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Differential Electrostatic Interaction Patterns in SARS-CoV-1 and SARS-CoV-2 variants: A Molecular Dynamics Simulation Study

Ugochi Hope Isu
University of Arkansas-Fayetteville

Differential Electrostatic Interaction Patterns in SARS-CoV-1 and SARS-CoV-2 variants: A Molecular Dynamics Simulation Study

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Ugochi Isu
Federal University of Technology
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University of Arkansas

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Mahmoud Moradi, Ph.D.
Thesis Director

Douglas Duane Rhoads, Ph.D.
Committee Member

Dylan Girodat, Ph.D.
Committee Member

Bin Dong, Ph.D.
Committee Member

Abstract

The SARS-related coronavirus (SARS-rCoV) is a highly contagious virus that has raised significant worldwide health concerns. It caused outbreaks in 2002-2003 and more recently in 2019-2020 with SARS-CoV-2. SARS-CoV-2 is responsible for the COVID-19 pandemic, which has resulted in a significant global impact on health and the economy. The spike protein of the virus plays a critical role in its infectivity and transmission, and the receptor-binding domain (RBD) within the spike protein is of particular interest, as it is responsible for binding to the human angiotensin-converting enzyme 2 (ACE2) receptor. In this study, we used Molecular dynamics (MD) simulations to investigate the electrostatic interaction patterns in the active and inactive models of SARS-CoV-1, SARS-CoV-2, and several variants of SARS-CoV-2, including the Alpha, Beta, Delta, and Epsilon variants. MD simulations are a computational method that allows us to model the motion of atoms and molecules over time, providing insights into the structure and behavior of biological molecules. The findings indicate differential electrostatic interaction patterns between the RBD of SARS-CoV-1 and SARS-CoV-2 spike protein. The RBD of SARS-CoV-2 exhibited a slower conformational pattern, which could influence higher stability, potentially affecting its binding affinity with the ACE2 receptor. Additionally, the Delta variant demonstrated significant differences in electrostatic interactions compared to the original SARS-CoV-2 strain, particularly in the N-terminal domain (NTD) and RBD regions. These findings suggest that Delta variant mutations could affect the RBD's binding affinity to the ACE2 receptor, impacting transmission and virulence. Overall, this study highlights electrostatic interaction patterns in SARS-CoV-1, SARS-CoV-2, and variants, with implications for the development of long-term effective vaccines and therapeutics. Understanding the spike protein's molecular basis may enable designing more effective treatments and strategies to prevent the spread of these viruses.

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Introduction

The combination of empirical data and computer-generated simulations has become a standard procedure in modern chemistry and biology. This technique offers a comprehensive approach to examining and predicting properties of various systems, ranging from viruses to individual molecules [1, 2, 3, 4]. Recent technological advancements have facilitated the use of novel methodologies and extensive simulation programs, which enable scientists to monitor molecular behavior in real-time [5, 6]. Biomolecular simulation and modeling have made significant contributions to both basic and applied research. They have enabled scientists to address complex challenges such as understanding protein folding mechanisms, identifying therapeutic targets for common diseases, and developing new materials and medicines [7, 8, 9].

In recent years, a widespread application of the atomistic molecular dynamics (MD) simulation technique has been observed in the investigation of protein behavior in various explicit environments, as evidenced by several recent studies [10, 11, 12, 13, 14, 15, 16]. All-atom MD has gained popularity due to its ability to perform brief simulations of local conformational changes at the nanosecond level, which can provide a reasonable approximation of functionally relevant large-scale conformational transitions that occur over much longer timescales, as seen in most MD-based research [17, 18, 19, 20, 11].

The COVID-19 pandemic that has swept across the globe since its emergence in late 2019 has caused a health crisis of unprecedented proportions [21, 11, 22, 23, 24, 25]. This virus, known as SARS coronavirus-2 (SARS-CoV-2), is highly contagious and has infected millions of people worldwide [26, 27, 28, 29, 30]. Its impact has been felt across all facets of life, from healthcare systems struggling to cope with the influx of patients to businesses and economies grappling with the fallout of lockdowns and travel restrictions [31, 32, 33, 34, 35]. It is worth noting that SARS-CoV-2 is not the first virus of its kind to cause such a pandemic. In 2003, another closely related virus, SARS-CoV-1, emerged and caused a global outbreak [36, 37, 38]. Both viruses share similarities in their spike proteins, which enable them to enter human cells and replicate [11, 39, 40, 41, 36, 38, 42]. However, the spike protein of SARS-CoV-2 has been found to result in a higher infectious rate, which has led to a greater spread of the virus [11].

Moreover, the virus is constantly evolving, with new mutated variants emerging. These variants pose a significant challenge to the effectiveness of current vaccines and raise concerns about the ability of healthcare systems to manage future outbreaks [43, 44, 45]. The emergence of new variants has also highlighted the need for ongoing research and development of new treatments and vaccines to combat this deadly virus

[46]. Despite the challenges posed by the pandemic, there is hope on the horizon. The development and rollout of effective vaccines [47, 48] has been a game-changer, with millions of people vaccinated worldwide. However, the fight against the virus is far from over, and it is imperative that we continue to be vigilant and take all necessary precautions to prevent the spread of the virus. This includes wearing masks, practicing social distancing, and following guidelines from public health authorities. In addition, the COVID-19 pandemic caused by the SARS-CoV-2 virus has highlighted the importance of understanding the behavior of coronaviruses, particularly in the early stages of infection. While SARS-CoV-1 and SARS-CoV-2 share many similarities [11, 39, 40, 41, 36, 38, 42], some potential differences between the two viruses could have significant implications for their transmission and pathogenesis [11, 40].

The objective of this thesis is to investigate these differences in detail, with a particular focus on the differential dynamic behavior and electrostatic interactions of SARS-CoV-1 and SARS-CoV-2 prior to receptor binding. This study will explore other regions outside of the receptor binding domain (RBD) that may contribute to the transmissible difference between these two viruses. By analyzing the behavior of these viruses at the molecular level, we can gain a better understanding of their mechanisms of action and identify potential targets for therapeutic interventions. This information is particularly important given the ongoing evolution of SARS-CoV-2, which has led to the emergence of several variants with different characteristics [46]. Therefore, this study will also aim to investigate the behavior of the SARS-CoV-2 variants and compare them to the original virus. By doing so, we can determine whether the changes in the virus have any impact on its dynamic behavior and electrostatic interactions and whether these changes have implications for the transmission and pathogenesis of the virus. MD simulations [5, 49] will be utilized to achieve this study. The technique will allow us to explore some of the behavior of these viruses at the molecular level, providing insights into their mechanisms of action and potential therapeutic targets.

MD simulations have revolutionized the field of computational biology by allowing scientists to explore the dynamics and energetics of complex biological systems at an atomic level [50, 14, 13]. In particular, these simulations have proven to be a valuable tool for studying protein-protein interactions, including those involving the spike protein and the angiotensin-converting enzyme 2 (ACE2) receptor in SARS-CoV-1 and SARS-CoV-2 [51, 52, 53]. While MD simulations have been used extensively to study the interactions between these proteins, the role of electrostatic interactions in the behavior of these proteins prior to receptor binding and outside of the receptor binding domain (RBD) has not been fully explored. This is an important area of investigation, as it is likely that electrostatic interactions play a significant role in the transmission and

pathogenesis of these viruses. Electrostatic interactions are critical for the stability and function of proteins, and can strongly influence protein-protein interactions. In the case of the SARS-CoV-1 and SARS-CoV-2 viruses, it is possible that electrostatic interactions outside of the RBD may contribute to the differences in transmissibility and pathogenicity observed between these two viruses. By using MD simulations to explore the behavior of these viruses at an atomic level, we can gain a better understanding of the role of electrostatic interactions in their behavior. By investigating the differential behavior and electrostatic interactions of SARS-CoV-1 and SARS-CoV-2 prior to receptor binding and exploring the role of these interactions outside of the receptor binding domain, we can gain insights into the molecular mechanisms that contribute to the transmissibility and pathogenicity of these viruses. This knowledge can be leveraged to design more effective and specific inhibitors that can target these interactions and prevent the virus from infecting host cells. The development of such inhibitors would be a major step forward in the fight against COVID-19, as it would provide a new tool for clinicians to treat infected patients and prevent the spread of the disease. Furthermore, the results of this study could shed light on the evolution of SARS-CoV-2 and how new variants arise. As the virus continues to mutate, it is important to understand how these changes affect the virus's behavior and interactions with host cells. This knowledge could lead to the development of more effective disease prevention and control strategies, including the development of new vaccines and therapies that are tailored to specific variants of the virus.

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Chapter 1: A Comprehensive Review of SARS-CoV-1 and SARS-CoV-2 Spike Protein: Insights from Molecular Dynamics Simulations and Electrostatic Interactions Analyses

Background to SARS-CoV-1 and SARS-CoV-2

Severe acute respiratory syndrome coronavirus (SARS-CoV) is a highly infectious virus that belongs to the family Coronaviridae [1, 2], which is characterized by enveloped, positive-sense, single-stranded RNA viruses [3, 4]. The virus causes severe respiratory illness in humans, with symptoms ranging from mild to severe and in some cases, leading to fatal outcomes [5, 6, 7].

There are two known strains of the virus, SARS-CoV-1, and SARS-CoV-2 [8, 9], which have caused epidemics in 2002-2003 [10] and 2019-2021 [11], respectively. SARS-CoV-1 was first identified in Guangdong Province, China, in November 2002, and it quickly spread to other countries, leading to a global outbreak that lasted until 2003 [12, 10]. The virus was found to have originated in bats and was transmitted to humans through intermediate hosts, such as civet cats [13, 14, 15].

On the other hand, SARS-CoV-2, the virus responsible for the ongoing COVID-19 pandemic [16, 17], was first identified in Wuhan, China, in December 2019 [18]. The virus quickly spread globally, leading to a pandemic that has had a profound impact on human health and the global economy [19, 20]. The virus is also believed to have originated in bats, with transmission to humans occurring through an intermediate host [21, 22, 23].

SARS-CoV-1 and SARS-CoV-2 share many similarities in terms of their genetic structure and pathogenesis [24, 25]. Both viruses use the spike protein on their surface to enter host cells, primarily through binding to the angiotensin-converting enzyme 2 (ACE2) receptor [25, 26]. However, SARS-CoV-2 has a higher binding affinity to ACE2, which may contribute to its increased transmissibility compared to SARS-CoV-1 [27, 28]. In addition, SARS-CoV-2 can also be transmitted by individuals who are asymptomatic or presymptomatic [29], further contributing to its rapid spread.

In this literature review, we provide a brief overview of the background and characteristics of SARS-CoV-1 and SARS-CoV-2. This includes their origin, transmission, symptoms, and epidemiology. Understanding the characteristics of these viruses are crucial in developing effective preventive measures and treatment strategies for these highly infectious diseases.

Molecular Dynamics Simulation as a Tool for Studying Protein Interactions

Molecular dynamics (MD) simulation has become a powerful tool for studying the behavior of complex biological systems, including protein interactions [30, 26, 31, 32]. MD simulation is a computational method that allows researchers to simulate the behavior of atoms and molecules over time enabling a detailed understanding of their dynamics and interactions [33, 34, 35]. Proteins play a critical role in many biological processes, and the interactions between proteins are fundamental to the functioning of living systems [35, 36]. Protein-protein interactions are often complex, and their study requires sophisticated experimental techniques [37, 38]. However, MD simulation provides an alternative approach that can complement experimental techniques, providing a way to study the interactions of proteins *in silico* [39, 40]. The basic principle of MD simulation is to simulate the movement of atoms and molecules over time, using classical mechanics equations to calculate their positions, velocities, and energies [39, 41, 42]. In the context of protein interactions, this involves modeling the protein molecules and their solvent environment as a set of particles and then simulating their behavior using molecular force fields that describe the interactions between them [43, 44].

One of the strengths of MD simulation is its ability to capture the dynamics of protein interactions [45, 39]. Proteins are not static structures, but rather they undergo constant fluctuations and movements [46, 47]. These dynamics are important for understanding how proteins interact with each other and with other molecules. MD simulation can provide a dynamic view of protein interactions, allowing researchers to study how protein conformations change over time, how proteins associate and dissociate, and how they undergo conformational changes during binding [39, 48]. MD simulation can also provide detailed information on the energetics of protein interactions [49]. The force fields used in MD simulation calculations incorporate both bonded and non-bonded interactions, including electrostatics, van der Waals forces, and hydrogen bonding [43]. By simulating the interactions between proteins and analyzing the energy landscape, researchers can gain insight into the thermodynamics and kinetics of protein-protein interactions.

In recent years, MD simulation has been used to study a wide range of protein interactions, including protein-protein interactions [50], protein-ligand interactions [51], and protein-DNA interactions [52].

However, MD simulation also has its limitations. One of the biggest challenges is that MD simulation calculations can be computationally intensive, and simulating large systems over long timescales can be challenging [53, 40].

SARS-CoV-1

Severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) is a highly infectious virus that belongs to the Coronaviridae family [1]. It was first identified in November 2002 in Guangdong province, China, and subsequently caused a global outbreak that lasted until July 2003 [54, 10, 12]. The virus was transmitted from animals to humans, likely from civet cats to humans, and then spread through human-to-human contact [13, 14, 15].

SARS-CoV-1 is an enveloped, positive-sense, single-stranded RNA virus with a genome length of approximately 30,000 nucleotides [55]. The genome encodes for 14 proteins, including the spike (S) protein, which mediates viral entry into host cells [56]. The S protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor in human cells, facilitating viral entry into the cells [57, 58, 59]. Other proteins encoded by the genome include the nucleocapsid (N) protein, which packages the viral RNA genome, and the membrane (M) and envelope (E) proteins, which are involved in viral assembly and release [60].

SARS-CoV-1 caused severe respiratory illness in infected individuals, with symptoms such as fever, cough, and difficulty breathing. The virus had a mortality rate of approximately 10% [61, 62], with the elderly and those with underlying medical conditions more vulnerable to severe disease. Effective measures such as early detection, isolation, and treatment of infected individuals, as well as public health interventions, helped to control the spread of the virus and ultimately contain the outbreak [61, 62].

SARS-CoV-2

SARS-CoV-2 is a highly infectious virus that causes severe respiratory illness in humans [63, 64, 65, 66]. It is an enveloped virus with a single-stranded RNA genome that is approximately 30,000 nucleotides long and encodes for 27 proteins. Among these proteins, the spike (S) protein is the primary mediator of viral entry into host cells [2]. The S protein of SARS-CoV-2 shares a similar mechanism of entry with SARS-CoV-1 in that it binds to the angiotensin-converting enzyme 2 (ACE2) receptor in human cells [25, 24, 67]. However, the S protein of SARS-CoV-2 has mutations that allow it to bind to ACE2 more efficiently than SARS-CoV-1 [68, 64], potentially contributing to its higher transmissibility and infectivity.

In addition to the S protein, the genome of SARS-CoV-2 encodes for several other proteins, including the nucleocapsid (N) protein, which packages the viral RNA genome, and the membrane (M) and envelope (E) proteins, which are involved in viral assembly and release. Moreover, the genome of SARS-CoV-2

encodes for several non-structural proteins involved in viral replication and immune evasion, such as the RNA-dependent RNA polymerase (RdRp), helicase, and proteases [60].

The clinical manifestations of COVID-19, the disease caused by SARS-CoV-2, can range from mild to severe respiratory illness, with symptoms such as fever, cough, and difficulty breathing. In severe cases, the disease can progress to pneumonia, acute respiratory distress syndrome, and even death [5]. Due to its highly infectious nature and potential for severe disease, SARS-CoV-2 has caused a global pandemic since its emergence in late 2019.

Spike Glycoprotein

The spike glycoprotein of coronaviruses plays a crucial role in viral entry into host cells. The S protein binds to specific receptors on the surface of host cells, facilitating fusion between the viral envelope and the host cell membrane. In the case of SARS-CoV-2, the S protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor on human cells [69, 70, 71, 72]. This binding event triggers a conformational change in the S protein, allowing it to fuse with the host cell membrane and release the viral genome into the host cell.

The spike glycoprotein is a major target of the immune response, as it is the main surface antigen of the virus that is recognized by the immune system [73]. Antibodies produced by the immune system can bind to the spike glycoprotein and neutralize the virus by preventing its attachment to host cells. The spike glycoprotein is also a major target of T cells, which can recognize and eliminate infected cells that present viral spike glycoprotein fragments on their surface [74].

Structure of SARS-CoV Spike Glycoprotein

The spike glycoprotein is a type 1 membrane protein that mediates entry into host cells [75]. The spike glycoprotein of SARS-CoV-1 and SARS-CoV-2 consists of two subunits, S1 and S2, which are important for virus-host interactions [69, 76].

The S1 subunit contains the N-terminal domain (NTD) and receptor-binding domain (RBD) (Figure 1B), which interacts with the host cell receptor, angiotensin-converting enzyme 2 (ACE2), and is responsible for determining the host range and tissue tropism of the virus. On the other hand, the S2 subunit contains the fusion peptide and two heptad repeat regions (HR1 and HR2), which are essential for mediating membrane fusion. The overall structure of the spike glycoprotein is a large, heavily glycosylated protein that forms a

trimeric complex (Figure 1A) on the surface of the virus. Each monomer of the spike glycoprotein comprises a central core structure, a receptor-binding domain, and a fusion peptide [77, 78]. The spike protein's RBD plays a crucial role in binding to the host cell receptor ACE2 [79, 80]. The RBD is located on the protein's surface and comprises approximately 200 amino acid residues that are essential for ACE2 binding. Among these residues are those located in a loop area called the receptor binding motif (RBM), as shown in Figure 1B, which directly interacts with ACE2 [26, 80]. The binding of the RBD to ACE2 is a crucial step in viral infection, allowing the virus to enter the host cell and start replicating [80, 81]. Consequently, RBD is an attractive target for vaccines and therapeutics as interrupting the interaction between RBD and ACE2 may prevent viral entry and infection [82]. Although the RBD of SARS-CoV-2 is highly similar to that of SARS-CoV, some key differences in the amino acid sequence may affect binding to ACE2 [81, 83]. Thus, understanding the RBD's structure and function is critical for developing effective treatments and vaccines for COVID-19. The N-terminal domain (NTD) is a region of the spike protein of the severe acute respiratory syndrome coronavirus (SARS-CoV) that is located in the S1 subunit, adjacent to the receptor-binding domain (RBD). The NTD is a highly glycosylated region of the spike protein that contains approximately 200 amino acids. It is characterized by a β-sheet structure that is stabilized by disulfide bonds [84]. Recent studies have suggested that NTD may play a role in immune evasion by the virus [85]. The NTD has been shown to contain epitopes that can elicit neutralizing antibodies, but these antibodies may not be as effective against SARS-CoV variants that have acquired mutations in the NTD. This is thought to be due to the high degree of variability in the NTD region, which may allow the virus to escape recognition by the immune system. In addition to its role in immune evasion, the NTD may also contribute to the stability and function of the spike protein [86]. Studies have suggested that the NTD may help to stabilize the RBD and facilitate its interaction with the ACE2 receptor [85, 87]. While the NTD of the SARS-CoV spike protein is not as well understood as the RBD, it is an important region of the protein that plays a critical role in the function and virulence of the virus. Further research into the structure and function of the NTD may provide valuable insights into the development of effective vaccines and therapeutics to combat SARS-CoV and other related coronaviruses.

The structure of the spike glycoprotein of both SARS-CoV-1 and SARS-CoV-2 has been determined using X-ray crystallography and cryo-electron microscopy (cryo-EM) techniques [88, 89, 90]. The structure of the spike glycoprotein is similar between the two viruses, with some notable differences. For instance, the RBD of the SARS-CoV-2 spike glycoprotein adopts a more "up" conformation compared to SARS-CoV-1

which could explain the higher affinity of SARS-CoV-2 for ACE2 [91, 92].

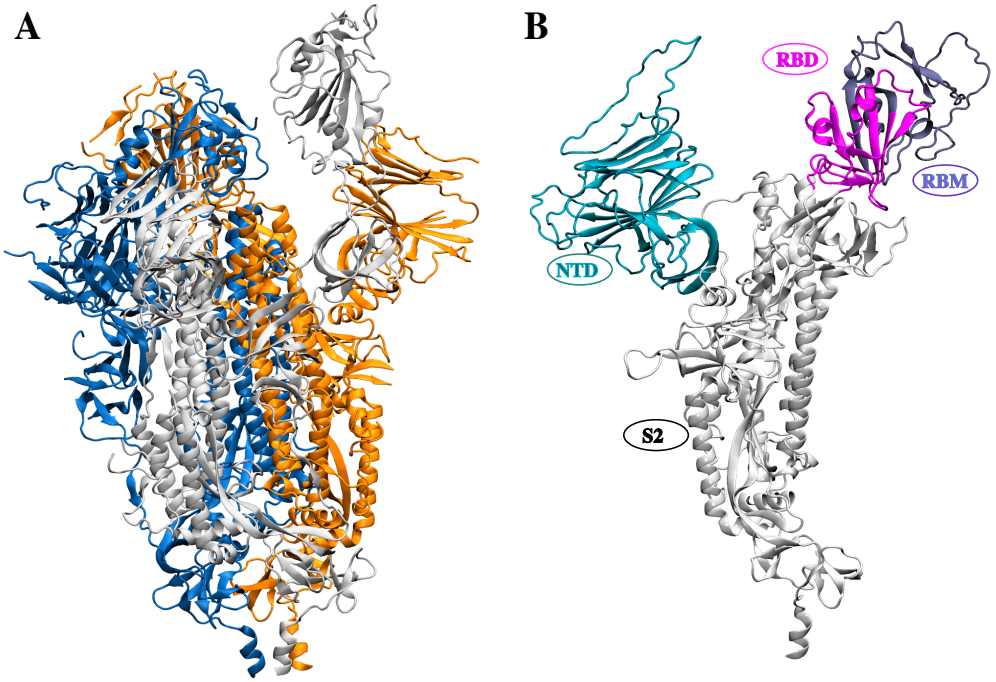

Figure 1: (**A**) The trimeric structure of the Spike protein of SARS-CoV is depicted in this figure, obtained from the protein data bank (PDB ID: 6VYB). The three individual protomers are presented in orange, blue, and white colors. (**B**) This figure displays a single protomer of the Spike protein of SARS-CoV-2. It highlights the receptor binding domain (RBD) in magenta, the receptor binding motif (RBM) in purple, the nucleotide binding domain (NTD) in cyan, and the S2 region in white.

Electrostatic Interactions in Protein Structures

Proteins are macromolecules that perform a wide range of essential functions in living organisms. These functions are often mediated by the interactions between different amino acid residues within the protein structure [93]. One important type of interaction is electrostatic interaction, which arises due to the distribution of electric charges on the protein's surface [50].

In proteins, electrostatic interactions can have a significant impact on protein stability, folding, and function [50]. The presence of electrostatic interactions in a protein structure can also affect its conformational dynamics. Electrostatic interactions between residues can lead to repulsion or attraction forces that can influence the stability and flexibility of the protein structure [94]. For example, electrostatic interactions can contribute to the stability of protein structures by forming salt bridges, which are strong electrostatic

interactions between oppositely charged residues. These salt bridges can stabilize the folded structure of the protein by bringing together oppositely charged amino acid residues that are separated in the unfolded state [50].

In addition to their functional roles, electrostatic interactions can also contribute to protein misfolding and aggregation. This is because charged residues on the protein surface can interact with other charged molecules, such as ions or other proteins, leading to the formation of protein aggregates that can be toxic to cells [95].

The electrostatic interactions between charged residues in a protein can be either short-range or long-range. Short-range electrostatic interactions occur between charged residues that are in close proximity to each other, while long-range electrostatic interactions occur between charged residues that are far apart in the protein structure [50, 96].

Another important type of electrostatic interaction in proteins is the formation of hydrogen bonds. Hydrogen bonds occur between a hydrogen atom bonded to an electronegative atom and another electronegative atom in the protein structure. These interactions can stabilize protein structures by forming stable secondary structures such as alpha-helices and beta-sheets [97].

Computational methods such as molecular dynamics simulations [98] and electrostatic potential calculations [99] are widely used to study the role of electrostatic interactions in protein structures. These methods can provide insight into the nature and strength of electrostatic interactions and their effects on protein stability, conformational dynamics, and protein-protein interactions [50].

Overall, electrostatic interactions are an essential component of protein structure and function. By understanding the role of electrostatic interactions in protein structures, researchers can gain insights into the molecular mechanisms of protein folding, stability, and function, which has implications for the design of new drugs and therapies targeting protein-protein interactions.

Current Research Findings on Electrostatic Interactions in Spike Glycoproteins

According to recent research studies, the stability and function of spike glycoproteins are significantly influenced by electrostatic interactions [100, 101, 102].

A study published in the journal Science in 2005 described the crystal structure of the spike protein RBD of SARS coronavirus in complex with its cellular receptor, ACE2 [76]. The study identified the key amino acid residues involved in the binding interaction and characterized the conformational changes in

the spike protein that facilitate viral entry. They revealed a hinge-like motion in the RBD of SARS-CoV-1, which allows it to switch between a ”closed” and ”open” conformation, facilitating binding to ACE2. The researchers noted that the interaction between the spike protein RBD and its cellular receptor ACE2 is mediated by both hydrogen bonds and van der Waals interactions, which play important roles in maintaining the specificity and stability of the complex. The study provided insights into the mechanism of SARS-CoV-1 infection and revealed their implications for the development of antiviral therapies and vaccines against SARS-CoV-1 and other related coronaviruses [76].

A more recent study in 2020 by Wang *et al.*. investigated the role of electrostatic interactions in the binding of the SARS-CoV-2 spike protein to the human ACE2 receptor, which is the initial step in viral entry into host cells [103]. The researchers used computational techniques to analyze the interaction between the virus spike protein and its host receptor ACE2 and discovered that the binding interface is made up of both hydrogen bonds and hydrophobic interactions. The study showed that the binding interface consisted of a hydrophobic region and a weak hydrogen-bonding network. They also found that a mutation from a hydrophobic residue in SARS-CoV to Lys417 in SARS-CoV-2 created a salt bridge, which resulted in greater electrostatic complementarity. Additionally, the substitution for four out of five proline residues in a short loop enhanced hydrophobic packing and led to a conformation shift in the binding groove, believed to have contributed to the increased infectivity of SARS-CoV-2 compared to SARS-CoV. However, the disruption of hydrophobic contacts in the complex of the SARS-CoV-neutralizing antibody 80R indicated that it could not recognize SARS-CoV-2. The study suggested that both hydrogen bonding and hydrophobic interactions are important for the efficient binding of SARS-CoV-2 to the ACE2 receptor and offered insights into the molecular mechanisms of viral entry and infection [103].

Taka *et al.* conducted molecular dynamics simulations in 2021 to identify critical residues that stabilize the interaction between the receptor-binding domain (RBD) of the SARS-CoV-2 Spike Glycoprotein and the Human ACE2 Receptor [104]. They found an extended network of salt bridges, hydrophobic and electrostatic interactions, and hydrogen bonds between the two molecules. In particular, the RBD of SARS-CoV-2 Spike Glycoprotein showed hydrophobic interactions with PD residues F28, L79, M82, and Y83 through F486 and Y489, and with T27 through L455, F456, Y473, and A475. High-frequency salt bridges and hydrogen bonds were observed between K417-D30 and E484-K31 and among N487-Y83, T500-D355, and Q493-E35. Moderate-frequency hydrogen bonds were observed among Y449-D38, Q498-K353, T500-Y41, Y505-E37, and Q493-E35. Furthermore, residue pairs Y453-H34, N487-Q24, T500-Y41, N501-K353,

Q493-K31, and Y449-Q42 showed electrostatic interactions throughout the simulations. The authors categorized interactions as high or moderate frequency based on their occurrence in 49% or more, and 15-48% of the total trajectory, respectively [104]. Fernando *et al.* [102] observed that mutating the residues reduced the work required to unbind the S protein from ACE2 and that the hydrophobic end of RBD was the main anchor site, which was the last to unbind from ACE2 under force. They suggested blocking the hydrophobic surface of RBD with neutralizing antibodies as an effective strategy to prevent S-ACE2 interactions [102].

Studies on the Effect of Spike Protein Variants on Viral Infection and Transmission

The spike protein of the SARS-CoV-2 virus is the primary target of vaccines and therapeutic antibodies [105, 106]. The spike protein mediates viral entry into host cells by binding to the ACE2 receptor on the surface of human cells [25, 59, 64]. However, the emergence of new variants of the virus with mutations in the spike protein has raised concerns about the effectiveness of current vaccines and treatments [107, 108].

Several studies have investigated the effect of spike protein variants on viral infection and transmission [109, 110, 111, 112]. The World Health Organization (WHO) has identified several variants of concern, including the Alpha, Beta, Gamma, and Delta variants, which have become dominant in different parts of the world. These variants have mutations in the spike protein that may affect its interaction with the ACE2 receptor and its recognition by neutralizing antibodies [109].

In vitro studies have shown that some of these variants have increased infectivity compared to the original strain of the virus. For example, the Alpha variant has been shown to be 50% more transmissible than the original strain, while the Delta variant is thought to be twice as transmissible as the Alpha variant [110, 111]. These studies suggest that the mutations in the spike protein may increase the efficiency of viral entry into host cells, leading to more efficient viral replication and transmission.

In addition to increased infectivity, some studies have also suggested that spike protein variants may be more resistant to neutralizing antibodies generated by vaccination or previous infection. A study conducted by Wang et. al. found that the Beta and Gamma variants were more resistant to neutralizing antibodies than the original strain of the virus and that some antibodies were completely ineffective against these variants [112]. This suggests that current vaccines and therapies may be less effective against these variants, potentially leading to breakthrough infections and the need for booster shots [112].

However, other studies have shown that the existing vaccines are still effective against most variants of the virus, including the Delta variant [113]. A study from Morbidity and Mortality article reported

that the vaccines were highly effective against the Delta variant, providing over 90% protection against hospitalization [113].

Overall, the studies on the effect of spike protein variants on viral infection and transmission suggest that these variants may have increased infectivity and may be more resistant to neutralizing antibodies generated by vaccination or previous infection. However, the existing vaccines are still effective against most variants of the virus, including the Delta variant, and breakthrough infections are relatively rare. Ongoing surveillance and research will be needed to monitor the emergence of new variants and to develop strategies to prevent and treat COVID-19.

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Chapter 2: Dynamic Differences between SARS-CoV-1 and SARS-CoV-2 spike glycoprotein through electrostatic interaction analysis

Abstract

The highly infectious coronavirus SARS-CoV-2, responsible for the COVID-19 pandemic, shares sequence identity and binding patterns with SARS-CoV-1, which caused an epidemic in 2003. However, electrostatic interactions are critical to the spread of SARS-CoV-2 and may be responsible for its heightened transmissibility. To address this issue, we propose studying events prior to the binding of the receptor binding domain to the angiotensin-converting enzyme 2 (RBD-ACE2) in comparison to SARS-CoV-1, to develop therapeutic agents that can inhibit binding and prevent the spread of COVID-19. Our study utilized cryogenic electron microscopy (Cryo-EM) structures of active and inactive models of SARS-CoV-1 and 2, and investigated conformational changes and differences in electrostatic interactions prior to ACE2 binding. Our analysis revealed that SARS-CoV-1 differs significantly in their active and inactive state. Within the conserved residues of the RBD, we found that salt bridge interactions (R273-D290, R328-D578) were more stable in SARS-CoV-2 than in the conserved residues of SARS-CoV-1 (K258-D277, R315-D564), along with the same pattern in hydrogen bond networks. We attribute this to the active model of SARS-CoV-1 undergoing a conformational change that causes the RBD to move towards the N-terminal domain (NTD) (D24-K365), forming a semi-closed state. These findings can inform the design of therapeutic agents that can inhibit binding and prevent the spread of COVID-19 despite the emergence of new variants. By targeting the differences in electrostatic interactions between SARS-CoV-1 and 2, we can develop drugs that are effective against both viruses.

The defense will be conducted virtually on Teams, and I have provided the link below for your ease of access. Furthermore, I have enclosed a copy of my thesis for your

Disclaimer: The Journal of Biological Chemistry has published some of the figures that are reported in this chapter. (Govind Kumar V, Ogden DS, Isu UH, Polasa A, Losey J and Moradi M. Prefusion spike protein conformational changes are slower in SARS-CoV-1. Journal of Biological chemistry (2022). https://doi.org/10.1016/j.jbc.2022.101814) [1].

Introduction

The ongoing COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a global health crisis [2, 3, 4, 5, 6, 7, 1, 8, 9, 10, 11, 12, 13]. The virus enters host cells through its spike (S) protein, which binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell surface [14, 1, 15, 16, 17]. The molecular mechanisms underlying the interaction of the S protein with ACE2 are crucial for the development of effective therapies and vaccines [18, 19, 20]. Electrostatic interactions play a significant role in protein-protein interactions and are particularly important in receptor-ligand binding [21, 22, 23, 18, 24]. The S protein of SARS-CoV-2 is known to interact with ACE2 through electrostatic interactions [25, 26, 27, 28]. However, the contribution of electrostatic interactions to the binding affinity and the differential electrostatic interaction patterns between SARS-CoV-1 and SARS-CoV-2 have not been extensively investigated prior to the binding of the host cell receptor.

Molecular dynamics simulations have emerged as a powerful tool for investigating the molecular basis of protein-protein interactions [29, 30, 31, 32]. MD simulations can provide insights into the dynamics and energetics of protein-protein interactions and identify key residues and interactions involved in the binding process [33, 34, 31]. In this study, we employed MD simulations to investigate the electrostatic interaction patterns of the S proteins of SARS-CoV-1 and SARS-CoV-2 in their apo form. Our goal was to compare the electrostatic interaction patterns of the two viruses and identify any differences that may contribute to the increased infectivity of SARS-CoV-2. Previous studies have investigated the S protein-ACE2 interaction using MD simulations [18, 4, 32, 35]. These studies have identified key residues and interactions involved in the binding process and have provided insights into the conformational changes that occur during the binding process. However, the contribution of electrostatic interactions to the binding affinity and the differential electrostatic interaction patterns between SARS-CoV-1 and SARS-CoV-2 in their apo form have not been extensively investigated.

The goal of this research is to accurately describe, at an atomic and thermodynamic level, the structural and dynamic differences between SARS-CoV-1 and SARS-CoV-2 S proteins under different conditions. We envisage that the use of microsecond-level all-atom MD simulations in conjunction with statistical mechanics-based enhanced sampling techniques would provide a detailed, reliable account of the S glycoprotein prefusion mechanism. The rationale for the proposed research is that a proper understanding into the molecular differences between SARS-CoV-1 and SARS-CoV-2 S glycoprotein membrane prefusion

state will be a huge advantage in the design and possible modifications of therapeutic agents to combat the spread of COVID-19. Our study builds on previous work by specifically focusing on the role of electrostatic interactions in the absence of ACE2 and comparing the electrostatic interaction patterns of SARS-CoV-1 and SARS-CoV-2 in their apo form. We hypothesize that the electrostatic interaction patterns of the two viruses will differ in their apo form, which may contribute to their differential infectivity. Our study aims to provide a comprehensive understanding of the differential dynamic behavior and electrostatic interactions observed in SARS-CoV-1 and SARS-CoV-2 prior to receptor binding. Additionally, we consider hot spot regions outside the receptor binding domain (RBD), which could contribute to the transmissible difference between the two viruses despite their striking similarities.

The findings of our study have important implications for the development of therapeutics and vaccines against SARS-CoV-2. A better understanding of the molecular mechanisms underlying the S protein-ACE2 interaction in the absence of ACE2, and the role of electrostatic interactions in this process, may enable the design of more effective and specific inhibitors to target this interaction.

Materials and Methods

For our research project, we employed the All-Atom Molecular Dynamics (MD) Simulations technique [36, 31], which is a highly advanced computational analysis method. This method involves analyzing every atom in the molecule at regular intervals to determine its conformational dynamics [37, 38]. To ensure the accuracy of our simulations, we considered the electrostatic interactions between amino acid residues, and used the NAMD program to conduct our simulation. [39, 40]. The simulation of proteins consists of three essential steps: initialization, equilibration, and production [39, 41, 42]. In the initialization step, the positions and momenta of the atoms are loaded from the previous simulation. During equilibration, the simulation runs until equilibrium is reached, enabling the system to settle down and achieve stability. Finally, in the production step, the simulation is finalized and prepared to continue [39]. This process enabled us to gain insights into the complex molecular behavior of the SARS-CoV-1 and SARS-CoV-2 spike proteins. Our use of this advanced computational approach has allowed us to provide a detailed and thorough analysis of the differential behavior of the SARS-CoV-1 and SARS-CoV-2 spike proteins. Our results suggest that electrostatic interactions play a vital role in the stability of the active SARS-CoV-2 spike protein compared to the active SARS-CoV-1 spike protein. Overall, our findings provide important insights into the molecular

mechanisms underlying the behavior of these two viruses and could inform future research efforts aimed at developing effective treatments and therapies.

Molecular Dynamics Simulation

The cryo-EM structures of the spike proteins of SARS-CoV-2[43] and SARS-CoV-1 [44], with both active and inactive forms, were obtained from the Protein Data Bank (PDB) [45]. Using CHARMM-GUI [46, 47], simulation systems were built and engineered residues were mutated back to their wildtype counterparts. The systems were solvated with TIP3PBOX water and neutralized with 0.15M NaCl. Each system underwent several steps, including energy minimization, relaxation, and equilibration, to ensure accurate analysis. All simulations were performed using the NAMD2.13 [39, 40] simulation package with the CHARMM36 all-atom additive force field. The simulations were carried out with a time step of 2 fs at 310 K, using a Langevin thermostat to maintain temperature and the Nose-Hoover Langevin piston method to maintain pressure at 1 atm [48]. The missing residues and loops on all systems were generated using Modeller [49]. 5μs MD simulations were performed for all four systems, with additional simulations for the active SARS-CoV-1 and SARS-CoV-2. The simulations were run on TACC Longhorn initially, with production runs on Anton2 [50] for each model, allowing the characterization of the conformational dynamics of the spike protein from both viruses.

Salt Bridge/Hydrogen Bond Formation/Disruption: Salt bridges are essential for maintaining the structural integrity and proper function of proteins [51, 52]. This study aims to identify key interactions that contribute to the stability of the SARS-CoV-2 spike protein or play a crucial role in the SARS-CoV-1 active conformational transition, using salt-bridge and hydrogen-bond analysis. The analysis was conducted for SARS-CoV-2 and SARS-CoV-1 systems, using the VMD Timeline plugin [53] at a cutoff distance of 4.0 Å to identify salt bridges. The cutoff distance is defined as the distance between the oxygen atom of the acidic residue and the nitrogen atom of the basic residue. If the distance between any oxygen and nitrogen atoms are within the cutoff distance (default 3.2 Å) in at least one frame, a salt bridge is considered to be formed. The VMD HBond plugin [53] was used for hydrogen bond analysis, with a donor-acceptor distance cutoff of 3.5 Å and an angle cutoff of 30 degrees. The study reports the observed salt-bridge and hydrogen-bond interactions in the simulations. These interactions are critical for understanding the stability and function of the spike proteins in the two coronavirus strains. This research sheds light on the differential behavior of these two strains and provides insight into potential targets for drug development and

therapeutic interventions. Overall, salt-bridge and hydrogen-bond analysis is an important tool for studying protein structure and function, particularly in the context of viral infections.

Results

Unbiased all-atom molecular dynamics (MD) simulations were performed for a duration of 5 microseconds (μs) on both inactive and active states of the SARS-CoV-1 and SARS-CoV-2 spike proteins. Based on our research, we discovered distinct differences between the active and inactive states of SARS-CoV-1 in terms of their electrostatic interactions, particularly within their RBD. Notably, we observed a significant salt bridge interaction between D312 and K514 in the inactive state that is absent in the active state (Figure 2 [1]). Furthermore, our analysis suggests that the active spike protein of SARS-CoV-1 undergoes a spontaneous, large-scale conformational transition that renders it essentially inactive. Our analysis of salt bridge interactions revealed this through a unique set of interactions that are specific to the active SARS-CoV-1 spike protein. In particular, we observed that the residues D23 and D24 in the N-terminal domain (NTD) form relatively stable salt bridges with K365 in the receptor binding domain (RBD) of the spike protein in its active state, whereas these salt bridges are not formed in the inactive state (as illustrated in Figure 3 [1]). We postulate that an inter-domain electrostatic interaction between the NTD and RBD may be responsible for the inactivation of the active spike protein. Our confirmation of the inactivation mechanism for the SARS-CoV-1 spike protein is based on several pieces of evidence. Firstly, we observed that the salt bridge interaction between residues D23 and D24 in the NTD and K365 in the RBD is only present in the active protomer (protomer A) of SARS-CoV-1. We did not observe this interaction in the active trajectory of SARS-CoV-2, nor in the inactive trajectories of either SARS-CoV-1 or SARS-CoV-2. Moreover, the lack of conservation of residues D23 and D24 in the SARS-CoV-2 spike protein further supports the specificity of this interaction to SARS-CoV-1.

Additionally, we carried out analysis of residues that are conserved in both SARS-CoV-1 and SARS-CoV-2 spike proteins. Our observations revealed significant differences in some of the intradomain electrostatic interactions. For example, in the RBD, we noted that R328 and D578 formed a stable salt bridge in both active and inactive SARS-CoV-2 spike proteins, while the corresponding residues in SARS-CoV-1 (R315 and D564) did not form a salt bridge in the SARS-CoV-1 spike proteins as seen in Figure 4 [1]. Similarly, within the NTD, R273 and D290 formed a salt bridge in both active and inactive SARS-CoV-2

spike proteins, whereas their corresponding residues in SARS-CoV-1 (K258 and D277) did not form any salt bridge (as illustrated in Figure 5 [1]). The interactions between D600 and K836 in SARS-CoV-1 and the conserved residues D614-K854 in SARS-CoV-2 (Figure 6) depict a notable variation in the behavior of the S2 region of these viruses. These differences emphasize the distinct structural and functional characteristics of the spike proteins in SARS-CoV-1 and SARS-CoV-2.

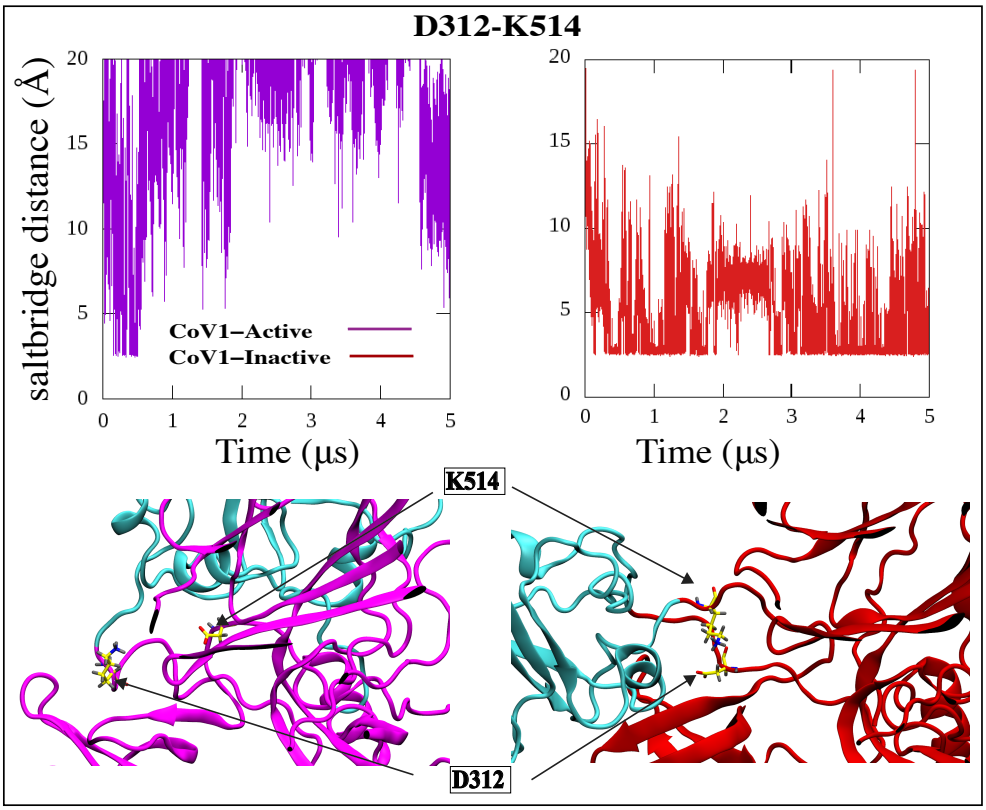


Figure 2: The dynamic behavior of the salt bridge interaction in the RBD of SARS-CoV-1 differs between its active and inactive states. Specifically, the bond between ASP312 and LYS514 is formed in the inactive state, but absent in the active state.

Taken together, these electrostatic interactions may contribute to the relative stability of the active SARS-SARS-CoV-2 spike protein compared to the active SARS-CoV-1 spike protein. Our study provides valuable insights into the role of electrostatic interactions in the conformational dynamics of spike proteins from different coronaviruses.

Discussion

Our research focused on unraveling the distinct behaviors of the spike proteins of two different coronaviruses, namely SARS-CoV-2, and SARS-CoV-1. Specifically, we sought to explore the impact of salt

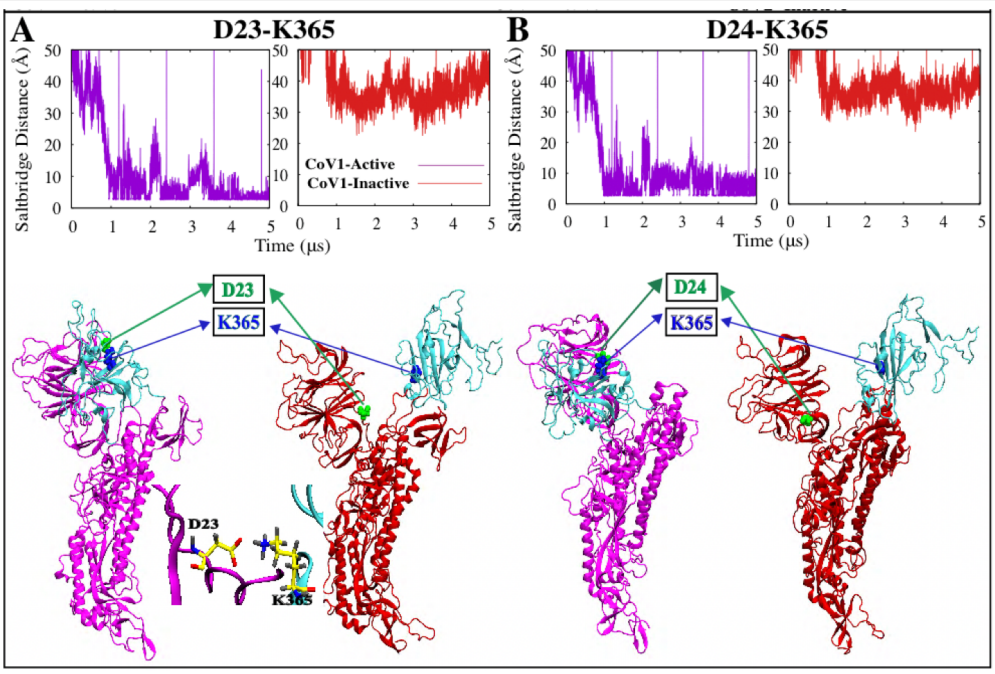


Figure 3: The SARS-CoV-1 spike protein undergoes a transition to a pseudoinactive conformation facilitated by distinct salt-bridge interactions between the N-terminal domain (NTD) and receptor-binding domain (RBD) of the active protomer. In simulations of the SARS-CoV-1 spike protein, the distances between the D23/D24–K365 salt bridges (A/B) were analyzed over time. The initially active SARS-CoV-1 protomer A forms salt bridges between D23/D24 (green) of the NTD and K365 (blue) of the RBD, which are visualized in panels C and D. These salt bridges are unique to the pseudoinactive state of SARS-CoV-1 and are absent in the SARS-CoV-2 spike protein [1].

bridges on the structure and functionality of these proteins. Our findings revealed a striking difference in the behavior of the active SARS-CoV-1 spike protein as compared to its inactive form (Figure 2). We observed that the active spike protein of SARS-CoV-1 undergoes a significant and spontaneous conformational shift as indicated by Figure 3 [1] unlike the active form of SARS-CoV-2 that continuously remained stable [1]. Our study sheds new light on the stability and conformational changes that take place in the spike proteins of both SARS-CoV-2 and SARS-CoV-1. By analyzing salt bridges, we identified unique interactions between the N-terminal domain (NTD) and receptor-binding domain (RBD) that contribute to the stability of the active SARS-CoV-1 spike protein. Our investigation highlighted that specific residues, D23 and D24 in the NTD, form relatively stable salt bridges with K365 in the RBD in the active state of SARS-CoV-1 (Figure 3 [1]). However, these electrostatic interactions are not present in either the inactive state of SARS-CoV-1 or the active or inactive states of SARS-CoV-2. Our findings have significant implications for understand-

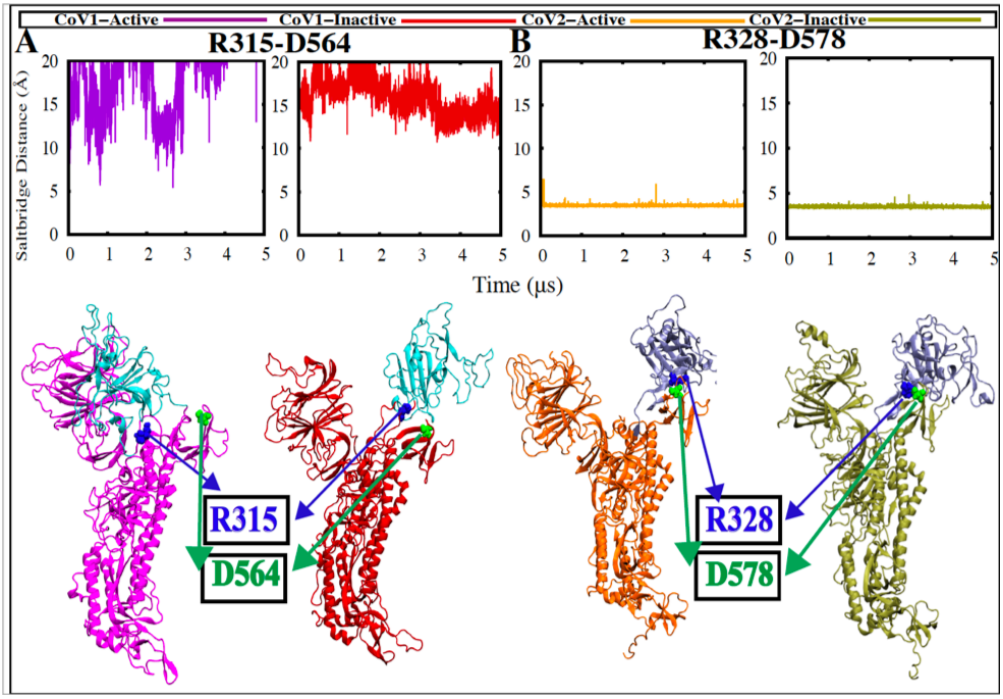


Figure 4: The maintained stability of SARS-CoV-2 is demonstrated by a distinct salt bridge interaction within the RBD region. In SARS-CoV-2, there is a distinctive and stable salt bridge interaction exclusively present in the RBD region, which involves the residues R328 (blue) and D578 (green). On the other hand, the equivalent conserved residues (R315 and D564) in SARS-CoV-1 do not form a salt bridge [1].

ing the conformational changes that occur during the activation and inactivation of the spike protein. We propose that the electrostatic interaction between the NTD and RBD is responsible for the inactivation of the active SARS-CoV-1 spike protein. This finding implies that the transition from the active to the inactive state involves a complex interplay of electrostatic interactions between different domains of the spike protein. This, in turn, suggests that a more comprehensive understanding of these intricate interactions is necessary for developing targeted therapeutic interventions to combat these deadly viruses.

In addition to salt-bridge analysis, the study also conducted hydrogen bond analysis to provide further insight into the specific salt-bridge and hydrogen-bond interactions that are critical to the stability and function of the proteins. The findings revealed striking differences in some intradomain electrostatic interactions between SARS-CoV-1 and SARS-CoV-2 spike proteins. The differences suggest that the stability of the SARS-CoV-2 spike protein is maintained by a different set of interactions compared to the SARS-CoV-1 spike protein. Specifically, the study observed that some conserved residues within the RBD and NTD form stable salt bridges in both active and inactive SARS-CoV-2 spike proteins. However, the corresponding

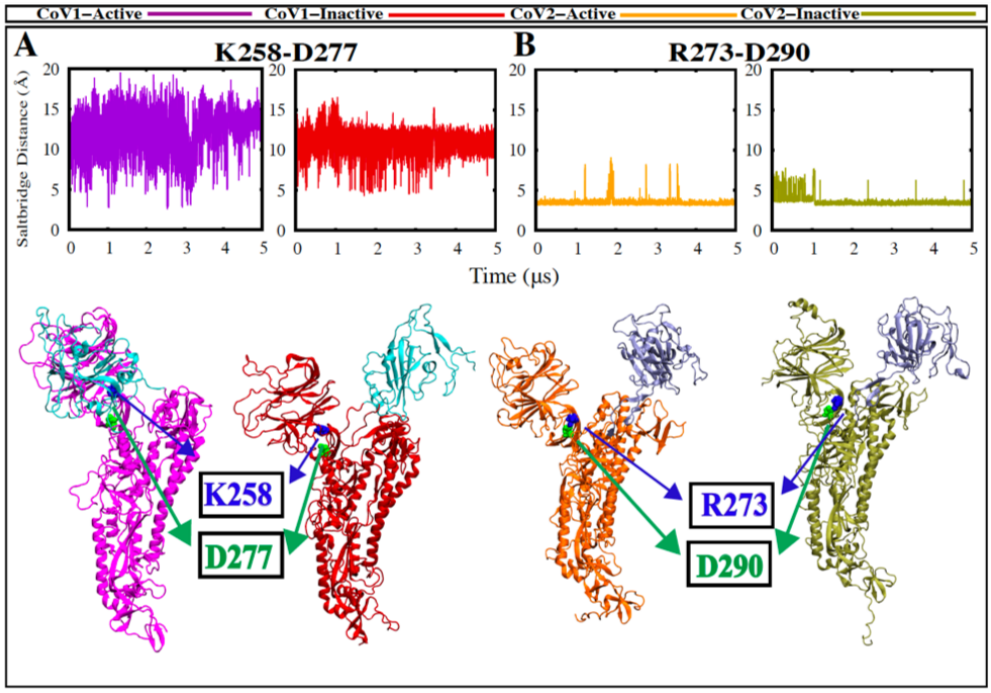


Figure 5: The maintained stability of SARS-CoV-2 is further evidenced by a unique salt bridge interaction within its NTD region. SARS-CoV-2 exhibits a stable and specific salt bridge interaction between R273 and D290 in the NTD region, whereas the corresponding conserved residues (K258 and D277) in SARS-CoV-1 do not form a salt bridge [1].

residues in SARS-CoV-1 spike proteins did not form salt bridges (Figure 7) [1]. This difference in electrostatic interactions between the two proteins suggests that the role of the RBD and NTD in the stability and function of the SARS-CoV-2 spike protein may differ from that of the SARS-CoV-1 spike protein.

Conclusion

The findings from this study provide insight into the specific salt-bridge and hydrogen-bond interactions that play a critical role in the stability and function of the SARS-CoV-1 and SARS-CoV-2 spike proteins. The differences in interactions between the two spike proteins suggest that the stability and function of the SARS-CoV-2 spike protein are maintained by a different set of interactions compared to the SARS-CoV-1 spike protein. The unique interaction between the NTD and RBD in the active SARS-CoV-1 spike protein could potentially be targeted for the development of novel therapeutic strategies against COVID-19.

Our results clearly demonstrate that the active SARS-CoV-2 spike protein undergoes a different set of electrostatic interactions when compared to the active SARS-CoV-1 spike protein. We observed that

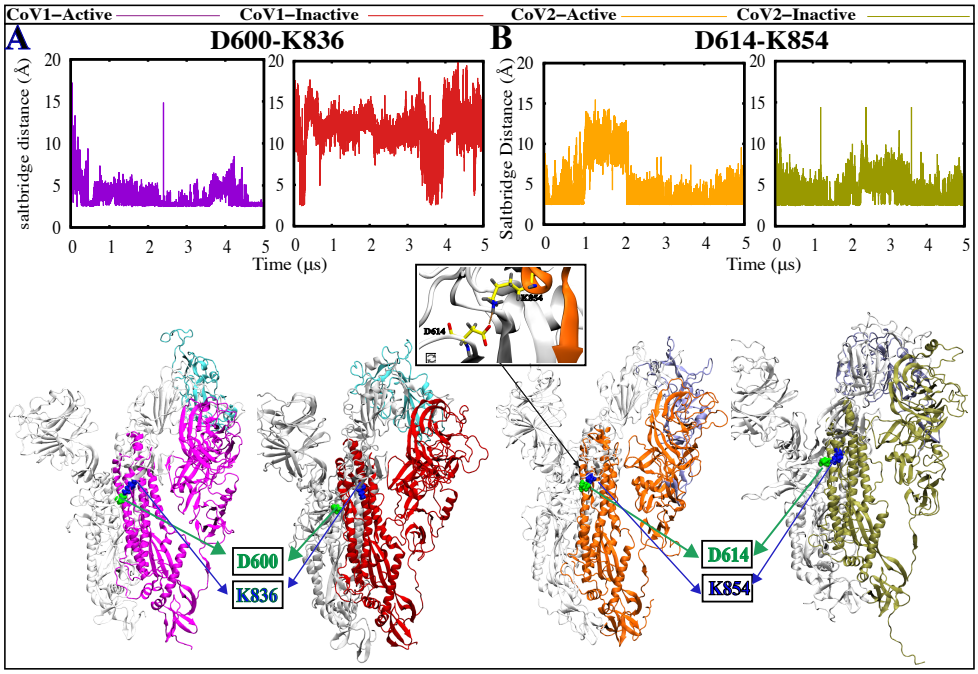
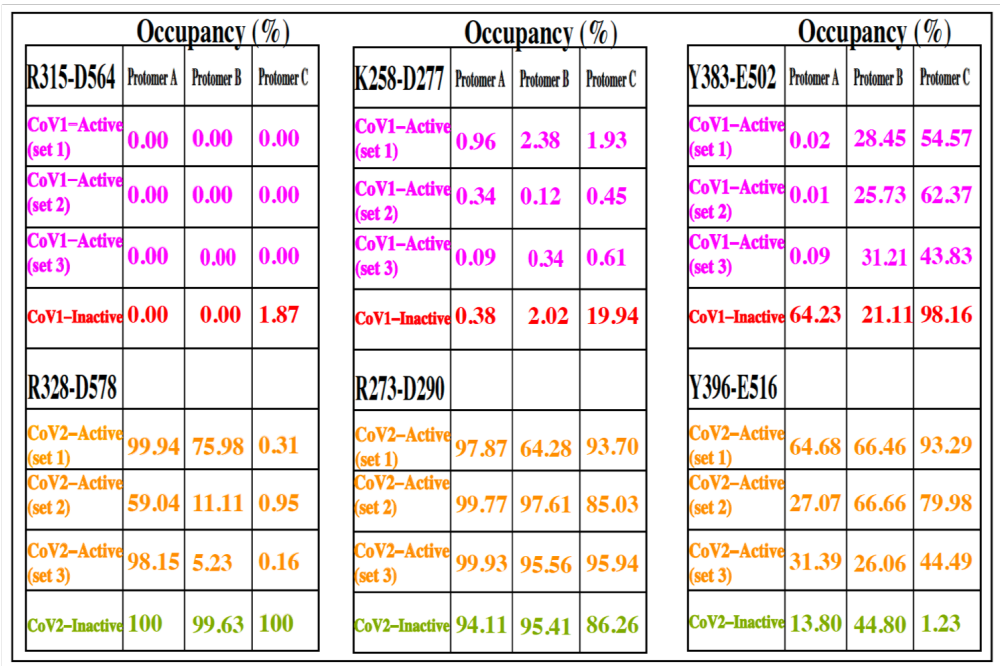


Figure 6: Distinctive behavior is observed in the interactions of conserved residues within the S2 region of both SARS-CoV-1 and SARS-CoV-2. This is exemplified by the formation of a salt-bridge interaction, which occurs mainly in the active state of SARS-CoV-1. However, in SARS-CoV-2, this interaction is unstable in the conserved residues and absent in the inactive state of SARS-CoV-1.

specific residues in the SARS-CoV-1 spike protein form stable salt bridges in the active state, which are absent in the inactive state. In contrast, we found that conserved residues in the SARS-CoV-2 spike protein form stable salt bridges in both the active and inactive states [1]. Overall, our analysis suggests that the unique set of electrostatic interactions in the active SARS-CoV-2 spike protein contributes to its relative stability when compared to the active SARS-CoV-1 spike protein. These findings are consistent with other analyses conducted in our laboratory and provide a further understanding of the structural and functional differences between SARS-CoV-1 and SARS-CoV-2 spike proteins. Our study highlights the importance of studying the electrostatic interactions within proteins to gain a better understanding of their structure and function. Further research into the role of electrostatic interactions in other viral proteins may provide valuable insights into the development of therapeutics and vaccines for viral diseases.



Occupancy (%)			
R315-D564	Protomer A	Protomer B	Protomer C
CoV1–Active (set 1)	0.00	0.00	0.00
CoV1–Active (set 2)	0.00	0.00	0.00
CoV1–Active (set 3)	0.00	0.00	0.00
CoV1–Inactive	0.00	0.00	1.87
R328-D578			
CoV2–Active (set 1)	99.94	75.98	0.31
CoV2–Active (set 2)	59.04	11.11	0.95
CoV2–Active (set 3)	98.15	5.23	0.16
CoV2–Inactive	100	99.63	100

Occupancy (%)			
K258-D277	Protomer A	Protomer B	Protomer C
CoV1–Active (set 1)	0.96	2.38	1.93
CoV1–Active (set 2)	0.34	0.12	0.45
CoV1–Active (set 3)	0.09	0.34	0.61
CoV1–Inactive	0.38	2.02	19.94
R273-D290			
CoV2–Active (set 1)	97.87	64.28	93.70
CoV2–Active (set 2)	99.77	97.61	85.03
CoV2–Active (set 3)	99.93	95.56	95.94
CoV2–Inactive	94.11	95.41	86.26

Occupancy (%)			
Y383-E502	Protomer A	Protomer B	Protomer C
CoV1–Active (set 1)	0.02	28.45	54.57
CoV1–Active (set 2)	0.01	25.73	62.37
CoV1–Active (set 3)	0.09	31.21	43.83
CoV1–Inactive	64.23	21.11	98.16
Y396-E516			
CoV2–Active (set 1)	64.68	66.46	93.29
CoV2–Active (set 2)	27.07	66.66	79.98
CoV2–Active (set 3)	31.39	26.06	44.49
CoV2–Inactive	13.80	44.80	1.23

Figure 7: The percentage of occupancy of hydrogen bonds indicates the presence of specific intra-domain interactions within the RBD and NTD regions. The repeated simulations (set1, set2, and set3) exhibited similar trends, indicating the increased stability of SARS-CoV-2 and the unique behavior of active SARS-CoV-1) [1].

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Chapter 3: Structural Dynamics of Prefusion Spike Protein of SARS-CoV-2 and its Variants

Abstract

Several drugs and vaccines have been created to target the SARS-CoV-2 spike protein, but the effectiveness of these treatments in the long term is uncertain due to the emergence of new SARS-CoV-2 variants and mutations, which cannot be investigated solely through structure-based methods. The number of identified variants of SARS-CoV-2 is rapidly increasing, and some variants are more transmissible and capable of evading the immune system than others. The static information on the wild-type spike protein structure is insufficient to understand the evolving infection process of coronavirus. To address this issue, molecular dynamics (MD) simulations were employed to investigate the active and inactive states of the spike protein in different SARS-CoV-2 variants, including the engineered spike protein associated with the Moderna vaccine. The simulations revealed crucial mutations that cause changes in the structural and conformational dynamics of the spike protein. Some of these mutations lead to significant conformational changes in spike protein variants. These mutations could be responsible for the observed differential dynamic behavior, potentially contributing to higher transmissibility and immune evasion. From our equilibrium simulations, we observe ionic interactions (within the RBD and within the NTD) that could be significant in the observed dynamic behavior of the variants.

Introduction

The COVID-19 pandemic caused by SARS-CoV-2 has resulted in a global health crisis [1, 2], with the emergence of new variants posing a significant threat to public health [3, 4, 5]. Among the four structural proteins of SARS-related coronaviruses, the spike (S) protein plays a critical role in viral attachment, fusion, and entry processes, making it a prime target for developing vaccines, entry inhibitors, and antibodies [6, 7, 8, 9]. The SARS-CoV-2 virus uses its spike protein to infect human cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptors on the host cell surface [10, 11, 12, 13]. Recent research has shown that the SARS-CoV-2 spike protein has a higher affinity for human ACE2 in comparison to the SARS-CoV-1 spike protein [14, 15, 16, 17], which is partly due to their differential conformational dynamics. It is crucial to comprehend how the coronavirus spike proteins are activated and how their efficacy may vary depending on the variant in order to create effective coronavirus vaccines and therapeutics that will remain effective

over time [18, 19, 20, 21]. The interaction between the RBD and ACE2 receptor is a multi-step process that involves conformational changes in the RBD. The RBD initially exists in a ”closed” conformation that does not allow for ACE2 binding. However, upon binding to the ACE2 receptor, the RBD undergoes a conformational change and switches to an ”open” conformation, allowing for tighter binding between the RBD and ACE2 [22, 23].

Despite considerable efforts to develop safe and effective drugs and vaccines to target SARS-CoV-2 spike protein, there is an information gap on their long-term efficacy due to mutations of the virus and the emergence of new SARS-CoV-2 variants [24, 25]. The rapidly expanding and evolving pandemic has led to enormous casualties and economic crashes worldwide over the last two years [26, 27, 28, 29]. It is, therefore, imperative to address this information gap and conduct further research into the activation mechanism of coronavirus spike proteins, as well as the potential variant-dependent nature of these proteins, to enable the development of effective drugs and vaccines with long-term efficacy against emerging variants. This research will be critical to overcoming the challenges posed by the evolving pandemic and mitigating the associated public health and economic impacts.

The COVID-19 pandemic has resulted in multiple mutation [30, 31, 32]. Among these mutations, certain variants have been highly successful in demonstrating increased transmissibility and a higher capability for immune system evasions [33, 34, 35, 36, 37, 38, 39]. However, static information regarding the structure of wild-type spike proteins is insufficient to comprehend the evolving infection process caused by the coronavirus.

To gain a better understanding of this evolving process, we have conducted molecular dynamics (MD) simulations [40, 41] to study the active and inactive states of the spike protein in various SARS-CoV-2 variants, including the wild-type (WT) [42], Alpha [43, 44, 45], Beta [45, 46], Epsilon [47, 48, 49], and Delta variants [50, 51, 52, 53, 54], as well as an engineered spike protein associated with the Moderna vaccine [55]. Our simulations have revealed that specific mutations contribute significantly to the structural and conformational dynamics of the spike protein.

Notably, the mutations shared by the Delta variant and some other variants may be responsible for the distinct dynamic behavior observed in our simulations, potentially leading to higher transmissibility and a greater potential for immune evasion.

Studying the dynamic behavior of different variants of spike protein is critical for the development of more effective vaccines and therapeutic agents. By understanding the activation mechanism of coronavirus

spike proteins and their potential variant-dependent nature, we can develop drugs and vaccines with long-term efficacy that are capable of effectively targeting SARS-CoV-2 spike protein. The insights obtained from our MD simulations can inform the design of novel drugs and vaccines that can better address the challenges posed by the evolving nature of the COVID-19 pandemic.

Materials and Methods

In this study, we have utilized the powerful tool of molecular dynamics (MD) simulations [40] to investigate the conformational dynamics of non-glycosylated spike protein variants of SARS-CoV-2 [42]. The models used for this investigation were based on the static structures of the active (PDB entry: 6VYB) [42] and inactive (PDB entry: 6VXX) [42] SARS-CoV-2 spike proteins obtained through Cryo-EM techniques. To obtain reliable and comprehensive results, a total of 4 microseconds of MD simulations have been carried out, encompassing six active and six inactive forms of SARS-CoV-2 wildtype and variants, including Alpha, Beta, Delta, Epsilon, and Moderna.

To prepare the protein for MD simulations, we used the CHARMM-GUI web server [56], which allowed us to generate solvated protein structures with the addition of 0.15mM NaCl. The CHARMM36m all-atom additive force field [57] was employed to simulate the protein systems, with each system containing approximately 800,000 atoms. We utilized NAMD 2.10/2.13 [58, 59] to perform the simulations with periodic boundary conditions in the NPT ensemble at a temperature of 310K using the Langevin integrator, and 1 atm pressure was maintained using the Nose-Hoover Langevin piston method [60]. Our approach to protein simulation consists of three primary steps: Initialization, Equilibration, and Production [61, 62]. During the initialization step, we loaded the positions and momenta of the atoms from the previous simulation. The equilibration step allowed the simulation to run until the system reached equilibrium, and finally, the production step simulated the system, which was then finalized and prepared for further analysis.

To further analyze our simulation data, we used the software package VMD (Visual Molecular Dynamics) [63] which provides various tools for visualizing and analyzing molecular dynamics trajectories. In particular, we used VMD plugins to conduct Hydrogen Bond and salt bridge analysis. For Hydrogen Bond analysis, we used a cutoff distance of 3.5Åand a cutoff angle of 30. For salt bridge analysis, we used a cutoff distance of 4Å and calculated the distance between the oxygen atoms of the acidic residues and nitrogen of the basic residues.

Our simulations have yielded important insights into the differential dynamics of the SARS-CoV-2 spike proteins in wildtype and various variants. The trajectories were analyzed using these methods, which have allowed us to identify key intermolecular interactions, such as hydrogen bonds and salt bridges, which play crucial roles in maintaining protein structure and stability. The results obtained through our simulations partially explain the differential dynamics of the variants (Alpha, Beta, Delta, Epsilon, and Moderna) relative to the wild type. Our findings provide a basis for further experimental studies and will aid in the design of potential therapeutics and vaccines.

Result

In the present study, we have investigated the behavior of various variants of SARS-CoV-2 spike protein, particularly the interaction between amino acid residues in its active and inactive protomers. Our data indicate that the Delta variant displays a significant conformational shift in its active state when compared to the other variants, as shown in the RMSD plots in Figure 8. Figure 9 and 10 shows the formation of a stable salt bridge between GLU 191 and LYS 206 (both residues within the NTD) in the active protomer A of the Delta variant of SARS-CoV-2, as observed in our study. This interaction is specific to the active protomer of the Delta variant and persists for a duration of 50 to 400 nanoseconds. We also observed the same interaction in the inactive protomer C (Figure 12) but for a shorter duration of approximately 100 nanoseconds. Notably, this interaction was not observed in the other variants or in the other inactive protomer B, as seen in Figure 11.

The formation of a stable salt bridge between GLU 191 and LYS 206 in the active protomer of the Delta variant suggests a potential role in the NTD regarding the virus's infectivity and replication. Such interaction may facilitate the stabilization of the active protomer, leading to increased viral replication and infectivity. Moreover, the observation of this interaction in the inactive protomer C indicates that it may also play a role in the conformational changes necessary for the transition between active and inactive states.

It is crucial to note that the formation of this interaction is unique to the Delta variant, and it is not observed in the inactive protomer B or other variants. This finding suggests that this interaction may be a distinguishing feature of the Delta variant, contributing to its higher transmissibility and virulence compared to other variants. Upon analysis of the receptor-binding domain (RBD) of the SARS-CoV-2 virus, we also observed a distinct salt bridge interaction between the amino acid residues D398 and R466. This interaction

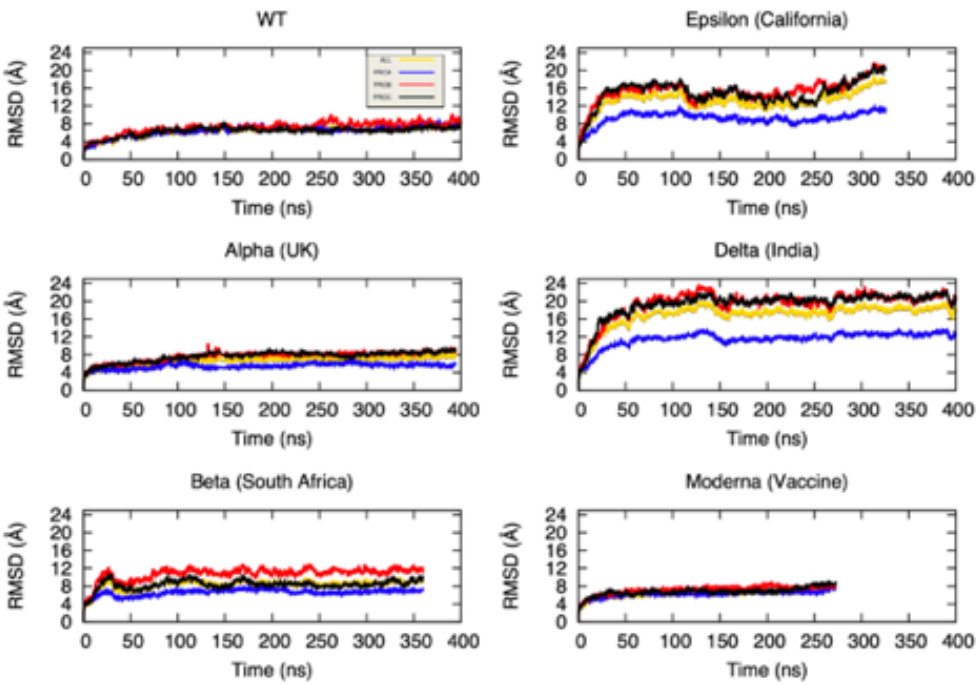

Figure 8: RMSD for variants of SARS-CoV-2 in an active state. Delta and epsilon variant (active state) shows more conformational changes that may be important in their activation mechanism and their differential transmisibility with other variants

occurs within the active protomer (protomer A), as shown in Figure 13, and is similar to the interaction observed within the N-terminal domain. We have found that this ionic interaction remains intact throughout the entire 400 nanoseconds of our simulation in both the delta variant and the wild type. However, we have not observed this salt bridge interaction in any of the other variants, including the alpha, beta, epsilon, and Moderna variants. Furthermore, when we examine the inactive protomers (protomers B and C), this interaction is not present in any of the variants except in the inactive protomer C of the delta variant. This finding further highlights the unique conformation of the delta variant compared to other variants.

Overall, our observation of the salt bridge interaction between D398 and R466 within the RBD of the SARS-CoV-2 virus provides valuable insight into the structural differences between the delta variant and other variants. These findings may have implications for the development of effective treatments and vaccines against SARS-CoV-2.

Discussion

In light of the high rate of viral mutations and the associated need for more effective therapeutics, an improved understanding of the conformational dynamics of the spike activation process is crucial, despite

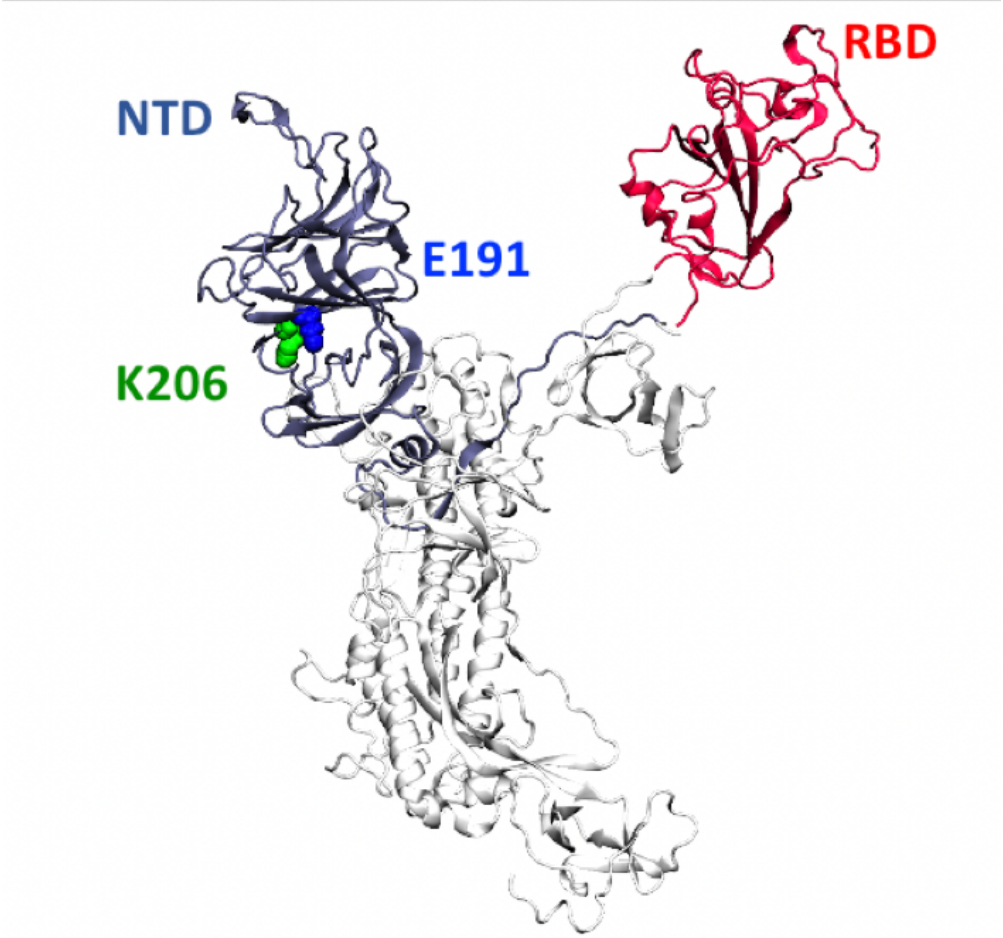

Figure 9: Visual representation of the stable salt bridge formed between GLU 191 and LYS 206 (both residues in NTD) in the active protomer A of the Delta variant of SARS-CoV-2.

current successes in mRNA vaccine development [64, 65, 66]. To this end, we have conducted an investigation into the structural and conformational dynamics of the activation process for SARS-CoV-2 spike proteins, both for the wild type and several of its variants, over timescales relevant to the conformational transitions. Our findings suggest that the activation process may differ significantly for the different variants of SARS-CoV-2 spike proteins. Such molecular details could be utilized to identify hotspots on the spike proteins as novel therapeutic targets for the development of COVID-19 drugs and vaccines. Moreover, this could pave the way for a reliable framework for structure-based drug design based on the inhibition of the activation process.

Our molecular dynamics simulations have revealed key insights into the differential dynamic behavior of SARS-CoV-2 spike proteins in the WT and its variants, including Alpha, Beta, Delta, Epsilon, and Moderna.

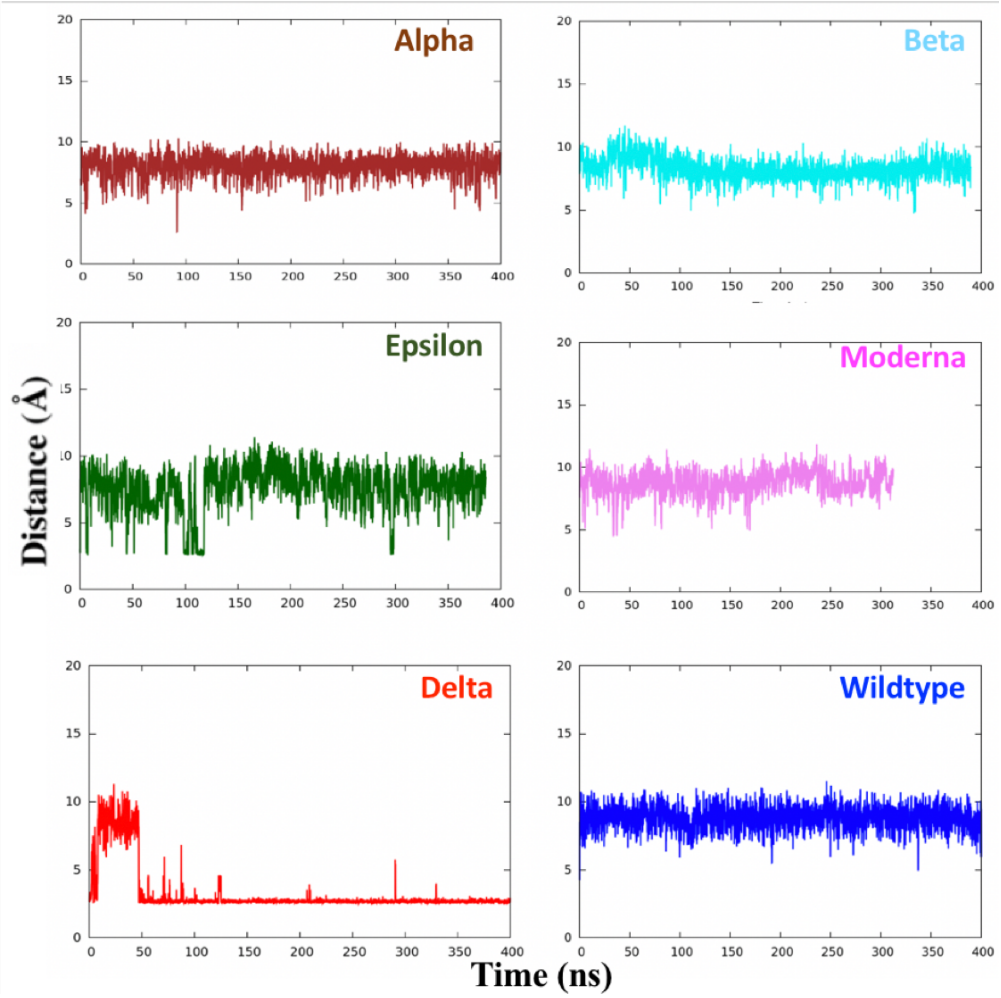

Figure 10: A time series plot displaying the stable salt bridge formed between GLU 191 and LYS 206 in the active protomer A of the Delta variant of SARS-CoV-2. Conversely, this interaction was absent in the other variants.

The data obtained from our preliminary research on the conformational dynamics of the non-glycosylated spike protein of Variants of SARS-CoV-2 [23] has led us to a new understanding that differs from previous explanations that have been influenced by static images of these proteins. Through our work, we have observed significant conformational shifts in the Delta variant of the spike protein when compared to other variants. These changes were observed in the active state of the protein but not in the inactive state, as illustrated in Figure 8. This finding challenges previous assumptions about the stability and dynamics of the spike protein in different variants of SARS-CoV-2. In particular, it highlights the importance of considering the dynamic behavior of these proteins, rather than relying solely on static images, to better understand their biological functions. Our results also suggest that the conformational shifts observed in the Delta variant may have important implications for the infectivity and virulence of SARS-CoV-2. This has also been in-

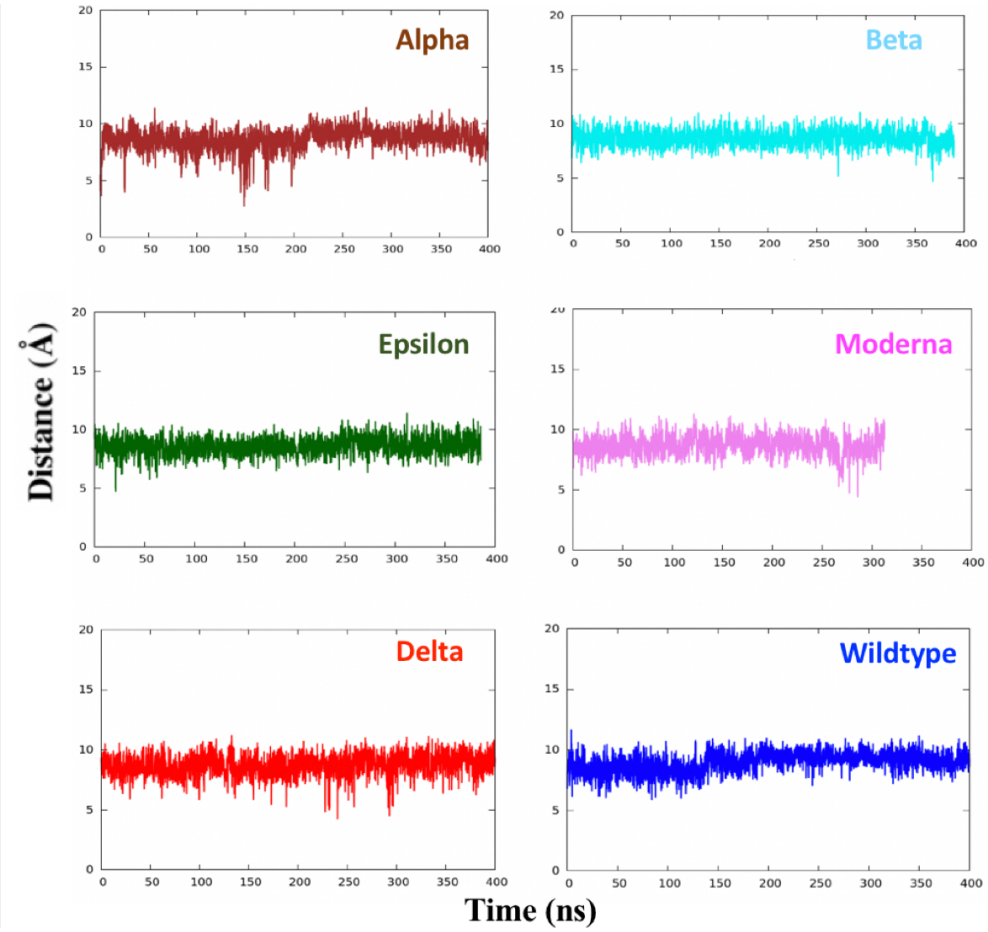

Figure 11: The time series plot depicts the minimum distance between GLU 191 and LYS 206 in the inactive protomer B, demonstrating the lack of interaction in the inactive protomer across all variants with similar behavior.

dicated in other studies [67]. Further research will be necessary to explore the mechanistic basis of these changes and to determine their impact on the pathogenicity of the virus. Through our investigations, we have delved into the possible role of specific ionic interactions that may play a crucial role in the observed dynamic behavior seen in the RMSD plots displayed in Figure 8. By studying the electrostatic interactions within the spike protein of the variants of SARS-CoV-2, we have discovered that these ionic interactions could potentially be influencing the conformational shifts observed in the Delta variant. Our research has revealed that the ionic interaction between GLU191 and LYS206 is significant in the differential conformational behavior of the variants, particularly the Delta variant. Our recent publication has emphasized that besides the receptor-binding domain (RBD), the N-terminal domain (NTD) also plays a crucial role in the activation and inactivation of the spike protein and its subsequent attachment to ACE2. As illustrated in Figure 10, the salt bridge between GLU191 and LYS206 in the NTD region of the Delta variant may prevent

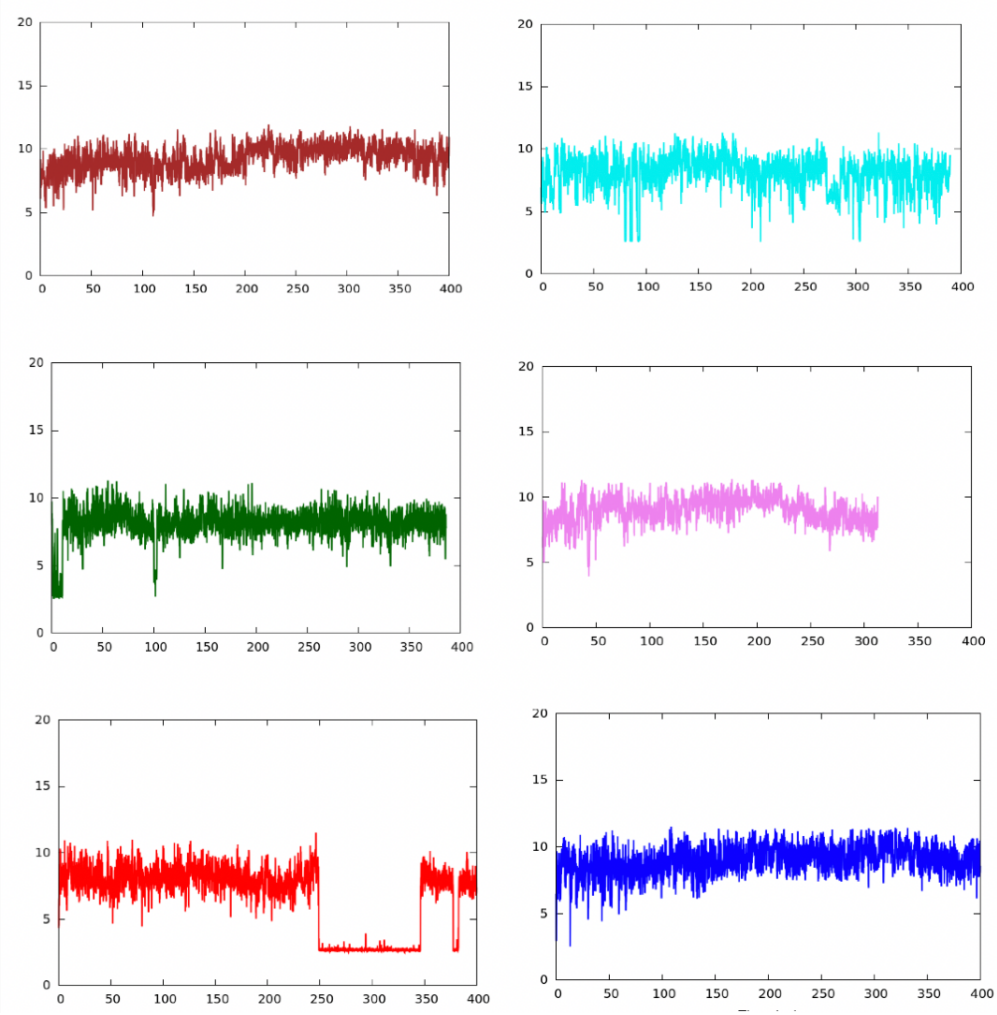

Figure 12: The time series plot illustrates that the inactive protomer C adopts a semi-active conformation in the Delta variant, indicated by the formation of a salt bridge between NTD residues (GLU 191 and LYS 206) in the inactive protomer C. This interaction is absent in the other variants.

the NTD from interacting with the RBD and thus ensure the availability of the RBD for binding to ACE2. This, in turn, may increase the transmissibility of the Delta variant.

The strong binding between the ACE2 receptor and RBD is believed to be a major factor in the highly infectious and transmissible nature of SARS-CoV [68, 69, 70, 71, 23]. Furthermore, mutations in the RBD have been detected in several concerning SARS-CoV variants, including Delta and Omicron, which could potentially alter the binding affinity between the RBD and ACE2 receptor, influencing the virulence and transmissibility of these variants [72, 73, 74]. Our findings revealed a specific electrostatic interaction formed in the RBD region of the active Delta variant. Additionally, our analysis of the RBD-NTD distance across different variants indicated that the Delta variant moves further away from the RBD domain compared

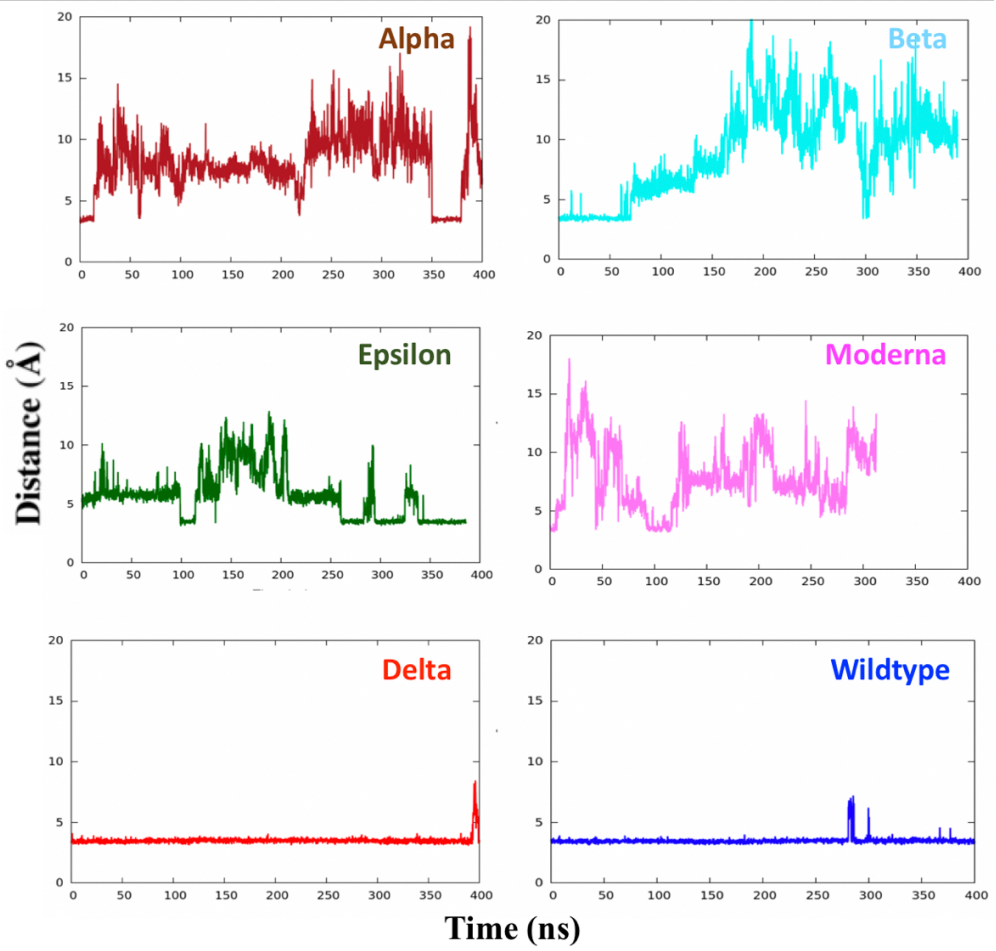

Figure 13: A time series plot illustrating the formation of a stable salt bridge between ASP398 and ARG466, which are residues present in the RBD of the active protomer A of both the Delta variant and the wildtype of SARS-CoV-2, while not forming in other variants.

to the Alpha variant and other variants, potentially contributing to its increased transmissibility.

Overall, our work underscores the importance of employing advanced computational tools, such as molecular dynamics simulations, to gain new insights into the dynamic behavior of biological molecules. By doing so, we can achieve a more comprehensive understanding of the molecular mechanisms underlying biological processes and ultimately pave the way for the development of novel therapeutic interventions.

Conclusion

In conclusion, the molecular dynamics simulations conducted on the SARS-CoV-2 spike protein and its variants have provided significant insights into the differential dynamic behavior of these proteins. The data generated from the simulations clearly indicate that the Delta variant exhibits a significant conformational

shift in its active state, which distinguishes it from the other variants. The simulations also highlighted the critical role played by the N-terminal domain (NTD) in the activation and attachment of the virus to the ACE2 receptor.

Furthermore, the study showed that the salt bridge between GLU191 and LYS206 in the NTD region of the Delta variant is critical in keeping the NTD away from the RBD, ensuring that the RBD is available for attachment to ACE2. This observation sheds light on the mechanisms behind the higher transmissibility of the Delta variant, which is a matter of global concern. The simulations have also shown that the RBD domain in the Delta variant moves away from the NTD more significantly than in the Alpha variant, providing another contributing factor to the Delta variant's higher transmissibility.

Overall, the study has provided a fundamental understanding of the dynamic behavior of the SARS-CoV-2 spike protein and its variants, shedding light on the key ionic interactions involved in the activation and attachment of the virus to the ACE2 receptor. The findings of this study could be useful in the development of novel therapeutics and vaccines against SARS-CoV-2 and its variants. The study underscores the importance of continued research efforts aimed at understanding the molecular basis of the virus's pathogenicity and transmission dynamics.

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Conclusion

The COVID-19 pandemic caused by the SARS coronavirus-2 has profoundly impacted the world, affecting every aspect of life. The virus is highly infectious, constantly evolving, and poses a significant challenge to the effectiveness of current vaccines. Despite these challenges, the development and rollout of vaccines have provided hope, and ongoing research and development are crucial to combat this deadly virus.

This thesis aimed to investigate the differential dynamic behavior and electrostatic interactions of SARS-CoV-1 and SARS-CoV-2 prior to receptor binding, with a specific focus on the hot spot regions outside of the receptor binding domain. The study also explored the behavior of the SARS-CoV-2 variants and compared them to the original virus to determine whether changes in the virus have any impact on its dynamic behavior and electrostatic interactions. To achieve this objective, molecular dynamics simulations were utilized.

The use of molecular dynamics simulations in this study allowed for a detailed investigation of the behavior of SARS-CoV-1 and SARS-CoV-2 at the molecular level. This approach facilitated the observation of changes in the electrostatic interactions and dynamic behavior of the viruses, providing insights into potential targets for therapeutic interventions. By utilizing this technique, researchers were able to explore the dynamic behavior of the viruses in a way that would not have been possible through experimentation alone.

The results of this study have significant implications for developing new treatments and vaccines to combat the COVID-19 pandemic. The findings provide valuable insights into the behavior of SARS-CoV-1 and SARS-CoV-2, highlighting potential targets for the development of therapeutics. Additionally, the study's findings regarding the behavior of SARS-CoV-2 variants will be crucial in informing the ongoing development and improvement of vaccines to address the evolving nature of the virus.

Furthermore, the ongoing evolution of SARS-CoV-2 underlines the need for continued research and development to combat this deadly virus and prevent future pandemics. It is crucial that everyone takes responsibility and follows guidelines to prevent the spread of the virus and protect ourselves and our communities.

In summary, the use of molecular dynamics simulations in this study allowed for a detailed investigation of the dynamic behavior and electrostatic interactions of SARS-CoV-1, SARS-CoV-2, and SARS-CoV-2 variants. The findings have significant implications for the development of new treatments and vaccines to combat the COVID-19 pandemic, highlighting potential targets for therapeutic interventions and informing

the ongoing development and improvement of vaccines. Ongoing research and development are necessary to combat this deadly virus and prevent future pandemics, and it is up to each individual to take responsibility and follow guidelines to protect ourselves and our communities.