Butwal Campus Journal, Vol. 7, No. 2: 137-148, December 2024
Research Management Cell, Butwal Multiple Campus, Tribhuvan University, Nepal
DOI: DOI: https://doi.org/10.3126/bcj.v7i2.73234

Tensile Strength of Delta Variants in SARS-CoV-2 Spike Protein with Human ACE2 Receptor

Jhulan Powrel
Butwal Multiple campus
E-mail: jhulam.powrel@bumc.tu.edu.np

Abstract

This research work aims to understand the mechanism by which SARS-CoV-2 and its variants bind to human body proteins in order to investigate their binding. Our research focuses on the tensile strength of spike protein mutated-RBD with human ACE2 in molecular level using molecular dynamics simulation via the approach of solving equations of motion. The method of steered molecular dynamics (SMD)is applied to study the strength of bonding interaction and tensile strength of two proteins. The present study is of delta variant using molecular dynamics. We have estimated the forces required for breaking the hydrogen bonds between SARS CoV-2 and hACE-2. The contact surface area between SARS CoV-2 and hACE-2 is found to be 875 $Å^2$ and the tensile strength between the two protein is found to be 80 to 189 pN/ $Å^{2.}$

Key Words: SMD, COVID-19, SARS CoV-2, RBD, hACE-2, mutated delta variants.

Introduction

The COVID-19 pandemic caused by highly contagious severe acute respiratory syndrome corona virus-2 (SARS-CoV-2), after its emergence in December 2019, has significantly affected the civilization. This disease has caused unprecedented severe impact on modern civilization in every areas of life. Almost all the countries in the world have been severely affected by the COVID-19 pandemic. Nepal also has faced the severe consequence of this pandemic. The lock-down condition necessitated by this pandemic has caused tremendous challenges in the public health, economy, education and many other sectors of the nation.

The continuous emergence of new variants has added challenge for the efficacy of the vaccines and drugs which have been designed especially for the SARS- CoV-2 virus initially emerged in 2019 (Figure 1). The rapid mutation rate in corona viruses is one of the challenges for world health agencies and is also problem in effectiveness of already discovered vaccines as well as RT-PCR tests (Nikaeen, Abbaszadeh, & Yousefinejad, 2020). The continuous emergence and rapid spread of new variants of the virus has increased the transmission of the virus, severity of the infection, risk of re-infection and the reduction in the protection provided by vaccination (Khateeb, Li, & Zhang, 2021). Although, there is continuous increase in immunity against this virus in global population, the new variants have allowed the virus to keep spreading and enhancing its replication capacity (koirala et al., 2022).

There is no single medicine or vaccine that could be effective against all these variants of SARS-CoV-2 viruses. For this, new in-depth understanding of interactions of SARS-CoV-2 virus with host cell receptor is necessary to address these challenges posed by the rapidly emerging variants of SARS-CoV-2 virus.

It is natural for virus to undergo mutation during replication. Being positive-sense single stranded RNA virus, SARS-CoV-2 is more prone to mutations than other DNA viruses. Although most of the mutations in virus have little effect on its properties, some may cause significant changes in the transmission and severity of the disease and efficacy of the vaccines and medicines. Different variants of virus differ from each other due to the mutation in their genomic sequence. Since the spike protein mediates the entry of virus to the host cell and is the major target of neutralizing antibodies (Hoffmann M et al., 2020, Shang J et al., 2020). the mutations in the spike protein and especially in the inter-facial region of the RBD have direct impact on the ability of virus to bind with the host cell and antibody recognition. The mutation in RBD region significantly affect the transmission and severity of the infection of the disease caused by the virus. World Health Organization (WHO) has classified the different variants of SARS-CoV-2 virus according to their characteristics as variants of concern (VOC), variants of interest (VOI), variants under monitoring (VUM) etc. (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants). Among them the variants of concerns are under global surveillance because of the significant increase in infectivity, transmissibility, severity of disease. These variants have shown lesser efficacy of vaccines and increased evasion of host immune system. The five notable variants of concerns (VOC) designated by WHO are Alpha variant (B.1.1.7), Beta variant (B.1.351), Gamma variant (P.1), Delta variant (B.1.617.2) and the Omicron variant (B.1.1.529, BA.1, BA.2). These variants share several mutation in the spike protein. The Alpha variant (B.1.1.7) has several mutation in spike protein, out of them the notable RBD mutation is N501Y, in which the Asparagine (N) residue in 501 position of spike protein RBD is mutated into Tyrosine (Y) (Wise, 2020). Similarly, in Beta variant (B.1.351) the prominent mutations are K417N, E484K, and N501Y in the RBD of the spike protein (Naveca et al., 2021). The Gamma variant (P.1) has total of 10 mutations in spike protein and out of them three mutation K417T, E484K, and N501Y in RBD are important to change the binding with hACE2. Similarly, in Delta (B.1.617.2) the L452R and T478K mutation in RBD cause the significant severity of the infection (Vaughan, 2021). The omicron variant (B.1.1.529) and its sub-variant BA.2 have the extraordinary speed of spread across the globe. It has unprecedented 15 mutations G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y and Y505H on the RBD of spike protein. Out of them, 10 mutations lie on the binding interface of the RBD with the hACE2 receptor protein (*Yan R et al., 2020)* . Most of these residues are involved in the binding of the wild type virus with the receptor cell through various polar and hydrophobic

interactions. These multiple RBD mutation change the binding affinity of the RBD to hACE2 receptor thereby affecting the spread and infectivity of the virus (Chen, Wang, Gilby, & Wei, 2022). Spike protein being the major antigen in current vaccines, It is essential to know how mutations in the RBD of spike protein change the binding of virus with host cell receptor. These mutations in the binding interface of the RBD are reported to increase the transmissibility of the virus, chance of re-infection and escape from the host immune system (Andreano et al., 2020). Therefore, it might be essential to modify the current vaccines and treatments to enhance their efficacy against new variants. For this, new in-depth insight is necessary to address these challenges posed by the rapidly emerging variants of SARS-CoV-2 virus. The Delta variant is (Lovelace, 2021; Organization et al., 2022; Zhao, Huang, Fu, & Abdullaev, 2022) another variant of SARS-CoV-2 emerging during the mid of 2020. The virus also causes COVID-19. It was first detected in India in late 2020. The Delta variant was named on 31 May 2021 and had spread to over the 179 nation by 22 November 2021. The World Health Organization (WHO) indicated in June 2021 that the Delta variant was becoming the dominant strain globally (Lovelace, 2021). It has the mutations in the gene encoding the SARS-CoV-2 spike protein (Lovelace, 2021). Besides other variant it is also causing serious illness with similar symptom as of SARS CoV2 of 2019. Several studies on the delta variant estimated the thermal, mechanical and transport properties quantitatively by MD (molecular dynamics). This study ultimately focusing the delta variant in molecular level. Although, it is the estimation of mechanical strength between the SARS-CoV2-hACE2 dimer complex. The maximum stress a material can stand before it breaks is called the breaking stress or ultimate tensile strength. or. Breaking stress is the maximum force that can be applied on a cross sectional area of a material in such a way that the material is unable to withstand any additional amount of stress before breaking. The breaking stress is fixed for material, but breaking force varies and depends on the area of cross-section.

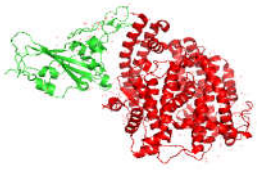


Figure 1

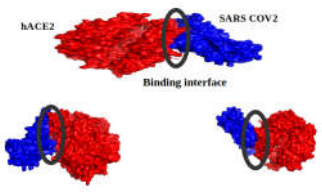


Figure 2

Many works were performed during last decade on SARS CoV and its variants. The continuous
effort on molecular dynamical study of drug material are in good practice. Practice on single drug medicine still unable to control all the variants for understanding the atomic and molecular or in nano scale. Zhou et al., in March, 2020; reported that SARS-CoV-2 virus has homology with the bat corona virus isolate RaTG13 strain (MN996532) by 96 %, but has not more than 80 % homology with other isolation of bat SARS-like corona virus (2020). Han et al., in 2022, studied the transmissible of the virus and omicron is considered as the fastest transmission variant, which has the RBD based mutations that allows efficient binding with human receptor protein (Han et al., 2022). Koirala et al., 2021, investigated the three major binding sites in SARS CoV-2 (Koirala et al., 2021). Shahcheraghi et al., in 2021, tested the effectiveness of vaccines such as pfizer, moderna and the found the 95% effectiveness against the viruses (Shahcheraghi et al., 2021). The effectiveness of such vaccines for the new variants is to be examined (Shahcheraghi et al., 2021; Excler et al., 2021). Kumar et al., 2022, suggested the important effect of other corona viruses variants such as kappa, eta, lotta, zetta, theta etc. Bhutta et al., 2021 studied the effect of Delta variants increase the death rate mainly in the south Asian countries (Bhutta et al., 2021). Kumar et al., 2022, found that Omicron has greater number of hydrophobic amino acid residues in its spike protein, which enhances the binding with hACE2 (Kumar et al., 2022). Yun et al., in 2020, studied the effectiveness of existing drugs as the emergency fulfillment.
Among the different variant, this study focusses the binding affinity of SARS Cov2 and human ACE-2 receptor using the steered molecular dynamics (SMD).

Material and Methods

Classical Molecular dynamics is the method used in studying the molecular level binding affinity of SARS Cov2 and hACE-2 receptor.

System set up

A molecular structures, PDB IDs 6LZG is taken for the molecular dynamics simulations The delta varient with PDB id of 6LZG is obtain from RSCB.ORG. Input file of 6LZG is made by CHARMM-GUI (Lee et al., 2016) was used to prepare the pdb and psf files of the complex. Later on, the complex structures was solvated in water (TIP3P) (Jorgensen et al., 1983) and electrically neutralized by mixing sufficient NaCl. We have added the NaCl in the system with concentration 0.15 M by using CHARMM-GUI. A cubical box of dimensions 100× 100 × 100 $Å^3$ was prepared for NPT simulation of the complex SARS-CoV-2 CTD/hACE2. Furthermore, an orthorhombic simulation box is prepared in order to estimate the rupture forces of the complex by changing the dimensions to 250 × 90 × 90 $Å^3$.

Molecular dynamics simulation

Molecular dynamics (MD) simulations is performed using NAMD (Phillips et al., 2005) simulation package. The CHARMM36m (Phillips et al., 2005) force field was

used for the considerd simulations. The Particle Mesh Ewald (PME) (Harvey M. J., 2009) was used for the long-range interactions with a cut off at 12.0 Å. The energy minimization was performed for 10 ns, using the conjugate gradient and line search algorithm (Khanal S.P., 2019) The system was then equilibrated at 310 K for 10 ns with a time step of 2fs. Finally, the production run was conducted for 100 ns simulation under NPT conditions by using Langevin dynamics with a damping constant of 1 ps ^{1}.

SMD (Steered Molecular Dynamics)

To perform the SMD, sample is chosen from steered molecular dynamics (SMD) trajectories. During SMD, CTD of SARS-CoV-2 was pulled with constant velocity pulling method. In this process, the alpha carbons of hACE2 protein were taken as the fixed atoms and alpha carbons in CTD part of spike protein of the systems is taken as the dummy atoms. CTD of spikes was pulled from their center of mass (COM) along the negative x-direction with constant velocity $v = dx/dt$ in water and ions. Then the SMD atom experiences the force ,

$$F=k(vt-\Delta x),$$

providing the external potential energy,

$$U(x.t) =(1/2)k(vt-\Delta x.n^)$$

where, k (=5 kcal mol ^{1} Å ^{2}) is the spring constant and gives the stiffness of the applied harmonic restraining force, and $\Delta x(t) = x(t) - x_0$, is the displacement of SMD molecules from initial position x_0 to instantaneous position x (t) and nˆ is the unit vector along the direction of pulling.

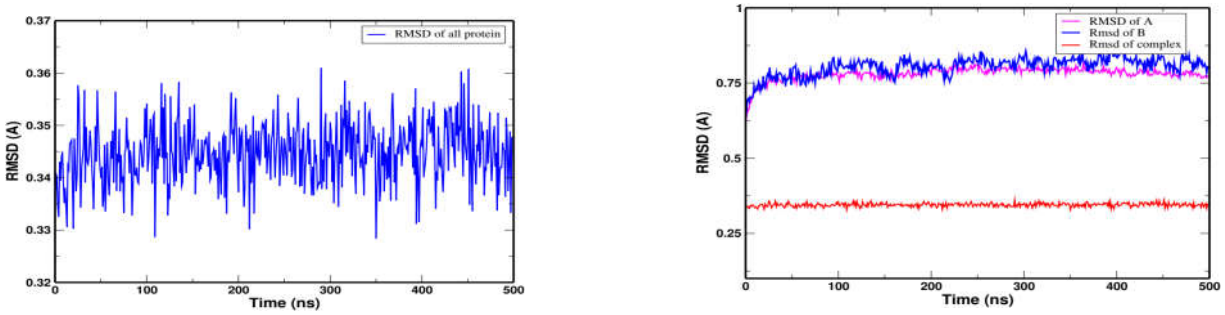
Result And Discussions

Molecular dynamics (MD) simulation have been carried out to understand the conformational stability, elastic behavior and tensile strength of the SARS CoV2/hACE2 complex. Properties are compared between these molecule proteins (SARS CoV2/hACE2 complex). We discuss the findings of this work in this section. Root Mean Square Deviation (RMSD) of SARS CoV2 ,hACE2 and their complex are compared at first and then we present the different non-bonded interactions. Finally, we discuss the SMD simulation.

RMSD Analysis

At first we have studied the RMSD of delta variants so as to depict the structural conformation as in Figure3 a). From the Figure 3 a and b), it is seen that the RMSD found stable after 20 ns of simulation time scale in SARS CoV2/hACE2 complex. However, SARS CoV2 and hACE2 protein individually becomes stable after 100 ns of simulation run. Meanwhile human-ACE2 have slightly higher flexibility than SARS CoV2. The average values of RMSD are 0.8 Å (more Salt bridge than PROB) , 0.78 Å and 0.345 Å for SARS CoV2, human-ACE2 and the complex respectively (Figure 3 b)), which means all the protein are in the stable form.

are stable structures.



a) b)

Figure 3: RMSD of a) all components b) sars cov2/hace2 complex

Hydrogen bond (H-bond) Interaction

Hydrogen bonding is an important interaction in the formation of single chain as well as in interaction of different chains in quartenary structure of a protein. This type of bonding system not only forms the single protein structure but also in the formation of complex structure between two or more proteins or ligands. Several biological characteristics of the molecule depends on the structure of the molecule, which can be governed by the pattern of hydrogen bonding.

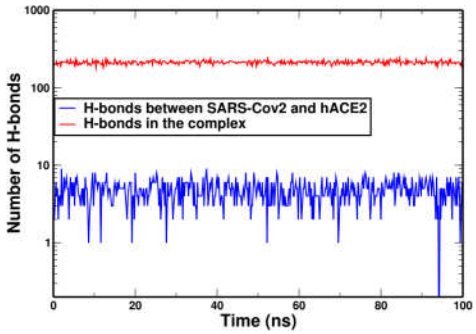


Figure 4: Hydrogen bonding in delta variant

In between the SARS Cov2 and hACE2 protein in average 9 hydrogen bonds consistently appears. The complex is consistently carrying 210 H-bondings in the system. due to this bonding two protein comes in contact with certain contact area as shown in Figure2.

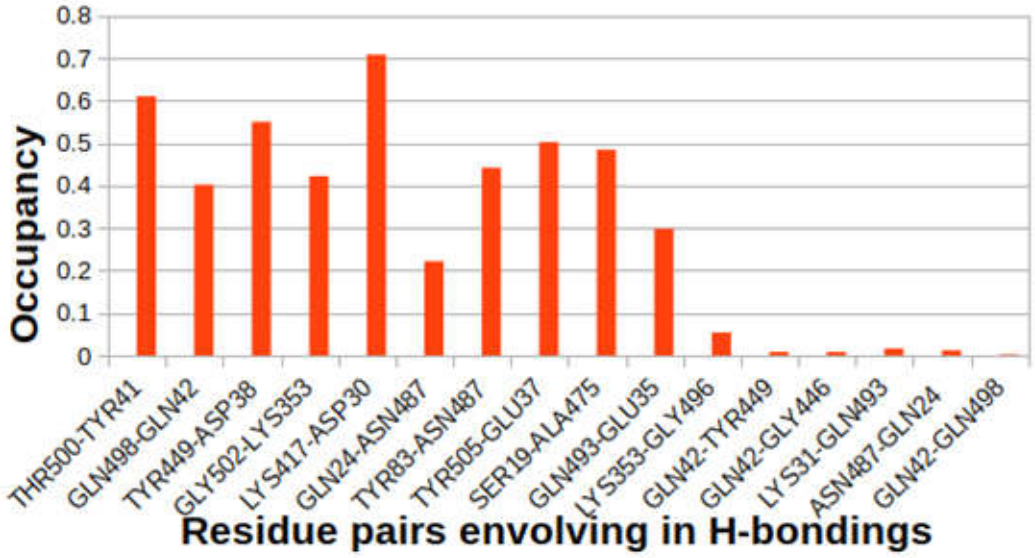


Figure 5: Hydrogen bindings in delta variant

However 16 pairs of residues showing the H-bonding in between the SARS CoV2-hAce2 with occupancy as in Figure 4. LYS417-ASP30, THR500-TYR41 and TYR449-ASP38 have shown the maximum occupancy of 70 %, 60 % and 55 % in the binding contribution by H-bindings in the dimer. Total of 832 residue pairs are contacted with H-bonding in the complex system of delta variant (Figure 5).

SASA of Delta Variant

SASA is another factor that governs the hydrophobic nature as well as the tensile strength of binding between the complex. The SASA of SARS CoV-2, hACE-2 and that of complex protein are estimated
to calculate the contact area of hACE2 and SARS COV2 RBD by the relation of,

Contact Area (S) = $S_1 + S_2 \quad S_{12}$

.

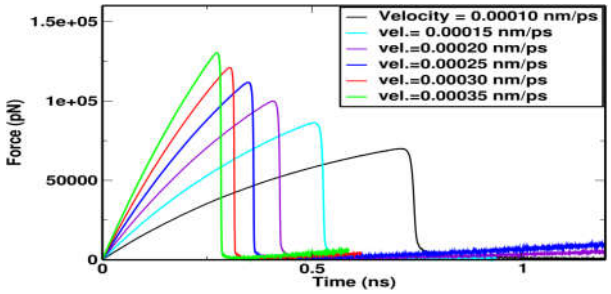
Table 1: SASA of components of delta varient in Å 2

SASA of	hACE2	SARS CoV2	Complex	Contact area=$S_1+S_2-S_{12}$
	$S_1Å^2$	$S_2Å^2$	$S_{12}Å^2$	$SÅ^2$
Delta	27000.00	11000.00	36250.00	875.00

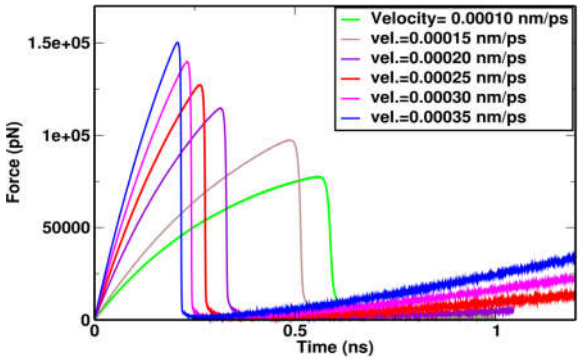
Estimation of Tensile Strength in Delta Varient of SARS CoV2/hACE2

The tensile stress is estimated by knowing the binding force in between the two molecules. By using

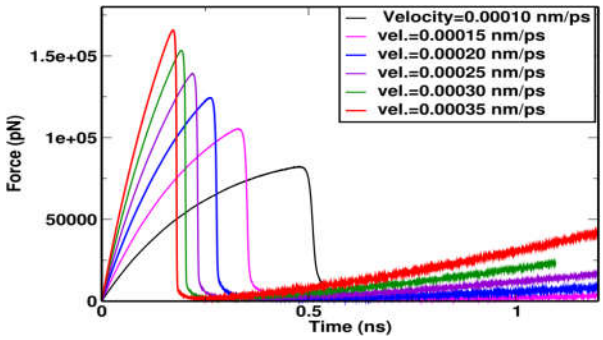
the molecular dynamics simulation the forces required to break the interaction between the sub unit in the complex protein dimer of delta variant of SARS CoV2/hACE2 are estimated. The estimated force and the SASA as well as contact area of the different component units gives the tensile stress in the dimer complex. The hACE2 molecule is translated away from the SARS-CoV2 RBD protein. Corresponding trajectories are plotted as in the Figure 6 a), b) and c). The first peak of the graphs gives the force required in breaking the biding at the interface.



a)



b)



c)

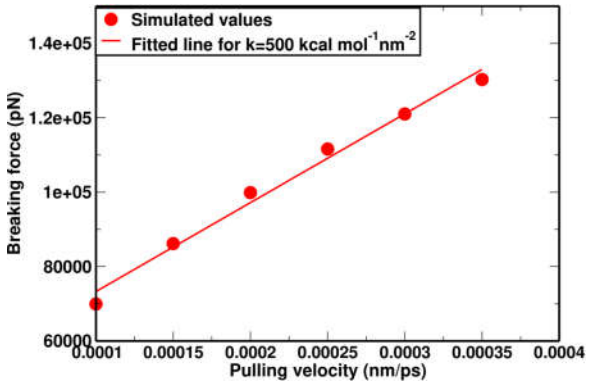
Figure 6: SMD graph of hACE2-SARS CoV2 dimer protein for verious pulling velocities at a) k=500 kcal $mol^{-1}nm^{-2}$, b) k=800 kcal $mol^{-1}nm^{-2}$ and c) k=1100 kcal $mol^{-1}nm^{-2}$

The average of the peak value of forces are tabulated for different spring constants of 500, 800 and 1100 kcal $mol^{-1}nm^{-2}$as in the Table2.

Table 2: Forces required for breaking the H-bonds in the hACE2-SARS CoV2 dimer protein with k=500 kkcal $mol^{-1}nm^{-2}$, k=800 kcal $mol^{-1}nm^{-2}$ and k=1100 kcal $mol^{-1}nm^{-2}$.

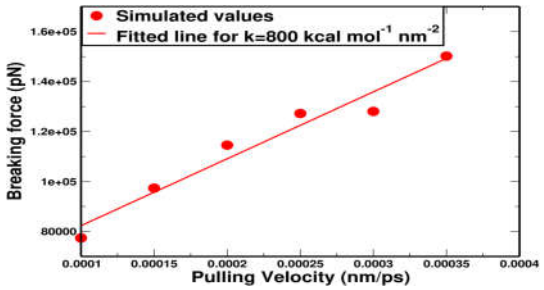
For Velocity(nm/ps)	Force required (pN) for		
	k=500 kcal $mol^{-1}nm^{-2}$	k=800 kcal $mol^{-1}nm^{-2}$	k=1100 kcal $mol^{-1}nm^{-2}$
0.00010	69958.54	77401.30	82163.60
0.00015	86194.29	97345.06	105401.50
0.00020	99880.75	114566.09	124378.63
0.00025	111607.81	127216.63	139106.27
0.00030	121032.27	128064.75	153302.45
0.00035	130281.63	150193.72	165701.18

The peak values of breaking forces and corresponding pulling velocities are plotted in graph which gives the linear increment of rupture force with pulling velocity. The Figure 7 a), b) and c) are the graph obtained for different spring constant values of k=500, =800 and 1100 kcal $mol^{-1}nm^{-2}$ respectively.



a)

b)



c)

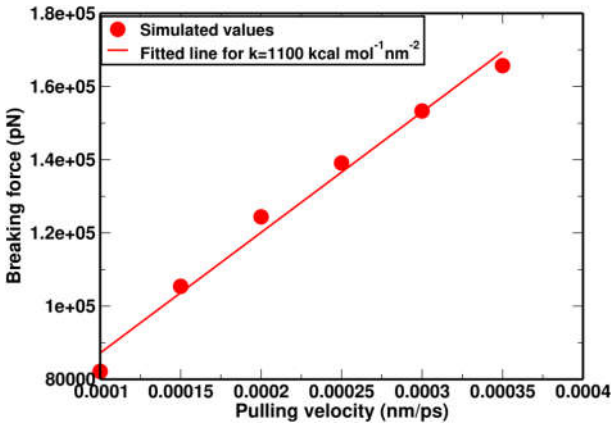


Figure 7: Forces required for breaking the H-bonds in the hACE2-SARS-CoV2 (Delta Varient) dimer protein with a) k=500 kcal mol ^{1} nm ^{2}, b) k=800 kcal mol ^{1} nm ^{2} and c) k=1100 kcal mol ^{1} nm ^{2}.

Tensile Strength in SARS CoV2-hACE2 RBD receptor protein is estimated as in the Table 3.

Table 3: Forces required for breaking the H-bonds in the hACE2-SARS CoV2 dimer protein with k=500 kcal mol^{-1}nm^{-2}, k=800 kcal mol^{-1}nm^{-2} and k=1100 kcal mol^{-1}nm^{-2}.

For Velocity (nm/ps)	Tensil strength (pNÅ^{-2})		
	For k=500 kcal mol^{-1}nm^{-2}	for k=800 kcal mol^{-1}nm^{-2}	For k=1100 kcal mol^{-1}nm^{-2}
0.00010	79.95	88.46	93.94
0.00015	98.51	111.25	120.46
0.00020	114.15	130.93	142.15

0.00025	137.55	145.39	158.98
0.00030	138.32	146.36	175.20
0.00035	148.89	171.65	189.37

The Table 2 indicates the rupture forces for different values of spring constant as well as pulling velocities. Higher the pulling velocities higher is the rupture force. Also the rupture force is increased as the values of spring constant k is increased. The required breaking force maximum for the value of k =1100 kcal $mol^{-1}nm^{-2}$ and is 165701.18 pN. The minimum force required in breaking the hydrogen bond is 69958.54 pN. Again the SASA analysis gives the contact area between hACE-2 and SARS CoV2 (Table 1). The maximum force per unit contact area indicates the tensil strength in between hACE2 and SARS Cov2 (Table 3). The minimum tensile strength is obtained for pulling velocity of 0.00010 nm/ps with a value of 79.95 pN $Å^{-2.}$, whereas the maximum strength is obtained for pulling velocity of 0.00035 nm/ps with maximum tensile strength of 189.37 pN $Å^{-2}$. These values of tensile strength (Table 3) indicates greater mechanical attachment of hACE2 with SARS CoV2.

Conclusions and Concluding Remarks

Besides hydrogen bondings a single salt bridge of ASP30-chainP-PROA-LYS417-chainP-PROB appear in between the two complex. Tensile strength, of observed to be very high (79.95 x10 2 MPa) and similar of some of the metallic alloys. The strength of SARS CoV2/hACE2 interface is lying between 7995 MPa to 18937 MPa which is equivalent to the strength of stronger alloy to that of carbon fiber.

References

Andreano, E., Piccini, G., Licastro, D., Casalino, L., Johnson, N. V., Paciello, I., . . . others (2020). Sars-cov-2 escape in vitro from a highly neutralizing covid-19 convalescent plasma. BioRxiv .

Callister, W. D. (2007). Materials science and engineering: An introduction john wiley & sons. Inc.: Utah.

Chen, J., Wang, R., Gilby, N. B., & Wei, G.-W. (2022). Omicron variant (b. 1.1. 529): infectivity, vaccine breakthrough, and antibody resistance. Journal of chemical information and modeling, 62 (2), 412–422.

Ercolessi, F. (1997). A molecular dynamics primer. Spring college in computational physics, ICTP, Trieste, 19 .

Harvey M J, Giupponi G and Fabritiis G De. (2009). ACEMD: accelerating biomolecular dynamics in the microsecond time scale Nat. Methods 5 1632–9

Hoffmann M .et al (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor Cell 181 271–80.

Jorgensen W. et al. (1983). Comparison of simple potential functions for simulating liquid water J. Chem. Phys. 79 926–35

Koirala, R. P., Thapa, B., Khanal, S. P., Powrel, J., Adhikari, R. P., & Adhikari, N. P. (2021). Binding of sars-cov-2/sars-cov spike protein with human ace2 receptor. Journal of Physics Communications, 5 (3), 035010.

Khanal S P, Kandel Y P and Adhikari N P 2019 Transport properties of zwitterion glycine, diglycine, and triglycine in water AIP Adv. 9065303–10

Khateeb, J., Li, Y., & Zhang, H. (2021). Emerging sars-cov-2 variants of concern and potential intervention approaches. Critical Care, 25 (1), 1–8.

Lee J et al 2016 CHARMM-GUI input generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM simulations using the CHARMM36 additive force field J. Chem. Theory Comput. 12 405–13

Lovelace, B. (2021). Who says delta is becoming the dominant covid variant globally. CNBC.

Nikaeen, G., Abbaszadeh, S., & Yousefinejad, S. (2020). Application of nanomaterials in treatment, anti-infection and detection of coronaviruses. Nanomedicine, 15 (15), 1501–1512.

Organization, W. H., et al. (2022). Surveillance, case investigation and contact tracing for monkeypox: interim guidance, 25 august 2022 (Tech. Rep.). World Health Organization.

Phillips, J. C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., . . . Schulten, K. (2005). Scalable molecular dynamics with namd. Journal of computational chemistry, 26 (16), 1781–1802.

Powrel, J., & Adhikari, N. P. (2022). Elastic property of sickle and normal hemoglobin protein: Molecular dynamics. AIP Advances, 12 (4), 045308.

Shang J et al 2020 Clinical characteristics of coronavirus disease 2019 in China Nature 581 221–4.

Tina, K., Bhadra, R., & Srinivasan, N. (2007). Pic: protein interactions calculator. Nucleic acids research, 35 (suppl 2), W473–W476.

Vaughan, A. (2021). Delta to dominate world. Elsevier.

Xiao, B.-L., Ning, Y.-N., Niu, N.-N., Li, D., Moosavi-Movahedi, A. A., Sheibani, N., & Hong, J. (2019). Steered molecular dynamic simulations of conformational lock of cu, zn-superoxide dismutase. Scientific reports, 9 (1), 1–10.

Yan R et al. 2020 Structural basis for the recognition of the 2019-nCoV by human ACE2 Science 367 1444–8

Zhao, X., Huang, K., Fu, J. S., & Abdullaev, S. F. (2022). Long-range transport of asian dust to the arctic: identification of transport pathways, evolution of aerosol optical properties, and impact assessment on surface albedo changes. Atmospheric Chemistry and Physics, 22 (15), 10389–10407.