not interact with the ACE2 receptor in closed conformation. The lines emerging from the bars located at the base of the plot are in two colors, red and cyan representing interactions with antibody alone and with both antibody and ACE-2 receptors, respectively. These lines merge into two wide ribbons continuing the same color representation which follows throughout the plot. The wide ribbon represented in red color further splits into smaller ribbons based on the number of variants and same goes with cyan except for the closed conformation. Correlation lines were drawn from the bars located at the base of each lobe to the amino acid residues in the inner rim of each lobe and the number of lines depends on the number of variants interacting with the specific amino acid residue.

MOLECULAR DYNAMICS SIMULATIONS: Molecular dynamics (MD) simulations were performed for the variant spike protein trimer homology models as described previously[49]. Briefly, the spike protein trimer homology model was pre processed using Maestro (Schrödinger, LLC, New York, NY) followed by the system builder using Desmond[50] (D. E. Shaw Research, New York, NY). System was built with an SPC water model and membrane. MD simulations were performed initially for 1 ns to

check the stability of the system by analyzing the trajectory. The protein C_α atom RMSDs and amino acid residue root mean square fluctuations (RMSF) were also analyzed.

Results

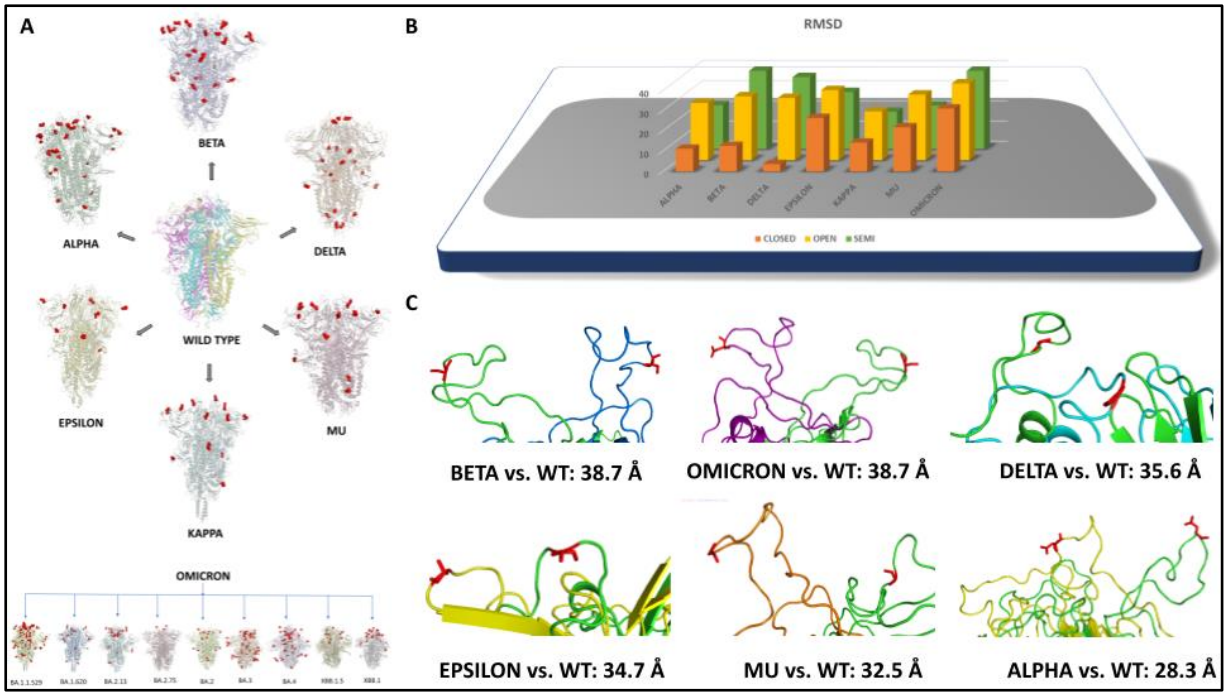
Table 1. List of SARS CoV-2 variants analyzed in this study along with the amino acid substitutions and deletions (Δ)[24, 51-56].

Variant	Reported substitutions and deletions (Δ)
Alpha	N501Y, A570D, D614G, P681H, T716I, S982A, D1118H ΔH69-V70, ΔY144
Beta	L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G, A701V ΔLAL242-244
Delta	E484Q, L452R, T19R(V70F), T95I, G142D, R158G, L452R, T478K, D614G, P681R, D950N, E484Q, L452R
Epsilon	L452R, D614G, W152C.
Kappa	E154K, L452R, E484Q, P681R.
Mu	P681H, YY144-145TSN, E484K, R346K.
Omicron: BA.1.1.529	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, A67V, T95I, G142D, L212I, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F Δ69-70, Δ143-145, Δ211
Omicron: BA.1.620	E484K, S477N, H245Y, R246I, P681H, V126A, P26S, T1027I, D1118H. ΔHV69/70, ΔY144, ΔLLA241/243
Omicron: BA.2	K417N, T478K, N501Y, D614G, H655Y, P681H.
Omicron: BA.2.13	L452Q, S371F, S373P, T376A, T478K, E484K, D614G, P681R.
Omicron: BA.2.75	G446S
Omicron: BA.3	V505H, N786K, T95I, N211I, N856K, V213R, A67V, G142D, Q954H, N969K, D614G, H655Y, N679K, P681H, N764K, D796Y, G339D, S373P, S375F, D405N.
Omicron: BA.4	L452R, F486V, T19I, Q954H, N969K, D614G, H655Y, N679K, P681H, N764K, D796Y, G339D, S371F, S373P, S375F, T376A ΔP26, ΔH69, ΔV70
Omicron: XBB.1	V83A, Q183E, R346T, L368I, V445P, F486S, S486P, G252V, F490S, G446S, N460K. ΔY144
Omicron: XBB.1.5	S486P, R346T, N460K.

THE HOMOLOGY MODELS OF VARIANTS: Homology models of all the variant spike protein trimers, including the wild type, in this study listed in Table 1 were built and analyzed using the SWISS MODEL server. As shown in Figure 2A, each variant model has multiple amino acid substitutions (red spheres) and deletions. The homology models for most of the omicron sub-variants were also built for a detailed evaluation in this study. The quality of each model trimer was assessed by the Ramachandran plots of each spike protein timer. As shown in Figures S1 and S2 the overall quality of all the homology models was reasonably acceptable because most of the amino acids (>95%) were within the standard contours of the plots with a few residues in the generously allowed regions. The outliers were <0.5% in the closed and semi-open models and <1.5% in the open conformation models. Hence these variant models were further considered with confidence for the structural analysis and C_α RMSD analysis compared to the wild type spike protein timer model. Variants gamma, iota, lambda, theta, zeta and eta were excluded from this study due to low model qualities.

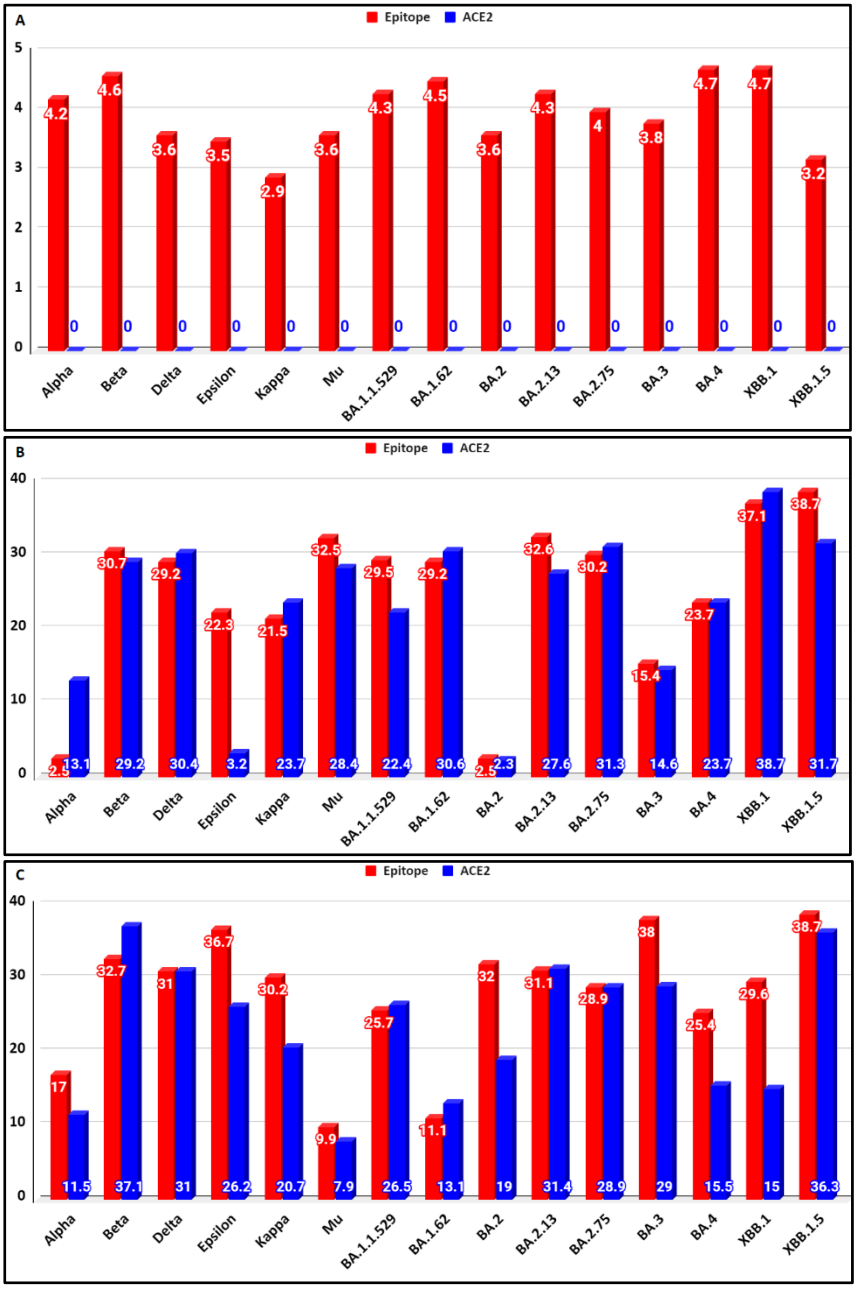
THE VARIANTS SHOW MAJOR STRUCTURAL DEVIATIONS COMPARED TO THE WILD TYPE: All the variant spike protein trimers in this study exhibited significantly large RMSD values in their C_α atoms compared to the C_α atoms of the wild type spike protein trimer. As shown in Figure 2B the open and semi-open conformations of the variants displayed more RMSD values compared to the closed conformation of the same. The omicron (parent strain) and the epsilon variants showed highest RMSD values in all three conformations while the alpha, beta and kappa variants showed low RMSDs in the closed conformations. Although the delta variant displayed the least RMSD values in the closed conformation, the corresponding semi-open and open conformations displayed highest RMSD values. As depicted in Figure 2C the highest RMSD values were seen ranging from 28.3 Å to 38.7 Å.

Figure 2. Homology models of SARS CoV-2 spike protein trimers and their structural deviations. A) The homology models of wild type and the variants along with mutations highlighted with red spheres. B) A 3-D histogram depicting the RMSD values of variants in all three conformations superposed onto the wild type. C) The highest RMSD sites of variant spike protein timers (variable colors) superposed onto the wild type (green color).



It is noteworthy that all of the variant spike protein timer models maintained an overall shape similar to the wild type without falling apart in spite of high RMSD values and hence they can be considered evolutionarily fit. Spike protein trimer being a membrane protein, the lipid bilayer may also contribute towards the stability of the overall shape and structure in the variants in spite of the mutation-induced major structural deviations. Most of the major structural deviations were mapped to the RBD part of the spike protein trimers that is critical in interacting with either antibodies or the ACE2 receptors suggesting that the variants may have differential affinity to both the neutralizing antibodies generated from vaccination and the ACE2 receptors. Hence we further investigated the effect of these structural deviations on the epitopes in order to predict the vaccine efficacy against the variants evaluated in this study.

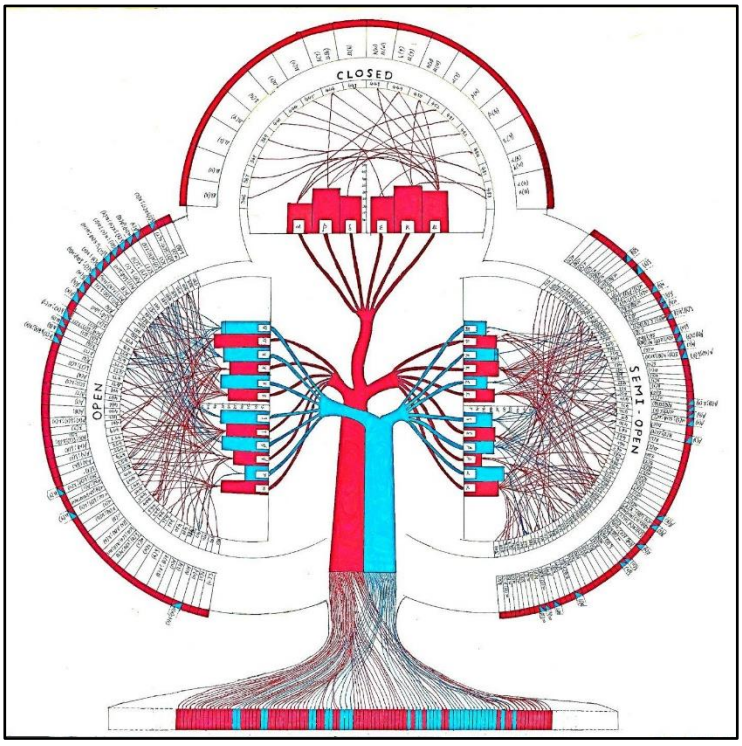
Figure 3. Strain-specific highest C_α RMSD values of epitopes (blue bars) and ACE2 binding sites (red bars) in variants. Panels A, B and C represent the closed, open and semi-open conformations, respectively. In all 3 panels the epitope RMSDs are seen commonly while the RMSDs of ACE2 binding site are seen only in the open and semi-open conformations. All 3 panels confirm significant structural deviations in epitope and ACE2 binding sites.



STRAIN-SPECIFIC STRUCTURAL DEVIATIONS IN THE EPITOPES AND ACE2 BINDING SITES OF VARIANTS: The strain-specific highest C_α RMSD values of the variants covering the epitope area and ACE2 binding sites are depicted in Figure 3 in red and blue colors, respectively for all three conformations. As displayed in Figure 3A the epitope RMSD values range from the lowest value of 2.9 Å (Kappa variant) to the highest value of 4.7 Å (Omicron variants, BA.4 and XBB.1) with a difference of 1.8 Å. Although the individual strain-specific epitope RMSD values were higher than 2 Å each, the difference between the highest and lowest epitope RMSD values was observed to be <2 Å suggesting that the strain-specific epitope C_α RMSD values of variants in this study were within 2 Å in their closed conformation. However, significantly high RMSD values were seen in the open and semi-open conformations of the same variants (Figures 3B and 3C). As illustrated in Figure 3B, with the exception of the alpha variant, all the variants in this study exhibited very high epitope RMSD values ranging from 15.4 Å (Omicron BA.3) to 38.7 Å (Omicron XBB.1.5). Similarly, the semi-open conformation of the variants exhibited very high epitope RMSD values ranging from 9.9 Å (Mu variant) to 38.7 Å (Omicron XBB.1.5).

The ACE2 binding sites on variants were mapped only to the open and semi-open conformations because the ACE2 does not bind to the spike protein trimers when they are in the closed conformations. Accordingly, the RMSD values in the closed conformations were taken as zeros (Figure 3A). However, as shown in Figure 3B the open conformation of the variants exhibited RMSD values ranging from 2.3 Å (Omicron BA.2) to 38.7 Å (Omicron XBB.1). Similarly Figure 3C shows the RMSD values of semi-open conformation ranging from 7.9 Å (Mu variant) to 37.1 Å (Beta variant). These results suggest that major structural deviations in the epitopes and ACE2 binding sites were seen in a strain-specific manner due to difference in the mutation/deletion profiles of the variants in this study (Table 1).

Figure 4. Hand-drawn clover leaf plot showing the correlations between mutation-induced RMSD with epitopes and ACE2 binding sites of variants. The correlation lines emerging from the RMSD bars connect to the corresponding amino acid position (innermost rim) of the variants (outer rim) in all three lobes either exclusively representing the epitope RMSDs (red color) or RMSDs in both epitopes and ACE2 binding sites together (cyan color).



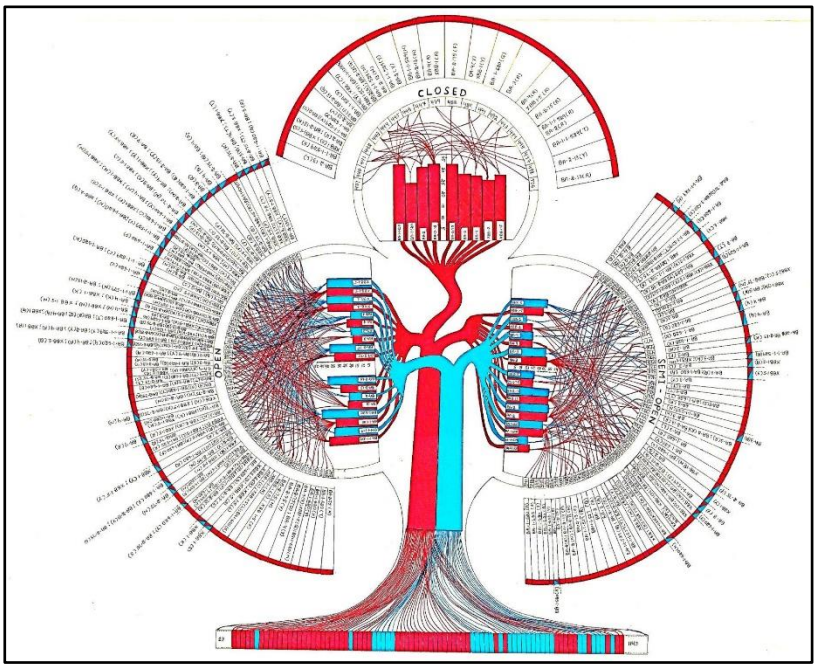
CLOVER LEAF PLOTS: The highest structural deviations in epitopes and ACE2 receptor binding sites were analyzed in detail and represented in Figure 3 for all three conformations of the variant spike protein trimers. However, we believe that a quick glance of individual RMSDs would give a more comprehensive knowledge. Hence correlation plots were hand-drawn in the shape of a clover leaf representing the three conformations as the three lobes of the plot. Figures 4 and 5 represent the clover leaf plots of variants (other than omicron variant) and exclusive omicron sub-variants, respectively. The primary goal of these clover leaf plots was to offer a quick reference to both the RMSD values and the mapping of epitopes and ACE2 binding sites on the strain-specific spike protein trimers of all the variants in this study.

CLOVER LEAF PLOT OF VARIANTS EXCLUDING THE OMICRON VARIANT: The clover leaf plot shown in Figure 4 displays the correlations of all the variants, other than the omicron variant, in this study. The base of this plot contains 63 amino acids that are represented in red color and are involved in the epitope site of the variant spike protein trimers. Thirty two amino acid residues represented in the cyan color are involved in both epitope site and the ACE2 receptor binding site of the variant spike protein trimers. Accordingly, 63 red correlation lines and 32 cyan correlation lines originated from the base merging into two major ribbons that lead into the center of the plot as the stalk of the clover leaf. These ribbons were further branched into the three lobes of the plot with 6 branches entering each lobe for the 6 variants (alpha, beta, delta, epsilon, kappa and mu). The cyan color ribbon does not branch into the upper lobe because ACE2 does not bind to the closed conformation of spike protein timer. The top lobe (closed conformation) of the clover leaf plot shown in Figure 4 the red color ribbon divides into 6 branches each connecting to the bottom of the bars of the RMSD histogram. Each individual branch connects to a single bar in the histogram representing the average C_α RMSD values (y-axis, Å) of amino acid positions given on the innermost rim of the lobe that are mapped to the epitopes (red color) and the ACE2 binding sites (cyan color) of the variant spike protein trimers. The outer rim of each lobe contains the specific variant and the amino acid present at the corresponding position given on the innermost rim. Thus, each line originating from the top of the bars in the RMSD histograms branches out to the innermost rim identifying the strain-specific position and the amino acid of the variants.

In the top lobe representing closed conformation, the leftmost bar representing alpha variant's average epitope RMSD shows three red lines originating from the top of the same bar that spread out to connect with the innermost rim at positions 452, 487 and 488. The corresponding labels can be clearly seen in the outer rim confirming that the positions 452, 487 and 488 correspond to tyrosine, glutamic acid and glycine, respectively, in the alpha variant. Similarly in the left lobe (open conformation) both the red and cyan colored ribbons divide into 6 branches each reaching to the bottom of the histogram containing average RMSD bars for epitope (red) and ACE2 binding sites (cyan). From the top of each bar in this histogram, further correlation lines emerge connecting to the innermost rim of the left lobe. In the case of alpha variant, the red color bar from the histogram shows 7 lines emerging from the top of the bar connecting to the amino acid positions 441, 445, 447, 449, 450, 451 and 503 that contain serine, aspartic acid, lysine, glycine, glycine, asparagine and aspartic acid, respectively. While the positions 441, 445, 447 and 503 on the innermost rim are connected with the alpha variant only, the positions 449, 450 and 451 are shared by the lines originating from other variants as well. Position 449 on the innermost rim is shared by 3 red lines from alpha (glycine), delta (tyrosine), epsilon (tyrosine) and 1 cyan color line from mu (tyrosine). Position 450 is shared by 4 red lines originating from alpha (glycine), epsilon (asparagine), kappa

(asparagine) and mu (asparagine). Position 451 is shared by 2 red lines originating from alpha (asparagine) and beta (cysteine). The right lobe representing the semi-open conformation contains red and cyan color bars for the alpha variant in the average RMSD histogram. A total of 35 lines, 31 red lines and 4 cyan color lines originated from the top of the red bar and cyan bar, respectively, that branched out to different locations connecting to the innermost rim. Out of the 35 positions connecting the alpha variant, 13 positions were shared with other variants. As explained above for the alpha variant, a similar pattern can be expected for the remaining variants in all three conformations of the clover leaf plot.

Figure 5. Hand-drawn clover leaf plot showing the correlations between mutation-induced RMSD with epitopes and ACE2 binding sites of omicron variants. The base of this plot represents the amino acid residues that are correlated to either the epitope RMSDs (red color) or both epitope and ACE2 receptor binding sites (cyan color) in three3 lobes (open, close and semi-open). All correlation lines merge from the base as major ribbons that split into smaller ribbons entering into the three lobes.



CLOVER LEAF PLOT OF THE OMICRON SUB-VARIANTS: The base of the clover leaf plot for the omicron sub-variants shown in Figure 5 contains a total of 84 amino acid positions out of which, 55 are in red color representing the epitope mapping and 29 are cyan colored representing both epitope and ACE2 binding sites on the omicron sub-variant spike protein timer models. The 84 lines merge into red and cyan color ribbons and extend to the center of the plot where they branch out into the lobes connecting to the base of the average RMSD histograms in a strain-specific manner. The top lobe representing the closed conformation has only the red color branches because ACE2 cannot bind to the closed conformation of the spike protein trimers. In the left and right lobes that represent the open and semi-open conformations, respectively, of the omicron sub-variant spike protein trimers both red and cyan color branches are seen connecting to the histograms. Further, each sub-variant-specific bar from the histograms is connected to the innermost rim of the lobes via correlation lines that are either in red color or cyan color. Innermost rim positions can be shared among the omicron sub-variants in this clover leaf plot.

MOLECULAR DYNAMICS SIMULATIONS OF THE ALPHA VARIANT: In order to understand the structural deviations in detail, we made an attempt to perform MD simulations of the alpha variant spike protein trimer in the closed and open conformations. The model was inserted into a lipid

bilayer with physiological pH and salt concentration. However, a typical 1 ns MD simulation for alpha variant took almost >48 hours using 8 GPUs. Due to high computational demand and lack of access to high performance cluster computing facilities, we did not perform further simulations for other variants. As shown in Figure S3 the average RMSD over 1 ns was <3.2 Å for the closed conformation while the open conformation model exhibited almost 5 Å RMSD in the same time period. Similarly, the highest RMSF values of the closed and open models over 1 ns MD simulations were 4 Å and 5 Å, respectively. Although 1 ns MD simulation data is not enough to make any conclusions, this data correlates with our data analysis with static models shown in Figure 3. These MD simulations will be performed in future and hence they fall out of the scope of this study.

Discussion

In the current study we performed an extensive *in silico* evaluation of the substitution- and/or deletion-induced structural deviations in the SARS CoV-2 spike protein trimer models in a strain-specific manner to predict their effect on current vaccine efficacy. Our study focused on correlating the RMSDs in variants (alpha, beta, delta, epsilon, kappa, mu and omicron sub-variants) with their epitopes and ACE2 binding sites, with respect to the wild type, in order to predict the possibility of antibody binding in the skewed epitopes. Both the epitope and ACE2 mapping on the variants were performed using the superposition method instead of docking because docking results did not yield reasonably explainable models due to high RMSDs in the epitopes and the ACE2 binding sites of the variants compared to the wild type. Although we made an attempt to perform MD simulations using the alpha variant (Figure S3), we couldn't extend the MD simulations for reasonable time periods (25 ns to 50 ns) to obtain conclusive results for the variants due to high computational demand for such simulations.

It has been previously shown that properly stored spike protein undergoes unfolding with diminishing immunogenicity resulting in complete loss of antigenic properties[57]. Along these lines, we predict that amino acid substitution- and/or deletion-induced structural changes in the spike protein trimers of the variants may also result in decreased or diminished immunogenicity due to skewed epitopes resulting in the loss of binding affinity for the neutralizing antibodies that are produced in response to the current vaccines. As seen in Figures 3, 4 and 5 the C_α RMSD values of the open and semi-open conformations of the variants in this study range from 15 Å to 40 Å. Such structural deviations are detrimental to the epitope area and for antibody binding[46]. In such cases one can certainly predict a total loss of antibody binding resulting in the failure of the vaccination. However, if such variants are able to bind the ACE2 receptor in spite of large structural deviations, then they become evolutionarily fit as evidently seen during the COVID-19 pandemic.

Based on our *in silico* analysis in this study, we predict a significant loss of antibody binding affinity to the skewed epitopes for all variants (except alpha) and all the sub-variants of omicron (except BA.2). The exceptions for alpha and omicron BA.2 were given because of their low epitope RMSD values in the open conformation (Figure 3B). However, we considered all the RMSD values that are >2 Å as real structural deviations. Hence, the loss of antibody binding affinity to the alpha variant and the omicron BA.2 sub-variant could still happen but it may not be as significant compared to the other variants in this study. We believe that a single coronaviral particle may not necessarily have only one type of the spike protein distributed uniformly on the viral surface. If a host cell was infected by two or more different variants of the coronavirus then the chances for the existence of multiple variant spike proteins and their incorporation into the new virions during the budding process are high. In such cases, a viral particle with differential variant spike protein trimers may have different binding affinity values

towards the neutralizing antibodies. For example, if a single viral particle is decorated with spike protein trimers in open conformation with alpha (epitope RMSD: 2.5 Å) and omicron-XBB.1.5 (epitope RMSD: 38.7 Å) then the chances for the neutralizing antibody to bind that trimer are low due to the high RMSD in the omicron-XBB.1.5. Hence the efficacy of vaccines may fail towards the hybrid even though they may have good efficacy towards the pure alpha variant.

Different approaches for passive immunization were evaluated for COVID-19 such as the convalescent plasma therapy. However, usage of a strategy that can produce nanobodies for passive immunization has not been implemented. It has been proposed previously that nanobodies have better access to the conserved epitopes, in spite of structural deviations, compared to the normal human antibodies suggesting that nanobody-therapeutics may have better neutralizing efficacies than the normal human antibodies[58]. Especially when encountering with variants that exhibit high structural deviations, even though if the natural epitope is skewed, a nanobody might identify other epitopes and gain access to them. Manmade monoclonal antibodies were shown to retain their efficacy towards variants such as the omicron even when the normal human antibodies fail to neutralize such variants[59]. Our laboratory is currently in the process of evaluating nanoparticles with immobilized nanobodies and/or monoclonal antibodies that target the SARS CoV-2 spike protein as an alternative strategy to the convalescent plasma therapy to treat COVID-19.

Recently it has been shown that the SARS CoV-2 variants of concern (VOCs) have originated from different ancestral origins but evolved under the selection pressure of mass vaccination and immunocompromized patients harboring certain VOCs[60]. In this context, López-Cortés *et al.* have mentioned that the vicinity of specific glycosylation in the RBD of the omicron variant has considerable RMSDs compared to the other variants and that such changes are possible within immunocompromized patients that were unable to clear the viral load in spite of vaccination and/or treatments. It is well known that protein glycosylation plays a major role in immune regulatory networks[61] and any changes in such glycosylation sites can and will have consequences on the efficacy of current COVID-19 vaccines. Along these lines, novel approaches such as the *in silico* design and efficacy prediction of multi-epitope vaccines candidates for COVID-19 have been reported earlier[62]. The multi-epitope vaccines may have some advantages over the conventional vaccines because the broadly neutralizing antibodies can target at least one of the several epitopes in spite of structural deviations in the RBD. However, detailed animal studies are needed for such *in silico* approaches.

The strain-specific structural deviations in the RBD of the spike protein trimers can not only challenge the efficacy of the current COVID-19 vaccines but also can significantly affect the binding affinities of new experimental small molecules that bank on the conserved binding pockets of the RBD[63]. In our current study, most of the amino acids lining the conserved druggable binding pockets on the RBD showed >15 Å C_α RMSD which is significant to skew the drug binding pocket. These results suggest that small molecule drug design is probably better suited for other targets of the SARS CoV-2 such as the RNA-dependent RNA Polymerase and the viral protease that are less prone to the mutations compared to the spike protein/RBD. Evidently from the evolutionary point of view, changes in the structural genes such as spike protein/RBD can be compensated more often resulting in variants, than changes in the critical genes such as RdRP and protease because impaired function of RdRP or protease is lethal to the viral replication.

Taken together, the future vaccine or passive immunization strategies for COVID-19 prevention/treatment should focus on the amino acid substitution- and/or deletion-induced major structural deviations in the SARS CoV-2 spike protein trimers. One can follow a combination therapy containing a combination of different strategies that has been

well established and successfully used for HIV/AIDS. Such combination therapy should potentially include nanobodies and monoclonal antibodies in addition to the traditional vaccines.

Conclusion

The current study clearly demonstrates that there are significant structural changes in the epitopes of the SARS CoV-2 variants that may challenge the antibody binding leading to the loss of antibody binding affinity to these altered epitopes resulting in either decreased/loss of efficacy of the current vaccines. Whether the vaccine is a whole virus attenuated type or nucleic acid-based, the structural changes in the epitopes may always challenge their efficacy. Therapeutic interventions such as nanobodies or monoclonal antibodies that can be passively administered can be considered as potential viable alternative options in case if the current vaccine efficacy is lost due to the mutation-induced structural deviations in the epitopes.

Conflict of Interest Statement:

The authors declare no conflict of interest in this study.

Funding Statement:

This study received no external funding.

Acknowledgements:

We thank the Yedidi Institute of Discovery and Education for collaborative research support.

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Supplementary Information

Figure S1. Ramachandran plots for variants alpha, beta, delta, epsilon, kappa and mu including the wild type spike protein trimer homology models in three different conformations (closed, open and semi-open). Apparently most of the amino acid residues fall within the contours falling within the Ramachandran allowed regions.

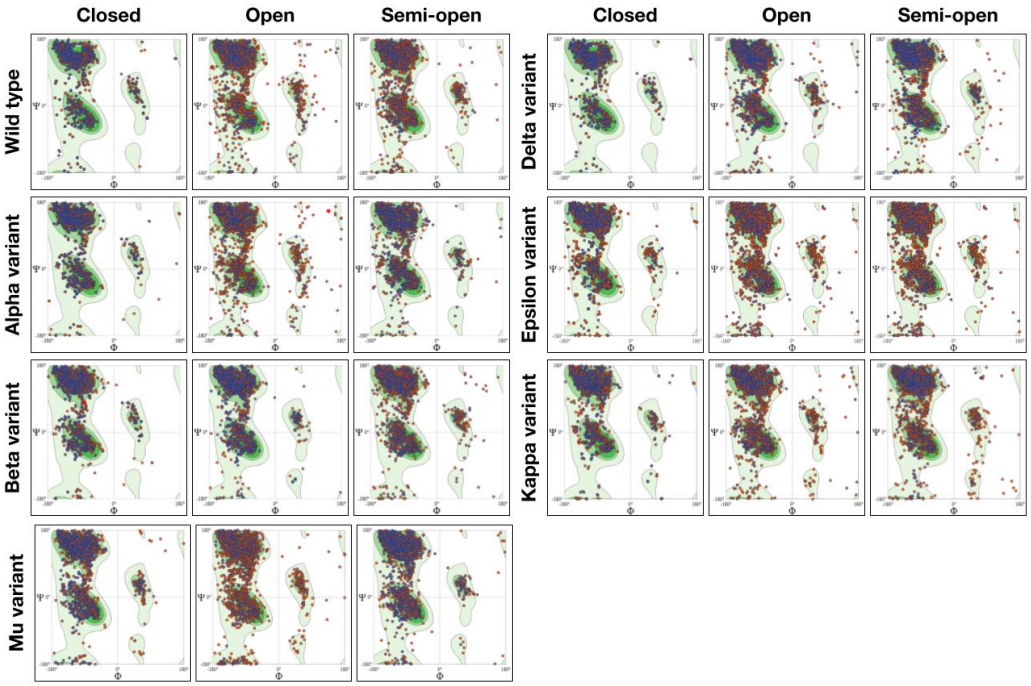

Figure S2. Ramachandran plots for omicron sub-variants including the wild type spike protein trimer homology models in three different conformations (closed, open and semi-open). Apparently most of the amino acid residues fall within the contours falling within the Ramachandran allowed regions.

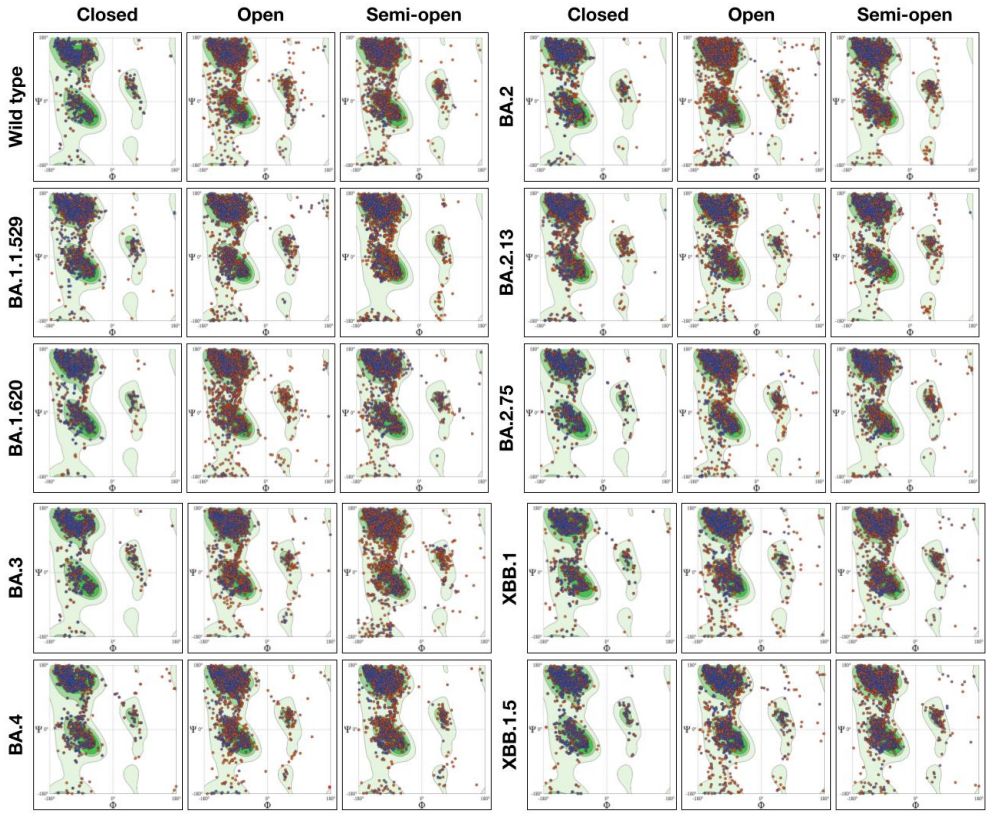

Figure S3. Molecular dynamics (MD) simulations of the alpha variant spike protein timer homology model in closed and open conformations. Both MD simulations were performed up to 1 ns. The top row shows RMSD variation in C_α (blue color), heavy atoms (light olive color) and sidechains (dark olive color) over 1 ns. The bottom row shows RMSF of amino acid positions taken on the x-axis in the alpha variant. Both RMSD and RMSF values of the open conformation are higher compared to the closed conformation.