Omicron, Delta, and Beta, Deadliest among SARS-CoV-2 Variants: An *In Silico* Analysis

By

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A dissertation submitted to the Department of Mathematics and Natural Science as a partial fulfillment of the criteria for the degree of Bachelor of Science in Microbiology

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Declaration

It is hereby declared that,

1. The thesis, "Omicron, Delta, and Beta, Deadliest among SARS-CoV-2 Variants: An *In Silico* Analysis," was completed as part of a degree program at BRAC University in collaboration with the "International Centre for Diarrhoeal Disease Research, Bangladesh."

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Abstract

SARS-CoV-2 has a higher mutation rate since it is emerging in Wuhan, China, and has a high propensity for mutation as it contains RNA as its genome. Because of its high transmissibility and new variants constantly mutating, global health and populations worldwide are under severe threat. There are many mutations that cause structural changes and their transmission power and risk severity, but the spike protein mutation was mainly responsible for the higher transmission and risk severity of SARS-CoV-2. From several geographic locations, including Africa, Asia, Europe, Oceania, and North and South America, fifty sequences of SARS-CoV-2 structural and nonstructural proteins (NSPs) from five variants were retrieved. BioEdit is used to perform multiple sequence alignments and protein homology modeling were performed using the Swiss model. Then, using Pymol, the proteins' 3D structures were seen, and their structural analysis was performed by superimposing them against the Wuhan sequence. Their RMSD values were also noted. Sequence alignment showed several common mutations and a few uncommon regional mutations in each of the five variants, but only the Beta, Delta, and Omicron variants had a few unique mutations. Structural analysis of such unique mutations revealed that they caused structural deviations in Beta, Delta, and Omicron spike proteins. Those findings provide insight into the functional and structural changes and its effects in SARS-CoV-2 spike protein mutations in Beta, Delta, and Omicron and a spike protein vulnerability that could be utilized to obtain comprehensive protection against those variants. Additionally, these variants had higher death rates, higher hospitalization rates, and more illnesses, all of which had a significant correlation with the structural deviations caused by those particular mutations. This study can help with regional vaccine strain selection, virus pathogenicity testing, diagnosis, and treatment.

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List of Acronyms

ACE2	Angiotensin-Converting Enzyme 2
NSPs	Non-Structural Proteins
PLpro (NSP3)	Papain Like-Protease
ORFs	Open-Reading Frames
IFN	Interferon
ISG-15	Interferon-Stimulating Gene-15
NCBI	National Center for Biotechnology Information
WHO	World Health Organization
VOC	Variations of Concern
RdRp (NSP12)	RNA-Dependent RNA polymerase
RNA	Ribonucleic Acid
RMSD	Root Means Square Deviation
RBD	Receptor-Binding Domain
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2

Amino acid Abbreviation List

Name	Reference symbol
Alanine	A
Cysteine	C
Aspartic acid	D
Glutamic acid	E
Phenylalanine	F
Glycine	G
Histidine	H
Isoleucine	I
Lysine	K
Leucine	L
Methionine	M
Asparagine	N
Hydroxyproline	O
Proline	P
Glutamine	Q
Arginine	R
Serine	S
Threonine	T
Pyroglutamatic	U
Valine	V
Tryptophan	W
Tyrosine	Y

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Chapter 01

Introduction

Introduction

SARS-CoV-2 or Severe Acute Respiratory Syndrome Coronavirus 2 has already reached its pandemic levels and continues to pose a risk to our way of life. The virus's 29.7 kb genome is contained within a single-stranded, positive-sense RNA (Marra et al., 2003). There are six open reading frames (ORFs) in the genomic RNA. The SARS-CoV-2 ORF1ab gene encodes a polypeptide that is divided into sixteen nonstructural proteins (Yoshimoto, 2021). Among them, it is crucial to include three non-structural proteins: RNA-dependent RNA polymerase (RdRp) or NSP12, Helicase or NSP13 and papain-like protease (PLpro) or NSP3 and as well as a structural protein such as the spike protein (Yoshimoto, 2021).

The primary structural protein of SARS-CoV-2, spike glycoprotein, is extremely conserved in all human coronaviruses. It plays a vital role in viral attachment, receptor recognition, and host cell entry (Khan, Hussain, et al., 2022). Due to its vital importance, it is one of the most important targets for the COVID-19 vaccine and treatment (Huang et al., 2020). The protein PLpro, which contains 1945 amino acid residues, has a wide range of functions. The papain-like domain breaks nonstructural protein peptide bonds and blocks host cells from producing type 1 interferon (IFN), which has antiviral properties (Barretto et al., 2005; Lei et al., 2018; Narayanan et al., 2015). The interferon-stimulating gene-15 (ISG-15) protein is cleaved by PLpro, preventing host cells from correctly responding to antiviral signals (Morales & Lenschow, 2013; Shin et al., 2020). RdRp contains 932 amino acids, is one kind of RNA polymerase that synthesizes viral RNA (Yoshimoto, 2021). For viral replication, RdRp is required. It serves as the main surface site where antiviral drugs bind in order to prevent or minimize viral replication via remdesivir binding (Chan et al., 2020). The enzyme helicase, which has 601 amino acids, also has RNA 5′-triphosphatase and NTPase activity (Ivanov et al., 2004; Shu et al., 2020; Yoshimoto, 2021).

All three of these nonstructural proteins are crucial for host cell genome replication and propagation, making them ideal candidates for antiviral therapeutic targets (Wu et al., 2020). Nevertheless, the development of SARS-CoV-2 strains that are more contagious and occasionally more severe than the Wuhan, China-origin strain has periodically shattered hopes for new vaccinations (Korber et al., 2020). World Health Organization (WHO) classified SARS-CoV-2 variants Alpha, Beta, Gamma, Delta, and Omicron as variations of concern (VOC). These played a key role.in boosting their virulence and transmissibility or reducing the efficacy of treatments and vaccinations. The worldometer estimates that SARS-CoV-2 is responsible for 6,549,724 deaths (till October 1, 2022). Numerous studies have been done to determine how severe the respiratory diseases are in their various variants, especially Beta, Delta, and Omicron. A higher hospitalization rate, illness severity, and death were shown to be associated with the Beta and Delta variations compared to the Alpha and Gamma variants (Mohammad et al., 2021). There are some unique mutations found on the genetic profiles of Beta and Delta that may result in their structural and functional abnormalities. However, in comparison with earlier variants, Delta has been transmitted globally and has become the sole VOC since around September 2021 (Perez-Gomez, 2021). In addition, end of November 2021, a new variant of concern has been emerged which named Omicron (Khan, Randhawa, et al., 2022). Omicron infections typically resulted in less illness than infections with earlier variants. In a comparative analysis of the disease severity between the Omicron and Delta variants in the USA, it was found that the cumulative number of deaths in the Omicron wave was very similar to that seen in the Delta wave. Similarly, it was shown that during the peak outbreak of Omicron variant in Australia, UK and US, daily hospitalization cases and daily ICU cases were about one-time greater than during the Delta variant's most severe outbreak (Duong et al., 2022). However, the

recent scenario update demonstrates that Omicron is not a less serious threat than Delta. These findings suggest that Beta, Delta and Omicron types resulted in more severity of illness, and the risk of death because of the structural changes on their spike protein. It is essential for developing proteomic-based COVID-19 control strategies by understanding the SARS-CoV-2 proteome (Khan, Hussain, et al., 2022). That's why, the investigation of structural variations is required to connect the genomic patterns of corona viruses to the phenotypic attributes in order to show the binding discrepancies and antibody response for variants of concerns (Khan, Waris, et al., 2022). Such data of structural analysis then can be used to determine binding affinity with receptor binding sites and design potential therapeutics against the new variants (Khan et al., 2021). This study attempted to report mutations in all five VOCs from different countries, describe them, and then assess if these structural variations in nonstructural and structural proteins significantly affected disease severity and increased transmissibility. Our study focused on the correlation between the structural and mutational changes of these five variations and the corresponding mortality rate.

Chapter 02

Materials and Methods

Materials and Methods

2.1 Sequence Collection

In our study, we chose three major nonstructural proteins (NSPs), Papain-like proteinase (NSP3), RdRp (NSP12), and Helicase (NSP13), and one structural protein, spike protein, from five different types of SARS-CoV-2 variants: Alpha, Beta, Gamma, Delta, and Omicron. We retrieved sequences from the NCBI SARS-CoV-2 database with a minimum and maximum sequence length of 25,000 and 35,000, respectively (*NCBI SARS-CoV-2 Resources*). Moreover, the human was chosen as the host, and the pangolin lineages B.1.1.7, B.1.351, P.1, B.1.617.2, and BA.1, BA.2, and BA.3 were given for Alpha, Beta, Gamma, Delta, and Omicron, respectively. We used GenBank as the sequence type. Additionally, a criterion for nucleotide completeness was chosen. At first, we picked and downloaded fifty sequences from the NCBI SARS-CoV-2 database of four proteins of Alpha, Beta, Delta, Gamma, and Omicron variants. In this case, two different locations were chosen. From the origin country variants, 50% of the sequences were downloaded, and depending on NCBI availability the rest were downloaded from different geographic locations such as Africa, Asia, Europe, Oceania, North America, and South America. Though we downloaded the whole genome sequence of SARS-CoV-2 from the NCBI database, then retrieved the NSP13 sequence because it was not directly found in the database. Likewise, reference sequences of SARS-CoV-2 and the Wuhan strain (YP_009724389 for NSPs and YP_009724390 for the spike protein) were also retrieved from the NCBI SARS-CoV-2 database.

2.2 Mutational Analysis

The Clustal Omega program by using BioEdit software was used to evaluate multiple sequence alignments of these three NSPs and spike proteins of five variations. In nearly every sequence,

we found a few mutations that were widespread. We called these mutations "common mutations." Intriguingly, we also found a small number of mutations that were categorized as "uncommon regional mutations". These types of mutations are only observed in particular places in our research. Moreover, the term "unusual regional mutations" was only used to describe mutations that were found in more than 10% of all sequences.

2.3 Protein Modeling and Quality Assessment

At first, homology modeling of PLpro, RdRp, Helicase, and spike proteins of all five variants: Alpha, Beta, Gamma, Delta, and Omicron (NCBI ID UFA39486.1, UAL50113.1, QXF22993.1, UHK30205.1, and UJU86736.1 respectively) was performed using the SWISS-MODEL database (Waterhouse et al., 2018). In addition, 6wuu, 5rl6 and 7krn were used as PLpro, Helicase and RdRp template proteins respectively for each variant and 7n1u, 7n1q, 7sbt, 7sbo and 7cn4 were used as template spike proteins for alpha, beta, gamma, delta and omicron respectively to determine protein structure. Swiss-Model QMEAN (Quality model energy analysis) quality assessment was used to determine the quality of the modelled protein structures using QMEAN Z-score (Benkert et al., 2009), GMQE (Global Model Quality Estimation) (Mora Lagares et al., 2020) and Ramachandran Plot (Alam, 2021).

2.4 Structural Analysis

In order to identify the structural changes where mutations occurred, wild RdRp, Helicase, and spike proteins of SARS-CoV-2 (NCBI ID: YP 009724390.1; Wuhan, China) were superimposed individually with the modeled proteins. Then, RMSD values provided by pymol during superimposing were recorded. Root-mean-square-deviation (RMSD) is used to compare the structural similarity of two proteins by their best-superimposed atomic coordinates. (Zhou et al.,

2006). When comparing two structures, a lower RMSD signifies a stronger similarity, whereas a greater RMSD shows a more structural difference. RMSD Value less than 2 Å is considered an insignificant or minor structural deviation (Alam, 2021). Superimposing the wild structure was not possible as PLpro sequences were retrieved from the whole genome sequence.

Chapter 03

Results

Results

3.1 Common Mutations Found Worldwide

Our main focus was on three nonstructural proteins (NSPs), e.g., PLpro, RdRp, and Helicase, and one structural protein, e.g., the spike protein, of the five variants of concern from different countries. When we analyzed all fifty sequences, we found that there were some mutations present in all variants. In the RdRp case, P323L mutation was found in all five variants of all regions, but G671S mutation was only found in the Delta variants. Likewise, T183I, A890D, and I1412T mutations were found in the PLpro for the Alpha variants; K837N for Beta variants; S370L and K977Q for Gamma variants; A488S, P822L/S, P1228L, and P1469S for Delta variants; and K38R, L1266I, and A1892T for Omicron variants. However, out of all the proteins involved, the structural protein spike protein has undergone the most mutational changes. Omicron has an insertion mutation called ins214EPE. Due to the addition of the insertion mutations, BioEdit places the omicron spike mutations three positions later in the sequence alignment figures than those stated **(Figures 11, 13, and 18).** Only one of them, the D16G mutation, was present in the spike protein of each of the five variants. **Table 1** provides a complete list of all the mutations present in these five variants.

Table 1: Common Mutations in SARS-CoV-2 Sequences

Variant	Protein	Mutation
Alpha	Plpro	T183I, A890D, I1412T
Beta		K837N
Gamma		S370L, K977Q
Delta		A488S, P822L/S, P1228L, P1469S
Omicron		K38R, L1266I, A1892T
Alpha	RdRp	P323L
Beta		P323L
Gamma		P323L
Delta		P323L, G671S
Omicron		P323L
Alpha	Spike Protein	H69S, Y144V, V143del, N501Y, A570D, D614G, P681H, T716I, S892A, D1118H
Beta		L18F, D80A, D215G, LLA240del, K417N, E484K, N501Y, D614G, A701V
Gamma		L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F
Delta		T19R, T95I, G142D, E156G, L452R, T478K, D614G, P681R, D950N
Omicron		H69V, V70I, T95I, G142D, N211I, ins214EPE, G339D, S371L, S373P, S375F, K417N, K440N, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

3.2 Regional Uncommon Mutations of the Nonstructural and Structural Proteins

Not all over the world, certain uncommon mutations were found only in the sequences of some specific geographical locations. We referred to these mutations as uncommon or unique regional mutations, and they are reported in **Table 2.**

Table 2: Uncommon Regional Mutations Found in SARS-CoV-2 Sequences

Region	Variant	Protein	Mutation
USA	Alpha	RdRp	P227L
USA		Helicase	K159R
Djibouti	Beta	RdRp	T293I
USA		PLpro	E37D
USA		PLpro	A416V
USA	Gamma	Spike Protein	A688V
USA		PLpro	T1303I
India, USA	Delta	Spike protein	T95I
India		PLpro	P822L
India		Helicase	K159R
India	Omicron	PLpro	T1004I

According to a mutational investigation, these geographical mutations were primarily present in USA-based sequences **(Figure 1**). For instance, sequences collected from several US state revealed that the RdRp protein had a P227L mutation, the spike protein had a K1191N mutation, and the helicase had a K159R mutation in the Alpha variants. In the case of the Beta variant, the

RdRp protein from Djibouti contained the T293I mutation, but the PLpro from the USA contained the E37D and V416A mutations. In the spike protein and PLpro sequences of the Gamma variants from the USA, A688V and T1303I mutations were found, respectively.

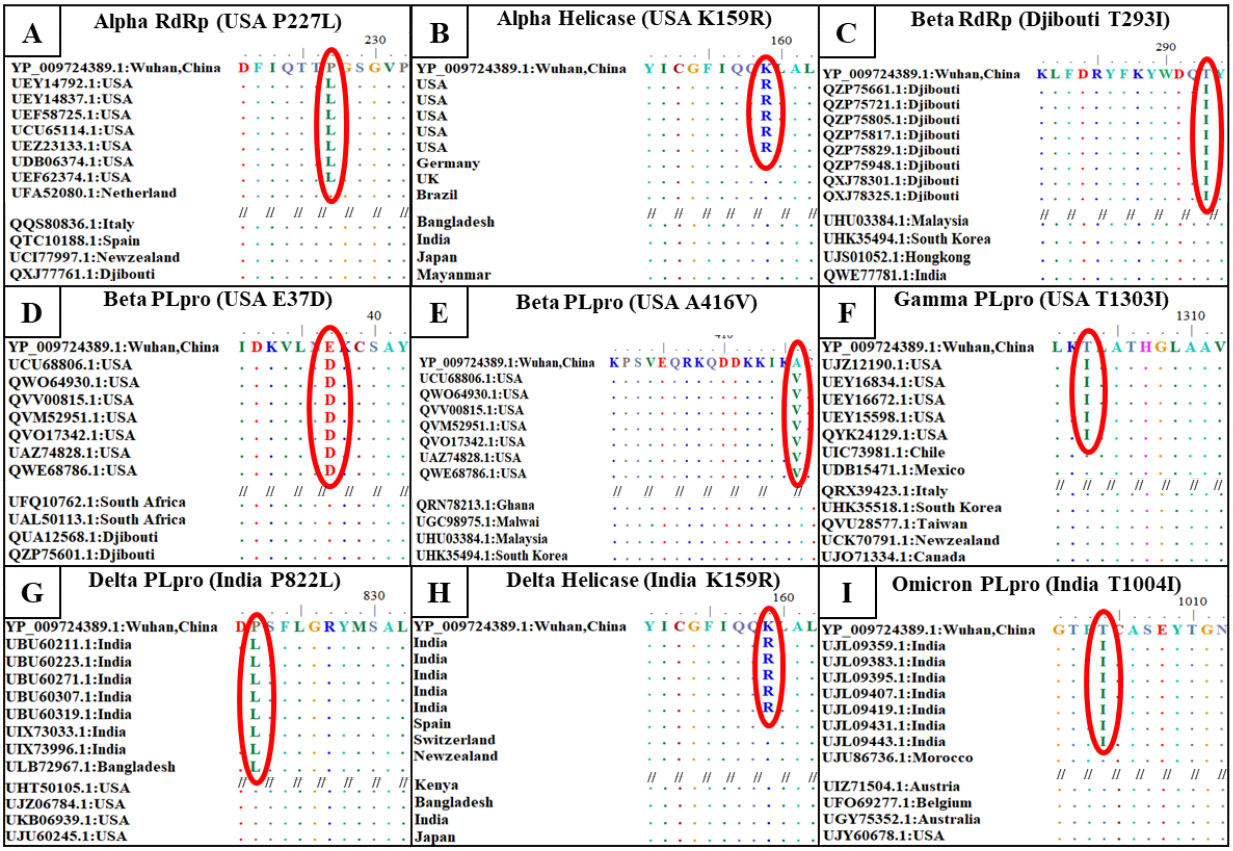

Figure 1. Uncommon regional mutational analysis of non-structural proteins (nsps). (A) P227L mutation in the RdRp protein of Alpha variant in the USA. (B) K159R mutation in the Helicase protein of Alpha variant in the USA. (C) T293I mutation in the RdRp protein of Beta variant in Djibouti. (D) E37D mutation in the PLpro protein of Beta variant in the USA. (E) A416V mutation in the PLpro protein of Beta variant in the USA. (F) T1303I mutation in the PLpro protein of Gamma variant in the USA. (G) P822L mutation in the PLpro protein of Delta variant in India. (G) P822L mutation in the PLpro protein of Delta variant in India. (H) K159R mutation in the Helicase protein of Delta variant in India. (I) T1004I mutation in the PLpro protein of the Omicron variant in India.

Spike protein of the Delta versions of the Indian and US sequences both had the T95I mutation. The Indian Delta sequences of Helicase and PLpro, respectively, contained P822L and K159R mutations. However, the T1004I mutation, which was found in the PLpro of the Indian sequences, is the only significant mutation in the Omicron variants.

3.3 Homology Modeling Quality Assessment

For quality assessment of homology models sequence identity, QMEAN Z-Score, GMQE values and Ramachandran Favoured Score for each variant spike protein are noted **(Table 3)**. Models were also visualized by Ramachandra plot **(Figure 2)**

Table 3: Homology Modeling Quality Assessment

Variant	Template	Sequence Identity	QMEAN Z-Score	GMQE	Ramachandran Favoured Score
Alpha	7n1u	100%	-1.77	0.72	92.63%
Beta	7n1q	100%	-1.67	0.73	93.24%
Gamma	7sbs	100%	-1.89	0.72	93.43%
Delta	7sbo	99.76%	-2.3	0.71	92.23%
Omicron	7cn4	94.93%	-2.24	0.7	93.00%

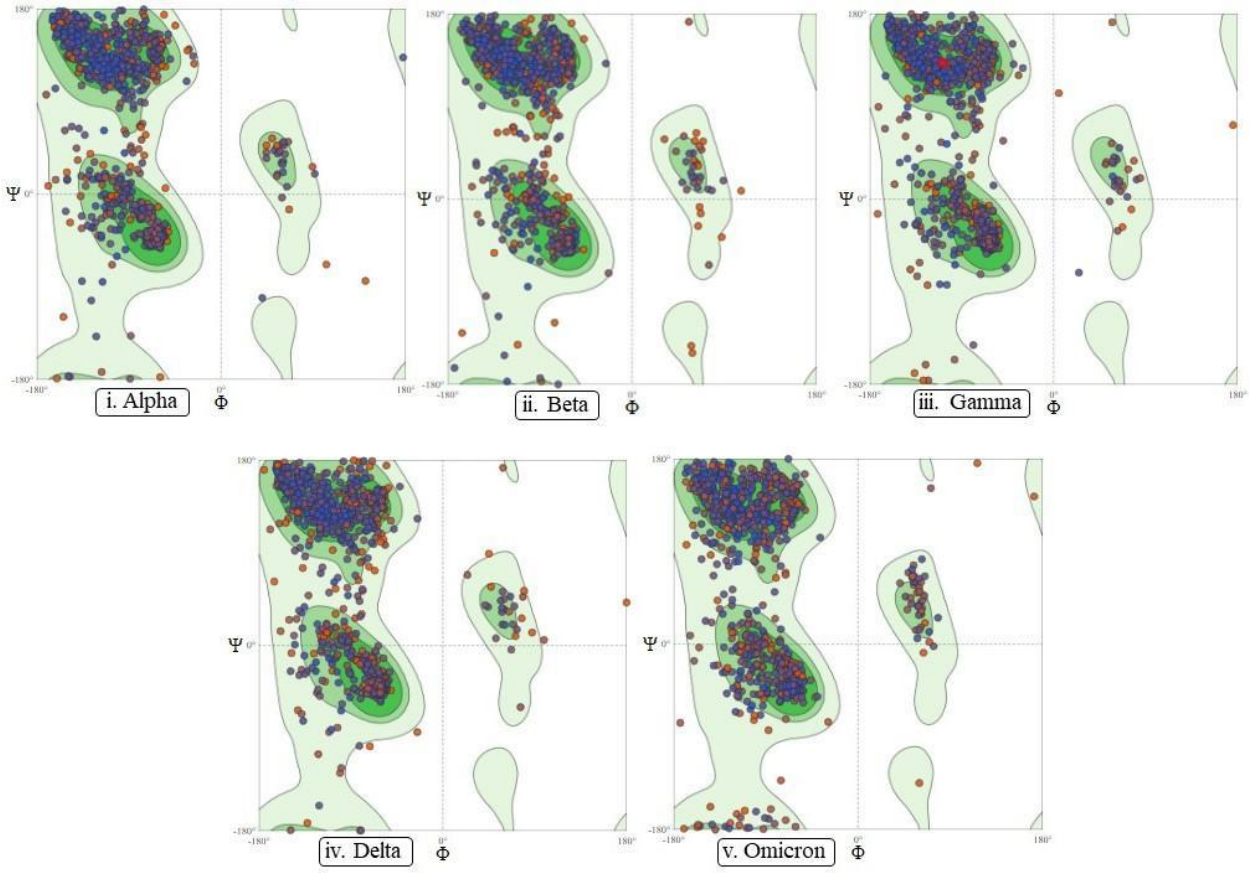

Figure 2: Spike protein models were also visualized through Ramachandran plot. Most of the amino acids are in the favored regions

3.4 Analysis of Structural Deviation by Measuring RMSD Value of RdRp and Helicase

By comparing the structural changes of proteins to reference structures, we were able to detect the structural changes that had occurred (Wuhan). In order to achieve this, we superimposed the RdRp and Helicase proteins on reference structures and calculated the root mean square deviation (RMSD) (NCBI ID: YP 009724390.1). The RMSD values for RdRp and Helicase across all variants were 1.09 and 0.85, respectively **(Table 4)**. However, an RMSD value lower than 2 Å is considered an insignificant or minor structural deviation. Therefore, the analysis of structural changes shows minor alterations.

Table 4: Impact of Uncommon Regional Mutations with Structural Deviations of Non-structural Proteins.

Nonstructural Protein	Superimpose		RMSD Value (Å)	Total Atom
RdRp	Alpha	Wuhan	1.094	7400
	Beta		1.094	7400
	Gamma		1.094	7400
	Delta		1.094	7400
	Omicron		1.094	7400
Helicase	Alpha	Wuhan	0.85	2323
	Beta		0.85	2323
	Gamma		0.85	2323
	Delta		0.85	2323
	Omicron		0.85	2323

3.5 Identification of Structural Deviation of Nonstructural and Structural Proteins

3.5.1 Analysis of Structural Deviation by Measuring RMSD Value of Spike Proteins

Spike proteins of variants were superimposed with reference proteins (NCBI ID: YP 009724390.1). The RMSD value for the spike protein of Delta variants was the highest (11.067). Likewise, RMSD values of Beta and Omicron were 10.055 and 10.083, respectively. On the contrary, the lowest RMSD values were recorded by Alpha (4.354) and Gamma (2.840). **(Table**

5). In addition, according to our findings, the spike protein of Delta variants showed higher structural deviations from Wuhan, followed by Beta and Omicron. Moreover, little structural variation is found in Alpha and Gamma variants.

Table 5: Impact of Common Mutations with Structural Deviation of Structural Proteins

Structural Protein	Superimpose		RMSD Value (Å)	Total Atom
Spike	Alpha	Wuhan	4.354	8413
	Beta		**10.055**	8525
	Gamma		2.84	8781
	Delta		**11.067**	8486
	Omicron		**10.083**	8507

3.5.2 Impact of Common Mutations and Uncommon Regional Mutations within the Structure of Spike Proteins

After comparing them to the reference structure, structural modifications were found in the Gamma (NCBI ID UEF38454.1) and Delta spike proteins (NCBI ID UBU60165.1) of the USA variants, which are caused by the A688V and T95I mutations. In the first instance, the removal of one methyl group caused changes at amino acid number 688 of the structure. In contrast, the hydroxyl group being removed and a few methyl groups being added in the second case resulted in a polar to nonpolar amino acid shift, which changed the structure at position 95 **(Figure 3)**. These could have an impact on receptor binding affinities. Additionally, structural alterations were found at position 95 of the Indian Delta spike protein in comparison to the reference structure **(Figure 3**).

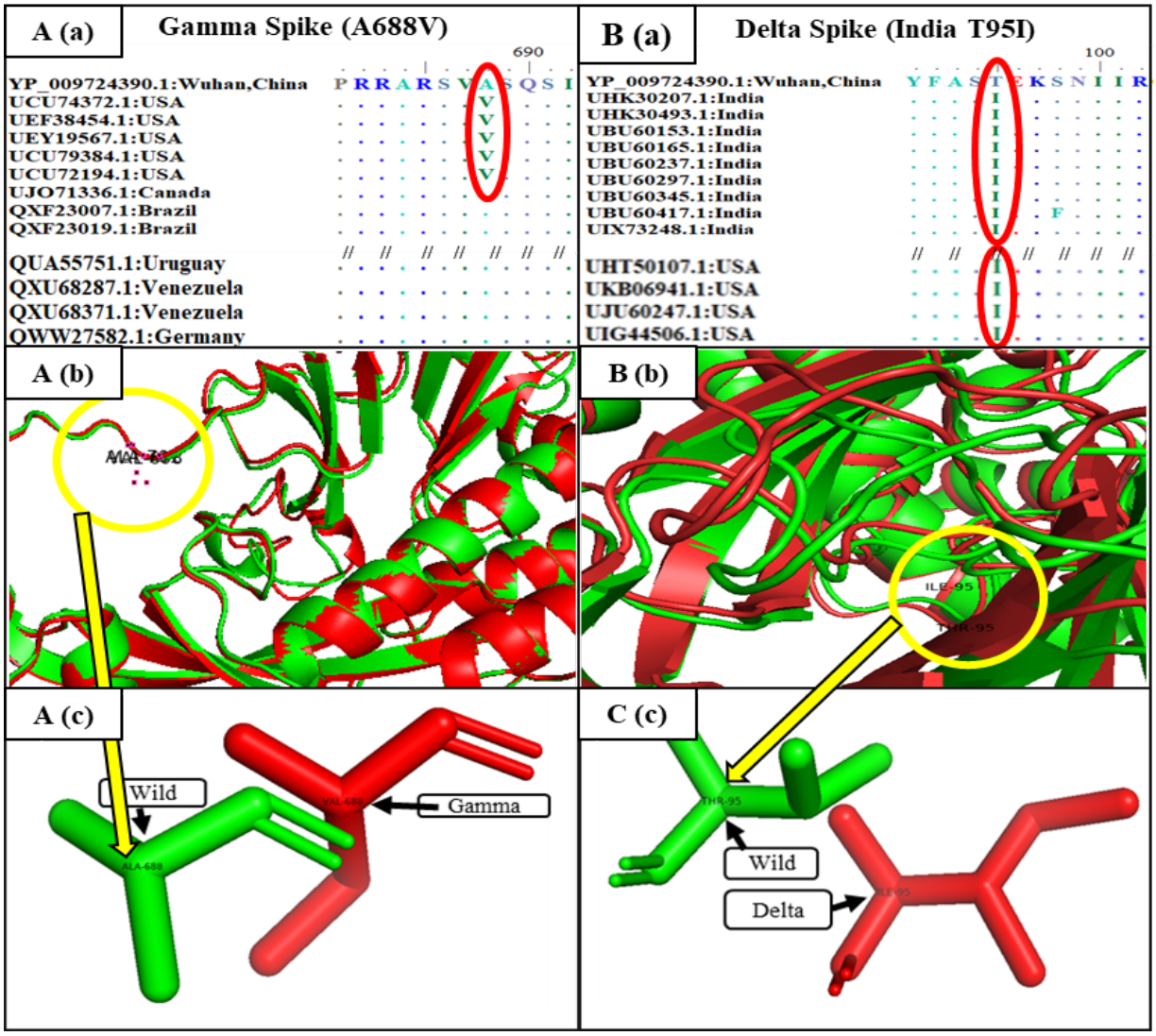

Figure 3. Uncommon regional mutational and structural analysis of structural proteins (spike proteins). A(a) A688V mutation in the USA's spike protein of Gamma variant. A(b) Structural deviations of A688V mutation in Gamma spike A688V, and A(c) Change in amino acid Alanine to Valine. B(a) T95I Mutation in the Delta spike protein in the USA. B(b) Structural deviations of Delta spike T95I, and B(c) Change in amino acid Threonine to Isoleucine.

3.5.3 Mutational and Structural Analysis of the Spike Proteins of Beta Variants

A mutational study of the spike protein Beta variants showed some novel mutations with structural changes **(Figures 4 and 5)**. In the spike protein of Beta variants, at position 80, we have found a structural alteration. Wild type of Beta variants that were found in Wuhan, the

negatively charged Aspartic acid was converted to the nonpolar amino acid Alanine **(Figure 4)**. Leucine-Leucine-Alanine found in the spike protein of Wuhan strain which was deleted in the spike protein of Beta variants.

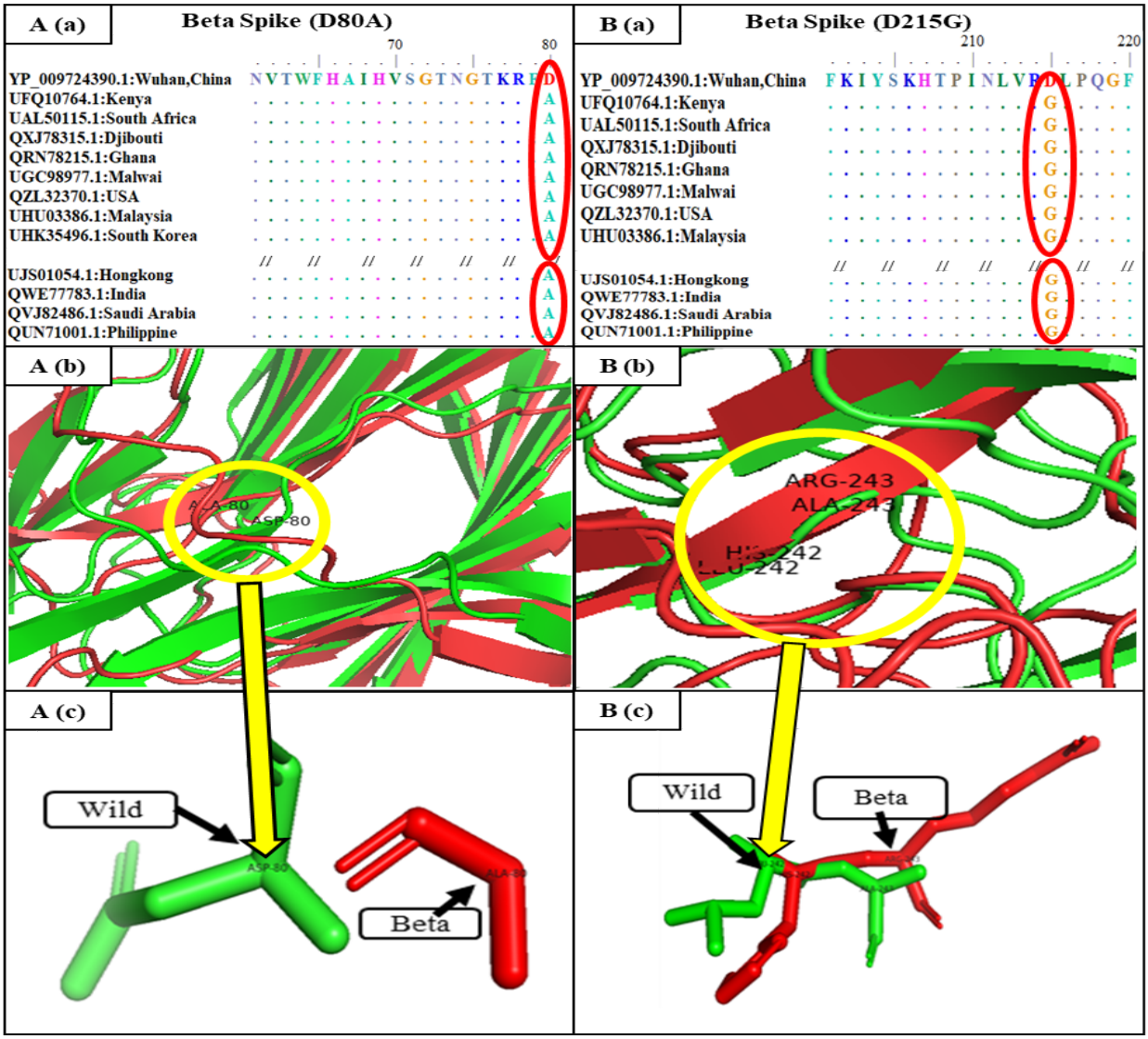

Figure 4. Mutational and Structural Analysis of the D80A and D215G mutations of the Spike Proteins of Beta Variant. A(a) D80A mutation in the spike protein of the Beta variant, A(b) Structural change in Beta spike D80A, and A(c) Amino acid alteration of Aspartic Acid to Alanine. B(a) D215G mutation in spike protein of Beta variant, B(b) Structural change in Beta spike LLA242del, and B(c) Amino acid alteration of Arginine to Alanine.

As a result of the deletion mutation, Leucine, which is a nonpolar amino acid, became Histidine (positively charged) at atom position 242, while Alanine, which is a nonpolar amino acid, became Arginine (positively charged). Due to the additional amine group inclusion, such modifications resulted in structural variations **(Figure 5)**. Besides that, a structural modification was also found at the position of the 215th atom **(Figure 4**). A carboxyl group was removed and hydrogen was added, resulting in a structural alteration when aspartic acid (which is negatively charged) was changed to glycine (which is non-polar). Moreover, atom position 701 also displayed structural changes. The Addition of methyl groups led to a minor modification in the structure of that atom where nonpolar amino acid Alanine replaced another nonpolar amino acid Valine, but this is neglectable **(Figure 5)**.

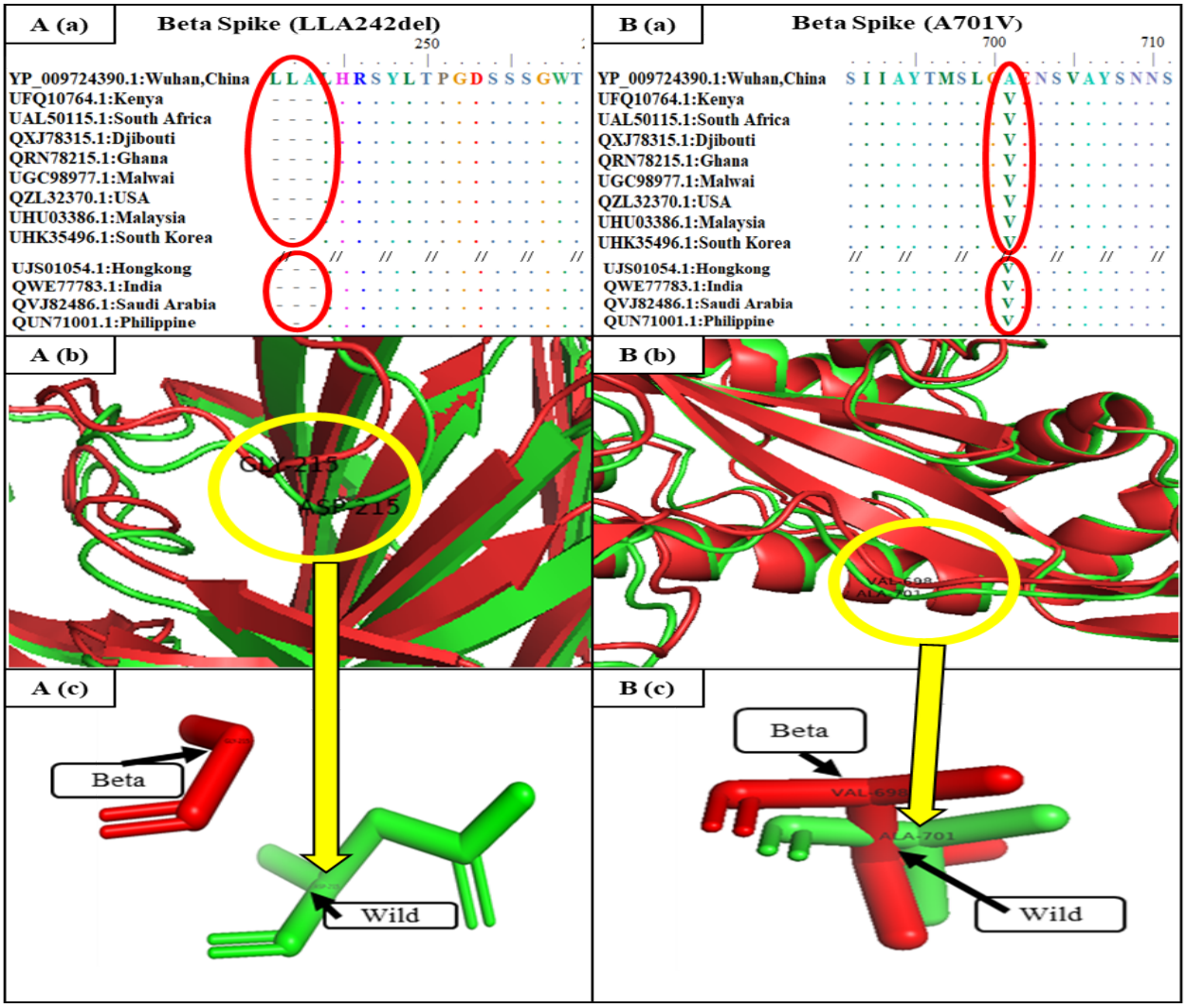

Figure 5. Mutational and Structural Analysis of the LLA242del and A701V mutations of the Spike Proteins of Beta Variant. A(a) LLA242del mutation in the spike protein of the Beta variant, A(b) Structural change in Beta spike LLA242del, and A(c) Amino acid alteration of Aspartic Acid to Glycine. B(a) A701V mutation in spike protein of Beta variant, B(b) Structural change in Beta spike A701V, and B(c) Amino acid alteration of Alanine to Valine.

3.5.4 Mutational Analysis and Structural Analysis of the Spike Proteins of Delta Variants

Similarly, we have also found some common mutations in the spike protein of the delta variants by mutational analysis. These are T19R, E156G, L452R, T478K, P681R, and D950N locations, respectively **(Figures 6, 7 and 8)**. The spike protein of Delta variants had undergone structural changes at position 19. The changes from Threonine, which is a polar amino acid, to positively charged Arginine resulted in a substantial structural mutation since the hydroxyl group was removed and amine groups were added **(Figure 6)**. In contrast, a carboxyl group was removed at position 156, which resulted in a conversion of Glutamic acid (which is negatively charged) to Glycine (which is nonpolar) and caused mutational changes to the structure of the spike protein of Delta variants **(Figure 6).** The addition of an amine group caused a mutation at position 452 of the atoms, where the amino acid Leucine (a nonpolar amino acid) was changed to Arginine (a positively charged amino acid), resulting in structural changes **(Figure 7**). But in position 478, Threonine, which is a polar amino acid, was converted to Lysine which is a positively charged amino acid. This mutation resulted in a structural alteration because the hydroxyl group was removed and amine groups were added **(Figure 7)**. Also, a structural alteration happened at position 681 where Proline, a nonpolar amino acid, changed to Arginine, a positively charged amino acid, due to the addition of amine groups **(Figure 8)**. Moreover, a mutation was also found in the spike protein of Delta variants at position 950 due to the removal of a carboxyl group and the addition of a methyl group **(Figure 8**). Here, Aspartic acid, which is a negatively charged amino acid, is converted to Valine which is a nonpolar type of amino acid.

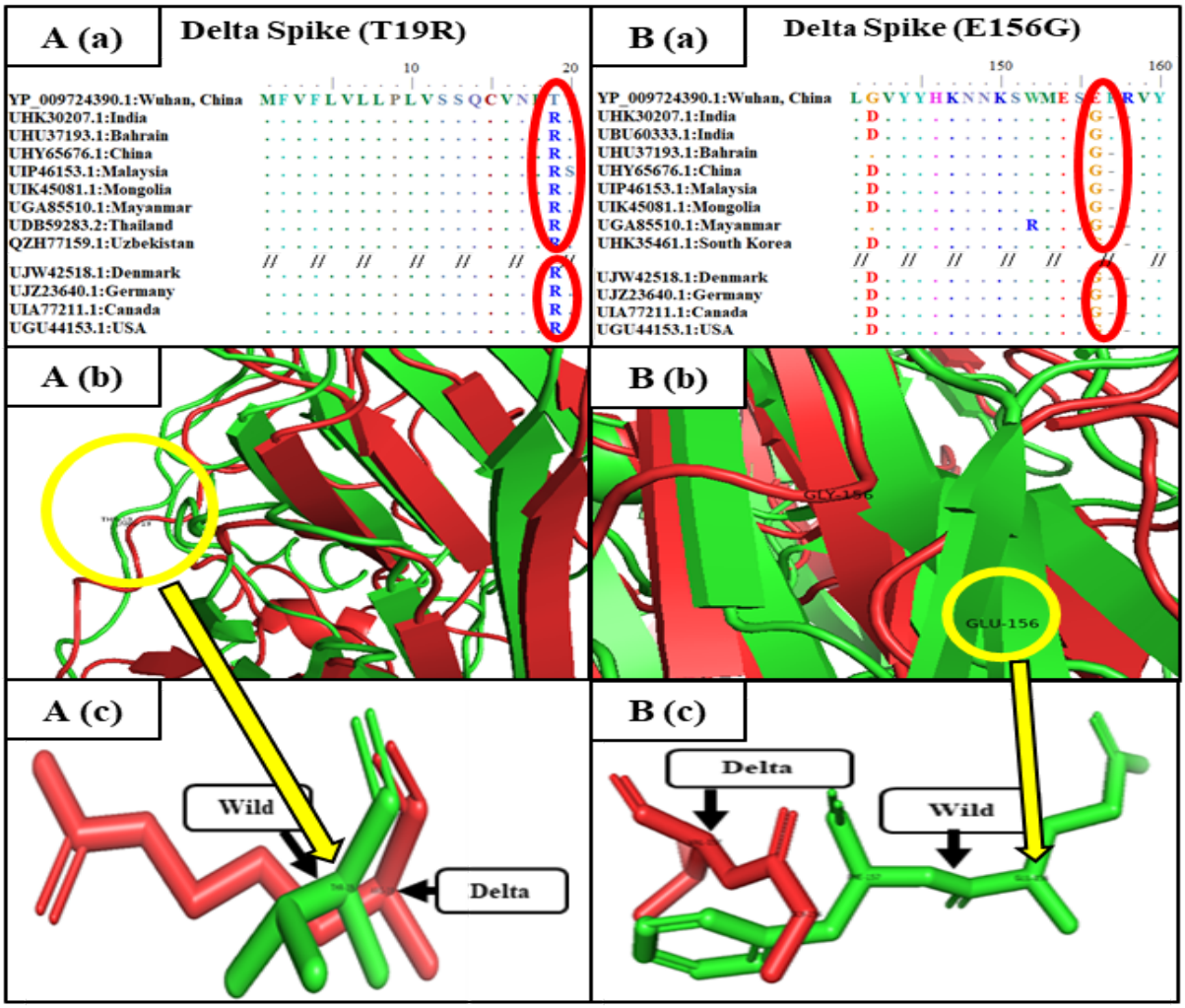

Figure 6. Mutational and Structural Analysis of the T19R and E156G Mutations of the Spike Proteins of Delta Variant. A(a) T19R mutation in the spike protein of the Delta variant, A(b) Structural change in Delta spike T19R, and A(c) Amino acid alteration of Threonine to Arginine. B(a) E156G mutation in spike protein of Delta variant, B(b) Structural change in Delta spike E156G, and B(c) Amino acid alteration of Glutamic acid to Glycine.

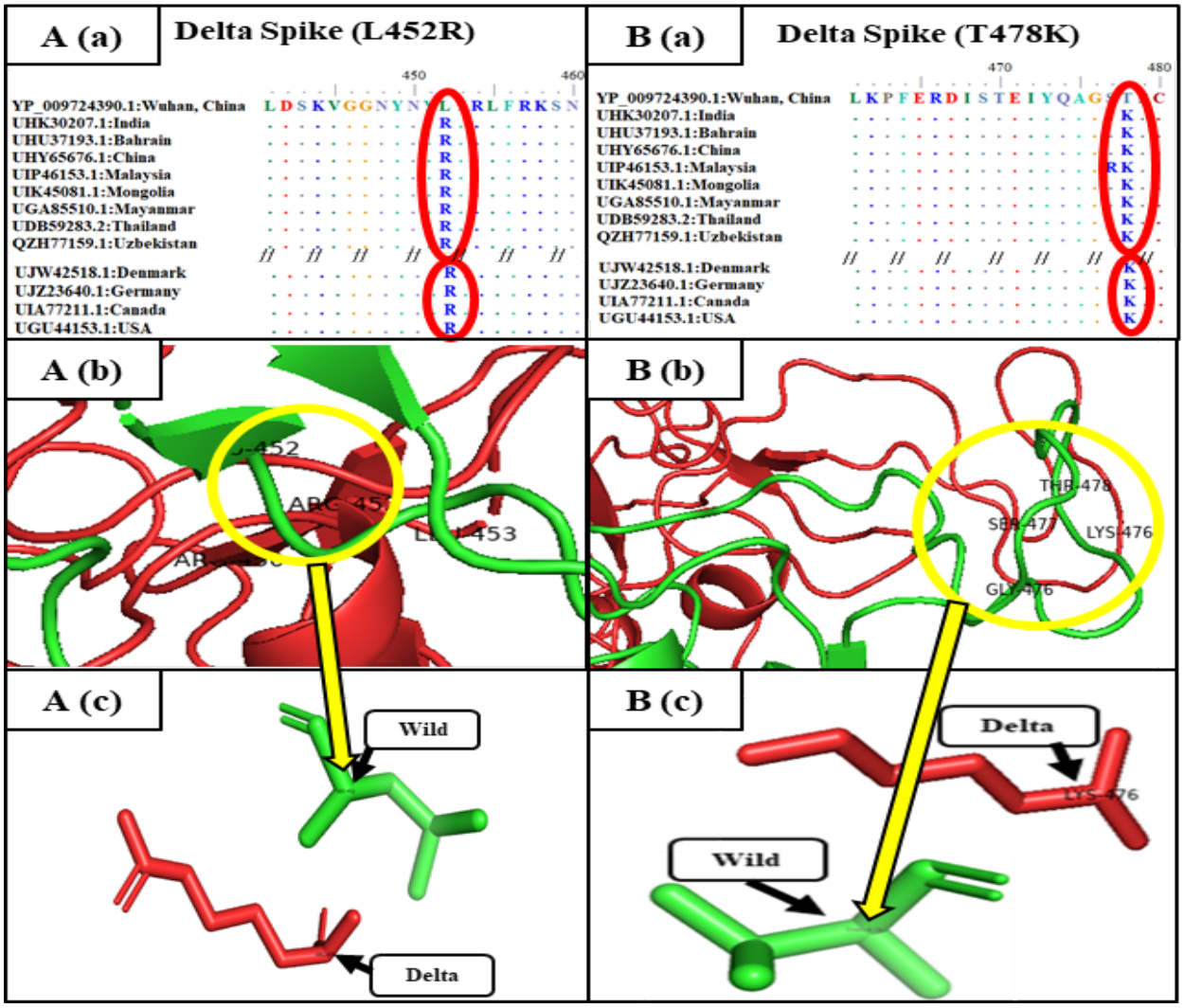

Figure 7. Mutational and Structural Analysis of the L452R and T478K Mutations of the Spike Proteins of Delta Variant. A(a) L452R mutation in the spike protein of the Delta variant, A(b) Structural change in Delta spike L452R, and A(c) Amino acid alteration of Leucine to Arginine. B(a) T478K mutation in spike protein of Delta variant, B(b) Structural change in Delta spike T478K, and B(c) Amino acid alteration of Threonine to Lysine.

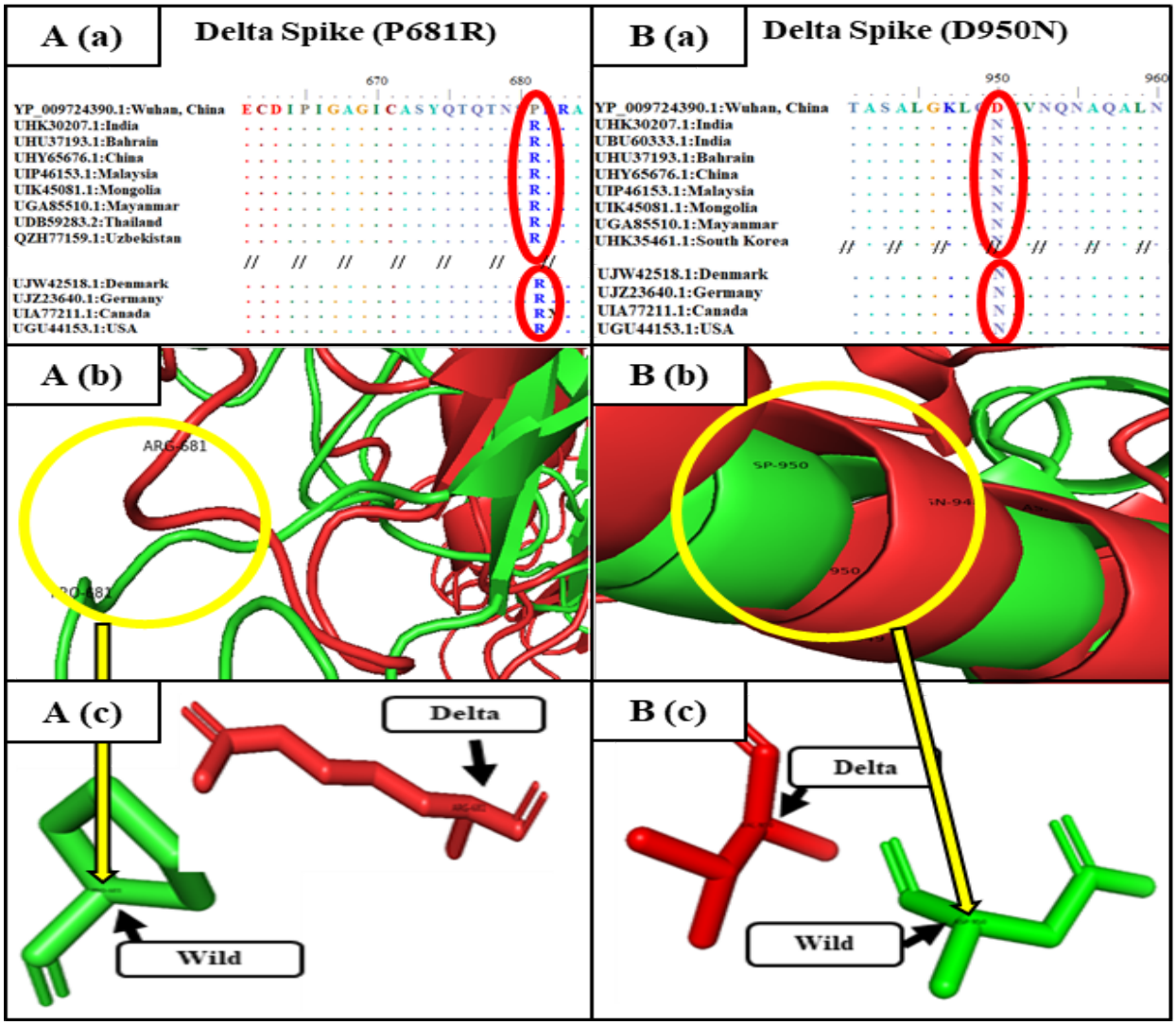

Figure 8. Mutational and Structural Analysis of the P681R and D950N Mutations of the Spike Proteins of Delta Variant. A(a) P681R mutation in the spike protein of the Delta variant, A(b) Structural change in Delta spike P681R, and A(c) Amino acid alteration of Proline to Arginine. B(a) D950N mutation in spike protein of Delta variant, B(b) Structural change in Delta spike D950N, and B(c) Amino acid alteration of Aspartic Acid to Asparagine.

3.5.5 Mutational Analysis and Structural Analysis of the Spike Proteins of Omicron Variants

In this study, a considerable number of shared mutations found in the spike protein of Omicron variants by mutational analysis. The frequent mutations identified in the Omicron spike were H69V, N211I, S371L, V70I, S373P, S375F, G339D, N440K, G446S, S477N, Q493R, G496S, Q498R, E484A, T547K, N679K, D796Y, N856K, N764K and N969K. At position 69, a noticeable mutation was found that is responsible for structural changes. Here, Valine substituted Histidine due to the removal of amine groups **(Figure 9).** Similarly, at position 211, structural modification was also found because amino acid changed from polar to nonpolar due to the removal of amine groups **(Figure 10).** Moreover, the addition of a carboxylic group caused a mutation in position 339 and structural modification occurred. Here, Glycine which is a nonpolar amino acid replaced by a negatively charged amino acid named Aspartic acid. **(Figure 11).** Furthermore, polar to nonpolar structural modifications were also found at positions 371, 373, and 375, Leucine, Proline, and Phenylalanine replaced Serine **(Figure 10, and 12).** In contrast, nonpolar to polar structural modifications were found at positions 446 and 496, where Glycine was replaced by Serine **(Figure 9).**

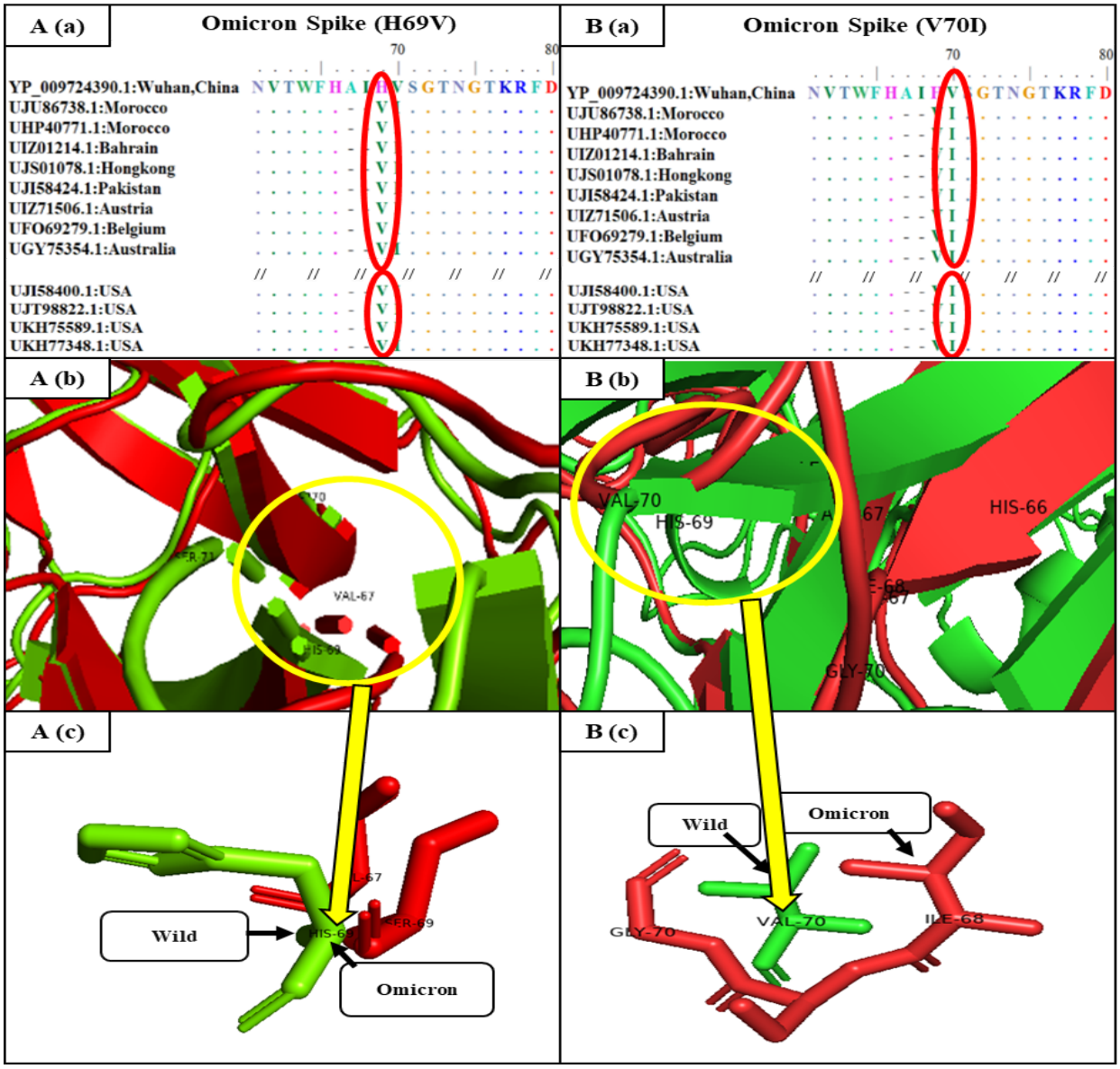

Figure 9. Mutational and Structural Analysis of the H69V and V70I Mutations of the Spike Proteins of Omicron Variant. A(a) H69V mutation in spike protein of Omicron variant, A (b) Structural Change in H69V mutation, A(c) Amino acid alteration of Histidine to Valine, B(a) V70I mutation in spike protein of Omicron variant, B(b) Structural Change in V70I mutation, B(c) Amino acid alteration of Valine to Isoleucine

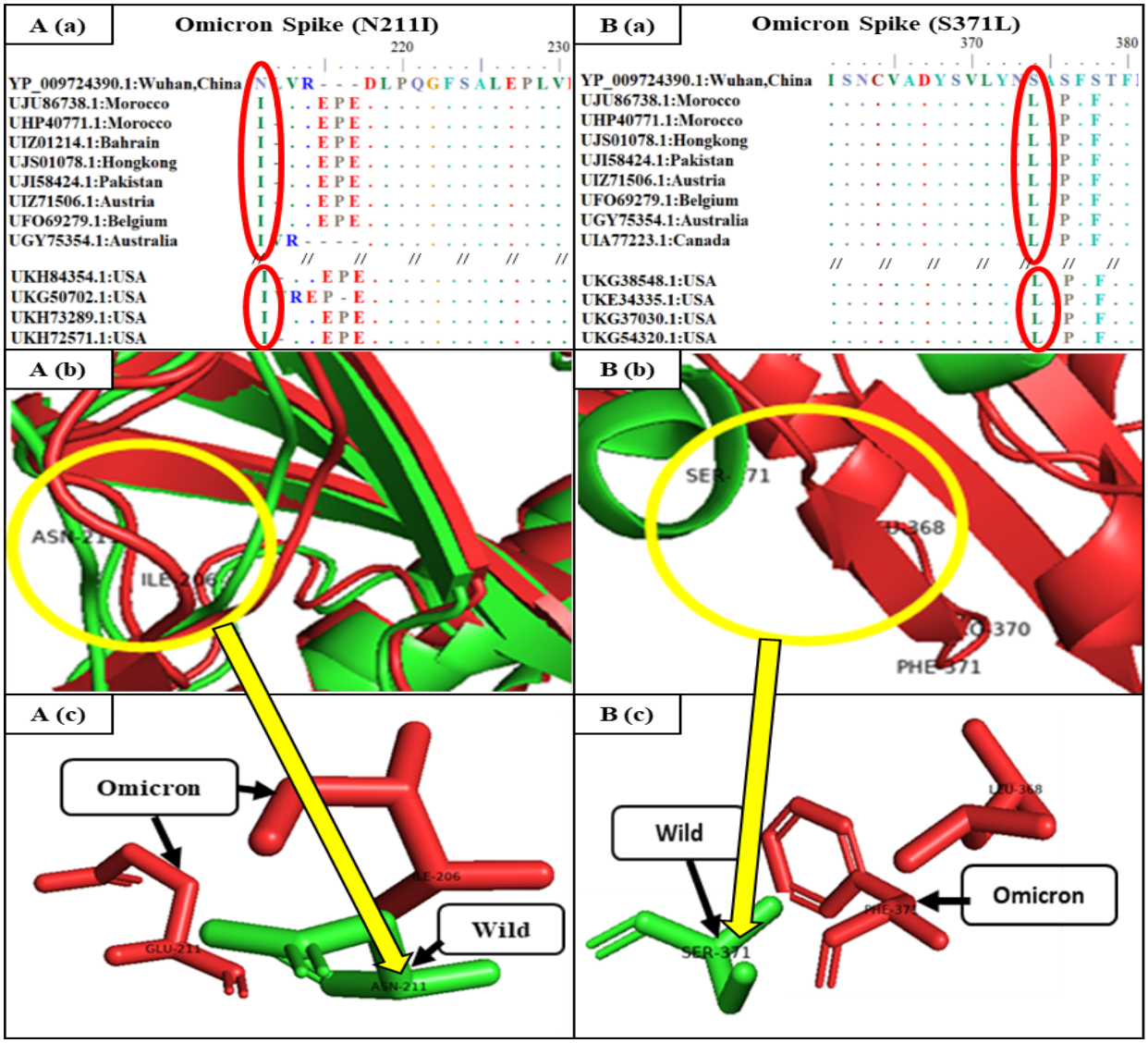

Figure 10. Mutational and Structural Analysis of the N211I and S371L Mutations of the Spike Proteins of Omicron Variant. A (a) N211I mutation in spike protein of Omicron variant, A (b) Structural Change in N211I mutation, A (c) Amino acid alteration of Asparagine to Isoleucine, B (a) S371L mutation in spike protein of Omicron variant, B(b) Structural Change in S371L mutation, B(c) Amino acid alteration of Serine to Leucine

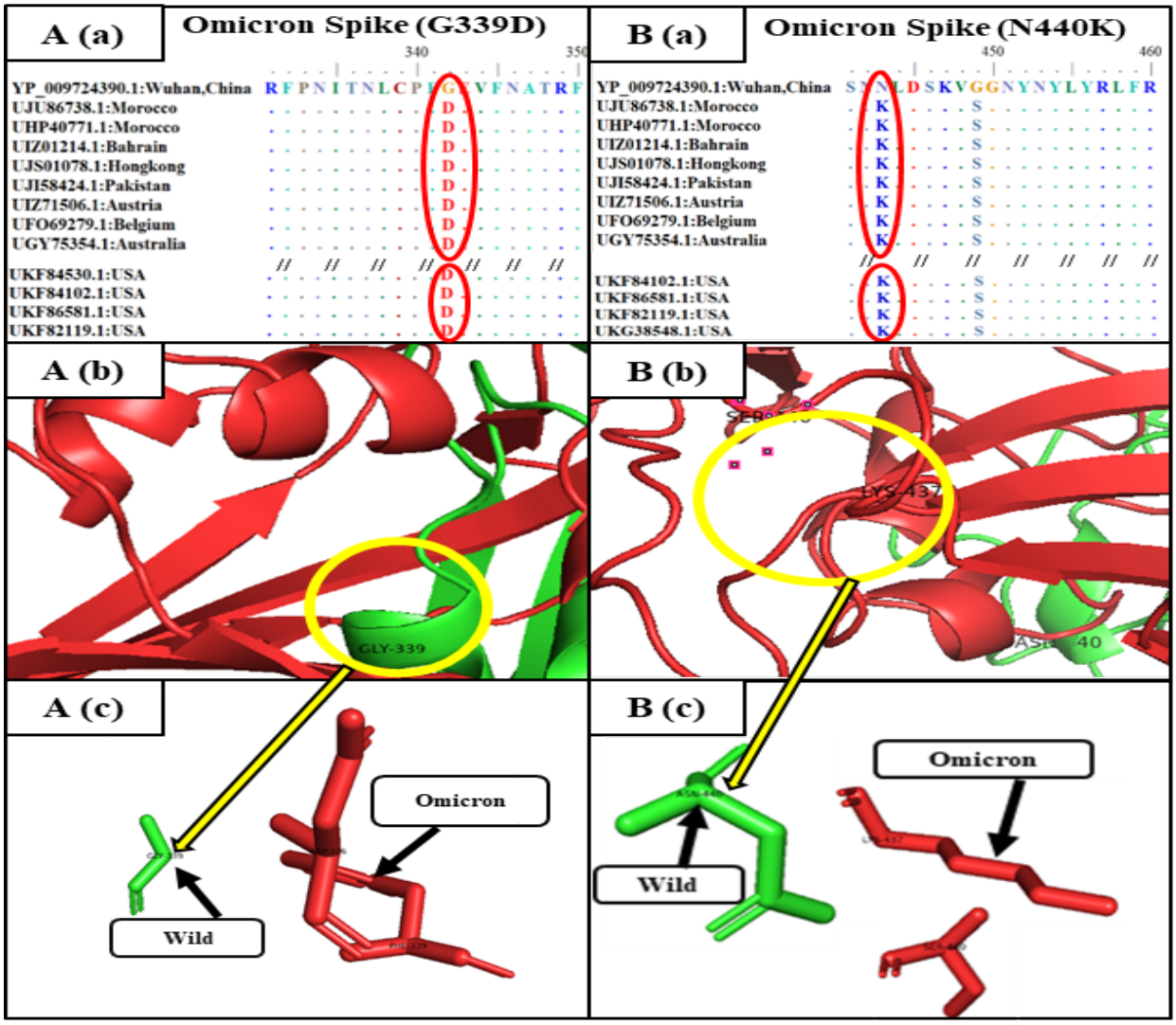

Figure 11. Mutational and Structural Analysis of the G339D and N440K mutations of the Spike Proteins of Omicron Variant. A(a) G339D mutation in spike protein of Omicron variant, A(b) Structural change in Omicron spike G339D, and A(c) amino acid alteration of Glycine to Aspartic acid. B(a) N440K mutation in spike protein of Omicron variant, B(b) Structural change in Omicron spike N440K, and B(c) Amino acid alteration of Asparagine to Lysine.

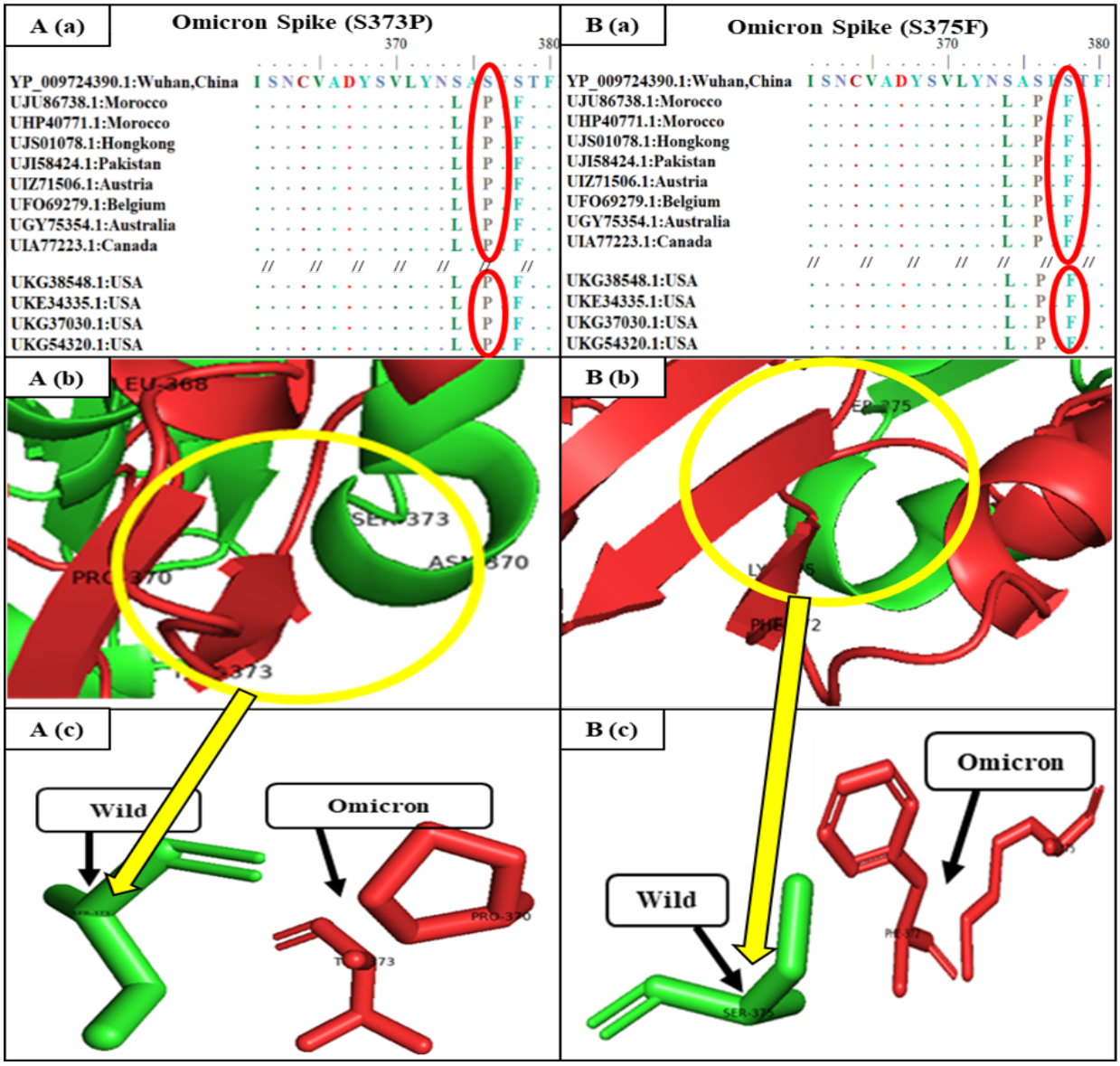

Figure 12. Mutational and Structural Analysis of the S373P and S375F Mutations of the Spike Proteins of Omicron Variant. A (a) S373P mutation in spike protein of Omicron variant, A (b) Structural Change in S373P mutation, A (c) Amino acid alteration of Serine to Proline, B (a) S375F mutation in spike protein of Omicron variant, B (b) Structural Change in S375F mutation, B (c) Amino acid alteration of Serine to Phenylalanine

Likewise, we have found another significant mutation at position 484 due to the carboxyl group being removed and a few methyl groups being added **(Figure 13)**. Glutamic acid, which is a negatively charged amino acid, is converted into Alanine which is a non-polar amino acid. Finally, at position 493 **(Figure 14)** and position 498 **(Figure 13)**, due to the hydroxyl group being removed and a few amine groups being added, structural modification occurred where a polar amino acid, Glutamine, changed to Arginine, a positively charged amino acid. Similarly, at position 547, the mutation took place from Threonine (a polar amino acid) to Lysine (a positively charged amino acid), which caused structural modification **(Figure 15)** because of the hydroxyl group being removed and a amine groups being added. Besides, other modifications were found at position positions 70 (nonpolar to nonpolar) and 477 (polar to polar) but this could be neglectable **(Figures 9, and 16).**

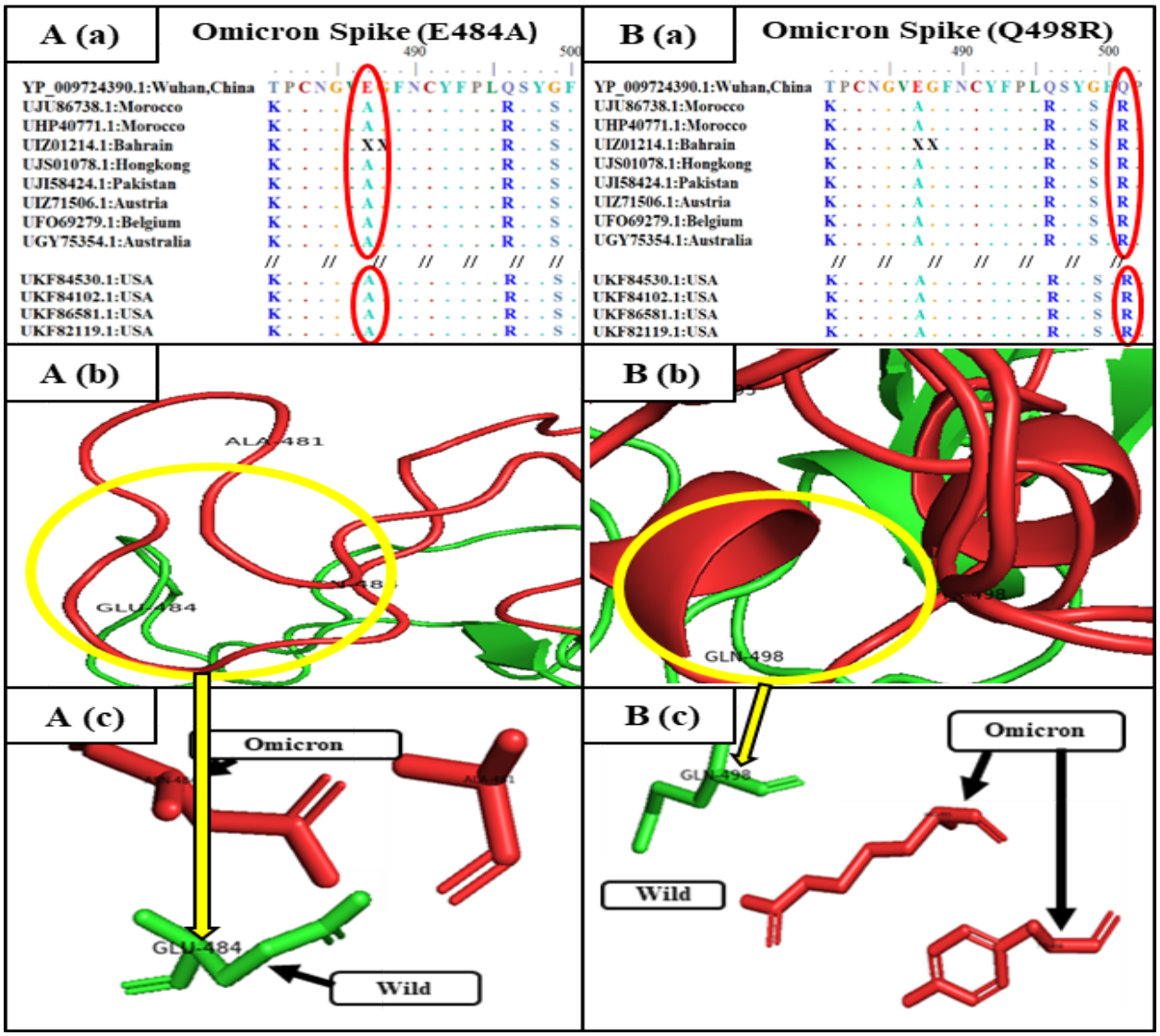

Figure 13. Mutational and Structural Analysis of the E484A and Q498R Mutations of the Spike Proteins of Omicron Variant. A(a) E484A mutation in spike protein of Omicron variant, A(b) Structural change in Omicron spike E484A, and A(c) amino acid alteration of Glutamic Acid to Alanine. B(a) Q498R mutation in spike protein of Omicron variant, B(b) Structural change in Omicron spike Q498R, and B(c) Amino acid alteration of Glutamine to Arginine.

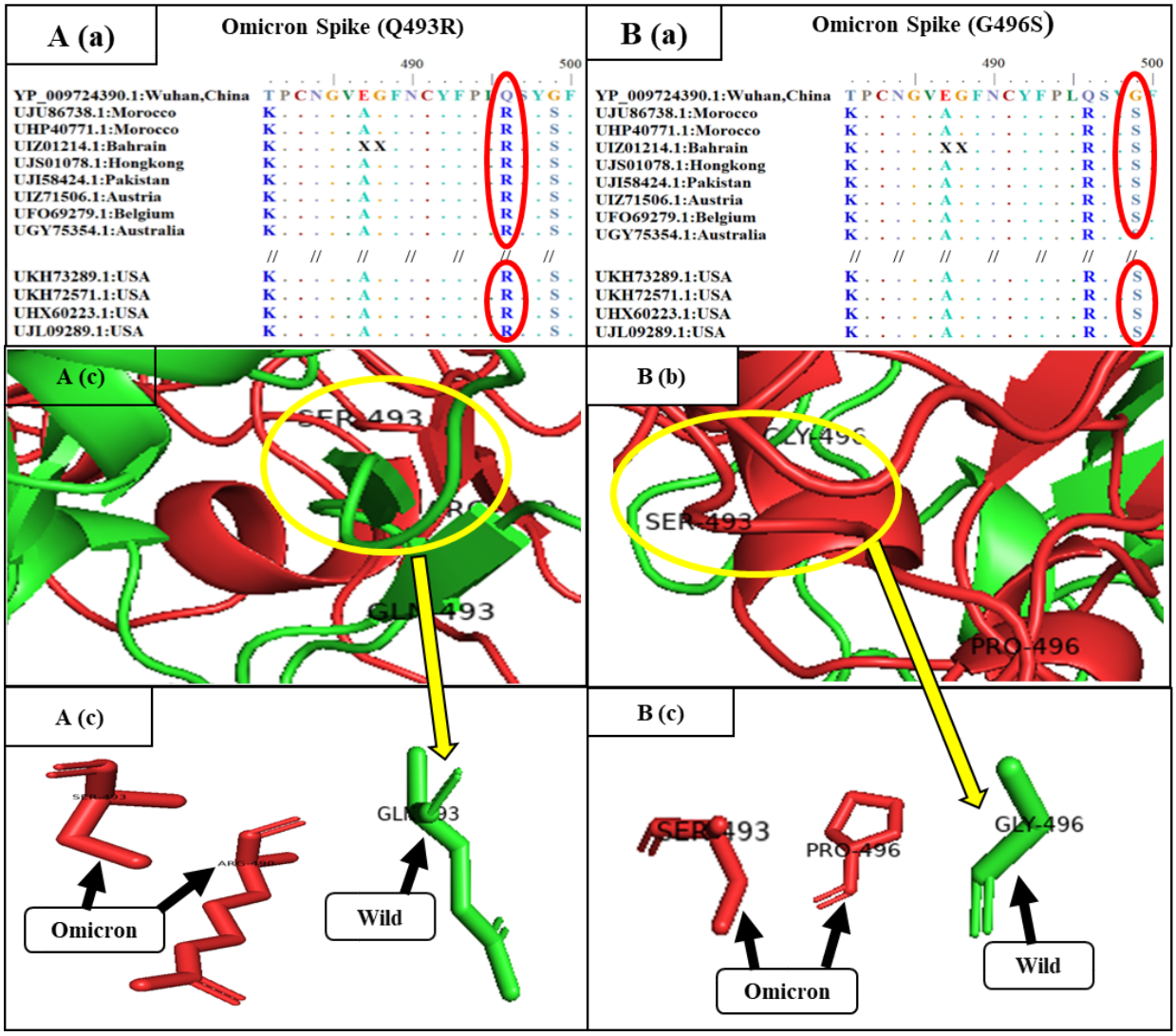

Figure 14. Mutational and Structural Analysis of the Q493R and G496S Mutations of the Spike Proteins of Omicron Variant. A (a) Q493R mutation in spike protein of the Omicron variant, A (b) Structural Change in Q493R mutation, A (c) Amino acid alteration of Glutamine to Arginine, B (a) G496S mutation in spike protein of the Omicron variant, B (b) Structural Change in G496S mutation, B (c) Amino acid alteration of Glycine to Serine.

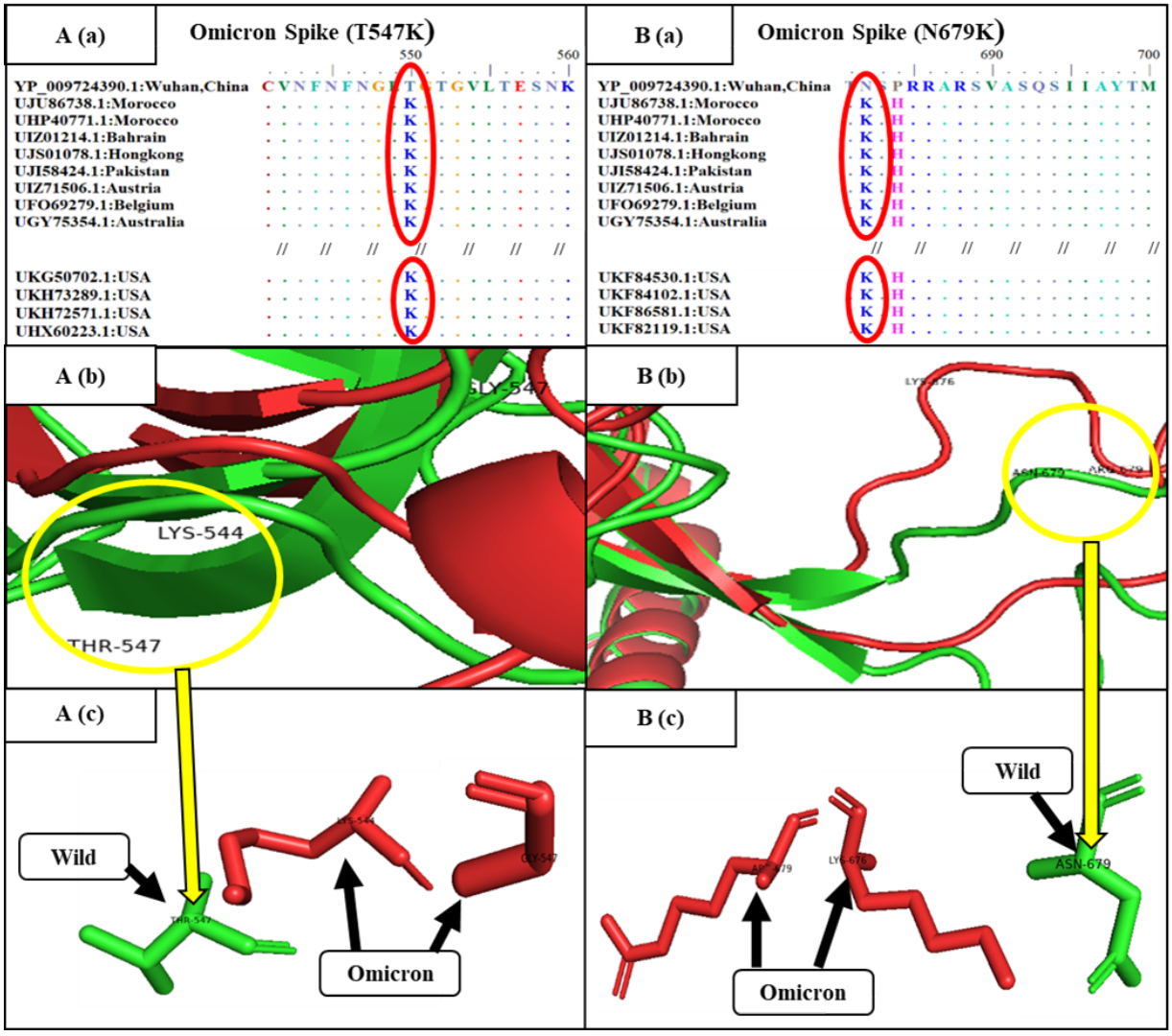

Figure 15. Mutational and Structural Analysis of the T547K and N679K Mutations of the Spike Proteins of Omicron Variant. A (a) T547K mutation in spike protein of Omicron variant, A (b) Structural Change in T547K mutation, A (c) Amino acid alteration of Threonine to Serine, B(a) N679K mutation in spike protein of Omicron variant, B(b) Structural Change in N679K mutation, B (c) Amino acid alteration of Asparagine to Lysine.

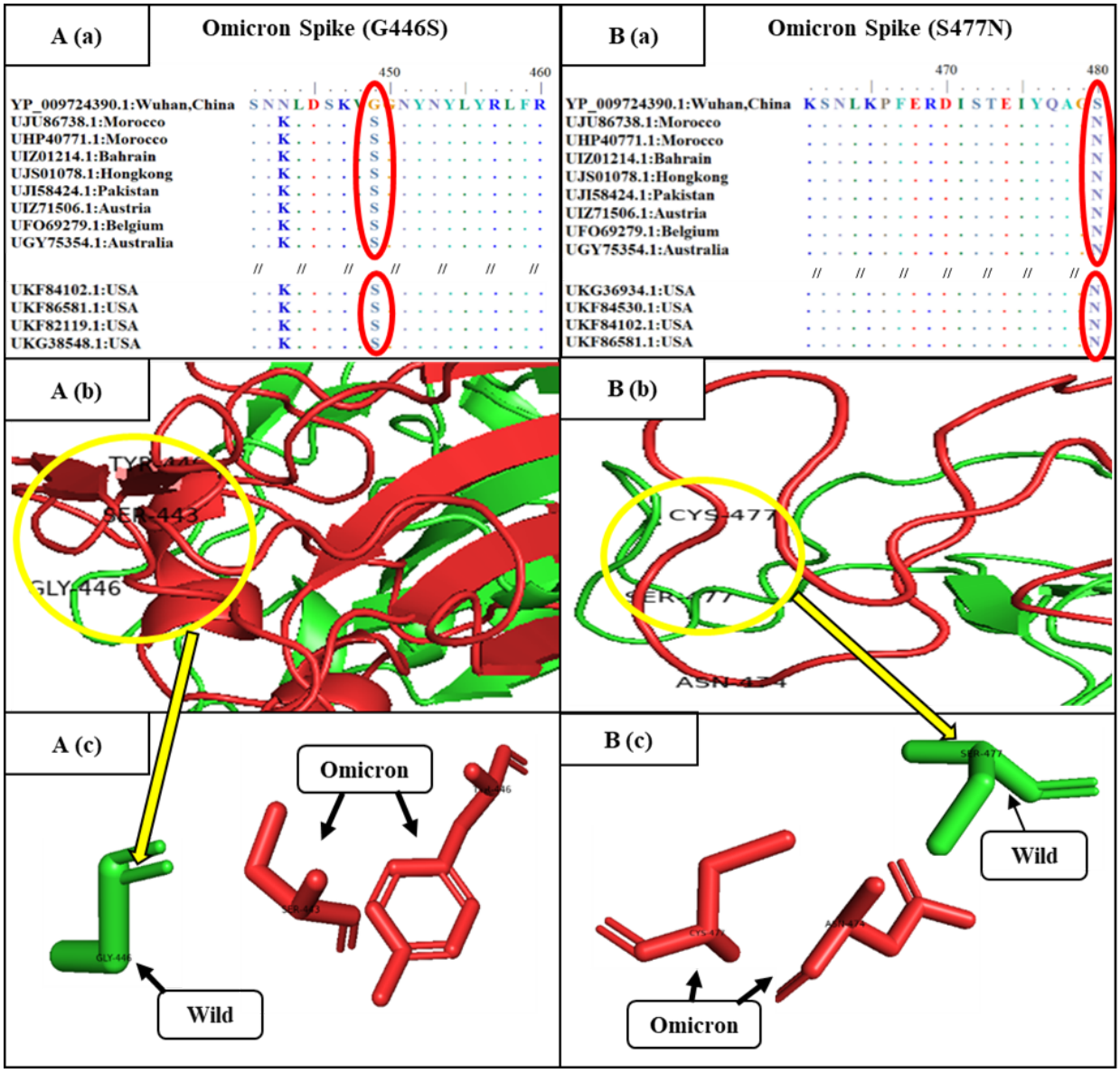

Figure 16. Mutational and Structural Analysis of the G446S and S447N Mutations of the Spike Proteins of Omicron Variant. A (a) G446S mutation in spike protein of Omicron variant, A (b) Structural Change in G446S mutation, A (c) Amino acid alteration of Glycine to Serine, B (a) S477N mutation in spike protein of Omicron variant, B (b) Structural Change in S477N mutation, B (c) Amino acid alteration of Serine to Asparagine.

Aspartic acid, a negatively charged amino acid convert to Tyrosine, a polar amino acid, which is responsible for structural changes at that position 796 because of the removal of a carboxyl group and the addition of a phenol group **(Figure 17).** In addition, structural change was found at positions 440, 764, 856, 679, and 969, where mutation took place from Asparagine (a polar amino acid) to Lysine (positively charged amino acid), which caused a crucial change in structure due to the removal of the keto group and the addition of amine groups **(Figures 11, 15, 17 and 18).**

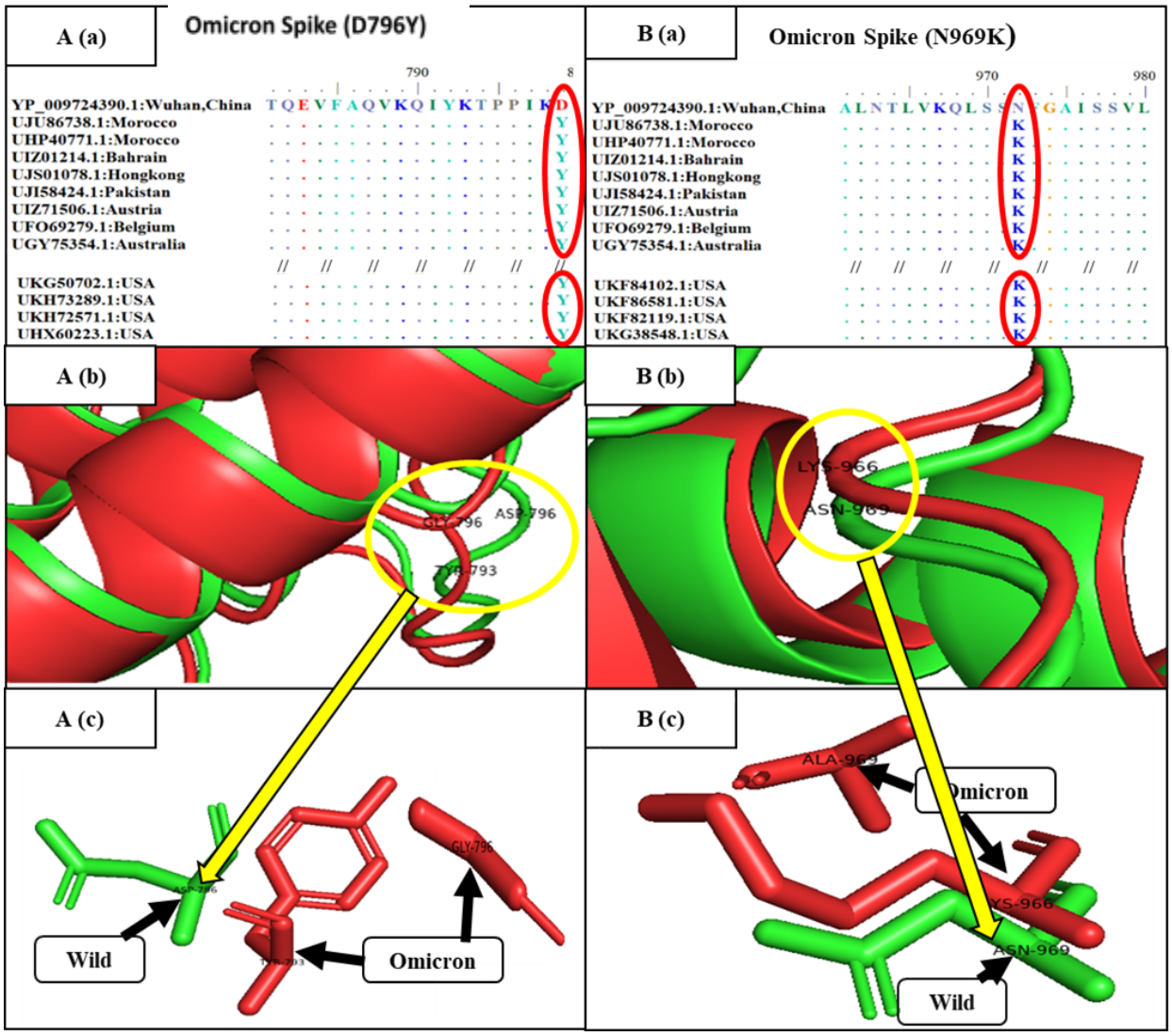

Figure 17. Mutational and Structural Analysis of the D796N and N969K Mutations of the Spike Proteins of Omicron Variant. A(a) D796Y mutation in spike protein of Omicron variant, A(b) Structural Change in D796Y mutation A(c) Amino acid alteration of Threonine to Serine, B(a) N969K mutation in spike protein of Omicron variant, B(b) Structural Change in N969K mutation, B(c) Amino acid alteration of Asparagine to Lysine.

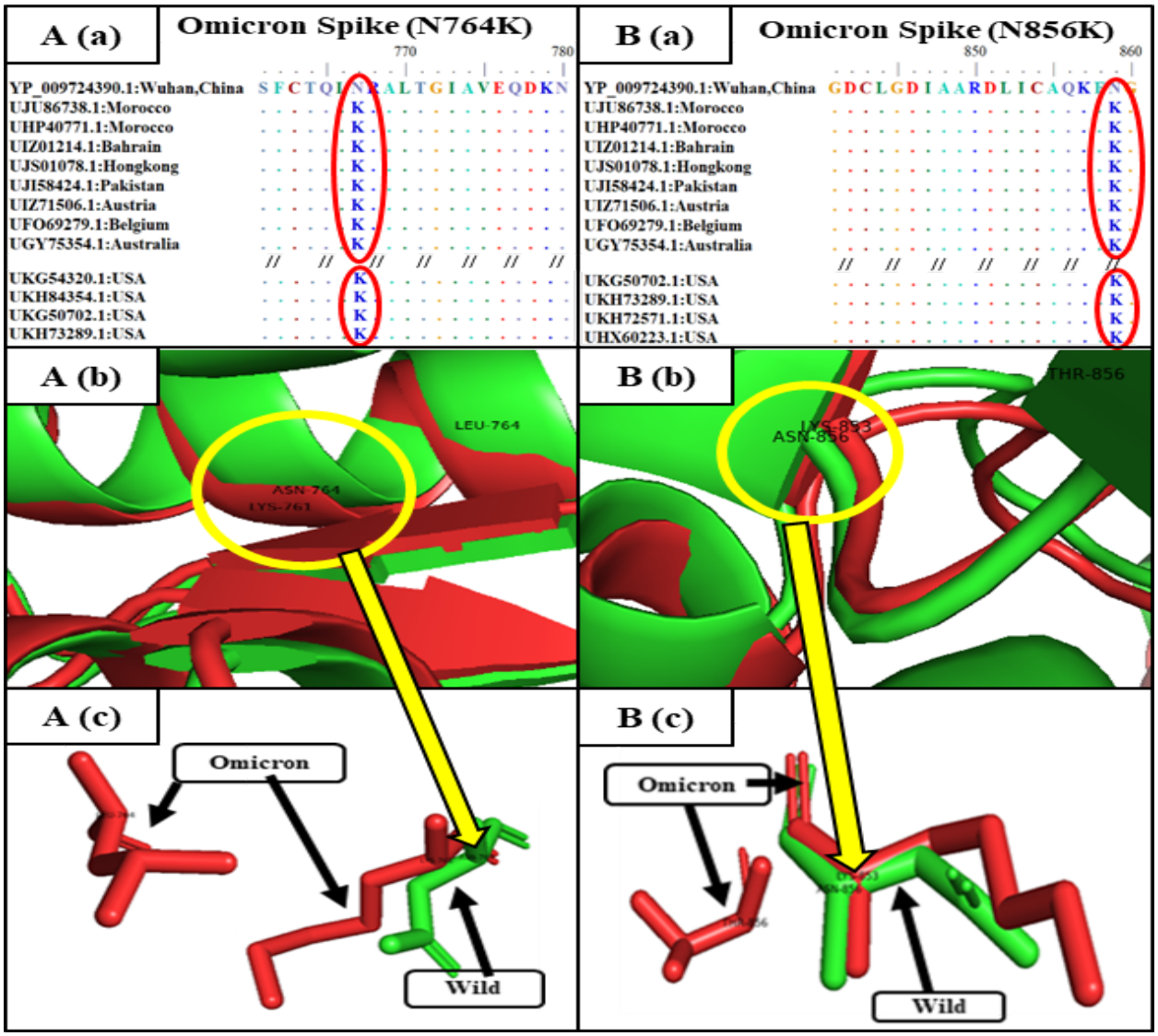

Figure 18. Mutational and Structural Analysis of the N764K and N856K Mutations of the Spike Proteins of Omicron Variant. A(a) N764K mutation in the spike protein of the Omicron variant, A(b) Structural change in Omicron spike N764K, and A(c) amino acid alteration of Asparagine to lysine. B(a) N856K mutation in spike protein of Omicron variant, B(b) Structural change in Omicron spike N856K and B(c) Amino acid alteration of Asparagine to lysine.

Chapter 04

Discussions

Discussions

Recently identified mutations in SARS-CoV-2 variants affect both the structure and activity of structural and nonstructural proteins. As a result, these variants make the diseases challenging to treat and manage (Mohammad et al., 2021). Numerous common mutations have been observed in prior investigations in all five SARS-CoV-2 VOCs. In this study, we concentrated on the mutational investigation of rare regional mutations identified in three nonstructural proteins, namely PLpro, RdRp, and Helicase, as well as one structural protein, the spike protein of five SARS-CoV-2 VOCs, namely Alpha, Beta, Gamma, Delta, and Omicron. Moreover, we also looked into the structural changes caused by a few special mutations among these unusual regional mutations and their connection to mortality.

We know that interferon-stimulating gene-15 (ISG-15) protein is cleaved off by PLpro, which leads to inappropriate antiviral signaling by host cells (Morales & Lenschow, 2013; Shin et al., 2020). Additionally, RdRp is a crucial surface site where an antiviral drug might halt or decrease viral replication (Chan et al., 2020). The role of helicases is to move along nucleic acid strands, unfold or separate the helical shape of double-stranded nucleic acids, and, in some cases, to obstruct protein-nucleic acid interactions. They do this by using the energy produced by nucleoside triphosphate hydrolysis (Abdelhaleem, 2009). According to a mutational investigation of these three nonstructural proteins, some unusual mutations found in a separate location may have an impact on the functions listed above. A recent example is the P227L mutation, whose prevalence may have grown during the most recent worldwide peak (Mazhari et al., 2021).

For the purpose of analyzing structural deviations, the RMSD values of PLpro, RdRp, and Helicase were too low. Because of this, we decided not to perform the structural analysis of nonstructural proteins. However, one of the key structural proteins of SARS-CoV-2 showed a higher RMSD value. For this reason, we performed a mutational study of two uncommon regional mutations in this protein.

Structures of spike proteins of each variant were assessed as good quality as QMEAN Z scores were below -2.5. In fact, Z-scores close to 0.0 represent a structure that is "native-like," and, as a general rule, a "QMEAN" Z-score below -4.0 denotes a model with low quality (Benkert et al., 2011). The GMQE scores, which provide an overall model quality evaluation between 0 and 1, were also used to validate the models' quality. Higher scores indicate higher expected quality. (*Introduction to SWISS-MODEL: Model Evaluation*). Ramachandran plot, a tool for visualizing energetically preferred locations for backbone dihedral orientations against amino acid residues in protein structure (*SWISS-MODEL Structure Assessment: Ramachandran Plots*). In our study GMQE scores of all models are between 0 and 1 and finding maximum amino acids in favoured region by visualizing Ramachandran plot and scores (Patil et al., 2019). Our structural investigation of the spike protein's rare regional mutations considered the possibility that the T95I mutation in the Indian spike protein, which results in structural change, may be the reason for the high viral load (Shen et al., 2021). Additionally, the P688V mutation and structural alterations of the Gamma Spike Protein found in the USA in our study could result in a significant rise in the rate of infection.

The RMSD values for the Beta (10.055), Delta (11.067), and Omicron (10.083) variations of spike proteins were also incredibly high in contrast to the reference structure. To find out whether there is a relationship between the structural change and the risk of severity, specific

mutations of the Beta, Delta, and Omicron spike proteins that resulted in significant structural changes were further evaluated.

Protein structure and function may change if amino acids convert from polar to nonpolar or positively to negatively charged, and vice versa (Chen et al., 2021). According to our findings, the D80A mutation, which is only present in the spike protein of Beta variants, has a structural modification that may allow it to evade neutralizing antibodies and reduce the effectiveness of the vaccination (Mohammadi et al., 2021). In this research, we structurally examined the Delta spike protein with the T19R mutation. This structural analysis showed that the side chain conformational alteration and decreased antibody interactions may be caused by the structure change (Kannan et al., 2021). Similar to this, in Delta variants the structural alterations identified in our research in the receptor-binding domain (RBD) spike's protein of SARS-CoV-2. The L452R mutation present in the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein may cause a reduction in the neutralizing activity of several monoclonal antibodies (Mohammad et al., 2021). Moreover, this mutation boosted spike protein's affinity for the ACE2 receptor which reduced the detection capability of the immune system (Aoki et al., 2021). Our research looked further into the structural alteration of the T478K mutation discovered in the Delta spike, which may be predicted to be responsible for raising the binding affinity between hACE2 receptor and RBD (the point at which SARS-COV-2 enters the human cell) and facilitating immune escape (Hagen, 2021).

Intriguingly, we also discovered that P681R, the most noticeable mutation observed solely in the Delta spike, results in structural change and may be the reason for the elevated cell-level infectivity. The fundamental cause of the infectivity of the Delta variant was postulated to be this

structural change, which happened directly at the site of furin cleavage of the variant. It played a critical role in enhancing S-protein cleavage (Adhikari et al., 2022).

Although, according to our findings, the structure of the delta spike protein changed as a result of the D950N mutation, no functional change had yet been noticed. In a similar way, LLA242del and D215G mutations in the beta spike protein, our investigation revealed a difference in the protein's structure but did not yet reveal any functional changes. Minor structural changes from nonpolar-to-nonpolar amino acids were seen in the Beta spike protein due to the A701V mutation.

In our investigation of Omicron spikes, structural alterations were also discovered that may result in improved immune escape capabilities (Cao et al., 2021). These type of mutations included G339D, S477N, E484A, S371L, K440N, G496S, S375F, Q498R, G446S, and Y505H. In addition, we also found that Omicron has the N679K mutation, which causes a structural change. This change incorporates basic amino acids near the furin cleavage site and may make it easier for the spike protein to split into S1 and S2, which may increase fusion and virus infection (He et al., 2021). Additionally, in our research, N764K, D796Y, and N856K mutations also led to structural variations, which helped increase stability in the Omicron variant (Cao et al., 2021).

However, a detailed study is needed to determine how structural changes in the spike protein of the variants cause disease severity, and continuous research is necessary to better comprehend the pathogenicity, diagnosis, and therapy of the virus geographical mutations.

Chapter 05

Conclusion

Conclusion

In the course of our investigation, we discovered very substantial structural mutation in the spike protein of the Omicron, Delta and Beta variants, which may significantly affect the rates of disease severity and mortality. These days, Omicrons are producing alarming rates of infection and mortality that are comparable to Delta variations. According to our study, these results indicates that Beta, Delta, and Omicron variants' effects on disease severity identified in our investigation may be significantly influenced by the structural deviation of the spike protein.

However, these new findings and in-depth analysis will be better able to judge the choice of vaccine against regionally dominant strains and treat them with those vaccines that are more specifically targeted.

Chapter 06

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