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# Computational Analysis of SARS-CoV-2 Genome Representing Intraspecific Variability

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***Abstract:*** ***The US National Institutes of Health, it is the successor to SARS-CoV-1, which was the virus that causedSARS outbreak in 2002-2004. SARS-CoV-2 is a virus of the species severe acute respiratory syndrome–related coronavirus (SARS-CoV-2) Genomic analysis of 566SARS CoV2 virus populations to identify mutations as substitutions, deletions, insertions,and single nucleotide polymorphisms(SNPs).Clustal,ClustalOmega and MAFFT in order to align the Indian 566 SARS-CoV-2 sequences. SARS-CoV-2 is an enveloped virus consisting of a positive sense, single-stranded RNA genome of approximately 30 kb. There will be 3 possible reading frames in each direction of the RNA. So total 6 possible reading frame or (6 horizontal bars) would be there for every RNA sequence. +1, +2, +3 and -1, -2 and -3 ( in the reverse strand) are the 6 possible reading frames. The graphical abstract of the human coronavirus NL 63 genome of BLAST is represented by a red bar, which shows the most similar sequences. Bit score is anotherbiostatistical indicator used in addition to the e-value in a blast output and where comparing the sequence similarities search i.e. pairwise multiple sequence alignment shows a way of arranging protein or DNA sequence to identify region or similarity in clustal omega***

***Keywords: Genomics, SARS-CoV-2, COVID-19,Coronavirus,Sequence alignment , Orf translation***

## I. INTRODUCTION

Baricitinib is a pill that seems to fight COVID-19 by reducing inflammation and having antiviral activity. The FDA stated that for patients who are hospitalized due to COVID-19 and require mechanical ventilation or need supplemental oxygen, Barictinib can be used in combination with Redecivir.There are several monoclonal antibody drugs available. These include the combination of bamlanivimab and etesevimab, the combination of two antibodies called casirivimab and imdevimab, and sotrovimab. These drugs are used to treat COVID-19 in people who are at a higher risk of serious illness from COVID-19. Many COVID-19 patients may have mild illness and can be treated with supportive care

1) *SARS-CoV-2 Analysis Using Blast:* The 2020 pandemic of COVID-19 has had a devastating impact on human health, economies, culturalpractices, and higher education, but it has also provided a unique opportunity to teach about microbes in a highly relevant context [1]. Modern technology enables people to make a powerful and rapid response to the research of this virus, which has never been seen in past virus outbreaks. Data have been made publicly available at a record rate, allowing open access to the latest results and helping to direct public health decisions [2]. The genome forSARSCoV2 (causative agent for COVID-19) was sequenced and made publicly available before most of the general public even knew it existed [3]. Professors and instructors teaching microbiology and other biology courses can capture the enthusiasm and true curiosity surroundingCOVID-19 by providing engagement with scientific literature and helping students find answers forthemselves[4]. The free online database allows students to access cutting-edgegenome data of the virus that causes COVID19 and SARS CoV2. Through a basic introduction togenomics and bioinformatics, students can create alignments, search for related ancestors, anddiscover unique mutations and sequences that distinguish COVID19 from other coronaviruses.Thisarticle introduces a COVID-19 case study created and used during the COVID-19 pandemic andenabling students to the compare genome sequences of different coronaviruses to betterunderstand the origin of SARS-CoV-2. Case studies have been used in active learning classrooms fordecades to help the learner connect course objectives with real world applications [5]. Some courses are purely case study oriented and demonstrate impressive learning and usefulcourse concept retention [6]. The emergence of COVID-19 surprised most people andwas very confused about the source of the virus. The purpose of this case study is as follows: tointroduce students to bioinformatics and genomics so that they can compare the genome sequencesof different coronaviruses to better understand the origin of SARS- CoV-2.
2) *SARS-CoV-2 Genome Analysis Using Multiple Sequence Alignment:* Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causes a diseaseCOVID-19 which originated in Wuhan, China. This disease has caused awave of pandemics worldwide. In this worrying situation, vaccination is undoubtedly aprimary prevention strategy to contain this virus.

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However, the vaccine development processis time-consuming and requires analysis of the genetic variability of virus populations inorder to develop effective and safe vaccines for heterogeneous populations [7].In this regard, Tung Phan has performed a genomic analysis to show the evolution of SARS-CoV-2 [8]. To continue this work, we performed genomic analysis of 566SARS CoV2 virus populations to identify mutations as substitutions, deletions, insertions,and single nucleotide polymorphisms(SNPs). Generally speaking, 1% of the populationaffected by substitution is referred to as SNP. We only studied non-synonymous mutationsbecause they are responsible for amino acid changes. In order to find genetic variation it isimportant to have multiple sequence alignments of reference sequences. On the other hand, itis well known that multiple sequence alignment techniques provide almost the best results.Therefore, for the same sequence pool, different alignment techniques can produce differentresults. Therefore, we used four different well-known multiple sequence alignmenttechniques viz. Clustal W [9],Clustal O [10] andMAFFT [11].Align the Indian 566 SARS-CoV-2 sequence. These alignmentresults are then used to identify the mutation list as substitutions, deletions, insertions, andSNPs. A consensus on these results is then created, called Consensus Multiple SequenceAlignment (CMSA), to have the final mutation list so that the benefits of all four alignmenttechniques can keep. It should be noted that identifying SNP helps to classify virus strains, sovaccine design and vaccine dose definition can be effectively carried out [12].Recently the metagenomic analysis using Next-Generation Sequencing (NGS) shows that theSARS-CoV-2 is a single-stranded enveloped RNA virus with a genome length of 27 to 32kilobases [13]. According to NCBI reports, SARS-CoV-2 has 11 codingregions, which encode ORF1ab polyprotein, spike (S) glycoprotein, envelope protein (E),membrane (M) glycoprotein, nucleocapsid (N) protein, and other accessory proteins ORF3a,ORF6, ORF7a ,ORF7b, ORF8 and ORF10. According to further reports, the open readingframe (ORF) can encode several non-structural proteins (NSP). The genomic orientation ofSARS-CoV-2 virus is shown in and their coordinates in supplementary. It is worthmentioning that the virus has a new strain, and the understanding of its genetic variability indifferent countries is still limited. This is another motivation for conducting this study on theIndian SARS-CoV-2 sequence.In December 2019, the outbreak of Severe Acute Respiratory Syndrome Coronavirus 2(SARS-CoV-2) caused severe pneumonia [14]. Since then, it has spread fromWuhan, China to Asia, Europe and the United States, becoming a global pandemic [15]. Severe cases beginning from Huanan Seafood Wholesale market in China whichconfirmed human pneumonia with the infection of a novel coronavirus and named as SARS-CoV-2 by International Committee on Taxonomy of Viruses Current reports singlenucleotide variants are found in many patients with SARS-CoV-2, which belongs to beta-coronavirus species.SARS-CoV-2 contains functional genomic ribonucleic acid (RNA),which is transcribed into structural protein as transmembrane spike (S) glycoprotein, whichuses host cell angiotensin converting enzyme to mediate virus entry into host cell and thenucleocapsid (N) protein holds the major nuclear viral RNA genome; the envelope (E) andmembrane (M) alone with spike protein form viral envelope [16]. Nonstructural RNA genome containing ORF1ab, ORF3, ORF6, 7a, 8, and ORF10 containshighly conserved information about genomic RNA synthesis and replication in ORF1ab andunclear-verified function in other ORF proteins [17]. The propagation(transmission) mechanism initiated by SARSCoV2 binds to the host cellmembrane receptor, and then induces membrane endocytosis into the host cell. ORF1 of theviral genome replicates it and synthesizes subgenomic RNA. At the sane time, N protein andnew genomic RNA assemble to form helical nucleocapsids with M protein inserted inendoplasmic reticulum (ER) and anchored Golgi of host cells [18]. Then the Eand M proteins start to trigger the budding process. S, together with the helix N on themembrane-bound ER, triggers the viral structural proteins required for translation andtransport to the Golgi apparatus. In the last cycle, virus particles are released throughexocytosis to end the life cycle and replication of the virus.

SARS-CoV- 2 is an enveloped virus consisting of a positive, single-stranded RNA genome of approximately 30 kb. Two overlapping ORFs, ORF1a and ORF1b are translated from positive-strand genomic RNA to produce continuous polypeptides that are cleaved into a total of 16 non-structural proteins (NSP). The translation of ORF1b is mediated by a -1 frameshift, which allows translation to continue beyond the stop codon of ORF1a. The negative-strand RNA intermediate is made from the viral genome and is used as a template for the synthesis of genomic positive-strand RNA and subgenomic RNA [19].Subgenomic RNA contains a common 5'leader sequence, 5'cap structure, and 3'poly (A) tail fused to different segments of the 3'end of the viral genome [20]. These distinct fusions occur during negative-strand synthesis at nucleotide core sequences called transcription-regulating sequences (TRSs), that are present at the 3′ end of the leader sequence and also preceding each viral ORF. Various sub genomic RNAs encode four conserved structural proteins- spike (S), envelope (E), membrane (M) and nucleocapsid (N) and also several accessory proteins. On the basis of sequence similarity to other beta coronaviruses, and specifically to SARS-CoV-2, the present annotation of SARS-CoV-2 includes predictions of y 6 accessory proteins (3a, 6, 7a, 7b, 8 and 10, NC_045512). Increased coverage was also observed at the 5' untranslated region (UTR) which reflecting the presence of 5'leader sequences in all sub genomic RNAs and genomic RNAs. The decrease in footprint density between ORF1a and ORF1b reflects the proportion of ribosomes ending with the ORF1a stop codon instead of moving the grid in ORF1b.

By dividing the footprint density in ORF1b by the density in ORF1a, we can estimate the frameshift efficiency to be 57% \u00b1 12%. This value is similar to the frameshift efficiency of mouse hepatitis virus (MHV) measured using Riboseq (48\u201375%) 3. Similar to the observations of MHV and Avian Infectious Bronchitis Virus (IBV) 3,11, we did not observe any obvious pauses of ribosomes before or at the grid movement site, but we found them in ORF1a and ORF1b Several potential pause sites. Except for ORF1a and ORF1b all other classical viral ORFs are translated from subgenomic RNA. Since the original RNAseq density represents the cumulative sum of genomic and subgenomic RNA, we use two methods to calculate the transcription frequency: deconvolution of RNA density, where the RNA expression of each ORF is calculated by dividing the RNAread density of the cumulative subtraction density by the ORF area Upstream; the relative frequency of RNA reads spans the preconductor connection of each classical subgenomic RNA. For most ORFs, there is a high correlation between these two methods, and of the two methods, N transcripts are the most abundant transcripts, consistent with other studies[21]. We next compared footprint densities to RNA abundance. For most viral ORFs, transcription frequency is almost completely correlated with footprint density, which indicates that the translation efficiency of these viral ORFs is similar (perhaps due to their almost identical 5'UTR); however, the three ORFs are outliers. The translation efficiency of ORF1a and ORF1b is significantly lower. This may be due to the different characteristics of their 5'UTR or the underestimation of their true translation efficiency, because some full-length RNA molecules can be used as templates for replication or packaging and therefore are not part of the translated mRNA library. The third outlier is ORF7b, for which we have identified very few body–leader junctions; however it shows relatively high translation, probably due to ribosome leaky scanning of the ORF7a transcript, as was suggested for SARS-CoV-2. Many transcripts derived from non-classical compounds have been identified as SARSCoV29,12. These connections include a combination of the leader and the 3'fragment at an unexpected position in the middle of the ORF (leader-dependent, uncanonical connection) or fusions between sequences, which have no similarities with the leader (leader-independent junction). The corona virus genomes encode five major open reading frames (ORFs), including a 5′ frameshiftedpolyprotein (ORF1a/ORF1ab) and four canonical 3′ structural proteins, namely the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins, which are common to all coronaviruses [22].

## II. METHODS AND MATERIAL

Computational analysis of sequence alignment is a computer programming for bioinformatics and data Management. NCBI focus on theoretical analytical and applied computational approach and widely used primary database standard protein, BLAST (blast p) programme search protein database using the protein query. A database is usually regulated by database management system. Together, the data and the database management system along with the applications that are related with them, are referred to as a database system. ORF Predictor facilitates annotation of expressed sequence tag-derived sequences particularly for large-scale EST projects.This tool finds Open Reading Frame for corresponding amino acid sequences and convert them into their single letter amino acid code and provides locations in the sequence. pairwise global alignment between the sequences makes it convenient to discover different mutation involved single nucleotide polymorphism. ORF Investigator is written in portable programming language and therefore available to users of all common operating systems. ORF Finder identify all open reading frame using standard genetic codes. Deduced amino acid sequence can be saved in many formats and searched against sequence database using the basic local alignment search tool server. The National Center for Biotechnology Information is the branch of the United States National Library of Medicine. It is accepted and funded by the government of the United States. The National Center for Biotechnology Information is situated in Bethesda, Maryland and was established in 1988. NCBI serves as an international resource for the scientific research community – providing approach to public databases and software tools for analyzing biological data, as well as performing research in computational biology. The NCBI is made up of multidisciplinary research and development teams composed of molecular biologists,biochemists, clinicians, Assemble scientific and medical research data from around the globe • Serve as the immense repository of the world's primary biological research data • Produce curate datasets to enhance the value and usabiliEntrez: The Entrez Global Query Cross Database Search System is a federated search engine, or web portal that allows users to search various individual health sciences databases. NCBI distributed the first variety of Entrez in 1991, composed of nucleotide sequences from PDB and GenBank, protein sequences from SWISS-PROT, translated GenBank, PIR, PRF, PDB.ty of the primary data.

## III. RESULT AND DISCUSION

Severe acute respiratory syndrome coronavirus 2(SARS CoV 2) is the virus that causes COVD-19 COVID-19(coronavirus disease 2019), the respiratory illness responsible for the epidemic.The molecular analysis of the sequence of genome organization by applying bioinformatics tools NCBI, BLAST, FASTA, CLUSTAL OMEGA, and ORF.

The "blastn" program is a general purpose nucleotide search and alignment program that is sensitive and can be used to align rRNA or tRNA sequences and also mRNA or genomic DNA sequences containing a mix of coding and noncoding regions. The web BLAST represents the basic local alignment search tool is an algorithm for comparing primary biological sequence information such as beta coronavirus protein sequence-Value is increased from default value, larger lists with more low scoring his can be reported based on quality of alignment. Human Coronavirus NL63 (HCoVNL63) is a coronavirus, especially from the genus Alpha coronavirus. The virus is an enveloped, positive-sense, single-stranded virus which enters its host cell by binding to ACE2. Modern technology enables people to make a powerful and rapid response to the research of this virus, which has never been seen in past virus outbreaks. The free online database allows students to access cutting-edge genome data of the virus that causes COVID-19 and SARS CoV-2.

>MK342133.1 Human coronavirus NL63 strain ChinaGD14 spike gene, partial cds

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Human coronavirus NL63 strain ChinaGD14 spike gene, partial cds | Human coronavi... | 3258 | 3258 | 100% | 0.0 | 100.00% | 1806 | MK342133.1 |
| Human coronavirus NL63 strain ChinaGD03, complete genome | Human coronavi... | 3258 | 3258 | 100% | 0.0 | 100.00% | 27516 | MK334044.1 |
| Human coronavirus NL63 strain ChinaGD12 spike gene, partial cds | Human coronavi... | 3253 | 3253 | 100% | 0.0 | 99.94% | 1806 | MK342131.1 |
| Human coronavirus NL63 strain ChinaGD02, complete genome | Human coronavi... | 3253 | 3253 | 100% | 0.0 | 99.94% | 27516 | MK334043.1 |
| Human coronavirus NL63 Fukushima_H219_2018 RNA, complete genome | Human coronavi... | 3244 | 3244 | 100% | 0.0 | 99.83% | 27516 | LC654455.1 |
| Human coronavirus NL63 strain ZJWZ41, complete genome | Human coronavi... | 3240 | 3240 | 100% | 0.0 | 99.78% | 27292 | MZ221193.1 |
| Human coronavirus NL63 isolate NL63/FRA-EPI/Caen/2015/19 replicase polyprotein 1ab (ORF1b) and spike p... | Human coronavi... | 3235 | 3235 | 100% | 0.0 | 99.72% | 4452 | KY862036.1 |
| Human coronavirus NL63 HCoVNL63_Fukushima_O230_2018 RNA, nearly complete genome | Human coronavi... | 3235 | 3235 | 100% | 0.0 | 99.72% | 27542 | LC756668.1 |
| Human coronavirus NL63 Fukushima_H257_2018 RNA, complete genome | Human coronavi... | 3235 | 3235 | 100% | 0.0 | 99.72% | 27563 | LC687394.1 |
| Human coronavirus NL63 isolate 4574A/CHN/16 spike protein gene, complete cds | Human coronavi... | 3231 | 3231 | 100% | 0.0 | 99.67% | 4068 | MG426004.1 |
| Human coronavirus NL63 Fukushima_O579_2019 RNA, complete genome | Human coronavi... | 3231 | 3231 | 100% | 0.0 | 99.67% | 27544 | LC687397.1 |

Figure 1:- Analysis of Human Corona virus NL63 Genome

1) *Graphical Representation of Human CoronaVirus NL63 Genome of Blast:* Each bar represent the portion of the another sequence that is similar to query sequence=target sequence

Human corona virus NL63 genome represent the Red bar. (Red Bar indicates:-most similar sequence),Length of sequence and BLAST program, It shows the conserved domains detected during the sequence similarity search. The query sequence is represented by the numbered red bar below the color key. Database hits are displayed based on the alignment score below the red (query) sequence. Out of the aligned sequences, the most related sequences are remains close to the query sequence.

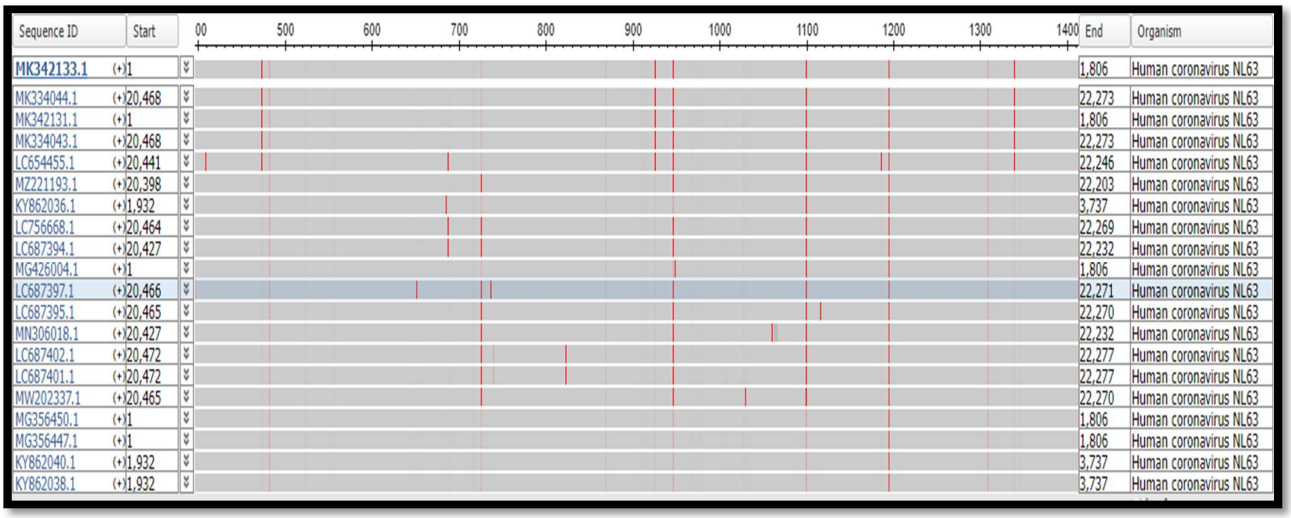



Figure 2:Multiple sequence Alignment viewer represent quality score of Biological sequence

*2) Full length Translation Result*

>lcl|Sequence_1 Frame +1

MKLFLILLVLPLASCFSTCNSNANLSMLQLGVPDNSSTIVTGLLPTHWICANQSTSVYSANGFFYIDVGNHRSAFALHTGYY
DVNQYYIY
VTNEIGLNASVTLKICKFGNTTFDFLSNSSSSFDCIVNLLFTEQLGAPLGITISGETVRLHLYNVTRTFYVPAAYKLTKLSVKC
YFNYSC
VFSVVNATVTVNVTTHNGRVVNYTVCDDCNGYTDNIFSVQQDGRIPNGFPFNNWFLLTNGSTLVDGVSRLYQPLRLTCL
WPVPGLKSSTG
FVYFNATGSDVNCNGYQHNSVADVMRYNLNFSANSVDNLKSGVIVFKTLQYDVLFYCSNSSSGVLDTTIPFGPSSQPYYC
FINSTINTTH
VSTFVGVLPPTVREIVVARTGQFYINGFKYFDLGFIEAVNFNVTTASATDFWTVAFATFVDVLVNVSATKIQNLLYCDSPFE
KLQCEHLQ
FGLQDGFYSANFLDDNVLPETYVALPIYYQHTDINFTATASFGGSCYVCKPHQVNLSLNGNTSVCVRTSHFSIRYIYNRVKS
GSPGDSSW
HIYLKSGTCPFSFSKLNNFQKFKTICFSTVAVPGSCNFPLEATWHYTSYTIVGALYVTWSEG




Figure 4: Above result show only Orf Translation

## IV. CONCLUSION

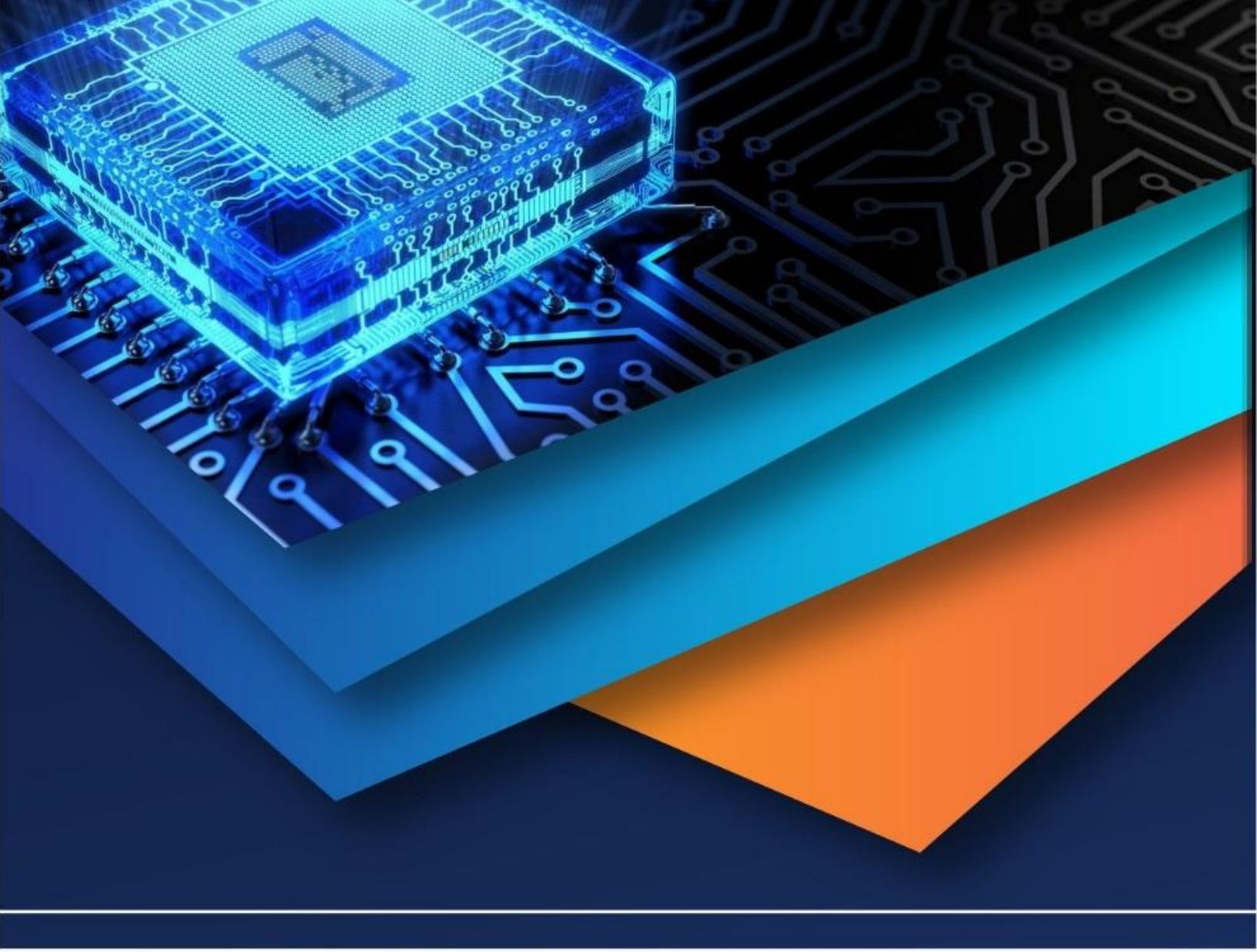
The computational analysis of SARS-CoV-2 describes the specificity of protein structure, function, phylogeny and interaction at both molecular and sequence levels. The Blast algorithm compares the database sequence with the query protein. BLASTE-value(anticipated value) is a parameter that specifies the number of successes that can be anticipated by chance when a searching a database of certain size.As the matching score increases the E-value decreases exponentially. There will be 3 possible reading frames in each direction of RNA. Data mining by genome alignment analysis to catch mutation induce severe deadly threat to human.In significantly observed that SARS-COV-2 RBD displayed remarkably the higher binding affinity to ACE2 receptors.The overall results of the database will play a more important role in future inspections against the coronavirus.

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