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Research article

Exploring the binding affinity landscape of SARS-CoV-2 variants: A Computational Approach

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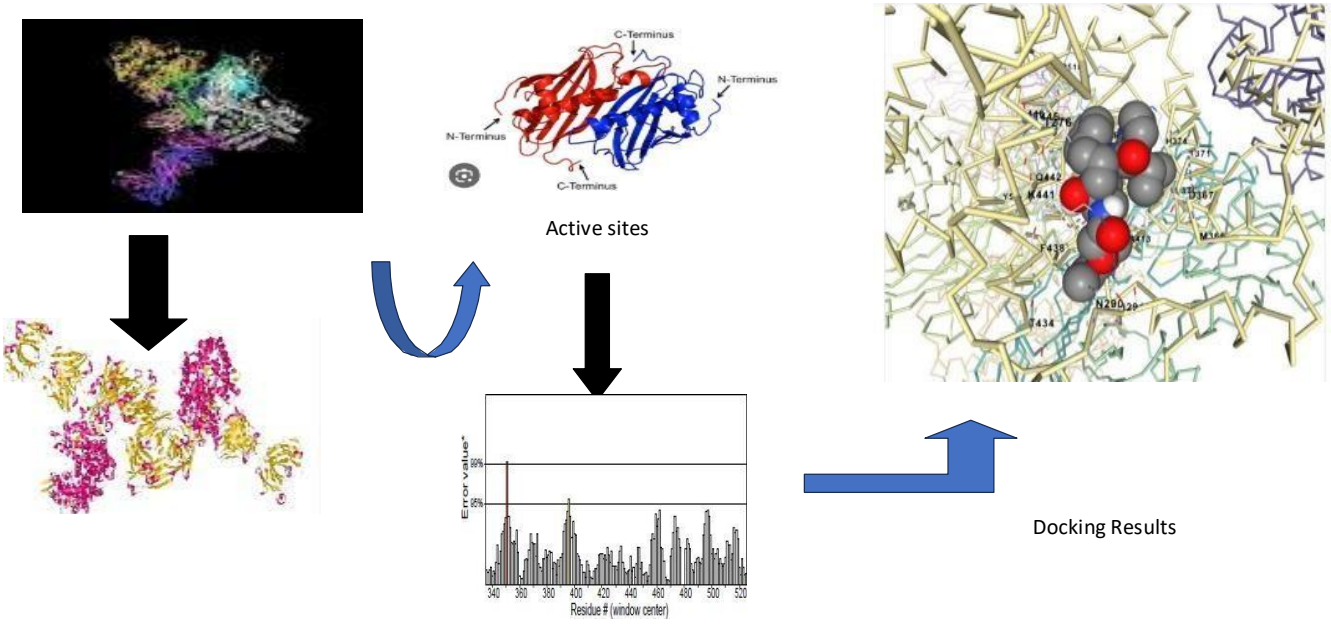
ABSTRACT

The emergence of SARS-CoV-2 variants, like Beta, poses a challenge due to potential changes in viral infectivity and immune escape. This study employs computational tools to analyze the interaction between the Receptor Binding Domain (RBD) of the SARS-CoV-2 Beta variant and the human Angiotensin-Converting Enzyme 2 (ACE2) receptor. Additionally, the potential inhibitory effect of an S304 Fab antibody fragment on this interaction is investigated. Protein structures were visualized using RASMOL/PYMOL and validated with ERRAT/PROCHECK. Docking simulations with the CB-DOCK server were performed to predict the binding mode and affinity of S304 Fab to the Beta variant RBD- ACE2 complex. Our results provide insights into the structural features of the Beta variant RBD-ACE2 interaction and predict potential binding sites for the S304 Fab fragment. The findings contribute to understanding viral entry mechanisms for the Beta variant and suggest S304 Fab as a likely candidate for further investigation as a therapeutic strategy.



Keywords: *Sars- CoV-2, ACE2, S304 Fab Fragment, RBD, Molecular Docking*

INTRODUCTION

The current scenario with the COVID-19 pandemic creates an urgent need for effective therapeutic involvement. The computational methods play a crucial role in drug discovery which allow researchers to analyze binding affinities of proteins with their structural modification and help in exploring potential drug candidates for future use. There are various databases and research tools available for research concerning drugs. Researchers have easy access to tolls and protein databases like PDB (Protein Data Bank), Compound databases like PubChem, and software like Schrodinger suite, Autodock, Autodockvina, and PyRx [1].

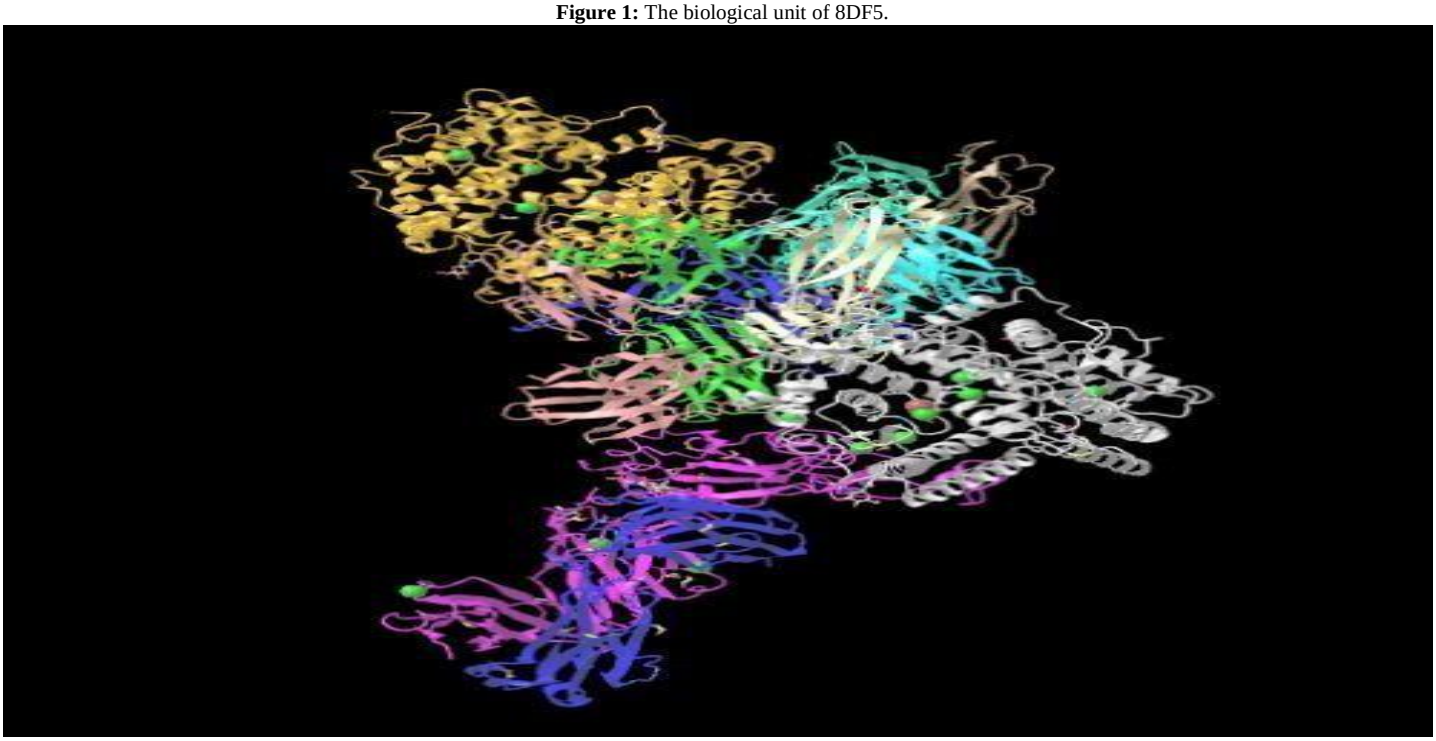
In this fight against respiratory viral infections, one of the most powerful tools that emerge is CADD (Computer Aided Drug Discovery), which allows researchers to virtually study the protein-ligand interactions and help discover potential drug candidates [8]. This paper delves into CADD and specifically focuses on studying structure-based docking of SARS-CoV-2 Beta RBD in complex with human ACE2 and S304 Fab fragments [2].

There are two approaches in computational methods one involves structure-based drug design (SBDD) and the other is ligand-based drug design (LBDD) [3]. Molecular docking is one of the key techniques in SBDD and the software simulates the interaction between a protein and a potential hit, a Ligand. This will predict the binding mode and protein affinity with the ligand. This in silico experimental technique aids in evaluating a vast number of candidate molecules efficiently with a single protein or set of proteins [4]. This paper majorly emphasizes the receptor-binding domain (RBD) of the SARS-CoV-2 virus spike protein and RBD is very important for viral entry inside the host cells. This RBD will bind to human angiotensin-converting enzyme 2 (ACE2) [19]. We were likely to investigate the potential of S304 Fab in disrupting this interaction of RBD with viral spike protein [16]. S304 Fab is a fragment of an antibody. We utilize CADD simulations and evaluate how well S304 Fab binds to the RBD-ACE2 Complex so that its potential as a therapeutic can be revealed. This approach can significantly fast-track the drug discovery process and support effective therapeutics against COVID-19 [5].

As per the recent studies conducted the receptor binding domain (RBD) of the spike protein (S-protein) emerged as a promising antigen to develop a specific antibody detection tool [9]. Antibodies that target RBD help in neutralizing SARS-Cov-2 as they block ACE2 binding and to date number of such antibodies have been discovered [12].

A solidarity trial was launched by WHO, during the pandemic time so that scientists could focus on testing the expectant treatments. Such drugs were opt-out for corroborating COVID- 19 treatments are remdesivir; chloroquine and hydroxy-chloroquine; lopinavir plus ritonavir; and lopinavir plus ritonavir and interferon-beta [6].

Figure 1: The biological unit of 8DF5.



The molecular graphic as obtained from Molecular Modeling Database [MMDB] (https://www.ncbi.nlm.nih. gov/Structure/MMDB/mmdb. shtml) is The multi-prolonged approach used the software tools beyond just docking in the present work. They are RasMol, PyMol, ERRAT, PROCHECK, and CB dock [10]. Rasmol and PyMol are used for the visualization and analysis of 3d structures. ERRAT & PROCHECK are the potential tools that will help assess the protein structure's quality and validity [11]. RMSD metric is used to

evaluate the conformational changes in the protein when it binds. CB-DOCK specifically helps to simulate the interactions between the molecules [6].

MATERIALS AND METHODS

This study implies the use of CADD methods, namely structure-based molecular docking, to examine how 8DF5—possibly a particular variation of the SARS-CoV-2 Beta RBD— interacts with human ACE2 and an antibody fragment called S304 Fab. Here is a possible breakdown of the approaches used:

Protein and Antibody Structure Preparation

We acquired the 3D structure of 8DF5 and retrieved it from the protein database that is PDB (protein data bank) &MMDB (Molecular modelling database). RASMOL AND PyMOL were used to visualize and analyze the structures in detail. Tools like ERRAT AND PROCHECK are employed to assess the validity and quality of the obtained structures and to ensure their suitability for docking simulations. This helps in reliability for the subsequent analysis.

Molecular Docking

For molecular docking, a docking program called CB-Dock was used to simulate the binding sites. In this, there are various parameters which were considered like molecular shape, electrostatic interactions, and hydrophobicity. This will predict the binding orientation and affinity of S304Fab to the 8DF5-ACE2 complex. During this stage, the specific binding site on the complex will be defined for S304 Fab to the target.

Analysis of Docking Results

Binding pose was predicted and estimated binding affinity was estimated by using docking simulations. The results were assessed for favourability and strength of interaction between S304 Fab and the target complex.

Conformational Analysis

RMSD was calculated to evaluate the structural similarity between the docked complex and the known structure. RMSD analysis helps in revealing how significantly the 8df5 structure and human ACE 2 alter upon binding to S304 fab. This will provide insights into the action mechanism of S304 Fab

RESULTS

The key findings obtained from the computational approach and structure-based docking simulations reveal the interplay between 8DF5, ACE2, and S304 Fab, an antibody fragment.

RasMol

Shapely is a Python library used in RASMOL and it is utilized for working with and analysing geometric objects. RasMol is a molecular visualization tool to understand molecular structures. Shapely is a Python library mostly used for manipulation in RasMol. It has specifically no direct role with RasMol [17]. It is mostly put into work for creating,

Analyzing, and manipulating geometries like angles, lines, and polygons primarily in 2-D space. Amino acid study is done using Shapely.

Figure 2: A visual representation showcasing the correlation between different colors and the structure of the protein can be summarized as a chart or a diagram illustrating how various colors symbolically represent different aspects or components of protein structures.

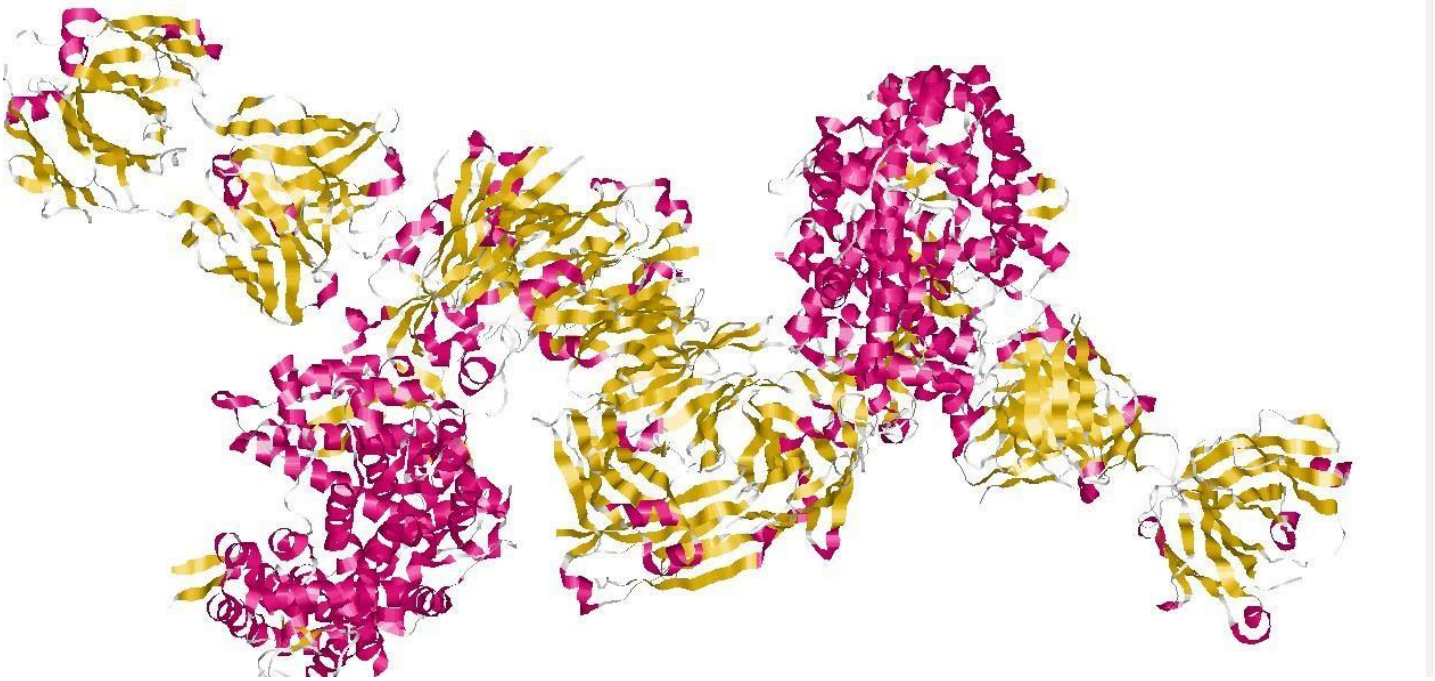


Table 1: Depicts the colour of different protein conformations

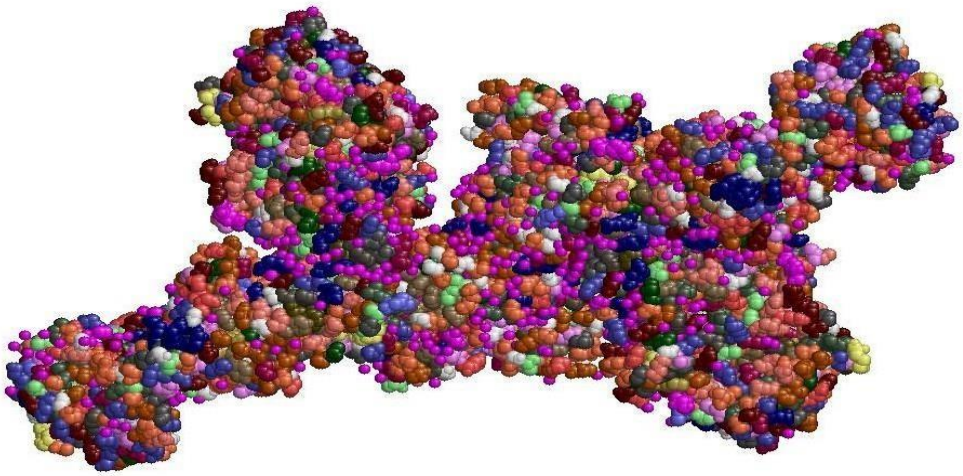
COLORS	PROTEIN STRUCTURE
Yellow	Alpha helix
Magenta	Beta pleated sheets
White	Residue

To get the total number of hydrogen bonds in the protein structure we used a command line inRASMOL and identified the total number of hydrogen bonds 2228

Command Line: RasMol> hbond on

It mainly depicts VanderWaals's sphere representation as a way to depict the spatial arrangement of atoms or molecules with molecular modeling. This space-fill model signifies each atom in the protein structure as a solid sphere within its radius corresponding to Vander Waals radius.

Figure 3: Depicting Spacefill in vanderwaal sphere



PyMol

Chain Identification in PyMol

Figure 4: Identification of Chain in PyMOL The total atoms present in the molecule: 26464 atoms

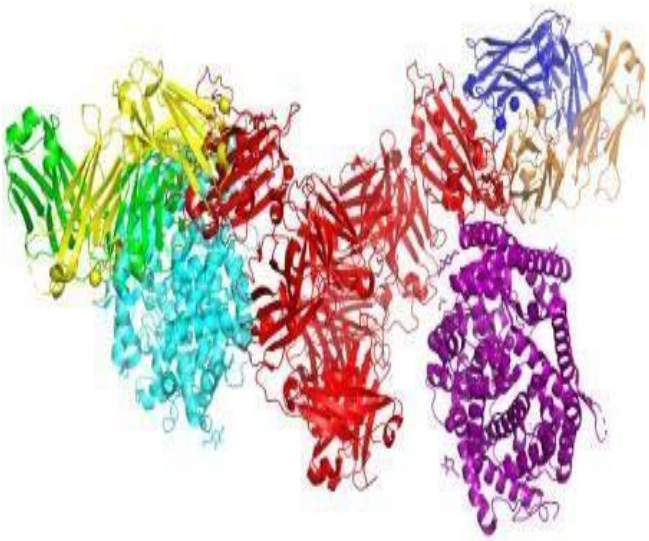
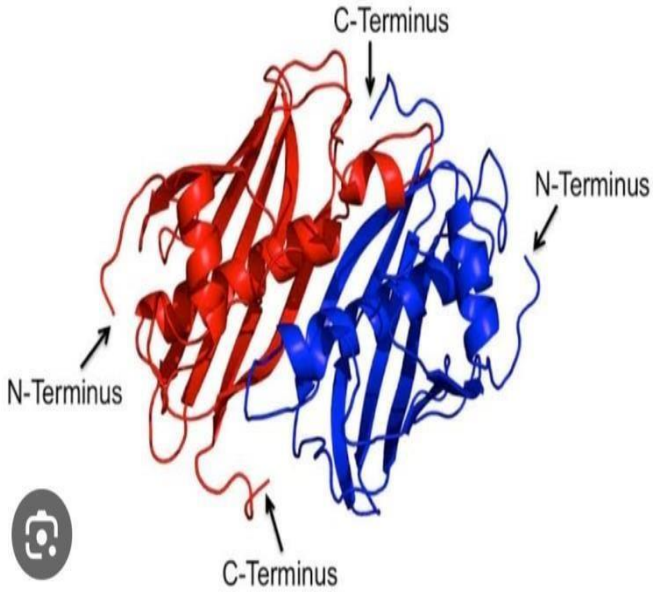


Table 2: Denotes Chain Identification using PyMol

COLOR	AA CHAIN	No of atoms	Command Line
Yellow	Chain A	1678 atoms	PyMOL>color yellow, Chain A
Green	Chain B	1641 atoms	PyMOL>color green, chain B
Blue	Chain C	1720 atoms	PyMOL>color blue, chain C
Orange	Chain D	1618 atoms	PyMOL>color orange, chain D
Cyan	Chain E	4904 atoms	PyMOL>color cyan, chain E
Purple	Chain F	5060 atoms	PyMOL>color purple, chain F
Red	Chain G	28 atoms	PyMOL>color red, chain G

Using PyMol, the active sites are denoted by red and yellow color for chain B and chain C of the protein [15].

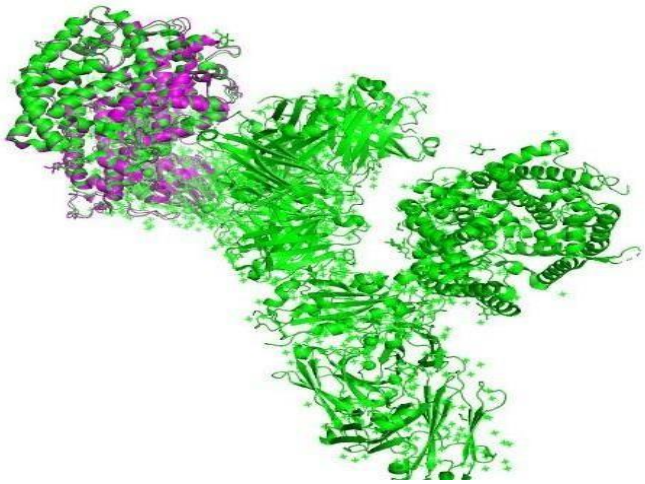
Figure 5: Representation of active sites in Chain B and Chain C



RMSD Values in PyMol

RMSD values are presented in Å and calculated for the protein structure. RMSD values are considered reliable indicators of variability when applied to very similar proteins, like alternative conformations of the same protein [18]. We calculated with CHAIN A. In PyMol there is a predefined scoring matrix that provides scores to various amino acid residue alignments [19]. It calculated the pairwise scores between all possible alignments for 8DF5 & 8fxb i.e. with 4988 residue and 1575 residues. The alignment score reported is 5622.500 in our study. The higher the score better the alignment. RMSD calculated for the given structures is 1.132 Angstroms for 4996 atoms for each protein and this is considered to be a good fit.

Figure 6: RMSD calculation of 2 protein samples (8DF5, 8fxb)



The residues from 8DF5 are colored in green and 8fxb are colored in Magenta in the PyMol scene.

Structure Validation

For the present study, we have done structure validation using ERRAT and PROCHECK.

ERRAT

ERRAT is a structure validation server. It works on analyzing the statistics of non-bonded interactions between different atom types and plots the value of the error function versus the position of a 9-residue sliding window, calculated by comparison with statistics from highly refined structures.

The ERRAT plot shows the error value of residue. The regions that exceed the error value will be rejected. There are error and non-error regions.

Figure 7: ERRAT analysis of Chain A with an Overall quality factor: of 95.695 where yellowand red denote the rejected portion of the error protein

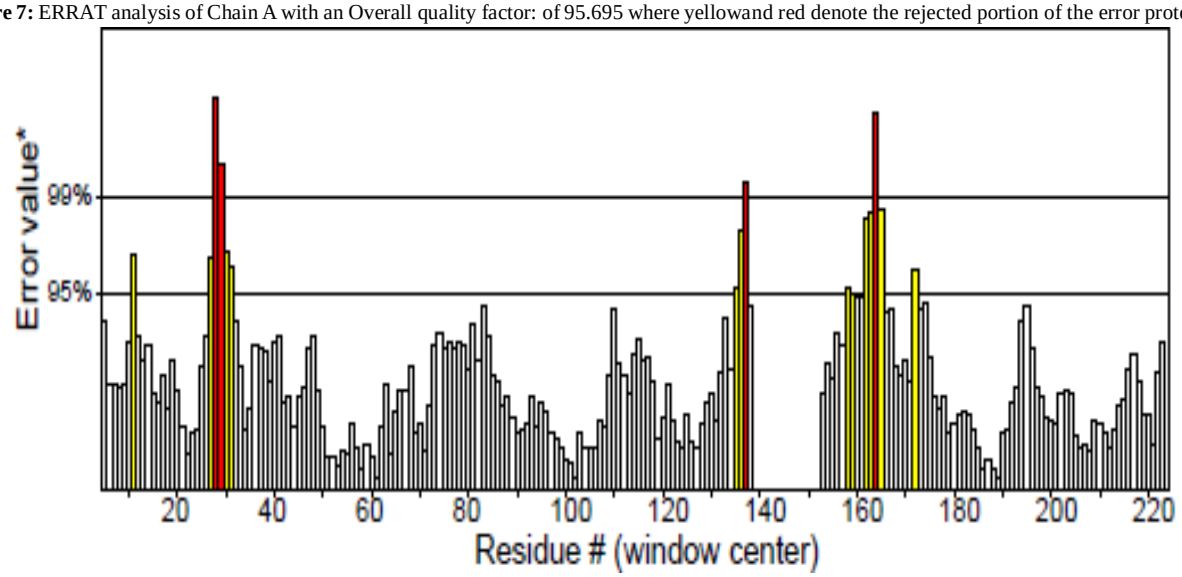
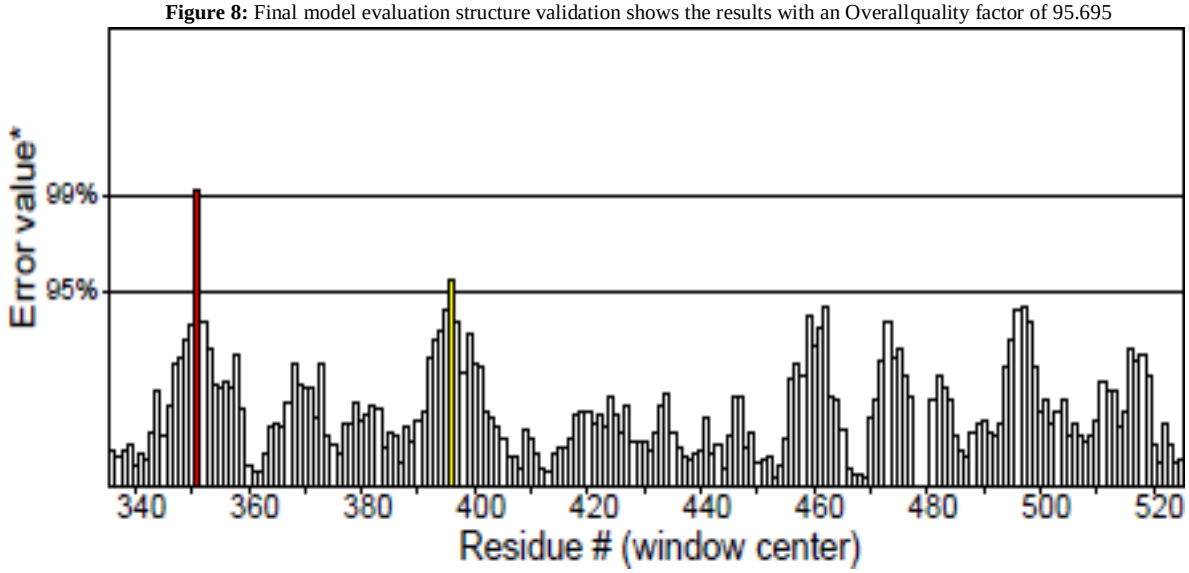


Figure 8: Final model evaluation structure validation shows the results with an Overallquality factor of 95.695



The black bar in ERRAT results showcases misfolded regions that are available at a distant sitefrom the active site. The Gray ones denote the error region between 95% to 99% and the whitebars represent low error regions of protein folding.

Procheck

It checks the stereochemical quality of a protein structure by analysing residue-by-residuegeometry and overall structure geometry. The results are generated in the form of a Ramachandran plot.

Figure 9: Ramachandran plot summary generated by procheck

```
+----------<<< P R O C H E C K  S U M M A R Y >>>----------+
|                                                          |
| /var/www/SAVES/Jobs/1629207/saves.pdb  1.5   3324 residues |
|                                                          |
*| Ramachandran plot:  91.9% core  7.9% allow  0.0% gener  0.1% disall |
|                                                          |
  +| All Ramachandrans:  34 labelled residues (out of3290)      |
   +| Chi1-chi2 plots:  11 labelled residues (out of1742)       |
    | Side-chain params:  5 better  0 inside  0 worse           |
    |                                                          |
   *| Residue properties: Max.deviation:  5.2  Bad contacts:  2 |
    *|  Bond len/angle:  5.9  Morris et al class:  1  2  2 |
     *|  20 cis-peptides                                      |
   | G-factors  Dihedrals:  -0.19 Covalent:  0.51  Overall:  0.10 |
    |                                                          |
   | Planar groups:  100.0% within limits  0.0% highlighted    |
    |                                                          |
     +--------------------------------------------------------------------+
     + May be worth investigating further.  * Worth investigating further.
```

VALIDATION SERVER showing the analysis of SARS-cov-2 Beta RBD in complex with human ACE2 and S304 Fab.

CB DOCK: Cavity Detection Guided Blind Docking

CB-Dock: This is the original version of the server. CB-Dock2: This is an improved version of the server that combines CB-Dock's methods with another technique called homologous template fitting. This can improve the accuracy of the predictions, especially for proteins that have similar structures to known proteins [20].

This tool is employed in computational work while working on protein-ligand docking simulations [21]. It predicts how well a ligand will bind to a protein. It facilitates molecular docking using multiple features like the detection of cavities, homologous template fitting, and template-based molecular docking. It is used for various purposes by developers and researchers. It finds its purpose in drug discovery, target findings, and drug design. This server is freely available to use online. CB dock is user-friendly and does not require extensive programming knowledge [3]. In working, we can upload the Ligand and protein files in a definite format, followed by blind docking. Upon completion of docking, we will obtain the pockets where homology modeling can be performed. After the results, the ligands can be ranked based on binding affinities. Lower the binding energy and strengthen the binding. This helps in HIT identification. The ligands with favorable binding affinities are considered potential drug candidates. For the study, we docked two ligands against the protein: Chloroquine and Lopinavir [15]. These are the most common drugs used for SARS-CoV-2, according to an extensive literature survey [22].

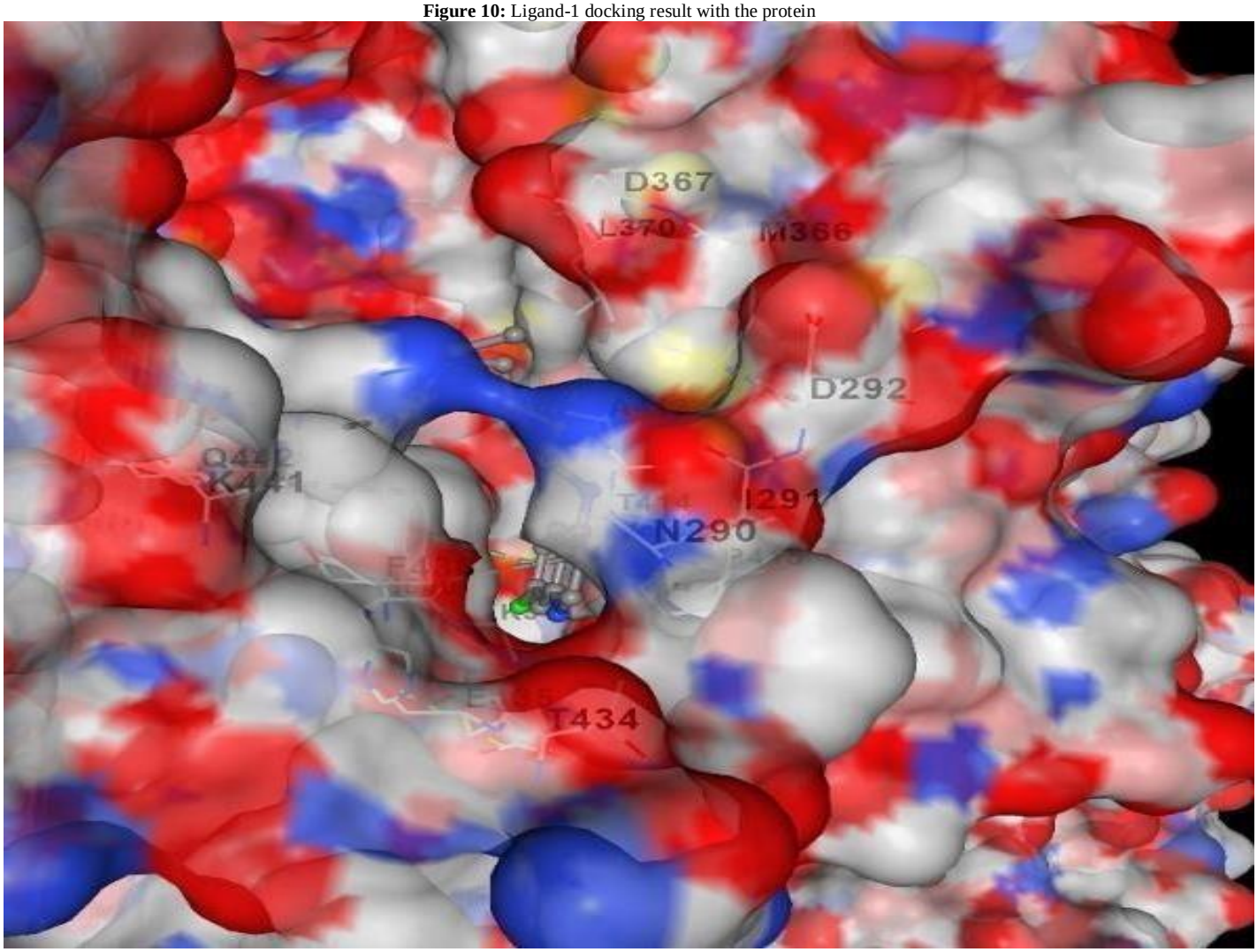
Ligand Chloroquinine

The PubChem ID is 2719 and the file is downloaded from PubChem and stored in MDL SD file format. The Ligand was docked with our protein and the following results were obtained.

Table 3: Docking results of Protein with Ligand-1

CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
◉ C2	-7.1	3094	-55, 68, 30	29, 23, 23
○ C1	-6.9	3127	-19, 3, 104	35, 23, 23
○ C4	-6.7	1424	-11, 36, 70	23, 30, 23
○ C5	-6.1	1374	-20, -24, 78	32, 23, 23
○ C3	-5.9	1985	-33, 33, -5	23, 23, 23

Figure 10: Ligand-1 docking result with the protein



Ligand Lopinavir

The PubChem ID is 92727 and the docking results are summarised as follows:

Table 4: Docking results of Protein with Ligand-2

CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
◉ C2	-8.6	3094	-55, 68, 30	24, 24, 24
○ C1	-8.3	3127	-19, 3, 104	35, 24, 24
○ C5	-8.1	1374	-20, -24, 78	32, 24, 24
○ C3	-7.8	1985	-33, 33, -5	24, 24, 24
○ C4	-6.3	1424	-11, 36, 70	24, 30, 24

Chain F: ARG273 PHE274 THR276 ASN277 TYR279 ASN290 ILE291 PRO346 MET366 ASP367 LEU370 THR371 HIS374 GLU375 GLY405 GLU406 SER409 LEU410 ALA413 THR414 PRO415 THR434 ASN437 PHE438 LYS441 GLN442 THR445 ILE446 ARG518 GLN522 PHE523 TYR587

Figure 11: Docking results with Ligand 2

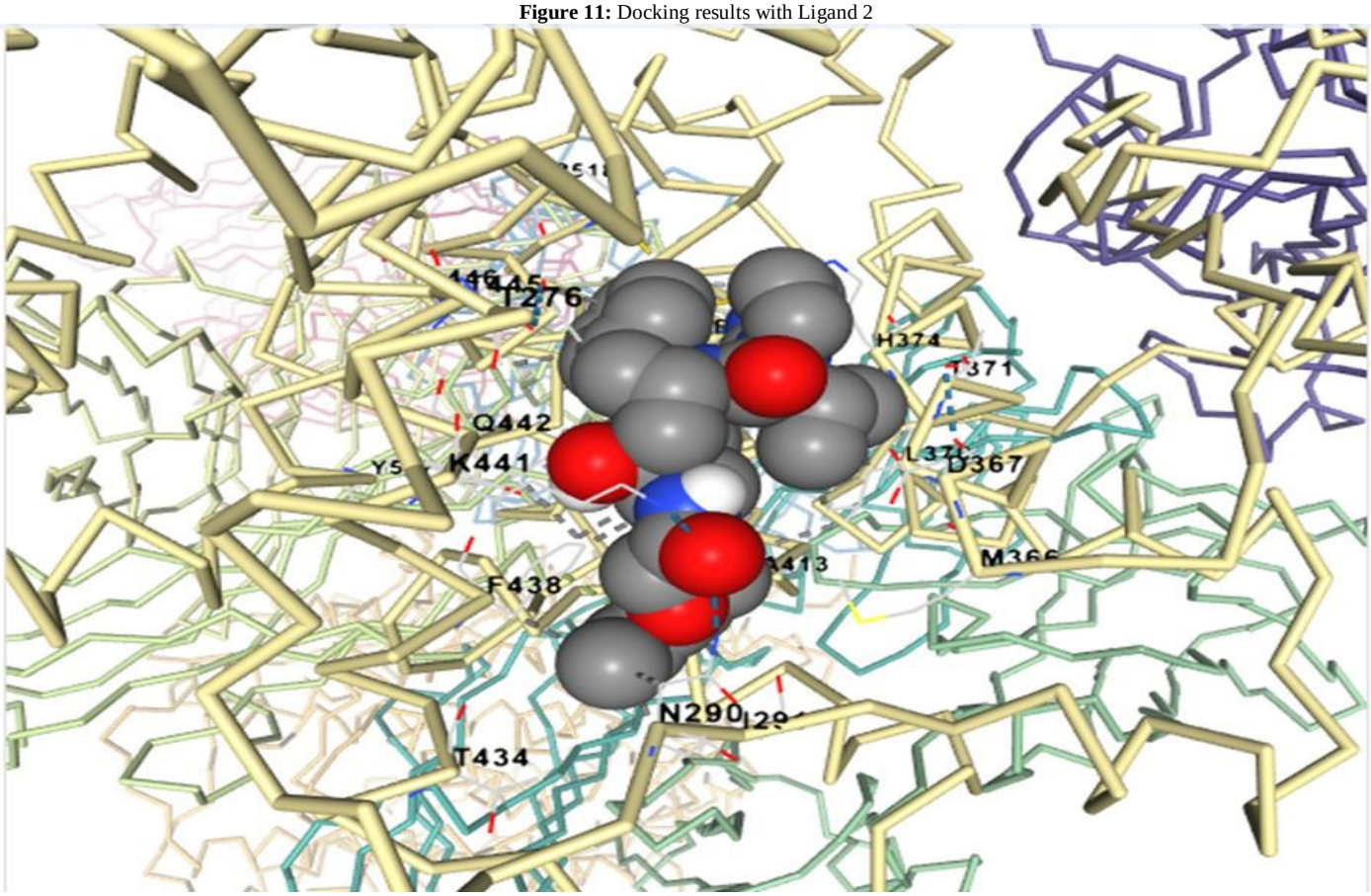
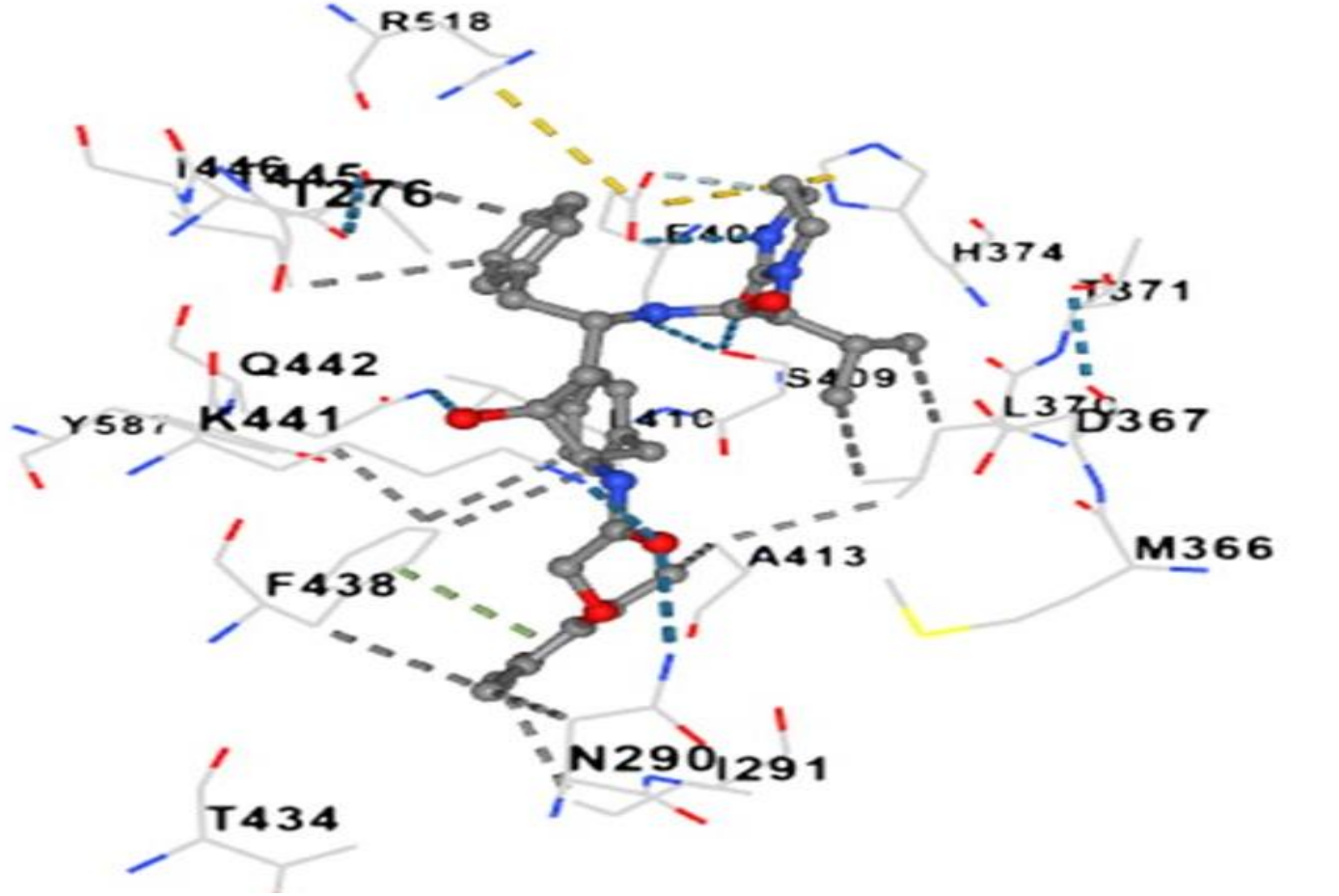


Figure 12: The figure depicts the binding sites between the ligand and the protein



CONCLUSIONS

Our computational analysis provides valuable insights into the molecular interactions of the SARS-CoV-2 Beta variant. This will enhance the understanding of potential therapeutic targets which may aid in the design of novel antiviral drugs. In the structure, the variant contains 4270 nitrogen atoms, 6134 oxygen atoms, 16357 carbon atoms, 2 zinc atoms, and 120 sulfur atoms. Several H bonds identified in our structure were studied using RasMol was 2228.

Our ERRAT scores (95.695) are above 90 so it denotes good quality protein structures. As higher the score more likely the structure is reliable and representative of real protein. The average difference between the corresponding atoms in the docked complex and the unbound structure of 8DF5, ACE2 & S304 in our case is represented by RMSD values. The RMSD Value in this case is in the range of 1-2 Angstroms which is generally considered a good indication for a good structural fit. The analysis shows that both samples have been infected with the SARS-CoV-2 virus, as indicated by the RMSD value of 1.132 Angstrom. This similarity in RMSD values suggests that the virus has comparably affected both samples. The specific number of atoms included in the RMSD calculation is 4996 to 4996 atoms for each structure respectively. Generally, lower docking scores represent stronger and more favorable binding interactions [7]. Here Ligand1 shows a score of -7.1 and Ligand 2 shows a score of -8.6. This signifies that Ligand1 has a predicted binding affinity with the target molecule. As per the docking result of Ligand 2, it binds with the C2 pocket and the results suggest a potential interaction between the ligand and pocket on the target molecule however further analysis and validation are needed to determine the biological significance of the interaction.

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