Computational approaches to understand the interactions of small bioactive compounds and cell surface receptors with the SARS-CoV-2 viral proteins

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By

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CHAPTER 13

Summary and Future Prospects

13.1 Overview of Results

The main theme of this thesis involves studying the inhibitory properties of small bioactive compounds against SARS-CoV-2 Mpro. Also, the impact of mutations on the extent of S protein binding to its ACE2 receptor and the study of the interaction profile of the RBD of S protein with other alternative receptors are two other significant topics covered in this thesis.

The thesis has been divided into three major parts.

The first part of the thesis comprises of a preliminary study on the salient structural features of the target proteins, Mpro and S protein to get insight into some physio-chemical features of those proteins. The study was performed entirely based on few bioinformatics tools like *Expasy Protparam*, *GOR IV*, *Clustal Omega* and *PONDR*. Results from **Chapter 4** depicts the thermostability, secondary structure prediction, conserved regions followed by the prediction of disordered regions of the Mpro and the S protein. Along with the salient structural feature analysis of the S protein, the extent of accessibility and inaccessibility of ACE2 receptor to three different states of Spike (open, closed, and intermediate) was examined. The results indicated that the open state of the S protein was the most accessible to the ACE2 receptor.

Within the second part of the thesis, development of small molecule inhibitors and peptide inhibitors against the Mpro were included. Numerous small molecules that could function as Mpro inhibitors have been proposed in the literature. Among those small molecules, compounds like Carnosol (CAN), Arjunglucoside-I (ARJ), and Rosmanol (ROS) has been identified from Indian spices as SARS-CoV-2 Mpro inhibitors. In **Chapter 5**, Potential of mean force (PMF) has been constructed in order to analyze the binding-unbinding (or association-dissociation) pathway of the Mpro-small molecule inhibitor complexes using umbrella sampling method. And the structural dynamics and characteristic features of binding of these small molecules demonstrated that the Mpro-small molecule inhibitor complexes had comparatively higher dissociation energy values than the alpha-ketoamide (AKA)-Mpro complex (positive control). Results indicated that the small compounds derived from Indian spices have a substantially stronger and more stable binding for Mpro, indicating that they may have the potential to function as Mpro inhibitors. Apart from the small molecules, peptides as inhibitors continue to be a class of promising candidates due to their low toxicity, high affinity, and similarity to endogenous ligand and can effectively and selectively inhibit the protein-protein interactions in viruses. Therefore, in the second part of the thesis, studied in detail in **Chapter 6**, we attempted to design peptides that can effectively inhibit the disease. Out of all 13 peptides, two of them (peptides 2 and 4) were screened out based on in-silico studies and put through MD simulation and results demonstrate that the two peptides bind to

the active site of the Mpro and it attains a higher stability upon binding to the peptides. Furthermore, a strong binding affinity between the Mpro and the target peptides was also identified, indicating that further research on the new designed peptides may aid the scientific community to establish them as viable drug candidates.

In the last i.e. the third part of the thesis, we have put forward the effect of mutations on the structural stability, molecular interactions, and binding properties of the RBD of S protein and ACE2 receptor for few 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 highlighted in **Chapter 7** depicts those mutations such L452R, E484Q, L452R, K417N and T478K, N501Y 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. Apart from the Spike and ACE2 interaction, this thesis also focuses on comparing the interaction profile of the RBD of the S protein with alternative receptors namely CAT and L-SIGN which may facilitate the virus entry. 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 depicted in **Chapter 8** illustrates that both the receptors have a stable interaction with RBD of S protein and binds with greater affinity than ACE2 receptor which provides 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 infectivity.

13.2 Future Prospects

(i) This thesis provides a comprehensive molecular-level analysis of inhibitors targeting the main protease (Mpro) through in-silico methodologies, including molecular docking and MD simulations. The findings contribute to the scientific understanding of potential drug candidates, and further validation of these inhibitors could facilitate their establishment as effective therapeutics against Mpro. Additionally, given the highly conserved nature of Mpro, these inhibitors may exhibit efficacy against future coronaviruses possessing a main protease with a high degree of sequence identity.

(ii) This thesis also explores the interaction dynamics and binding profiles of the spike (S) protein with ACE2 and other alternative receptors. Through computational analyses, this thesis investigates the effects of various mutations on its virulence and infectivity, providing insights that may aid researchers in designing novel therapeutic agents targeting the spike protein. Furthermore,

the findings offer a predictive framework for assessing the potential virulence of future variants harboring those significant mutations within the receptor-binding domain (RBD) analyzed in this thesis.

(iii) In addition to the alternative receptors examined in this thesis, existing literature indicates the presence of several other receptors that may facilitate viral entry into host cells. Future research could focus on a comprehensive analysis of these receptors, offering valuable insights into the mechanisms governing viral infectivity and transmissibility.