



***In Silico* Analysis of COVID-19 Variants: Structural Interaction with ACE2, TMPRSS2 and Pathogenic Implications**

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Abstract

Background: Compared to SARS-CoVs, the S-protein of SARS-CoV-2 has a significantly higher specificity and stability for binding to the ACE2 receptor and due to the structural variation of COVID-19, the development of drugs for each variant is complicated. **Objective:** This study investigates the three-dimensional structure of COVID-19 variants. **Methodology:** To predict the protein structure of COVID-19 variants, homology modelling was used to determine the tertiary structure. For modelling, the Swiss Model and BLAST were used, and to ensure reliability and validated with PROCHECK, Verify3D, and ERRAT. A Ramachandran plot was used to verify the quality of 3D structure. Models were validated through SAVES. Multiple Sequence Alignment was performed to identify similarities and differences between variants. **Results:** Homology Modelling indicates the sequence similarity of ALPHA, BETA, DELTA, andOMICRON variants with the template model observed as 94.48%, 94.64%, 95.11%, and 94.84%, respectively. Ramachandran plot signifies that residues are present in the most favorable region with a frequency of 90% for ALPHA, followed by BETA residues at 88%, DELTA residues at 99.9%, and OMICRON residues at 87%. **Conclusion:** In conclusion, Delta variant exhibits 80-90% higher transmissibility and can propagate infection more than twice as compared to the Alpha variant. [*Bangladesh Journal of Infectious Diseases*, June 2026;13(1):25-42]

Keywords: Homology modelling; structural prediction; COVID-19; multiple sequence alignment; pathogenesis

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Introduction

The Severe Acute Respiratory Syndrome (SARS-CoV-2), also known as COVID-19, started in Wuhan, China, in December 2019 and is widely disseminated in humans and other mammals¹. The two beta-coronavirus epidemics, SARS-COV and MERS-COV, affected more than ten

thousand (10,000) people over the course of the last twenty years, with SARS-COV having a mortality rate of 10% cases² even though coronavirus infections in humans are typically not severe. Alpha, beta, gamma, and delta coronaviruses are the four subgroups that make up the coronavirus family³. Before the SARS outbreak

in 2002-2003, it was believed that COVID-19 only infected animals. When the NMSARS-S protein binds to the ACE2 receptor, it undergoes subtle conformational changes that are believed to increase the S-protein's susceptibility to proteolytic cleavage between the S1 and S2 subunits⁴. Host cell proteases are involved in the proteolytic processing of ACE2, which may be crucial for SARS-COV-2 entrance and pathogenesis.

Recent research on S-protein, TMPRSS2, and ACE2 discovered the interface of their connections and identified various amino acid alterations that could potentially affect protein-protein interactions (PPI) by changing their structural stability⁵. The gene for the ACE2 receptor, which is encoded on chromosome Xp22.2, is a competent receptor for the Spikes (S) glycoprotein of SARS-COV-2⁶. Spike protein has multiple significant mutations. One is the N501Y mutation, which increases the mutant protein's ability to connect to cell receptors and spread the virus more easily⁷. The D614G mutation in the SARS-CoV-2 spike protein reduces S1 shedding and increases infectivity⁸. The beta variant was initially identified as a variant of concern in December 2020 and was first found in South Africa⁹. Beta carries the K417N mutation, along with the three mutations found in the Alpha variant (E484K, N501Y, and D614G), which may help the virus escape neutralizing antibodies from vaccination or prior infection¹⁰. First identified in India in May 2021, the D614G mutation, along with several additional mutations, are present in the delta variant. These include the P681R mutation, which is linked to enhancing fusogenicity and a higher risk of developing severe disease. T478K mutation, is thought to evade detection by the immune system, and the L452R mutation, which is thought to increase infectivity by having a modestly higher binding affinity for ACE2 receptors, and possibly prevent the virus from being destroyed by immune cells¹¹.

In November 2021, after South African scientists alerted WHO to an increase in COVID-19 cases in Gauteng, Omicron variant was reported for the first time in different countries with different reported mutations at N501Y, D614G, K417N, and T478K locations¹². Although the sequencing and epidemiological monitoring of Covid-19 variants have been the main focus of earlier research, there is still a significant knowledge vacuum about the structural and functional consequences of these mutations at the three-dimensional protein level. Without systematically comparing several variants in terms of structural and functional alterations,

many studies solely examine one variant or particular mutations.

Current computational studies frequently don't investigate variant-specific changes in protein-protein interactions or lack of validation. The objective of this research is to cover these gaps by doing a thorough structural and functional characterization of several COVID-19 variants. The current study performs the comparative analysis of ACE2 & TMPRSS2 through protein-protein interaction network visualization, Homology modelling, Multiple Sequence Alignment and 3D modeling of the different variants of COVID-19 using different in-silico tools.

Methodology

Data Collection: FASTA Sequence of Covid 19 wild type (PDB ID: 6VSB) along with ALPHA (PDB ID: 7EDH), BETA (PDB ID: 7VX1), Delta (PDB ID: 7V7V) and OMICRON (PDB ID: 7WVN) variants were downloaded from PDB Database (<https://www.rcsb.org/>)^[13]. The workflow of the study is shown in Figure I.

Homology Modelling: The downloaded FASTA sequences were uploaded to SWISS-MODEL (<https://swissmodel.expasy.org/>)¹⁴. For validation of the template structure, different methods of the SAVES (<https://saves.mbi.ucla.edu/>), like ERRAT, VERIFY 3D, and RAMACHANDRAN plots were used for checking the quality of 3D structures.

3D Structure Modelling: For 3D structure prediction and modelling SEQ and ALI files were generated in modeller (MODELLER, 10.4), (<https://salilab.org/modeller/>)¹⁵. Models with the lowest minimization energy and the lowest DOPE score were selected. The Chimera (CSF Chimera 1.8.1) (<https://www.cgl.ucsf.edu/chimera/>) was used for the interaction analysis of predicted models¹⁶. The PDB files of the target and predicted model were uploaded into chimera and superimpose. Based on the lowest root mean square deviation (RMSD) score, the best target model was selected.

Multiple Sequence Alignment: FASTA sequences of COVID-19 and Alpha, Beta, Delta and Omicron variants were uploaded to CLUSTAL-X (version-2.1) (<http://www.clustal.org/clustal2/>) for alignment¹⁷. For visualization of the Multiple Sequence Alignment, JALVIEW software (<https://www.jalview.org/>) was used¹⁸.

Protein-protein interaction (PPI): STRING database (<https://string-db.org/>) was used for the evaluation of protein-protein interactions¹⁹. FASTA sequences downloaded from Uniprot

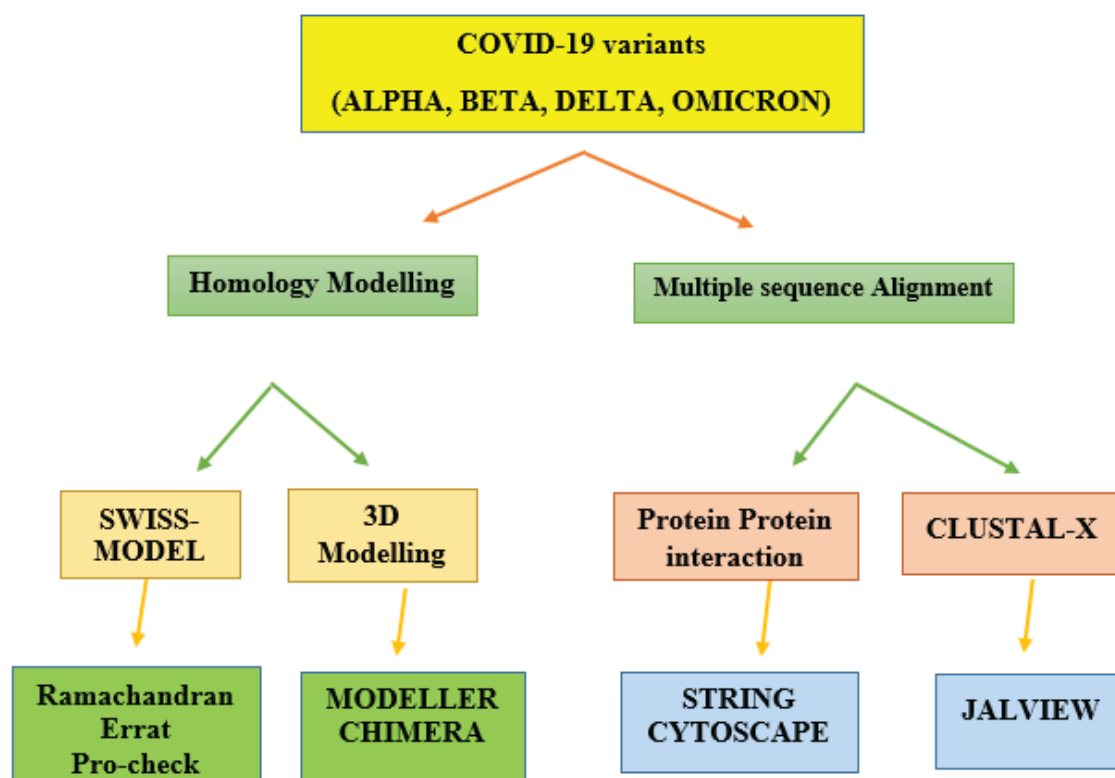


Figure 1: Workflow of the Computational Analysis of COVID-19; This approach demonstrates the comparison of ACE2 and TMPRSS2 through the visualization of protein-protein interaction networks (PPI), homology modelling, multiple sequence alignment, and 3D modelling of the many COVID-19 variants using various computational tool

(<https://www.uniprot.org/>) were uploaded to the STRING database¹⁵. Cytoscape tool (<https://cytoscape.org/>) was used for visualizing the protein-protein interactions²⁰.

from PDB database (<https://www.rcsb.org/>). Receptor binding domain sequences (RBD) ensures binding of ACE2 with protein. The FASTA sequence of Covid-19 variants with their PDB IDs are shown in Table 1.

Results

Study Data: FASTA sequences were downloaded

Table 1: The COVID-19 data with their PDB ID

Name	PDB IDs	FASTA sequences
Covid (wild Type)	6VSB	>6VSB_1 Chains A, B, C Spike glycoprotein Severe acute respiratory syndrome coronavirus 2 (2697049) MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPD KVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSNGTKRFDN PVLPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNA TNVVIKVCEFCNDPFLGVYYHKNNKSWMESEFRVYSSA NNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKI YSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLALHR SYLTPGDSSSGWTAGAAAYYVGYLQPRFTLLKYNENGTIT DAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIV RFPNITNLCPFGEVFNATRFASVYAWNKRISNCVADYSVL YNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQ IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNGY NYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYF PLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKS

Table 1: The COVID-19 data with their PDB ID

Name	PDB IDs	FASTA sequences
		TNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIA DTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLY QDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIG AEHVNNSYECDIPIGAGICASYQTQTNSPGSASSVASQSIIA YTMSLGAENSVAYSNNNSIAIPTNFTISVTTEILPVSMTKTSV DCTMYICGDSTECNLLLQYGSFCTQLNRALTGIAVEQDK NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIE DLLFNKVTLDAGFIKQYGDCLGDIAARDLICAQKFNGLT VLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLST ASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDIL SRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASAN LAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVF LHVTYVPAQEKNFTTAPAICHGDKAHFPREGVFSNGTHW FVTQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPE LDSFKEELDKYFKNHTSPDVDLGDISGINASVNIQKEIDRL NEVAKNLNESLIDLQELGKYEQGSYIPEAPRDGQAYVRK DGEWVLLSTFLGRSLEVLFGPGHHHHHHHSAWSHPQF EKGGS GGGS GGGS GGSAWSHPQFEK
Alpha variant	7EDH	>7EDH_1 Chains A, B, C Spike glycoprotein Severe acute respiratory syndrome coronavirus 2 (2697049) METDTLLLWVLLLWVPGSTGDVNLTRTQLPPAYTNSFTR GVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAISGTNGTK RFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSTQSLLI VNNATNVVIKVECFQFCNDPFLGVYHKNNKSWMESEFRV YSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDG YFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTL ALHRSYLT PGDSSSGWTAGAAAYVGYLQPRTFLLKYNE NGTITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQP TESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKISNCVA DYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRG DEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSK VGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEG FNCYFPLQSYGFQPTYGVGYQPYRVVLSFELLHAPATVC GPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQF GRDIDDTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQ VAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTR AGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSHRAAASV ASQSIIAYTMSLGAENSVAYSNNNSIAIPNFTISVTTEILPVSM TKTSVDCTMYICGDSTECNLLLQYGSFCTQLNRALTGIAV EQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSK RSFIEDLLFNKVTLDAGFIKQYGDCLGDIAARDLICAQKF NGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQI PFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQD SLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSV LNDILARLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI RASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAP HGVVFLHVTYVPAQEKNFTTAPAICHGDKAHFPREGVFS NGTHWFVTQRNFYEPQIITHTNTFVSGNCDVVIGIVNNTVY DPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVNI

Table 1: The COVID-19 data with their PDB ID

Name	PDB IDs	FASTA sequences
		QKEIDRLNEVAKNLNESLIDLQELGKYEQEFSGGGYIPEAP RDGQAYVRKDGEWVLLSTFLKGQDNSADIQHSRPLESR GPFEQKLISEEDLNMHTGHHHHHH
Beta variant	7VX1	>7VX1_1 Chains A, B, C Spike glycoprotein Severe acute respiratory syndrome coronavirus 2 (2697049) MFVFLVLLPLVSSQCVNFTTRTQLPPAYTNSFTRGVYYPDK VFRSSVLHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFANP VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLIVNNAT NVVIKVC EFQFCNDPFLGVYYHKNNKSWMESEFRVYSSA NNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKI YSKHTPINLVRGLPQGFSALEPLVDLPIGINITRFQTLHISYL TPGDSSSGWTAGAAAYYVGYLQPRFTLLKYNENGTITDAV DCALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPN ITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNS ASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIAP GQTGNIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNY LYRLFRKSNLKPFERDISTEYIYQAGSTPCNGVKGFNCYFPL QSYGFQPTYGVGYQPYRVVLSFELLHAPATVCGPKKSTN LVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADT TDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ GVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGA EHVNNSYECDIPIGAGICASYQTQTNSPGSASSVASQSIAY TMSLGVENSVAYSNNNSIAIPTNFTISVTTEILPVSMTKTSVD CTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKN TQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIED LLFNKVT LADAGFIKQYGDCLGDIAARDLICAQKFNGLTV LPPLLTDemiaQYTSALLAGTITSGWTFGAGAALQIPFAMQ MAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTA SALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILS RLDPPEAEVQIDRLITGRLQSLQTYVTQQ LIRAAEIRASANL AATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFL HVTYVPAQEKNFTTAPAICHGDKAHFPREGVVFVSNGTHWF VTQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPEL DSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRL NEVAKNLNESLIDLQELGKYEQSGGYIPEAPRDGQAYVRK DGEWVLLSTFLENLYFQGDYKDDDDKHHHHHHHHHH
Delta variant	7V7V	>7V7V_1 Chains A, B, C Spike glycoprotein Severe acute respiratory syndrome coronavirus 2 (2697049) MFVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPD KVFRSSVLHSTQDLFLPFFSNVTWFHAIHVS GTNGTKRFDN PVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLIVNNA TNVVIKVC EFQFCNDPFLDVYYHKNNKSWMESGVYSSAN NCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYS KHTPINLVRDL PQGFSALEPLVDLPIGINITRFQTLALHRSY LTPGDSSSGWTAGAAAYYVGYLQPRFTLLKYNENGTITDA VDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFP NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLVNS SASFSTFKCYGVSP TKLNDLCFTNVYADSFVIRGDEV RQIA PGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNY YRYRLFRKSNLKPFERDISTEYIYQAGSKPCNGVEGFNCYFP

Table 1: The COVID-19 data with their PDB ID

Name	PDB IDs	FASTA sequences
		FKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNE VAKNLNESLIDLQELGKYEQGSGYIPEAPRDGQAYVRKDG EWVLLSTFLENLYFQGDYKDDDDKHHHHHHHHHH FKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNE VAKNLNESLIDLQELGKYEQGSGYIPEAPRDGQAYVRKDG EWVLLSTFLENLYFQGDYKDDDDKHHHHHHHHHH

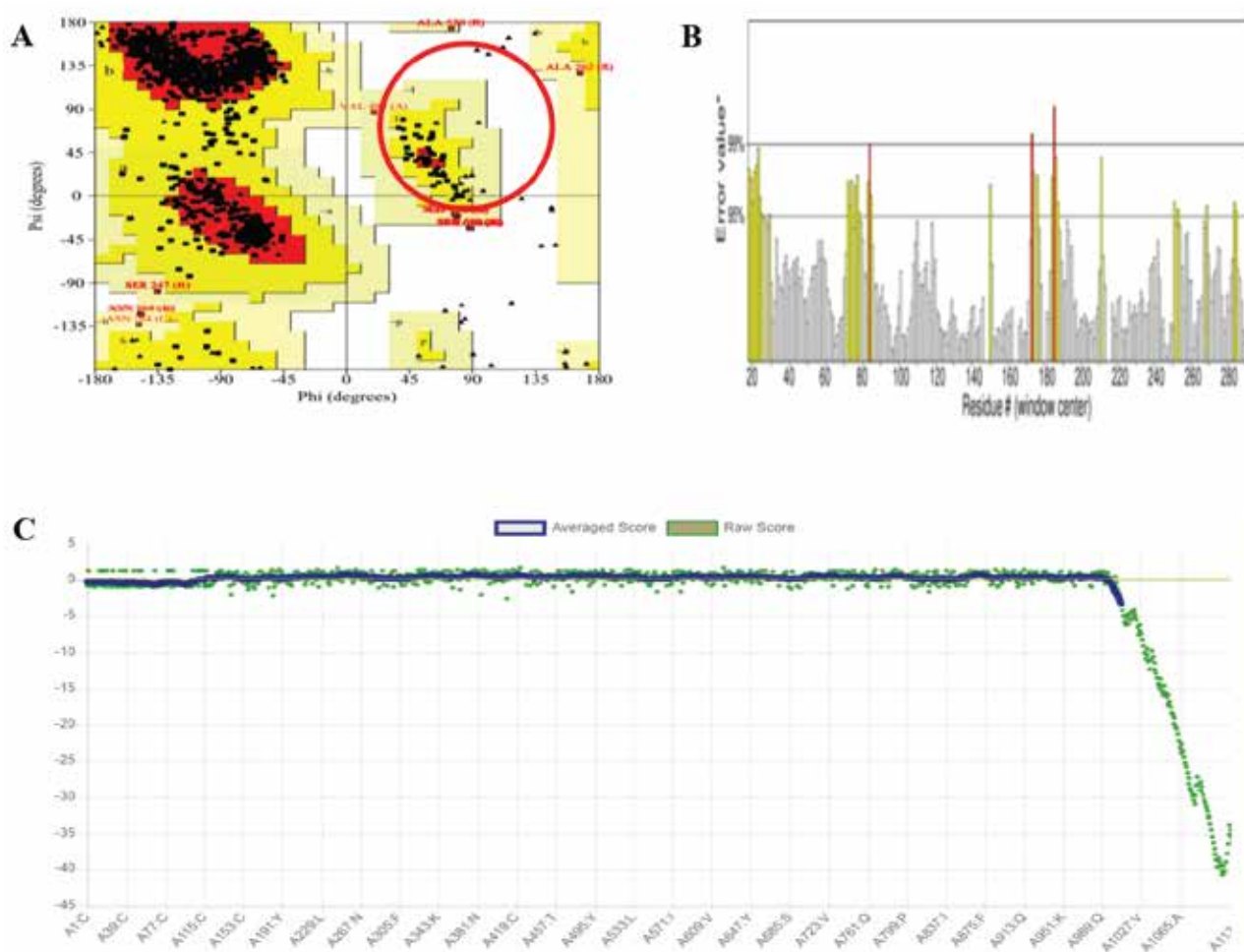


Figure II: Structure validation of Spike Protein (6VSB); (A) Ramachandran plot, 90% residues were showed in red highlighted region which is the most favorable region. Disallowed white region had 0.2% residue and 8.4% residue in the additional yellow allowed region. Glycine residues were 225, which is shown in triangles in the favorable region. The total number of residues is 3396. **(B) ERRAT Graph,** the line at 92% shows the warning and the line at 99% shows the error. If our residue occurs in the warning region, it's fine but if it exceeds the warning region, our structure is not good. In our graph, amino acids at 20 and 80 lie in the warning region, which means we consider our structure to proceed. While 180 shows the red error line **(C) VERIFY 3D,** shows 67.37% of the residues have an averaged 3D score ≥ 0.2 , which is shown by the blue circles, which is not a good value for a good structure.

Table 1: The COVID-19 data with their PDB ID

Name	PDB IDs	FASTA sequences
		LQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKST NLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGRDIAD TTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQ GVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGA EHVNNSYECDIPIGAGICASYQTQTNSRGSASSVASQSIIAY TMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKTSVD CTMYICGDSTECNLLLQYGSFCTQLNRALTGIAVEQDKN TQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIED LLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTV LPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQ MAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTA SALGKLQNVVNQNAQALNTLVKQLSSNFGAISSVLNDILS RLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANL AATKMSECVLGQSKRVDFCGKGYHLSFQPSAPHGVVFL HVTYVPAQEKNFTTAPAICHHDGKAHFPREGVFVSNGTHWF VTQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPEL DSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRL NEVAKNLNESLIDLQELGKYEQEFSGGGYIPEAPRDGQAY VRKDGEWVLLSTFLKQDQNSADIQHSRPLESRGPFEQKLI SEEDLNMHGTGHHHHHH
Omicron variant	7WVN	>7WVN_1 Chains A, B, C Spike glycoprotein Severe acute respiratory syndrome coronavirus (2901879) MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPD KVFRSSVLHSTQDLFLPFFSNVTWFHVISGTNGTKRFDNPV LPFNDGVYFASIEKSNIIRGWIFGTTLDSTQSLIVNNATN VVIKVCFFQFCNDPFLDHKNNKSWMESEFRVYSSANNCTF EYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTP IIVREPEDLPQGFSALEPLVDLPIGINITRFQTLALHRSYLT GDSSSGWTAGAAAYYVGYLQPRFTLLKYNENGTITDAVD CALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNIT NLCPFDEVFNATRFASVYAWNRKRISNCVADYSVLYNLAP FFTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQ TGNADYNYKLPDDFTGCVIAWNSNKLDSKVSNGYNYLY RLFRKSNLKPFERDISTEIQAGNKPCNGVAGFNCYFPLRS YSFRPTYGVGHQPYRVVLSFELLHAPATVCGPKKSTNLV KNKCVNFNFNGLKGTGVLTESNKKFLPFQFGRDIADTTD AVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGV NCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEY VNNSYECDIPIGAGICASYQTQTKSHGSASSVASQSIIAYTM SLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKTSVDCT MYICGDSTECNLLLQYGSFCTQLKRALTGIAVEQDKNTQ EVFAQVKQIYKTPPIKYFGGFNFSQILPDPSKPSKRSFIEDLL FNKVTLADAGFIKQYGDCLGDIAARDLICAQKFGLTVLPP LLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASA LGKLQDVVNHNAAQALNTLVKQLSSKFGAISSVLNDIFSRL DPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAA TKMSECVLGQSKRVDFCGKGYHLSFQPSAPHGVVFLHV TYVPAQEKNFTTAPAICHHDGKAHFPREGVFVSNGTHWFVT QRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDS

Table 2: Templates model against COVID-19 variants

Target PDB IDs	Sequence Similarity	Template PDB IDs
6VSB	92%	7CN8
7EDH	94.48%	7N1V
7VX1	94.64%	7WBP
7V7V	95.11%	7SBO
7WVN	94.84%	7CN4

Homology Modelling: In homology modelling, template models obtained through SWISS MODEL with high sequence similarity is shown in Table 2.

Homology modeling offers useful insights into the structural impact of COVID-19 variants, these limitations underscore the need for complementary experimental and computational approaches to ensure accurate and biologically meaningful predictions. While molecular docking studies (e.g.,

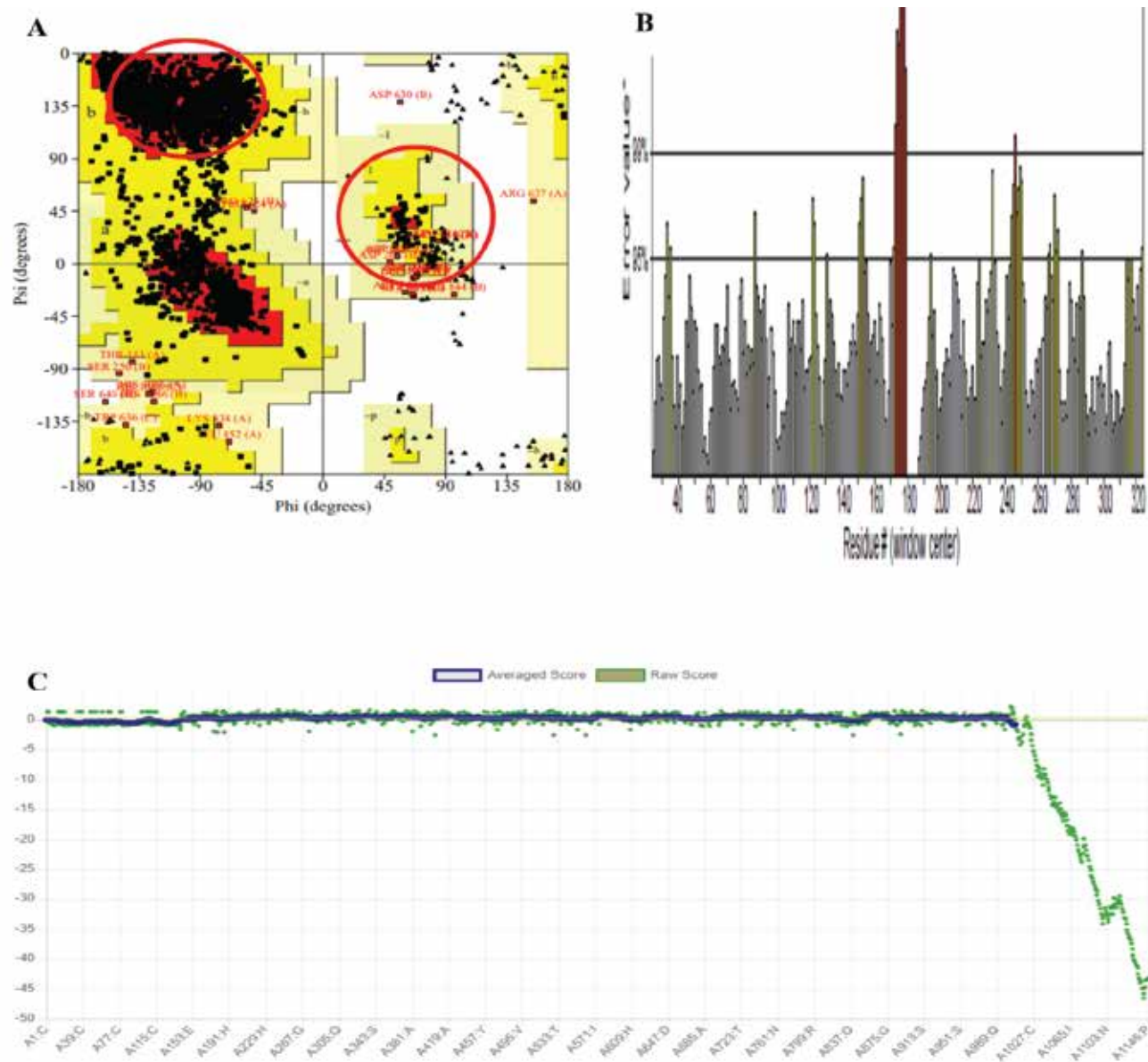


Figure III: Structure validation of ALPHA Protein (7EDH); (A) **Ramachandran plot** of the Alpha variant shows 88% residues in the highlighted red (favorable) region. The white disallowed region has 0.1% residue and 10.4% residue occurs in the yellow additional allowed region. Glycine residues are 228 which is shown in triangles in the favored region. The total number of residues is 3438. (B) **The ERRAT plot of the Alpha variant** shows an overall quality factor of 89%. It is and considered a high-quality model. In our graph of amino acids, yellow lines lie in the warning region, which means we consider our structure to proceed. While 180 shows the red error line (C) **VERIFY 3D of the Alpha variant** shows 64.57% of residues have averaged 3D score ≥ 0.2 , which is shown by the blue circles which is not good values for a good structure. For a high quality structure, 80% of the amino acids should have scored ≥ 0.2 .

ACE2-Spike interaction) can yield insights, homology modeling alone is unable to predict binding affinities or the effect of mutations on binding strength.

Structural Validation of SARS-COVID-19: For comparative homology modeling analysis, a template model against COVID-19 (PDB ID:6VSB) was uploaded to SAVES

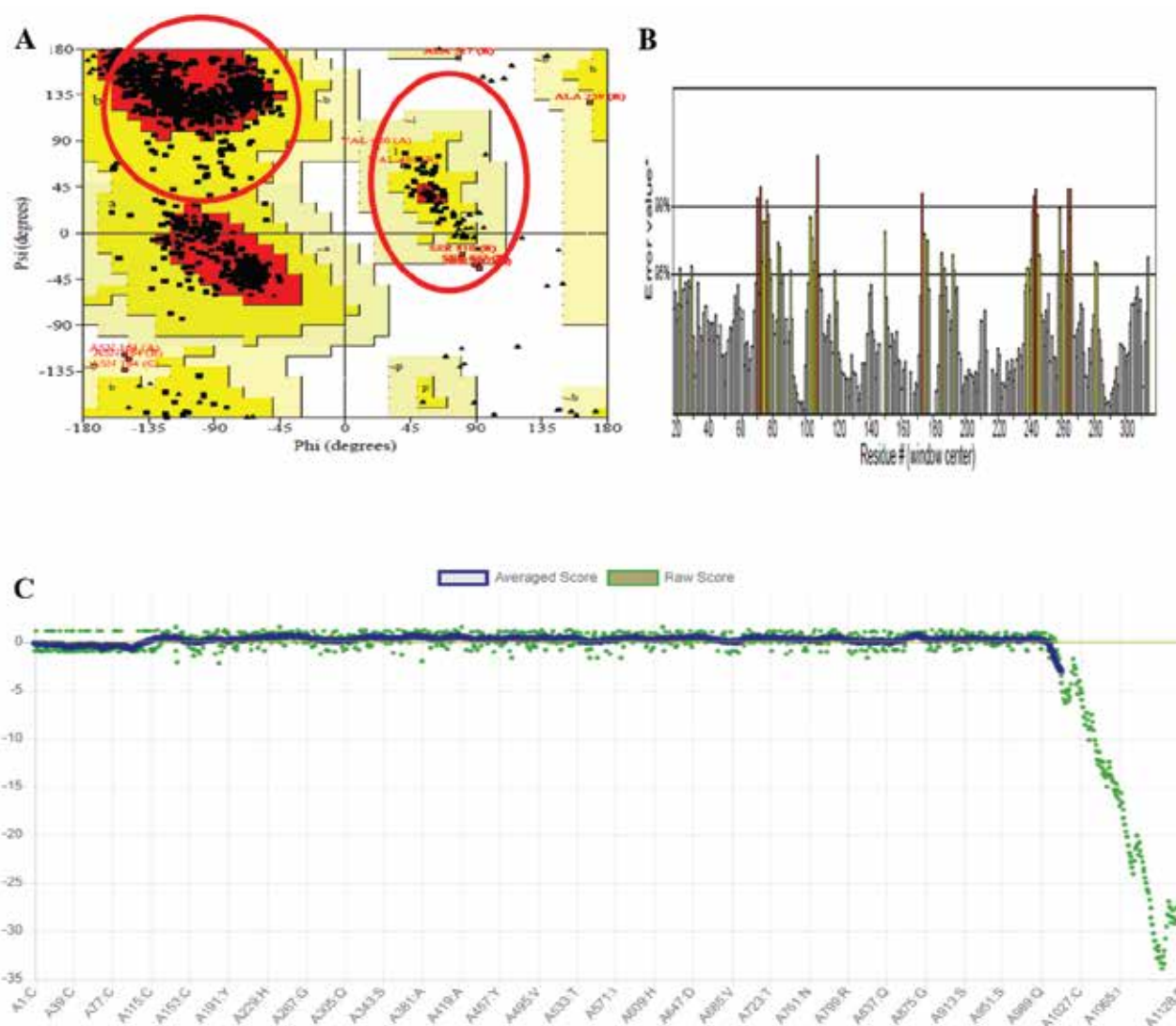


Figure IV: Structure Validation of BETA Protein (7VX1); (A) **Ramachandran plot** of the BETA variant shows 90.9 residues in the red highlighted region. The white disallowed region has 0.2% residues and 8.4% residues occur in the yellow additional allowed region. Glycine residues are 231 which is shown in triangles in the favored region. The total number of residues is 3387. (B) **The ERRAT plot of the BETA variant** shows that the overall quality factor is 91.0% which is considered a high-quality model. In our graph of amino acids, yellow lines lie in the warning region, which means we consider our structure to proceed. While 108 shows the red error line (C) **VERIFY 3D of the BETA variant** shows 67.7% of residues have averaged 3D score ≥ 0.2 , which is shown by the blue circles which are not good values for a good structure. For high-quality structure, 80.0% of the amino acids should have scored ≥ 0.2

(<http://nihserver.mbi.ucla.edu/SAVES/>). Ramachandran plot shows 90% residue in the most favorable region, which is good for the best model. The quality factor ERRAT graph is 92% and is

considered a high-quality model. VERIFY 3D shows 67.37% of the residues have an average 3D score ≥ 0.2 . For a high-quality structure, 80% of the amino acids should have scored ≥ 0.2 (Figure II).

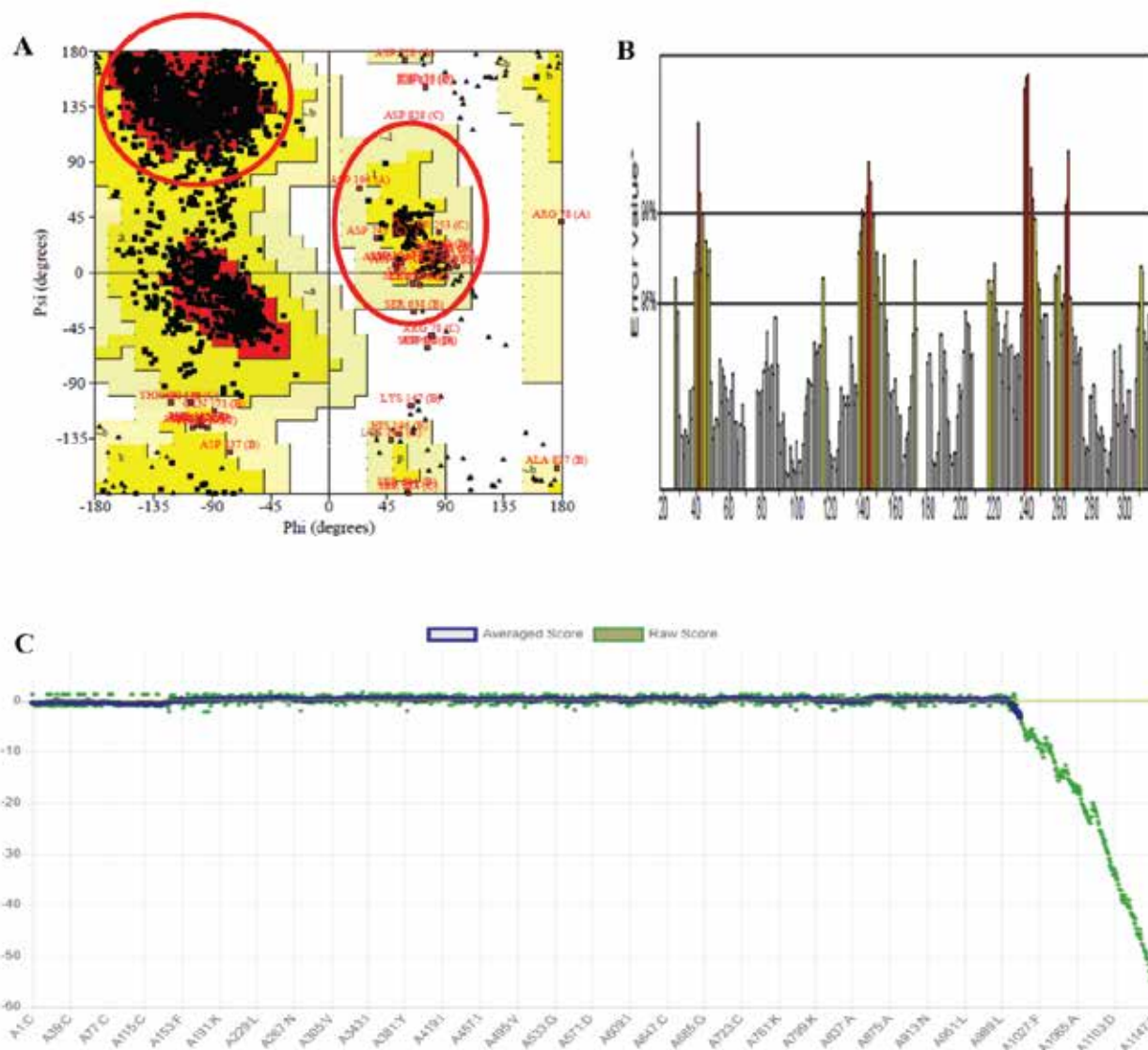


Figure V: Structure validation of DELTA Protein (7V7V); (A) Ramachandran plot of the DELTA variant shows 87.8% residues in the highlighted red region. The white disallowed region has 0.3% residue and 10.9% residue in the yellow additional allowed region. Glycine residues are 228, which is shown in triangles in the favored region. The total number of residues is 3441. **(B) The ERRAT plot of the DELTA variant** shows that the overall quality factor is 90% which means it is considered a high-quality model. In our graph of amino acids, yellow lines lie in the warning region, we consider our structure to proceed. While 240 shows the red error line. **(C) VERIFY 3D of DELTA variant** shows 64.63% of residues have averaged 3D score ≥ 0.2 , which is shown by the blue circles, which are not good values for a good structure. To achieve a high-quality structure, at least 80% of the amino acids must exhibit a score of ≥ 0.2 , ensuring the overall integrity and stability of the model.

Structure Validation of ALPHA, BETA, DELTA, and OMICRON: The PDB structure of the ALPHA variant (PDB ID:7EDH) was submitted for comparative homology modeling. Selected models of ALPHA, BETA, DELTA and OMICRON were

submitted for structural analysis to SAVES (<https://saves.mbi.ucla.edu/>) (Figure III, IV, V, VI).

Modelling of 3D structure: The structure was predicted by using MODELLER 3D

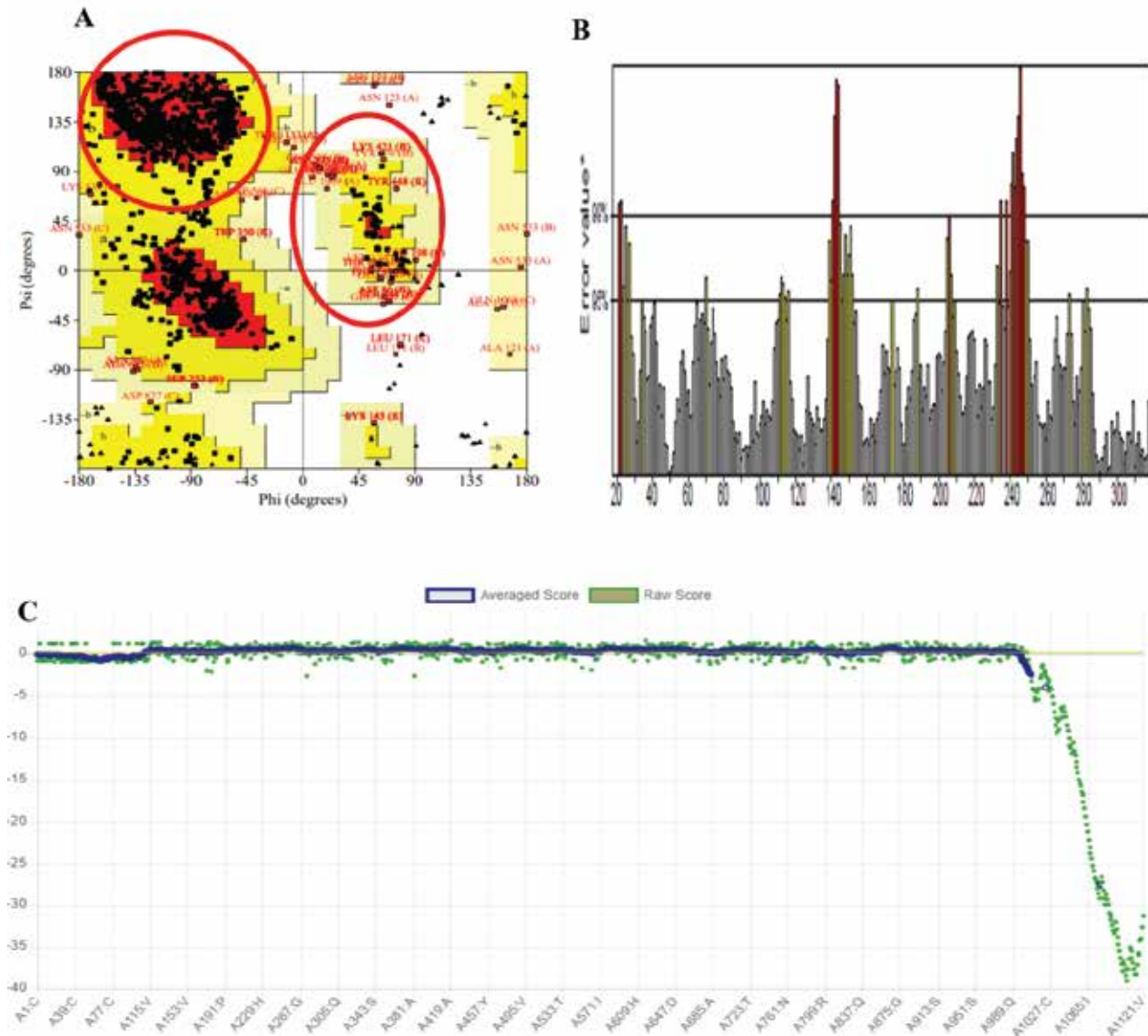


Figure VI: Structure validation of OMICRON Protein (7WVN); (A) **Ramachandran plot** of the OMICRON variant shows 84% residues in the highlighted red region. The white disallowed region has 0.1% residue and 13.4% residue in the yellow additional allowed region. Glycine residues are 216 which was shown in triangles in the favored region. The total number of residues is 3363. (B) **ERRAT plot of the OMICRON variant** shows an overall quality factor of 91%. It is considered a high-quality model. In our graph of amino acids, yellow lines lie in the warning region, which means we consider our structure to proceed. While 140 shows the red error line (C) **VERIFY 3D of OMICRON variant** shows 71.57% of residues have averaged 3D score ≥ 0.2 which is shown by the blue circles which are not a good value for a best structure. To ensure a high-quality structure, at least 80% of the amino acids should have a score of 0.2 or higher.

(<https://salilab.org/modeller/>)¹⁵. The model with the lowest Discrete Optimized Protein Energy (Dope) score has more similarity with the original model. The Root Mean Square Deviation (RMSD) value represents the average difference between the atoms of the corresponding two proteins. The lower RMSD values signify that the two structures will become more similar, is an indication of good structure. DOPE score and RMSD values of all variants (Alpha, Beta, Delta and Omicron) are

shown in Table 3 and the predicted 3D structure are shown (Figure VII).

Multiple Sequence Alignment: The Multiple sequence alignment (MSA) was produced by Clustal X (Clustal-X 2.1) (<http://www.clustal.org/clustal2/>)¹⁷ and the alignment was then visualized by using Jalview (<https://www.jalview.org/>)¹⁸ as shown in Figure VIII.

Table 3: DOPE score and RMSD values of predicted structures of all variants (Alpha, Beta, Delta, and Omicron)

Variants Name	PDB IDs	RMSD value	DOPE score
Covid (wild Type)	6VSB	0.751 A	-117690.05469
Alpha variant	7EDH	1.137 A	-101429.53125
Beta variant	7VX1	0.640 A	-241355.89062
Delta variant	7V7V	0.912 A	-222457.12500
Omicron variant	7WVN	1.175 A	-97589.40625

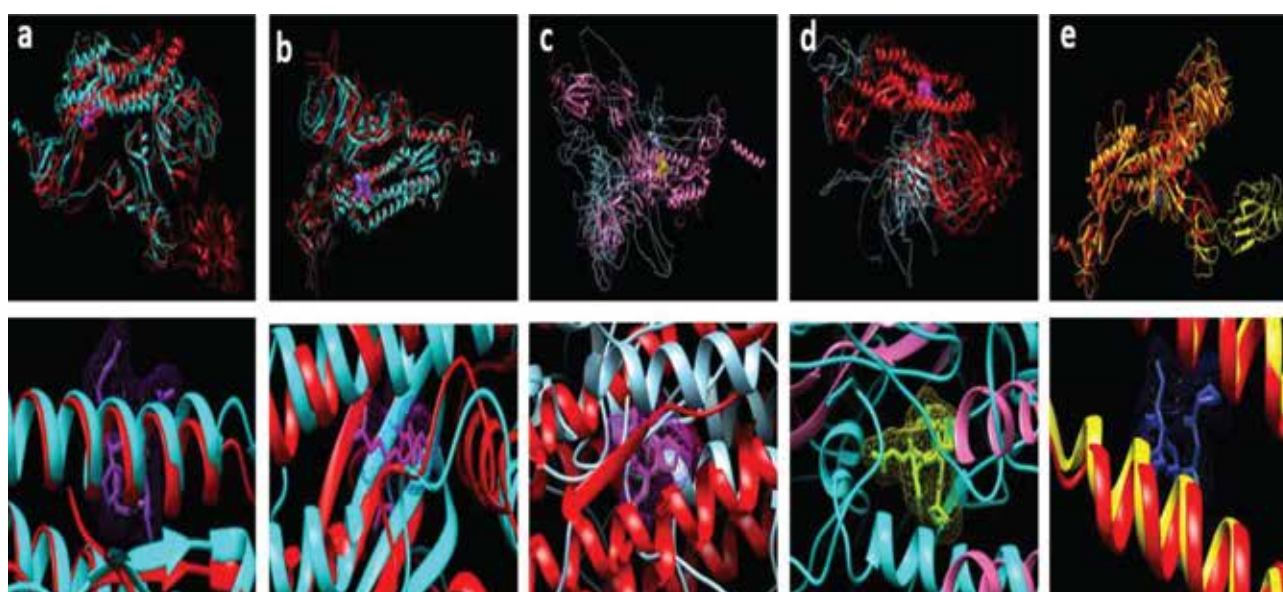


Figure VII: 3D Structure Modeling of COVID-19 Variants; (a) 3D Model of 6VSB (Spike Protein) shows red target structure and blue predicted structure. (b) 3D Model of 7EDH (ALPHA protein) also shows red target structure and blue predicted structure. (c) 3D of 7VX1 (BETA protein) shows a pink target structure and a blue predicted structure. (d) 3D Model of 7V7V (DELTA protein) shows red target and blue predicted structures. (e) 3D of 7WVN (OMICRON protein) shows yellow target and red predicted structures. In the target structure, missing residues were present, but no missing residues were found in the predicted structure. Moreover, a peptide bond is highlighted with every 3D structure, which provides stability to the protein structure.

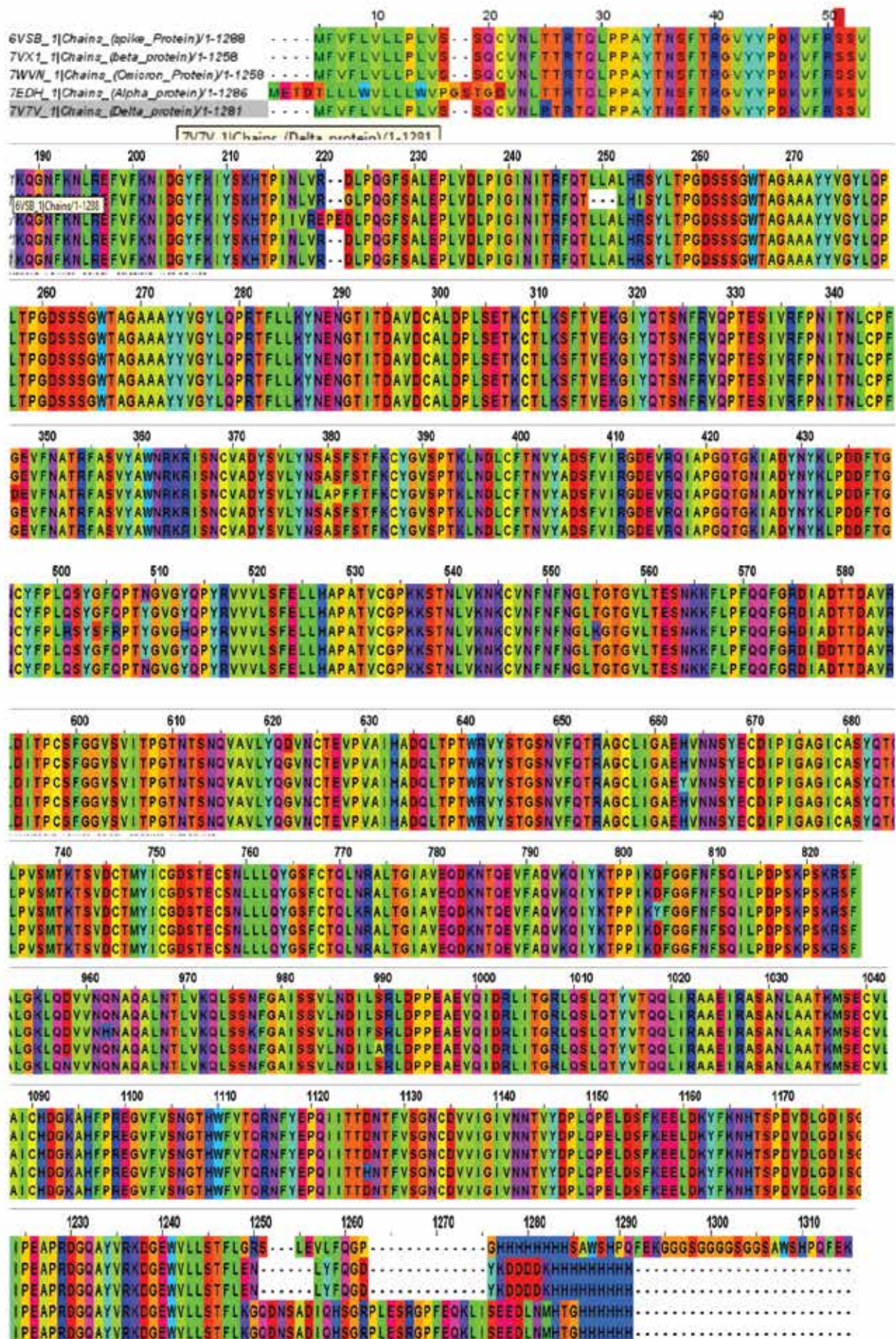


Figure VIII: Visualization of Multiple Sequence Alignment of all variants (Alpha, Beta, Delta, and Omicron); The sequence length was 1315 of all variants (Alpha, Beta, Delta, and Omicron). In MSA, Similarities were shown by the green color along the column. In the sequence of the OMICRON variant, a gap was observed at the position of 148,149,150 and represented by (-). Similarly, the gap was observed in Beta variant at positions No.221 & 222. Sequence changes brought about by insertions or deletions may affect viral characteristics like transmissibility, immune or replication efficiency are indicated by the existence of gaps. More mutational changes can be seen in omicron than in all other variants. Gaps were seen at position No.1291-1315 in Alpha, Beta, Delta, and Omicron. L residue at 379 positions in the Beta variant replaces S amino acid residue. Similarly, at position No. 1125, D residue was replaced by H in the Delta variant, which shows the point mutation. Many mutations occur in Omicron rather than Alpha, Beta and Delta variant.

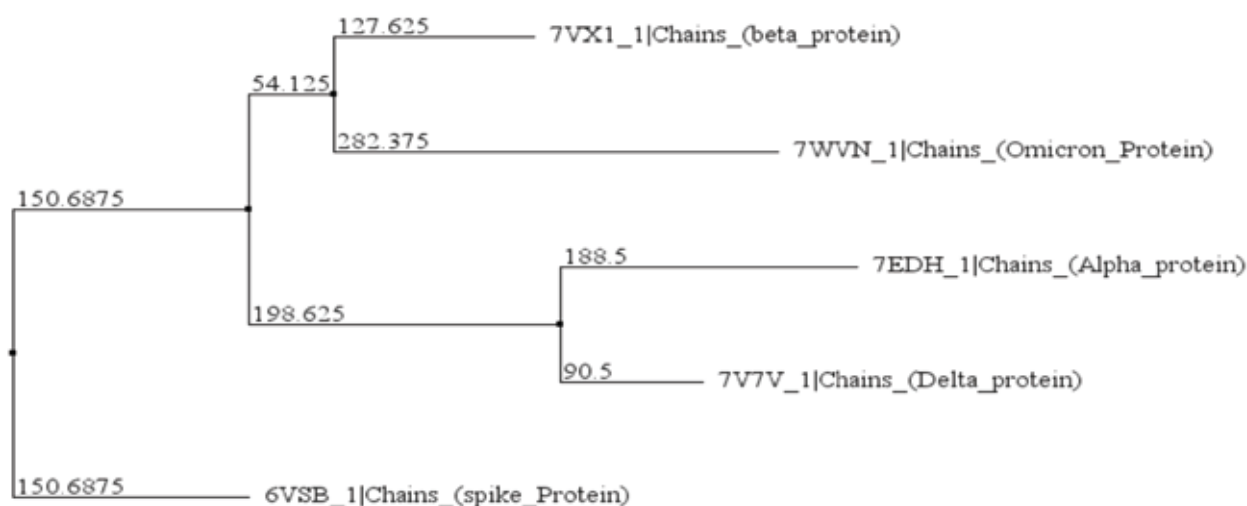


Figure IX: Phylogenetic tree of Alpha, Beta, Delta, and Omicron with calculated distance; The alpha (7EDH) variant was closely related to the delta (7V7V) variant having a distance of 198.625. Similarly, the omicron variant (7WVN) was closely related to the beta (7VX1) variant with a distance of 54.125. All four variants (Alpha, Beta, Delta, and Omicron) diverge from 6VSB, which is the wild type that originated in Wuhan. These were all represented by evolutionary distances. The phylogenetic tree branching points, where Omicron and Beta form one clade and Alpha and Delta form another, show the chronology and magnitude of mutation accumulation in each variation.

Protein-Protein interaction (PPI): For PPI, STRING (<https://string-db.org/>) tool was used. NCBI accession No. of ACE2 (NC_000023) and TMPRSS2 (NC_000021) were used. UniProt IDs of ACE2 (Q9BYF1) and TMPRSS2 (O15393) were

used. The Protein-Protein Interaction of ACE2 and TMPRSS2 was visualized by Cytoscape (<https://cytoscape.org/>). Graphs of the co-expressions of ACE2 and TMPRSS proteins were shown in Figure Xa & Xb.

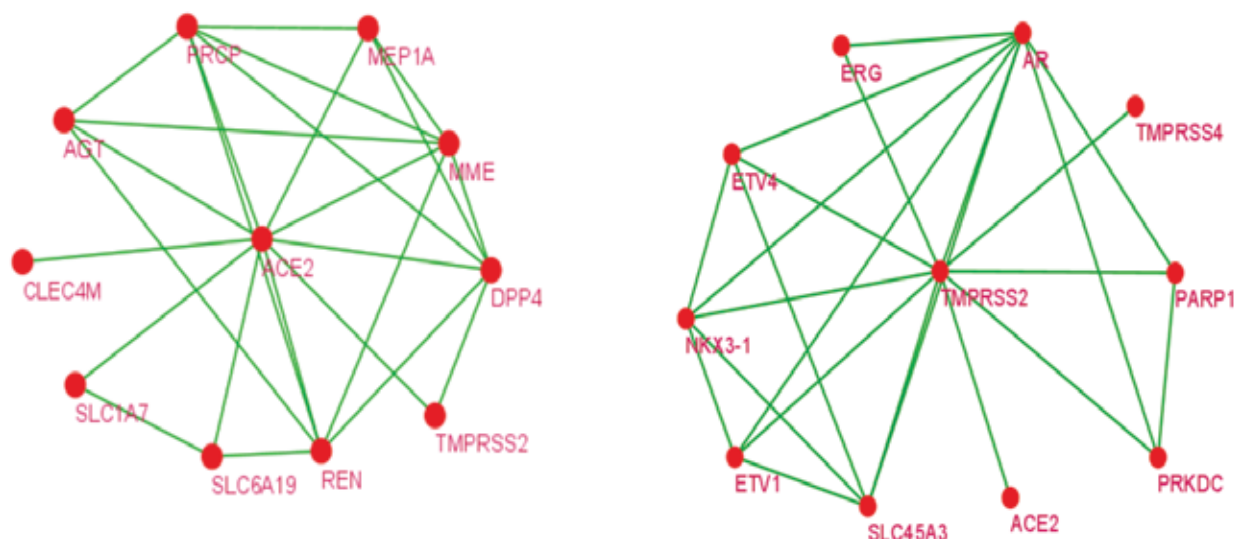


Figure Xa: Visualization of Protein-Protein Interaction of ACE2 and TMPRSS2 using Cytoscape; (A) Protein-Protein interaction network of ACE2. ACE2 shows interaction with SLC1A7, REN, DPP4, MME, CLEC4M, AGT, PRCP, and SLC6A19 and is mainly involved in protein digestion and absorption of KEGG pathway and key role in the Renin-angiotensin system KEGG pathway. (B) Protein-protein interaction of TMPRSS2; The protein-protein interaction network of TMPRSS2 revealed interactions with ETV1, ETV4, ERG, AR, SLC45A3, PRKDC, and PARP1. Additionally, TMPRSS2 is involved in the KEGG pathway of prostate cancer and plays a key role in transcriptional misregulation in cancer.

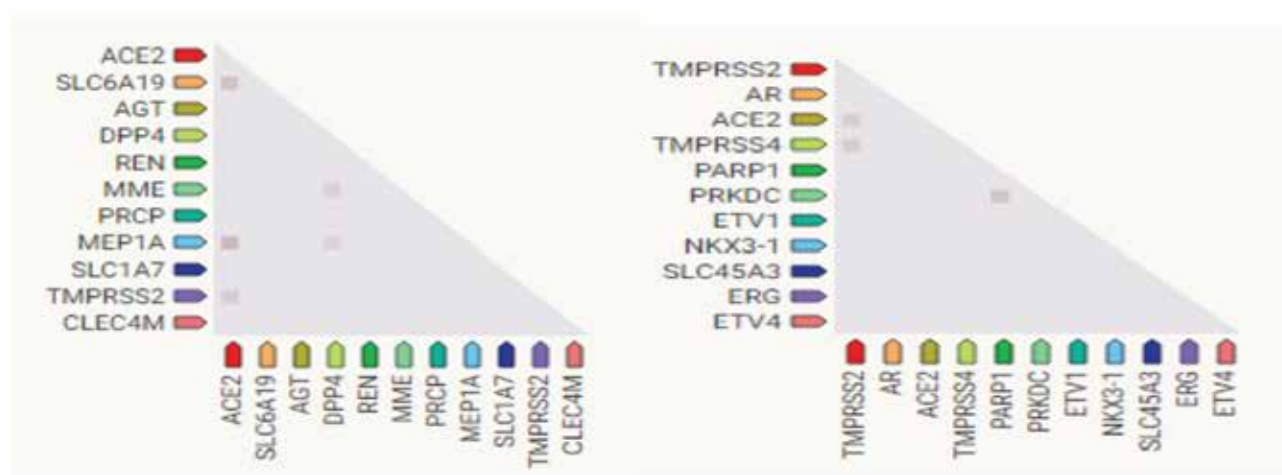


Figure Xb: Co-expressions Graphs of ACE2 and TMPRSS2; (A) Co-expression of ACE2 which indicate that ACE2 and MEP1A is highly co expressive ($p > 0.05$) (B) Co-expression of TMPRSS2, in which TMPRSS2 is highly co expressive with ACE2 expressive ($p > 0.05$). Co-expression of both proteins show variation from each other. TMPRSS2 have p-value between 0.07 and 0.09, while ACE2 have 0.05 to 0.999

Discussion

Different studies have reported the intricate relationship between the COVID-19 spike protein and ACE2, along with their mutation analysis, in an attempt to identify the key residues and penetration in the host cell²¹. ACE2 and TMPRSS2 are the two main host entry genes and their association, functional correlation and co-expression with other proteins are recently reported²². Viral spike protein priming is facilitated by TMPRSS2, which permits membrane fusion and viral entry into host cells, while ACE2 acts as the viral entry receptor¹¹. Variations in these gene's expression and activity can alter how susceptible a person or community is to infection, how severe the disease is, and how it turns out.

Genetic variations impact TMPRSS2 expression and can impact the virus's processing efficiency for cell entrance²¹. Few studies after comparing the nearest analogs of the COVID-19 protein sequence, reported that SARS associates with COVID-19 are similar to SARS in bats and pangolins and sharing 90-99% sequence identity^{23,24}. The current study reported that SARS-CoV-2 shows 92% sequence identity with 7CN8, 94.48% sequence identity of ALPHA variant with 7N1V, BETA variant shows 94.64% sequence identity with 7WBP, DELTA variant shows 96.11% sequence identity with 7SBO and OMICRON variant shows 94.84% sequence identity with 7CN4, demonstrating the good, predicted structure. Homology modeling of the SARS CoV-2 with PDB ID: 6NUR demonstrated the sequence identity of 97% with SARS-NSP12²⁵.

The Verify3D server reviewed the scores and determined that 91.0% of the residues had average 3D-ID scores ≥ 0.2 and passed, while the ERRAT server projected a quality factor of 95.0%. The model was validated by SAVES, confirming that the Ramachandran plot has 92.0% of the residues present in the allowed region, 7.2% were in the additional permitted region, and no residues were found in the disallowed region. Ramachandran plot exhibits the statistical distribution of torsion angles of a protein structure. In the current study, Ramachandran plot of SARS-CoV-2, ALPHA, BETA, DELTA, and OMICRON displays 90%, 88%, 90.9%, 87.8% and 84.0% residues in the most favorable region, respectively. ERRAT Graph of SARS-CoV-2, ALPHA, BETA, DELTA, and OMICRON indicates quality factor of 92%, 89%, 91%, 90% and 91% respectively. A study examined the structure-based sequence identity of SARS-CoV-2 using two non-structural proteins, Nsp13 and Nsp14. These proteins are crucial components of the

replication-transcription complex (RTC) and play a vital role in the infectious life cycle of SARS-CoV-2. With alignment, the RMSD value of Nsp13 is 0.259Å and Nsp14 showed RMSD value of 0.380 and a structure-based sequence identity of 94%²⁶.

The current study utilized the MODELLER software to determine the 3D structure of SARS-CoV-2 and variants, namely ALPHA, BETA, DELTA, and OMICRON. The predicted RMSD values for these variants were 0.751Å, 1.137Å, 0.640Å, 0.912Å, and 1.175Å, respectively. A model with the Lowest Dope Score has more similarity to the original model. The two structures' similarity increases with decreasing RMSD values, and therefore, it is considered a good structure. A study conducted a comprehensive analysis of the multiple sequence alignment (MSA) in different human coronavirus strains, indicating that MSA is conserved and can be easily accessed in all strains and the results were displayed using a color scheme, with red representing identical residues and yellow representing conserved residues²⁷. In the current study, multiple sequence alignment of all variants was performed. Overall, mutational changes were more prominent in the Omicron variant compared to other variants.

The green-highlighted region represents conserved residues, while the yellow-highlighted region indicates identical residues, as shown in Figure 8. TMPRSS2 has a p-value between 0.07 and 0.09, while ACE2 has 0.05 to 0.999 (Figure 11). A recently published study reported that ACE2 are strongly co-expressed with TMEM27, GLYAT, DDC, SLC13A1, and FMO1 genes having scores 8.97, 8.24, 6.46, 5.19, and 5.05, respectively and TMPRSS2 are strongly co-expressed with SLC44A4, C1orf116, TMEM125, KLK2 and HOXB13 genes having scores 12.35, 8.57, 7.12, 5.90, and 5.43, respectively²⁸. In this study, ACE2 is co-expressed with genes SLC1A7, REN, DPP4, MME, CLEC4M, AGT PRCP, and SLC6A1 having scores 0.99, 0.96, 0.98, 0.96, 0.89, 0.99, 0.92 and 0.92 respectively involved in many signaling pathways. TMPRSS2 is co-expressed with genes ETV1, ETV4, ERG, AR, SLC45A3, PRKDC, and PARP1 having scores 0.83, 0.73, 0.75, 0.98, 0.80, 0.83, and 0.84 respectively are involved in many regulating signaling pathways. All proteins in ACE2 showed interaction in severe acute respiratory syndrome (SARS).

Conclusion

The current study investigated different variants of COVID-19, their mutational changes, 3D structure modelling and protein-protein interaction. The

coronavirus beta variant is 50% more contagious than the primary strain. The Delta variant is 80 to 90% more highly contagious and can spread infection more than two times as quickly as the Alpha variant. Homology modeling of the SARS CoV-2 with PDB ID: 6NUR demonstrated the sequence identity of 97% with SARS-NSP12. The study ensures excellent accuracy and improves reliability by using a variety of computational tools for structure validation. It offers greater insights into infection mechanisms by using STRING and CYTOSCAPE to explore the interaction between ACE2 and TMPRSS2, the two important proteins in Covid-19 viral entry. In structure modeling, there were missing residues in protein 3D structure but after prediction, no missing residue was found in our predicted structure. In addition, the research investigated genes such as SLC1A7, REN, DPP4, MME, CLEC4M, AGT PRCP, and SLC6A1 that are highly co-expressed with ACE2. Additionally, genes such as ETV1, ETV4, ERG, AR, SLC45A3, PRKDC, and PARP1 were found to be highly co-expressed with TMPRSS2. The findings of the current study will open the door for genome and protein investigations of SARS-COVID-19 for further research and give important information for creating innovative, efficient, secure medications and personalized vaccines to treat COVID-19.

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None

Conflict of interest:

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

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Authors' contributions

Syed Ali Raza Shah; Conceptualization, Writing Original Draft, Supervision, Project Administration.: Iffat Nayila, Kushaf Mohsin, Sumaira Sharif; Preparation And Design The Project.: Syed Ali Raza Shah And Iffat Nayila; Resources, Review And Analyze The Experiments.: Imtiaz Mustafa And Zarish Zahid; Analysis, Revision Of The Draft. All Authors Have Read And Agreed To The Published Version Of The Manuscript.

Data Availability

All Data Generated For This Study Is Included In Article.

Ethics Approval and Consent to Participate

No Ethical Approval Was Required To Accomplish Writing Draft Of This Article. All Authors Gave Their Consent To Publish This Article

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References

- Sharma A, Tiwari S, Deb MK, Marty JL. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): a global pandemic and treatment strategies. *Int J Antimicrob Agents*. 2020;56(2):106054
- Pormohammad A, Ghorbani S, Khatami A, Farzi R, Baradaran B, Turner DL, et al. Comparison of confirmed COVID-19 with SARS and MERS cases-Clinical characteristics, laboratory findings, radiographic signs and outcomes: a systematic review and meta-analysis. *Rev Med Virol*. 2020;30(4):e2112
- Schoeman D, Gordon B, Fielding BC. Coronaviruses. *Encycl Infect Immun*. 2022;241
- Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. *Nat Rev Mol cell Biol*. 2022;23(1):3–20.
- Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181(2):271–80
- Fawzy MS, Ashour H, Shafie AAA, Dahman NBH, Fares AM, Antar S, et al. The role of angiotensin-converting enzyme 2 (ACE2) genetic variations in COVID-19 infection: a literature review. *Egypt J Med Hum Genet*. 2022;23(1):97
- Tian F, Tong B, Sun L, Shi S, Zheng B, Wang Z, et al. N501Y mutation of spike protein in SARS-CoV-2 strengthens its binding to receptor ACE2. *Elife*. 2021;10:e69091
- Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, et al. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. *Nat Commun*. 2020;11(1):6013
- Casella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, evaluation, and Treatment of Coronavirus (COVID-19). 2023. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024
- Zabidi NZ, Liew HL, Farouk IA, Puniyamurti A, Yip AJW, Wijesinghe VN, et al. Evolution of SARS-CoV-2 variants: implications on immune escape, vaccination, therapeutic and diagnostic strategies. *Viruses*. 2023;15(4):944
- Dhawan M, Sharma A, Priyanka N, Thakur N, Rajkhowa TK, Choudhary OP. Delta variant (B. 1.617. 2) of SARS-CoV-2: Mutations, impact, challenges and possible solutions. *Hum Vaccin Immunother*. 2022;18(5):2068883

12. Chekol Abebe E, Tirunch G/Medhin M, Behaile T/Mariam A, Asmamaw Dejenie T, Mengie Ayele T, Tadele Admasu F, et al. Mutational pattern, impacts and potential preventive strategies of omicron SARS-CoV-2 variant infection. *Infect Drug Resist.* 2022;1871–87
13. Rodrigues JPGLM, Teixeira JMC, Trellet M, Bonvin AMJJ. Pdb-tools: A swiss army knife for molecular structures [version 1; referees: 2 approved]. *F1000Research.* 2018;
14. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL : homology modelling of protein structures and complexes. 2018;46 (May):296–303
15. Webb B, Sali A. Comparative protein structure modeling using MODELLER. *Curr Protoc Bioinforma.* 2016;54(1):5-6
16. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. UCSF Chimera - A visualization system for exploratory research and analysis. *J Comput Chem.* 2004;25(13):1605-1612
17. Larkin MA, Blackshields G, Brown NP, Chenna R, Mcgettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD. Clustal W and Clustal X version 2.0. *Bioinformatics.* 2007;23(21):2947-2948
18. Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ. Jalview Version 2-A multiple sequence alignment editor and analysis workbench. *Bioinformatics.* 2009;25(9):1189-1191
19. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: Customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* 2021;49(D1):D605-612
20. Bateman A. UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Res.* 2019;47(D1):D506-D515
21. Pascarella G, Strumia A, Piliago C, Bruno F, Del Buono R, Costa F, Scarlata S, Agrò FE. COVID-19 diagnosis and management: a comprehensive review. *J Intern Med.* 2020;28(2):192-206
22. Rossi AD, de Araújo JLF, de Almeida TB, Ribeiro-Alves M, de Almeida Velozo C, de Carvalho Leitão I, et al . Association between ACE2 and TMPRSS2 nasopharyngeal expression and COVID-19 respiratory distress. *Sci Rep.* 2021;11(1):9658
23. Wacharapluesadee S, Tan CW, Maneecorn P, Duengkak P, Zhu F, Joyjinda Y, et al. Evidence for SARS-CoV-2 related coronaviruses circulating in bats and pangolins in Southeast Asia. *Nat Commun.* 2021;12(1):972
24. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol.* 2021;19(3):141–54
25. Athar F, Beg MA. Anti-HIV and Anti-HCV drugs are the putative inhibitors of RNA-dependent-RNA polymerase activity of NSP12 of the SARS CoV-2 (COVID-19). *Pharm Pharmacol Int J.* 2020;8(3):163–72
26. Gurung AB. In silico structure modelling of SARS-CoV-2 Nsp13 helicase and Nsp14 and repurposing of FDA approved antiviral drugs as dual inhibitors. *Gene Rep.* 2020;21:100860
27. Elfiky AA. Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study. *Life Sci.* 2020;253:117592
28. Hossain MS, Tonmoy MIQ, Fariha A, Islam MS, Roy AS, Islam MN, Kar K, Alam MR, Rahaman MM. Prediction of the Effects of Variants and Differential Expression of Key Host Genes ACE2, TMPRSS2, and FURIN in SARS-CoV-2 Pathogenesis: An In Silico Approach. *Bioinform Biol Insights.* 2021;15:11779322211054684.