CHAPTER 1

Motivation and Outline of the thesis

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1.1.Motivation of the present work:

The highly transmissible severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 in Wuhan, Hubei province, China, leading to the outbreak of coronavirus disease 2019 (COVID-19). This virus had a profound impact on global health and posed a serious threat to human civilization [1,2]. On March 11, 2020, the World Health Organization (WHO) officially classified COVID-19 as a global pandemic. Since then, the virus has continued to cause widespread devastation, triggering multiple outbreaks in various countries. Despite notable progress in clinical research that has deepened our understanding of SARS-CoV-2, many nations have experienced recurrent waves of infections over an extended period [3].

SARS-CoV-2 possesses a large, single-stranded, positive-sense RNA genome that is approximately 30 kb in length. This viral genome encodes 29 proteins, including four structural proteins and 25 putative non-structural and accessory proteins [4,5]. About one-third of the genome is responsible for coding structural proteins, such as the Spike glycoprotein (S), membrane (M), envelope (E), nucleocapsid (N), and several accessory proteins [6,7]. The remaining two-thirds of the genome encode non-structural proteins (NSPs) [6,7] **(Figure 1.1)**.

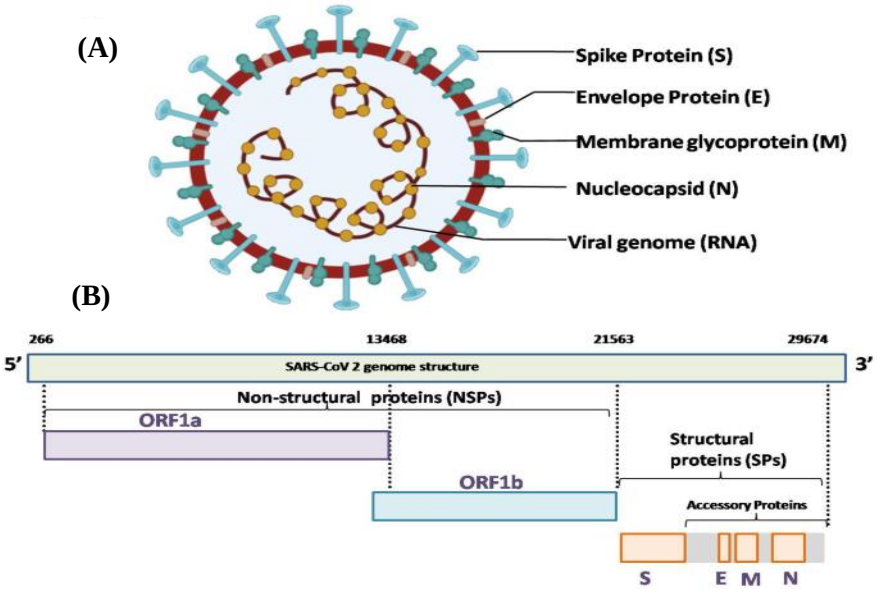

Figure 1.1. *Depicts the* ***(A)*** *structural elements of the virus, including the spike protein, envelope, membrane, and nucleocapsid proteins along with* ***(B)*** *the SARS-CoV-2 genome components (Taken from [8])*

The entry of SARS-CoV-2 into host cells is primarily facilitated by the spike protein (S protein), which binds to its key functional receptor, angiotensin-converting enzyme 2 (ACE2) [9]. A vital component of the virus, the receptor-binding domain (RBD) located in the S1 subunit of the spike protein, enables the virus to attach to host cell receptors, allowing infection to occur. Similar to other RNA viruses, SARS-CoV-2 undergoes genetic evolution and accumulates mutations, leading to the emergence of new variants that may differ from the original strains. The continuous emergence of these viral mutants, coupled with the absence of fully approved and highly effective treatments, created an urgent need to develop a reliable therapeutic solution for COVID-19. The pandemic had a significant global impact, prompting extensive efforts to formulate both preventive and therapeutic strategies. As a result of these efforts, several highly effective vaccines were successfully developed within an unprecedentedly short time frame [3]. Despite extensive global mass vaccination efforts and the rapid development of COVID-19 vaccines, creating effective therapeutics remains a significant challenge for the scientific community. This difficulty arises due to the continuous emergence of new SARS-CoV-2 variants, which have the potential to undermine previous advancements [10]. One of the key targets in the development of antiviral treatments for SARS-CoV-2 is the Main Protease (Mpro), also known as 3C-like protease [11,12]. Mpro plays a crucial role in viral replication by cleaving polyproteins (pp1a and pp1ab) at multiple sites, producing functional polypeptides essential for the virus's life cycle. Given its critical function and the absence of closely related human homologs, Mpro is regarded as a highly promising target for antiviral drug discovery [13]. Since the process of transforming a candidate compound into an approved drug is both time-intensive and expensive, computational approaches have been widely used to accelerate drug discovery. Various computer-aided techniques, including molecular docking, virtual screening, molecular dynamics (MD) simulations, and binding free energy analysis, can help identify potential drug candidates efficiently.

Small-molecule inhibitors offer several advantages, such as rational drug design and favorable oral bioavailability [14]. In contrast, peptides have often been overlooked as potential therapeutic agents, despite their high affinity and specificity for target proteins. Compared to full-length proteins, peptides are smaller and can be synthesized using reliable and cost-effective methods [15]. Therefore, this research focuses on the development of both small-molecule and peptide inhibitors targeting Mpro.

Since the onset of the pandemic, several SARS-CoV-2 variants have emerged, with Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) being

classified as variants of concern (VOCs) due to their potential impact on public health [16]. The Spike protein plays a critical role in viral entry by interacting with the host cell's ACE2 receptor, facilitating membrane fusion and infection. A comprehensive analysis of the binding interactions between the Spike protein and ACE2 across different variants can provide valuable insights into how specific mutations influence viral infectivity and pathogenicity. Understanding the effects of individual mutations on binding stability can also help predict the virulence of future variants carrying similar mutations. Additionally, as newly emerging variants introduce novel mutations, detailed knowledge of Spike-ACE2 interactions will be instrumental in guiding the development of effective therapeutic strategies against COVID-19. ACE2 serves as the primary functional receptor on cell surfaces that facilitates the entry of SARS-CoV-2 into host cells. It is highly expressed in the heart, kidneys, and lungs and is also present in circulation within the plasma [17]. Research indicates that although the lungs are the primary site of infection in COVID-19, they exhibit relatively low ACE2 expression. This suggests that additional co-receptors or alternative receptors may contribute to viral entry, thereby increasing infectivity. Scientists have identified several potential receptors for SARS-CoV-2 beyond ACE2, which are highly expressed in tissues affected by the virus [18,19]. Although numerous candidate receptors were examined during the pandemic, further in-depth investigations are necessary to gain a comprehensive understanding of their role in viral transmission and infection.

Hence, an effort has been made to address three broad areas about this particular topic in this thesis work: (1) To study the interaction and inhibitory properties of small bioactive compounds (natural compounds/peptides) with SARS-CoV-2 Mpro (2) To study the effect of mutations in the S protein on its interaction with ACE2 receptor and (3) To study the interaction profile of RBD of S protein with ACE2 primary receptor and other alternative receptors. To carry out this work we have mainly used Molecular Dynamics (MD) simulation with the AMBER 14 software package.

1.2. Outline of the Thesis:

Chapter 2 gives us a description of SARS-CoV-2, COVID-19 and its sign, symptoms and pathophysiology. This chapter also describes about the different variants of SARS-CoV-2 and the structure and function of Spike protein, and Main protease. It also includes the inhibition of main protease. **Chapter 2** also focuses on the Scope and objectives of this thesis.

Chapter 3 elaborates about the various computational techniques and the key principle of Molecular Dynamics (MD) simulation and other computational tools, including web servers and softwares used in this thesis.

Chapter 4 features the study on the salient structural features of SARS-CoV-2 Main protease (Mpro) and Spike (S) protein performed using few bioinformatics tools like *Expasy Protparam*, *GOR IV*, *Clustal Omega*, *PONDR*. **Chapter 4** also describes the conformational accessibility of different conformers of S-protein to the ACE2 receptor.

Chapter 5 emphasizes on the structural and binding features of small molecules identified from Indian spices namely Carnosol, Arjunglucoside-I, and Rosmanol that might act as inhibitors against SARS-CoV-2 main protease (Mpro) using computational methods and this study could be helpful in relevant to develop therapeutic candidates to combat SARS-CoV-2. Our findings suggests that these compounds have the capacity to inhibit Mpro and thus have the potential to act as therapeutic candidate against the disease.

Chapter 6 sheds light into the designing of potential target peptides that can act against the Mpro. Although small molecules dominate the field of drug market, peptide inhibitors still represent a class of promising candidates because of their high affinity, low toxicity and can effectively and selectively inhibit the protein-protein interactions in viruses. A total of 13 peptides were designed out of which two peptides (peptide2 and peptide4) were screened out and subjected to MD simulation and both the peptides were found to attain a stable and strong binding with the Mpro.

Chapter 7 seek to evaluate the effect of mutations on the structural stability, molecular interactions, and binding properties of the RBD of S protein and ACE2 receptor for the variants namely Double mutant, Delta, Delta-Plus and few omicron variants (BA.1, BA.2, BA.2.12.1, BA.4, BA.2.75, BA.2.75.2). Results depicted that few mutations occurring in the RBD enhances the overall stability of the S protein (RBD)-ACE2 complex and the increased affinity between them may result in higher virulence of the mutated strains than the wild-type strain.

Chapter 8 compares the interaction profile of the RBD of the S protein with alternative receptors namely CAT and L-SIGN. This study is based to the literature search that even though

the lungs are the organs that are most likely to infect in COVID-19, the comparatively low expression of the ACE2 receptor suggests that there may be alternative or co-receptors that facilitates the entry of the virus. Results illustrate that both the receptors have a stable interaction with RBD of S protein and binds with greater affinity than ACE2 receptor. This study provides us an insight that in addition to ACE2, there are other receptors that is facilitating the entry of the virus and might be a cause of higher virulence and infectivity.

Chapter 9 presents a summary of the significant findings and also the future prospects of this work.

1.3. Bibliography

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