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Next-Generation Sequencing Workflow Study Of SARS-Cov-2 Beta RBD in Complex with Human ACE2 With Biopython

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Abstract
In the present study, we focused on Next - Generation Sequencing analysis with Biopython to study the Beta SARS-CoV-2 variant RBD and Human ACE2 enzyme. The NGS workflow analysis includes computational tools MMDB, BioPython, BLAST, String, COBALT, InterPro, and PDB web server (PDBsum) for the present study. We employed a protein structural analysis to asses conserved domains which disrupt ACE2/SARS-CoV-2 Binding. Bio python was employed to automate the protein sequence for structure manipulation tasks. We performed MSA (Multiple sequence alignment) with COBALT and studied conserved residues that are essential for binding. Blast was used to search the homology and identify functionally relevant regions with a PDB web server that serves the purpose of visualization Protein domains and functions were classified with InterPro scan The findings of Interproscan of 8DF5 revealed peptidase and Collectrin –novel homolog of angiotensin-converting enzyme helps in the development of future therapeutic targets.

Keywords*: STRING network, PDBsum, InterPro Scan, Biopython, Homology, SARS-CoV- 2, Receptor Binding Domain, Angiotensin Converting Enzyme*

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I. Introduction

The emergence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in late 2019 and its subsequent rapid global spread have caused a devastating pandemic with unprecedented health and economic consequences. Understanding the mechanisms by which SARS-CoV-2 enters host cells is crucial for developing effective therapeutic strategies and vaccines. The viral spike protein plays a pivotal role in this process. This protein protrudes from the viral surface and mediates attachment to host cells through its Receptor-Binding Domain (RBD) which interacts specifically with the human Angiotensin-Converting Enzyme 2 (ACE2) receptor **(Wang et al., 2020)**.

The ongoing evolution of SARS-CoV-2 has resulted in the emergence of numerous variants with distinct characteristics. The beta variant has 21 mutations with 9 spike protein mutations in the genome. The key mutations beyond N501Y are E484K, K417N, orf1b deletion in the Receptor binding domain (RBD) and L18F, D80A, D215G, 242–244, R264I, A701V in the N terminal domain which have been linked to increased transmissibility and the potential to evade existing vaccines The affinity of Beta RBD binding to ACE2 receptors is also found to be 2.7- fold higher than that of the Alpha variant. The new coronavirus variant called KP.2 and KP1.1 nicknamed FLiRT which has been linked to rising cases of Covid-19 in the United States, United Kingdom, and South Korea, has been circulated in India since November 2023. These two are known as FLiRT variants because they are characterized by a phenylalanine (F) to leucine (L) mutation and an arginine (R) to threonine (T) mutation in the virus's spike protein **(Tegally et al., 2020)**.

Therefore, a detailed investigation of the interaction between the Beta variant RBD and human ACE2 is critical for understanding the unique properties of this variant and its potential impact on public health **(Kee, 2024)**. The present study proposes a next-generation sequencing (NGS) workflow analysis with Bio Python to comprehensively analyze the Beta variant RBD-ACE2 complex. Biopython, a powerful Python library specifically designed for bioinformatics tasks, will be used to automate various computational tools, streamlining the analysis process and enabling researchers to gain deeper insights into this crucial protein-protein interaction **[6].**

Main Objectives

The current work aims to achieve several objectives through an NGS workflow analysis using Biopython. First, it will investigate the structural characterization and comparative analysis of the Beta variant's RBD and ACE2 through protein-protein docking simulations. Additionally, the study will conduct a sequence-based analysis of the Beta variant's RBD compared to related coronavirus RBD sequences. Furthermore, it will explore the interactions between the Beta variant's RBD and human ACE2 using protein interaction databases for network analysis. The research will also provide in silico insights to examine potential changes in viral infectivity and immune escape mechanisms associated with the Beta variant. Ultimately, this study aims to contribute to developing targeted therapeutic strategies to combat the Beta variant and potentially other emerging SARS-CoV-2 variants.

II. Methods and Methodology

The present study employs Next-Generation Sequencing (NGS) workflow analysis with Biopython to comprehensively analyze the interaction between the Beta variant RBD and human ACE2. RBD-ACE2 complex was retrieved from the Protein Data Bank (PDB). Protein sequences for the Beta variant RBD and human ACE2 will be retrieved from public databases such as the National Center for Biotechnology Information (NCBI) Protein Data Bank (PDB) (**Wang et al., 2020**). Biopython, a user-friendly Python library, will be used to automate various bioinformatics tools, streamlining the analysis process. Bio Python's built-in functions for accessing online databases will be utilized (**Cock et al., 2009**).

Bioinformatics Analysis

RBD sequences are aligned so that mutations and variations will be studied by using Biopython and ClustalW. Blast and COBALT helped in the homology study and the functional domains associated with RBD & ACE2 sequences were studied using an InterPro scan. We employed String for Network analysis of our query protein (**Zhu et al., 2020**).

III. Results

Structural Characterization and Comparative Analysis

The protein has 12 molecules. The conserved domains on S309 FAB Heavy and FAB Light chains are different. FAB heavy chains have Conserved domains on Immunoglobin domain- containing proteins (**Lan et al., 2020**). It may function in cell adhesion and pattern recognition. The domain hits are Ig superfamily with an E-value of 3.07e-55 and IgC1_CH1_IgEG has an E-value of 3.98e-45. On the other hand, the FAB light chain has conserved domains as IgV_L_kappa and IgC1_L with E-values of 8.06e-64 and 5.87e-45 respectively. The lower the E-value, the better the hit (**Lan et al., 2020**).

Conserved domains on Chain E&F represent Angiotensin-converting enzyme 2 which is a carboxypeptidase that converts angiotensin I to angiotensin 1-9, and angiotensin II to the vasodilator angiotensin 1-7 (**Lan et al., 2020**). Peptidase_M2 & Collectrin are the domain hits. On Chain R which is spike proteinS1 the domain hit is CoV_Spike_S1_RBD superfamily.

The PDB Id 8DF5 has the conserved domain of PDB Id 8DF5 and has more hits on the IgV – (V set superfamily) and IgC1 (C1 set superfamily)

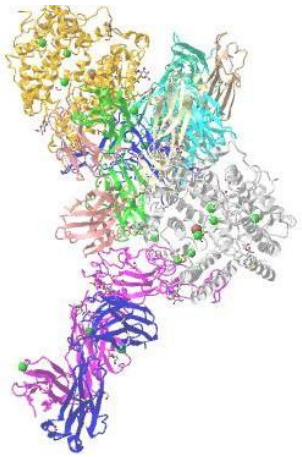

Figure 1: Representation of 3-D View of 8DF5 with conserved domains.

Sequence-Based Analysis

We ran BLAST to find a similar protein to our query protein 8DF5 and found similar proteins while using BLAST. Chain B of S309 Lambda chain with Accession Id 7XSW_B And Chain B, S309 antigen-binding (Fab) fragment, light chain [Homo sapiens]Accession Id 6WS6_B shows 100% percentage identity with 8DF5 (**Altschul SF et al.,1990**, **Pruitt KD et al., 2007**). Using the EMBL-EBI Clustal W program we understand that the sequences Chain shares 100% homology identity with Chain A, H & G of 8DF5 protein **(Larkin MA et al., 2007).**

The phylogenetic tree was studied using COBALT and it shows the evolutionary relationship of 8DF5-associated light chain variants of immunoglobulins with humans. In the tree two specific human light chains are accentuated:

- Chain B, neutralizing antibody light chain
- Chain B, COVOX-222 Fab light chain, **(Johnson et al., 2022; Liu et al., 2020)**

A shared evolutionary history and potential functional similarities are suggested by the clustering of the human light chains **(Roshan et al., 2021; Smith et al., 2019)**. Rapid evolution results are highlighted by diversification within the primates and an "unknown clade with primates" indicates the potential to ascertain the diversity of immunoglobulin light chains **(Thompson et al., 2023; Wang et al., 2024**).

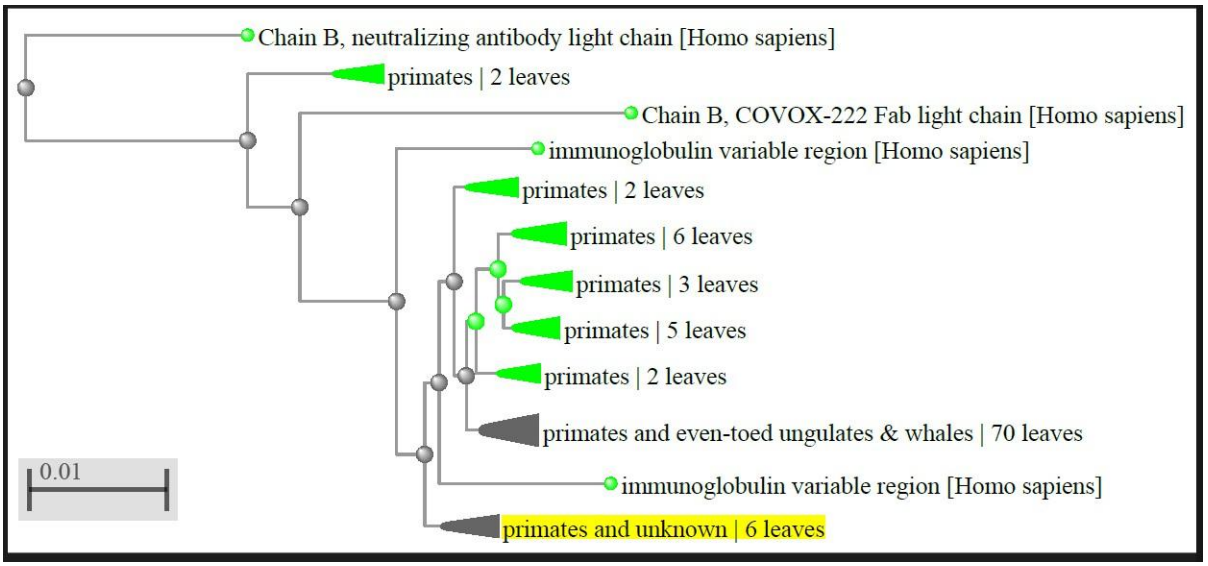

Figure 2: Phylogenetic tree focusing on evolutionary origin between human and primate lineage majorly associated with Ig variable region.

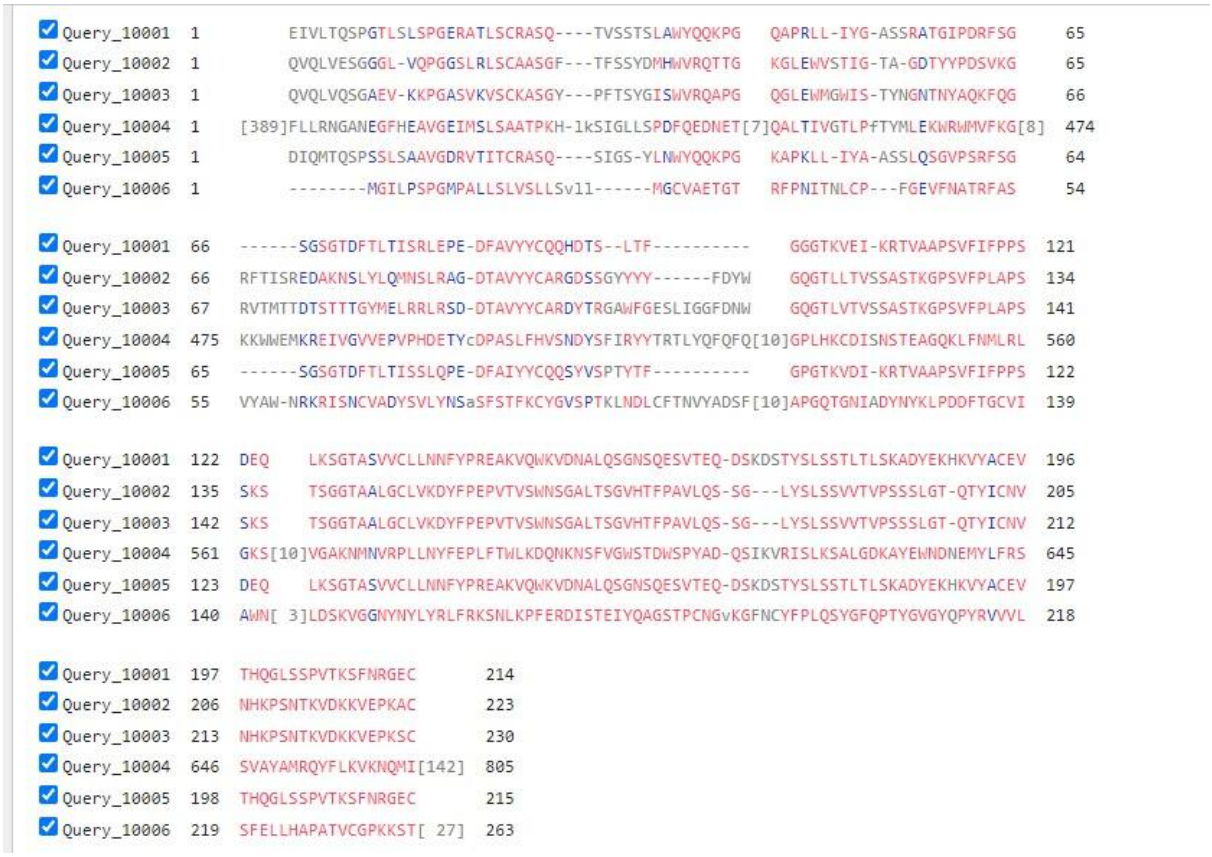

Figure 3: MSA with representations of amino acids involved in the sequences

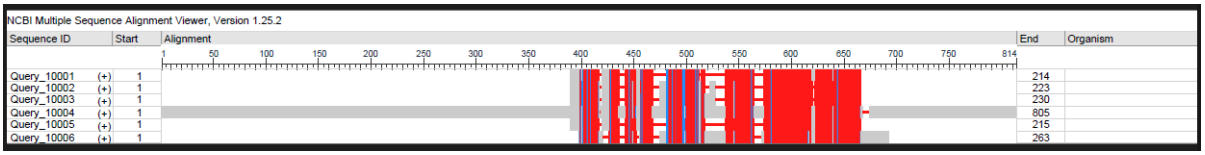

Figure 4: Multiple Alignment Results - 8DF5_2|Chains B, D|S309 Fab Light Chain| Homo - Cobalt RID A9402RXS212 (6 seqs)

This MSA denotes the variable region in red and conserved regions in the grey of our query protein. The former is essential for protein-specific activities and adaptability while the latter is required for protein function.

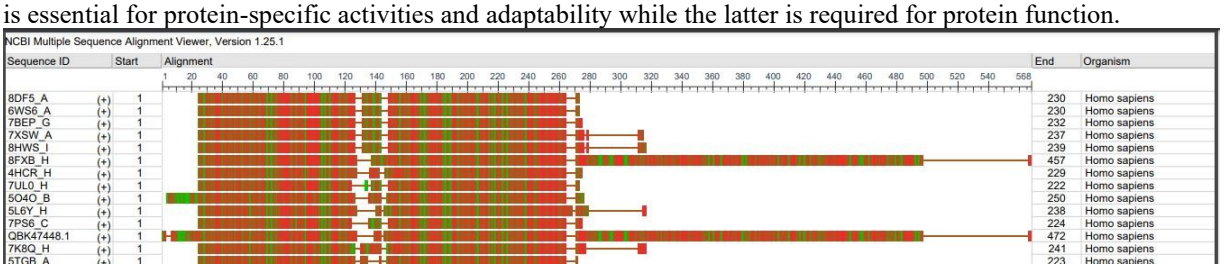

Figure 5: MSA results in COBALT by using the Membrane preference option in coloring.

The main 8DF5 PAGE in PDBsum can be accessed using the link [https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl]
PDB and Uniprot sequences differ at 3 residue positions marked by black crosses and can be accessed here for details [https://www.ebi.ac.uk/thorntonsrv/databases/cgibin/pdbsum/GetPage.pl?pdbcode=8df5&template=align.html&l=6.]
PDB sum offers a specific advantage in making comparison easier with already available experimental models/structures **(Laskowski, R. A. et al., 2021).**

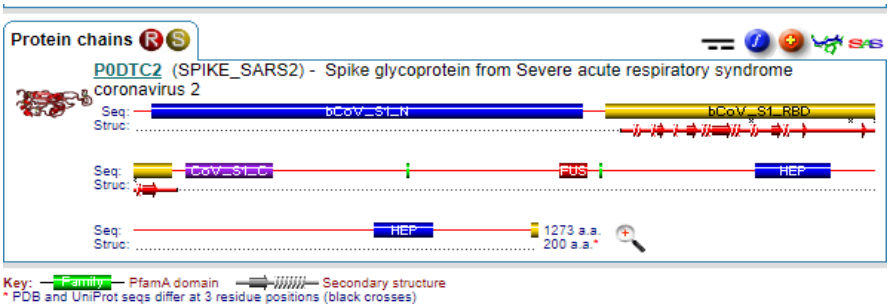

Figure 6: PDBsum page for the protein 8D5 with Uniprot accession P0DTC2 · SPIKE_SARS2.

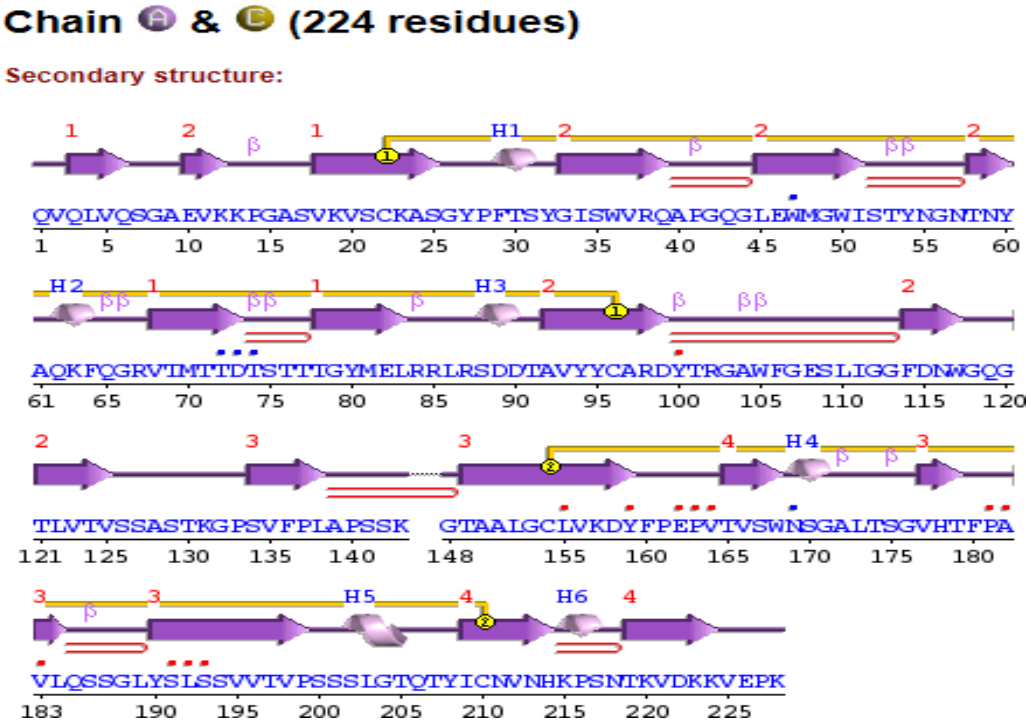

Figure 7. The schematic representation of Chain A and its identical Chain C with the residues. In this, Helices are labeled as H1 and H2 in blue, and sheets are labeled as A, B, and so on in red. The red and blue dots give information about residue contact, with blue indicating contact with metal and red indicating contact with a ligand. Yellow-colored bonds provide information on disulfide bonds, and motifs are shown as Beta in purple and hairpin bends in red.

Ligand analysis
Proteins tend to have many ligands bound to them which can be studied in several ways and using many devoted technologies. PDBsum allows us to study the same with its Ligand- specific column **(Laskowski et al., 2018; Swindells, 2020).**

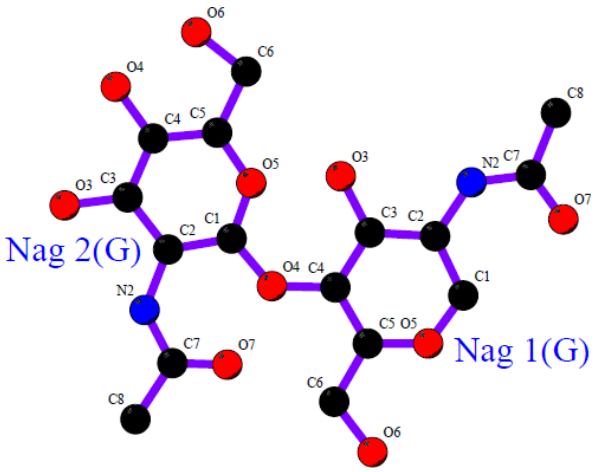

Figure 8: Ligplot for NAG-NAG ligand

The Ligand involved is Ligand NAG-NAG - 2-Acetamido-2-Deoxy-Beta-D-Glucopyranose [N-Acetyl-Beta-D-Glucosamine; 2-Acetamido-2-Deoxy-Beta- D- Glucose; 2-Acetamido-2-Deoxy-D-Glucose; 2-Acetamido-
2-Deoxy-Glucose; n-Acetyl-D-Glucosamine] with formula $C_8H_{15}NO_6$.
Ligand matches this enzyme's product L-phenylalanine with a similarity of 44.44%.
The Ligplot interaction involves NAG-NAG interaction with TYR 50. Ligand analysis helps in understanding that the NAG1 ligand forms hydrogen bonds and helps stabilize the interaction between the protein and the ligand. Many hydrophobic interactions are also involved and will assist in ligand attachment with the active site of protein **(Berg, 2019; Hsu et al., 2021).**

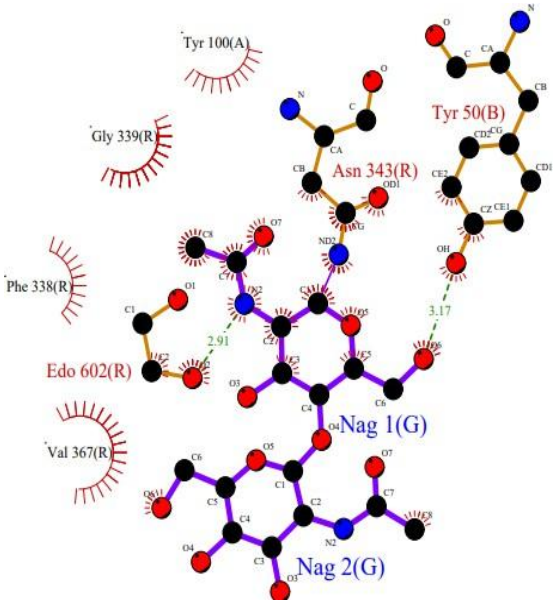

Figure 9: Ligplot Interaction of 8DF5. The protein is shown in blue, and the ligand is shown in green. The red lines indicate hydrogen bonds between the protein and the ligand. The black lines indicate hydrophobic interactions between the protein and the ligand.

By using Interpro scan analysis it can be revealed that the protein has multiple domains and motifs **(Jones, P et al., 2014)**. Peptidase M2 is confirmed while one more domain which is Collectrin-like is validated.

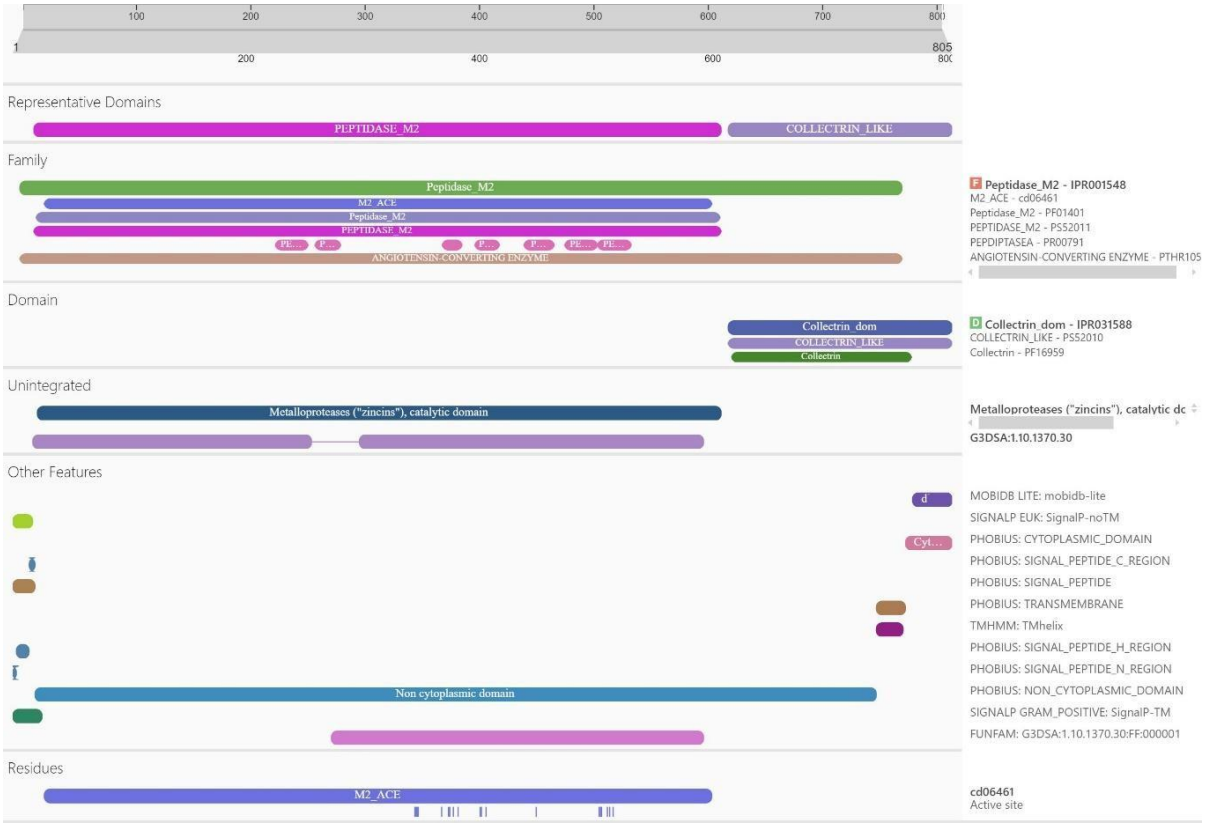

Figure 10: InterPro Scan Analysis of 8DF5

This will provide more granular insights into the function and structures of protein. The PANTHER GO terms associated with the protein help in understanding its role in the immune system.

Figure 11: Annotation of PANTHER GO terms in Interpro Scan.

The Biological process of the protein represents the "immunoglobulin-mediated immune response" (GO:0006959), Molecular Function denotes that the protein functions as an "antigen binder" (GO:0003823), and lastly, the Cellular component shows that it is located in "blood microparticles" (GO:0072562).

Network Analysis

STRING stands for Search Tool for the Retrieval of Interacting Genes/Proteins for Network Analysis.

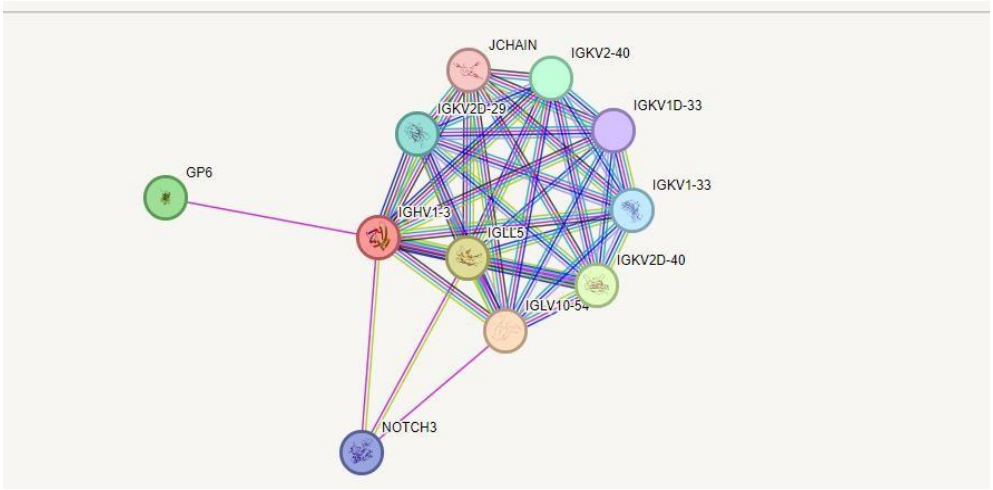

Figure 12: Depicting the protein-protein interaction (PPI) network of the 8DF5 protein using the STRING database.

In string, the evidence mode has differently colored lines with varied meanings. Seven types of evidence predict the associations and are as follows **[17]**:

Lines	**Evidence**
Green Line	Neighborhood Evidence
Red Line	Fusion Evidence
Blue Line	Cooccurrence Evidence
Purple Line	Experimental Evidence
Yellow Line	Text Mining Evidence
Black Line	Co-Expression Evidence.
Light Blue Line	Database Evidence

There are two modes in the STRING network, one is action mode and the other one is confidence mode. The line thickness in confidence mode dents the degree of confidence prediction of the interaction. Binding, activation, and other details of prediction will be represented by action mode. While clicking the node we can get several details about the protein. A detailed evidence breakdown is displayed when we click on an edge. Clicking on a node gives several details about the protein. Clicking on an edge displays a detailed evidence breakdown.

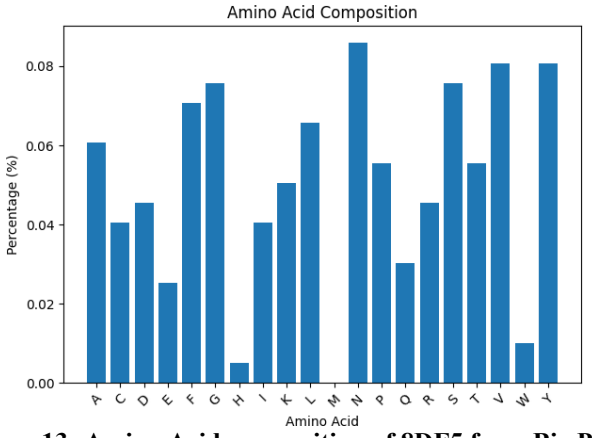

Figure 13: Amino Acid composition of 8DF5 from Bio.PDB

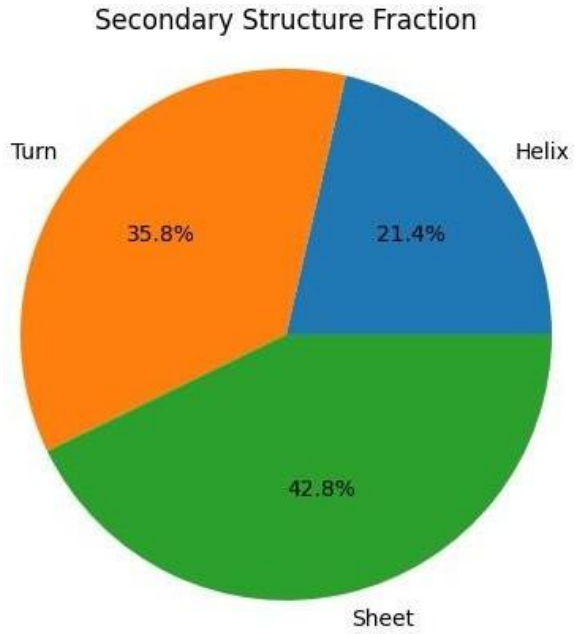

Figure 14: Plot of secondary structure fraction in Biopython using Bio. pdb

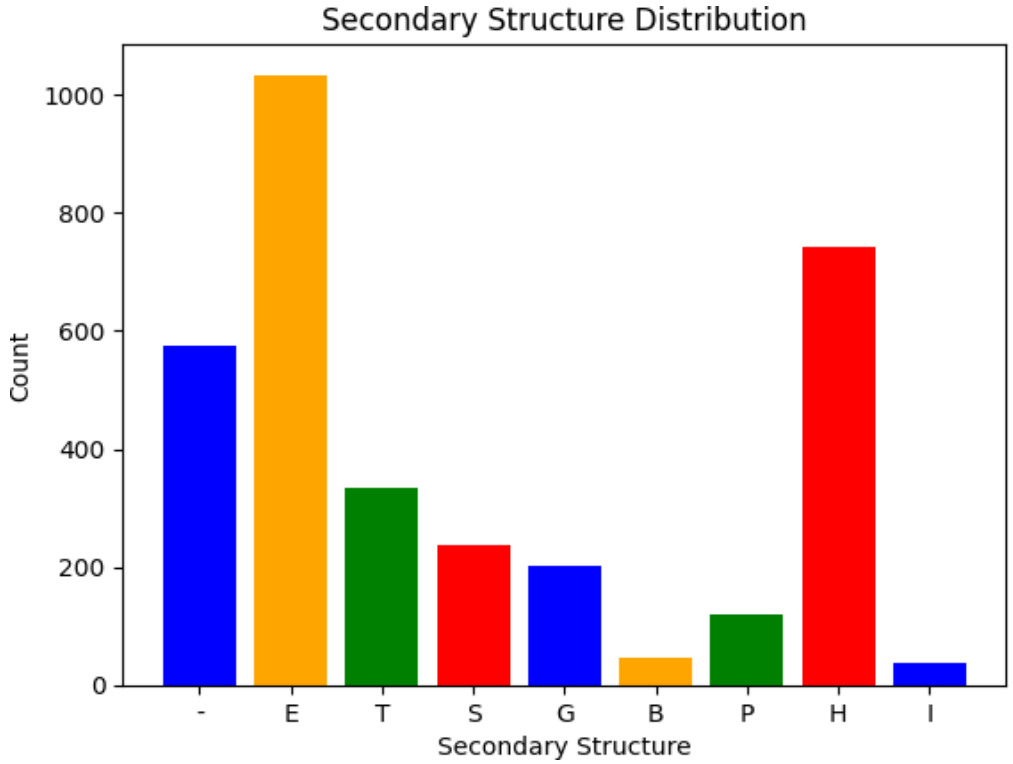

Figure 15: Graph depicting the secondary structure of 8DF5 From Bio. pdb in Bio python

IV. Conclusion

The present study employed a next-generation sequencing (NGS) workflow analysis with Biopython to comprehensively analyze the interaction between the Beta variant Receptor- Binding Domain (RBD) of SARS-CoV-2 and human Angiotensin-Converting Enzyme 2 (ACE2). An in-depth exploration of protein structures, their interactions, and functional domains was carried out by leveraging resources such as Biopython, MMDB, Blast, STRING, COBALT, INTERPro, and Pdbsum. A detailed computational approach is the centre of the present study which helps in analyzing the interaction between RBD and ACE2 enzyme leading to complex formation. The conserved residues in the study and their integration with NGS data along with bioinformatics tools will help in a deeper understanding of developing countermeasures and studying viral pathogenesis. Biopython facilitated the automation of various bioinformatics tools, streamlining the analysis process. The in-

silico analysis provided valuable insights into the Beta variant RBD-ACE2 complex by integrating these findings, the study has shed light on the potential alterations in viral infectivity and immune escape mechanisms associated with the Beta variant. This knowledge can be instrumental in developing targeted therapeutic strategies by designing specific drugs or inhibitors that can disrupt the Beta variant RBD-ACE2 interaction can be explored.

In conclusion, this study serves as a groundwork to advance the development of novel therapeutic approaches for future research work in SARS-CoV-2 and its interactions with human hosts.

Future Work

This study is focused on a single SARS-CoV-2 variant i.e. Beta variant and soon analysis expansion is required for a broader understanding of the virus and its evolution. Additional experimental data will help draw more peculiar conclusions which will help develop computational methods to study mutation and its role in protein function and stability. This is crucial for the development of more direct therapeutic therapies rather than indirect therapies.

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