**Structural Plasticity of Omicron BA.5 and BA.2.75 for Enhanced ACE-dependent Cell Entry**

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**Key Points**

* Amongst over 300 sub-variants of Omicron, BA.2.75 and BA.5 are currently the widest spread variants globally. Using protein-protein docking, protein structural graphs (PSG), and molecular simulation methods, we explored the binding variation, interactions pattern, and dynamic features of BA.2.75 and BA.5 in contrast to the wild type of SARS-COV-2.
* HADDOCK predicted the docking score for the wild type -111.8 +/-1.5 kcal/mol, for BA.2.75 -119.6 +/-4.5 kcal/mol while the BA.5 variant reported a docking score of -119.0 +/-5.5 kcal/mol.
* Further investigations revealed several variations in the hub residues, protein sub-networks and GlobalMetapath in these variants compared to the wild type.
* A very unusual dynamic for BA.2.75 and BA.5 was observed, and secondary structure transition was witnessed in the loops i.e., γ1 (474–485), γ2 (488–490), and γ3 (494–505) where the loop structure has adapted the beta-sheet structure.
* The total binding free energy (TBE) for the wild type was calculated to be -61.38 kcal/mol, while for BA.2.75 and BA.5 variants, the TBE was calculated to be -70.42 kcal/mol and 69.78 kcal/mol, respectively.
* The current study will aid the development of structure-based drugs/therapeutics against the circulating variants.

**Abstract**

The Omicron sub-variants BA.2.75 and BA.5 are the widest spread variants around the world. Using protein-protein docking, protein structural graphs, and molecular simulation methods, we explored the binding variation, interactions pattern, and dynamic features of BA.2.75 and BA.5 in contrast to the wildtype SARS-COV-2. The HADDOCK predicted the docking score higher for the wild type (-111.8 +/-1.5 kcal/mol) compared to BA.2.75 (119.6 +/-4.5 kcal/mol and BA.5 (-119.0 +/-5.5 kcal/mol). The dissociation constant (KD) was reported to be 5.20E-10 for the wildtype while for two sub-variants the KD was estimated to be 4.3E-09 and 3.8E-10 for BA.2.75 and for BA.5, respectively, further validated a tighter binding of studied variants compared to wildtype. Further investigations revealed critical variations in the hub residues, protein sub-networks and GlobalMetapath in these variants compared to the wildtype. Noteworthy, an unusual dynamic of BA.2.75 and BA.5 was observed, and secondary structure transition was witnessed in the loops including γ1 (474–485), γ2 (488–490) and γ3 (494–505) where the loop structure transitioned into a beta-sheet structure. The results showed that the flexibility of these three loops was increased by the mutations as an allosteric effect and this attributed to the enhanced bonding with nearby residues to connect and form a stable interaction. Furthermore, the additional hydrogen bonding contacts were revealed which steer the robust binding of these variants in contrast to the wildtype. The total binding free energy (TBE) for the wildtype was calculated to be -61.38 kcal/mol, while for BA.2.75 and BA.5 variants the TBE was calculated to be -70.42 kcal/mol and 69.78 kcal/mol, respectively. We observed that the binding of BA.2.75 is steered by the electrostatic interactions while the BA.5 additional contacts were due to the vdW energy. Taken together, it can be observed the spike protein is undergoing structural adjustments to bind efficiently to the hACE2 receptor and, in turn increase entry into the host cells. The current study will aid the development of structure-guided therapeutics against the circulating variants and may propose a roadmap to train future therapeutics against SARS-COV-2.

**Keywords:** Omicron; Variant; Binding; Simulation; MM/GBSA

**Introduction**

The SARS-CoV-2 identification as a causing agent of the COVID-19 disease, its alarming infectivity rate, and fast expansion across the globe compelled the World Health Organization (WHO) to declare it a pandemic [1, 2]. This pathogen has not only crippled the health systems of developed and developing nations but also put an enormous social and economic burden on common people [3]. The SARS-COV-2 is nested within the genus Beta (β) coronavirus of the family *Coronaviridae*. Two previous epidemics were also caused by the members of genus beta (β) coronavirus including SARS-CoV-1 and MERS-CoV in 2003 and 2012 respectively [4]. The number of infected persons reached to over 600 million and 6.47 causalities occurred due to SARS-CoV-2. These statistics indicate the gravity of the issue and humanity suffered from a very precarious condition due to the unavailability of proper treatment. Numerous vaccines have been developed for the control of the virus and diseases. Initially, the vaccination process was successful to decrease the causality rate. Several mutations in the genome of SARS-CoV-2 were responsible for the appearance of new variants, which were capable of evading immune response, having higher infectivity and tight binding with receptors [5]. The appearance of new variants has greatly contributed to prolongation of the pandemic, which has already entered into 5th wave [6].

The continuous evolution of SARS-CoV-2 led to the appearance of numerous variants, which are placed in various categories such as variant of interest (VOI), variant being monitored (VBM), and variant of concern (VOC) [7]. The earliest variant of SARS-COV-2 had a D614G mutation in spike (S) protein and was linked to a high transmission rate and helped in fast-global dispersion [8]. Numerous mutations have been identified in the S proteins such as K417N, L452R, T478K L452Q, F490S, E484K, N501Y, P681H, R346K, Y144T, Y145S, and 146N insertion [5, 15-16]. The main VOC reported so far are Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) [9, 10]. The mutations in these variants are responsible for higher infectivity and fast dispersion by evading immune response and enhances viral proliferation. The sub-lineages identified in the Omicron variant are named as BA.1, BA.2, BA.3, BA.4, and BA.5 [11-13]. Phylogenetically, the omicron variants are distantly related to other VOCs. A variant is termed VOC based on its higher virulence, fast transmission, greater infectivity, and offering resistance to vaccines and drug. Therefore, the omicron variant is a serious concern for public health because it harbours several mutations of previous VOC along with additional mutations, which enhance its infectivity, transmission, and penetration [14].

The identification of the first omicron variant was reported back in November 2021 from South Africa and was subsequently declared as VOC. The reports suggest the presence of this variant in Europe 10 days prior to its discovery in South Africa without any transmission link between these geographically distinct areas. In the S protein of Omicron variant BA.1 carried 35 mutations when compared with the Wuhan-Hu-1 reference genome, which results in 30 amino acid substitutions, insertion, and three deletions [15]. Amongst these mutations, RBD (binding site of the virus with host and target of neutralizing antibodies) harbours 15 mutations and can be a valuable target for anti-COVID therapies. The mutations such as Del69-70 (in Alpha), G142D (Kappa and delta), and T951 (Kappa and Iota) are also identified in the S protein of Omicron [5].

A total of nine RBD mutations in the S protein are present in the region which serves as the entry point of the virus by binding to human angiotensin-converting enzyme 2 (hACE2) receptor. Therefore, these mutations can alter the binding of ACE2-RBD, and tight binding can facilitate the entry of the virus into the cell by evading immune response or NAbs failure to detect them. Latest studies suggest that these mutations confer higher infectivity to the Omicron variant by avoiding immune response and thus facilitate the reinfection of the immunized population. Omicron BA.1 is up to six times more infectious compared to other variants [15].

Within Omicron, several distinctive sub-lineages are recognized including BA.1, BA.2, BA.3, BA.4, BA.5 amongst others. Initially, BA.1 sub-lineage prevailed the globe which was shortly surpassed by the BA.2 sub-variant and is the more pervasive version of the SARS-COV-2 around the globe. These two sub-lineages have many common mutations, however, 13 mutations in BA.1 are not present in BA.2 whereas BA.2 has 8 unique mutations [34]. Recently two novel sub-lineages were identified in South Africa and were also detected in many countries [35]. The S protein of these two sub-lineages is closely related to BA.2. Notably, the BA.4 and BA.5 exhibit a similar mutational landscape at 5′ genome region whereas a divergent pattern was observed at 3′ genome region. The higher infectivity, transmission and penetration are the qualities that make Omicron variants a serious threat to public health. Therefore, for the control of the current pandemic effective strategies are essential to reduce the infectivity and transmission associated with Omicron variants. Thus, the current study investigated the role of acquired mutations in the RBD of the recently reported variants i.e., BA.2.75 and BA.5 of the SARS-CoV-2 using structural modelling and simulation methods. Molecular docking and simulation explored the mechanism of interaction with the hACE2 receptor, which was further validated by binding free energy calculation. The current study provide invaluable insights into the development of structure-guided therapeutics against the circulating variants.

**Methodology**

**Retrieval of data and mutant modeling**

The Omicron variant sub-lineages BA.2, BA.4, and BA.5 were identified in South Africa and other countries. These sub-lineages are highly contagious, infectious, and have a fast transmission rate. We obtained the sequence of S proteins of these sub-lineages (ID: 6M0J) from UniProt and the exact location of mutations was marked [16]. The reference sequence (Wuhan; 6M17) was obtained from the database to compare it with the S protein of these sub-lineages [17]. The wildtype S protein was used as a template to model the mutant structure. AlphaFold 2.0 software was used for the modeling of novel mutations reported in RBD of the wild-type structure [18].

**Restraint docking of wild-type and mutant spike protein and hACE2**

Restraint docking of mutants and wild type with hACE2 was completed with the HADDOCK algorithm whereas the interface residues were defined based on earlier studies. Guru interface was used for visualization purposes to observe the interaction between hACE2 and RBD. HADDOCK server can facilitate the docking between the protein-protein or protein-RNA/DNA [19]. PDBsum online server and PyMOL software were used for the interaction pattern like electrostatic, hydrogen, and salt bridge [20, 21].

**Molecular dynamics simulation of wild-type and mutant complexes**

Molecular dynamics (MD) simulation results were obtained by using the AMBER20 package for the calculation of the dynamic behavior of both wild-type and Omicron sub variants RBDs with the hACE2 receptor by applying FF19SB force field [22-24]. System’s solvation was obtained by using an OPC water box showing higher accuracy in the FF19SB whereas system neutralization was completed by adding sodium counter ions. Energy minimization methodology was employed for the removal of bad clashes in each system by using 6000 cycles steepest descent algorithm and 3000 cycles of the conjugate gradient algorithm. System equilibration was performed at 1 atm pressure (with both weak restraint and without restraint) after heating it up to 300K. The production of each complex was accelerated through PMEMD.CUDA for a total of 500 ns each[24, 25]. The trajectories were processed using CPPTRAJ and PTRAJ modules[26]. For structural stability, root mean square deviation (RMSD) analysis as a function of time was performed using the following equation.

*(i)*

Where δi is the distance between atom i and either a reference structure or the mean position of the N equivalent atoms. This is often calculated for the backbone heavy atoms C, N, O, and Cα or sometimes just the Cα atoms.

RMSF (root mean square fluctuation) on the other hand, instead of indicating positional differences between entire structures over time, is a calculation of residues flexibility, or how a particular residue fluctuates during a simulation [33], mathematically the RMSF can be calculated by using the following equation.

*(ii)*

Where *T* is the time over which one wants to average and **r***i*ref is the reference position of particle *i*. This reference position will be the time-averaged position of the same particle *i*.

**Estimation of binding free energy**

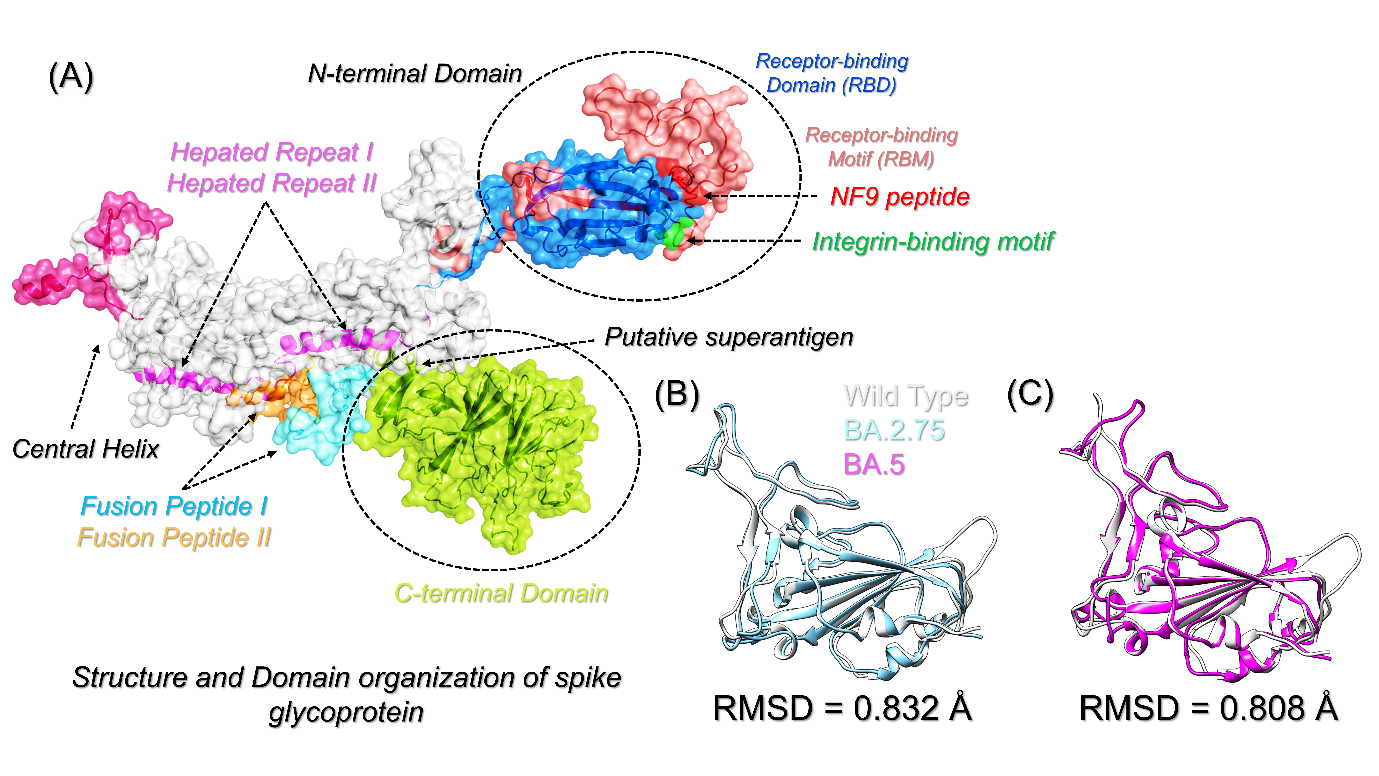
Based on the previous studies, the MM/GBSA approach was used for the estimation of the binding energies of wild-type and mutant complexes. Finally, the MM/PBSA.py script was utilized to obtain the total binding energy such as electrostatic, GB, SA, and vdW[27]. The following equation was used for energy calculation:

The above-mentioned component of the total free energy was calculated using the following equation:

**Results and Discussion**

**Structural Modeling and Analysis**

Despite enormous efforts, the prolonged pandemic is further aggravated by the evolution of novel variants that are acquiring mutations responsible for higher transmissibility and immune escape. Although all proteins in the genome are targeted by the virus for the essential mutations but the S glycoprotein is the primary and prime site for new mutations. Until now, many mutations such as E484K, K417N, D614G and many others are reported to cause significant changes in the viral dynamics and spread. The most contagious variant was reported to be Omicron (B.1.1.529) which has sub-variants that have created alarming situation recently around the globe. The sub lineages identified in the Omicron variant are named as BA.1, BA.2, BA.3, BA.4, and BA.5. Among these the BA.2.75 and BA.5 are still circulating around the world and increasing the infection rate. To demonstrate how the mutations in the BA.2.75 and BA.5 affect the protein’s function and its binding to the host receptor the current study uses structural modeling and atomistic simulations to decipher the structural basis for binding of RBD of these sub-variants with the hACE2. The S glycoprotein is the most essential protein required for entry into the host cell. The full-length S protein with the domain organization is shown in **Fig.1A**. Structures of the BA.2.75 and BA.5 RBDs were modeled using AlphaFold 2.0 with a pLDDT score >70 which shows the reliability of the structure prediction. Assessment of the wild-type RBD and mutant structures revealed an RMSD difference between the wild type and BA.2.75 of 0.832 Å while the BA.5 RBD revealed the RMSD difference of 0.808 Å. This shows significant structural deviation caused by the acquired mutations which may functionally alter the S protein. Hence, these structures were used for docking and simulation analysis to further understand the molecular mechanisms.

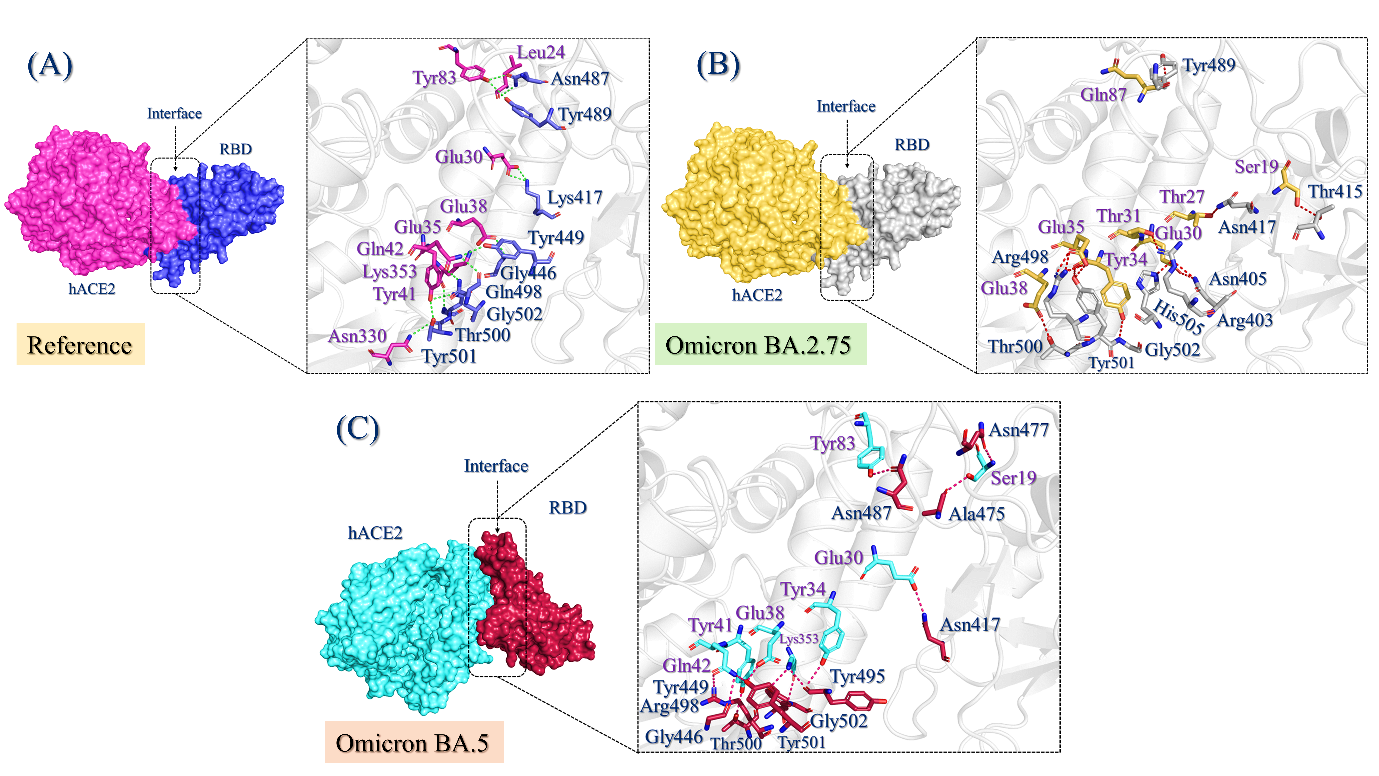
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**Fig.1: Structural organization, modeling, and comparison of the wild type, BA.2.75 and BA.5 RBDs. (A)** The complete structure of the S protein with different domains, **(B)** shows the superimposed structures of the wild type and BA.2.75-RBD while **(C)** show the superimposed structures of the wild type and BA.5-RBD.

**Binding Modes of Omicron sub-variants BA.2.75 and BA.5**

We also investigated the binding variations of the wild type and the omicron sub-variants i.e. BA.2.75 and BA.5. The HADDOCK restraint docking demonstrated differences in the docking scores of the wild type and these sub-variants. For instance, the docking score for the wild type has been previously calculated and reported to be -111.8 +/-1.5 kcal/mol [28]. On the other hand, the docking score for BA.2.75 was calculated to be -119.6 +/-4.5 kcal/mol while BA.5 reported a docking score of -119.0 +/-5.5 kcal/mol. The current findings show the tighter binding of these two sub-variants than the wild type. Moreover, comparison of these sub-variants with the parent strain (Omicron B.1.1.529) also reported a higher docking score. Previously the docking score for the Omicron B.1.1.529 variant was reported to be -118.3 +/-4.9 kcal/mol [28]. This show that the acquired mutations have helped these two sub-variants to further increase the binding towards the host receptor and increase the transmission. These results align with the experimental findings where the more efficient transmission and replication of BA.2.75 than BA.5 have been observed in hamsters [29]. To further understand the binding differences, we evaluated and compared the van der Waals (vdW) and electrostatic energies of each complex. The vdW for the wild type has been previously reported to be -48.1 +/-1.3 kcal/mol while the omicron B.1.1529 reported -53.6 +/-8.8 kcal/mol [29]. However, the BA.2.75 revealed a vdW of -47.9 +/-5.7 while the BA.5 showed a vdW of -56.1 +/-7.5 kcal/mol respectively. This further evident the optimized binding and enhanced transmission of these sub-variants in contrast to the wild type and the Omicron parent strain. The electrostatic energy for the BA.2.75 was reported to be -237.2 +/- 39.5 while the BA.5 reported electrostatic energy of -193.2 +/-20.9 kcal/mol respectively. Similarly, the alpha, beta, gamma, delta, and other variants have also been reported previously to demonstrate increased vdW and electrostatic energy [28, 30-32]. Interestingly increased in the electrostatic energy was observed in these two variants and this was considered as an important factor in the enhanced transmission by the previously highly transmissible variants of SARS-CoV-2 [28, 30-32]. The profound evidence reflect justifiable reasons for the enhanced transmission of these sub-variants.

A comparison of the binding at the atomic level was conducted to see the differences in the binding residues that facilitate the tighter binding and consequently increased transmission. The interaction pattern of the wild type strongly aligns with the previously published reports [28, 31, 32]. With the only salt bridge interaction and eleven hydrogen bonds the wild type presented a distinct pattern than the two sub-variants. The Tyr83 like the other reports targeted Asn487 and Tyr489 residues with hydrogen bonding. Moreover, Leu24 also coupled with Asn487 which was not observed in the BA.2.75 variant. Instead, the Gln87 of hACE2 established a hydrogen bond with Tyr489 in the BA.2.75 variant while in BA.5 variant Tyr83 interact with Tyr489 through a hydrogen bond. Moreover, Ser19 in BA.2.75 variant was also observed to interact with Asn477 and Ala475 residues from hACE2. It can be seen that the binding pattern is very distinct at this terminal in each complex thus implying different a strategy for interaction and entry to the host cell. The BA.2.75 established 11 hydrogen bonds and 2 salt bridges while the BA.5 variant established 13 hydrogen bonds only. The interactions between Ser19, Tyr83 with Asn487 and Ala475 are strongly conserved in the Omicron parent strain and BA.5 sub variant[28]. The Ser19-Ala475 contact has also been previously reported in the Alpha variant (B.1.1.7) complex carrying a singly mutation i.e. E484K only and other mutants [32]. On the other hand, the Tyr83 and Asn487 contact was also witnessed in C.1.2 variant of SARS-CoV-2[33]. In the BA.2.75 the Ser19 was observed to connect with Thr415 which demonstrates a divergent array of contact by this essential residue. Similarly, the wild type and BA.5 shared some more conserved interactions i.e. Glu30-Lys/Asn417 while the Asn417 in BA.2.75 enjoyed the interaction with Thr27. The variation in the binding pattern gives the speculation of increased binding particularly by these non-conserved contacts in the new sub variants which are currently widespread globally. In other variants such as B.1.1.7, B.1.651, P.1 and B.1.1.529 the interaction of Lys/Asn417 also demonstrated varied pattern[28, 31, 32, 34]. In lambda, Kappa, Mu and other variants the Lys417 was observed to interact with asparagine but not with glutamine[33]. Further variations were also observed in the interaction pattern such as Lys353 which establish several interactions with the wild type and previously reported variants is not present in these two sub variants. Interestingly the loss of contacts established by Lys353 was observed in the BA.2.75 sub variant while in the Omicron parent strain and BA.5 sub variant this residue was observed to establish contacts only with Tyr449[28]. The interaction pattern for the wild type includes hydrogen bonds among Glu30-Lys417, Glu35-Gln493, Glu38-Tyr449, Glu38-Gly496, Tyr41-Thr500, Tyr41-Thr500, Gln42-Gln498, Asn330-Thr500, Lys353-Gly502, Lys353-Gly496 and Lys353-Gln498. The only salt bridge Glu30-Lys417 was observed between the hACE2 and RBD. The hydrogen bonding interactions Ser19-Thr415, Glu30-Asn405, Glu30-His505, Glu30-Asn405, Thr31-Arg403, Tyr34-Gly502, Glu35-Arg498, Glu35-Arg498, Glu35-Tyr501, Glu38-Thr500 and Gln81-Tyr489 were observed in the BA.275 sub variant. The two salt bridges include Glu30-His505 and Glu35-Arg498 were observed for the first time in any variant. On the other hand, the BA.5 demonstrated interactions include Ser19-Asn477, Ser19-Ala475, Glu30-Asn417, Tyr34-Tyr495, Glu38-Tyr449, Tyr41-Thr500, Tyr41-Thr500, Gln42-Arg498, Gln42-Gly446, Tyr83-Asn487, Lys353-Gly502, Lys353-Tyr495 and Lys353-Tyr501. Interestingly the mutated residues are directly involved in all kinds of interactions in these sub variants making them capable of efficiently transmissible to the host. Several new interactions particularly by the mutated residues such as Arg498 and others were observed to be involved in interaction with the host receptor. The notion of high docking scores, vdW, electrostatic energy and the binding pattern revealed that these two sub variants determine a completely different pattern of binding in contrast to the wild type and other variants previously reported to increase the binding and transmission. The differences in the binding pattern between the wild type and the Omicron sub variants i.e., BA.2.75 and BA.5 revealed that the key substitutions are responsible for enhanced binding and the consequent transmissibility and infectivity. The interaction pattern of each of complex such as wild type, BA.2.75 and BA.5 are shown in **Fig.2A-2C**.



**Fig. 2: Interaction pattern of the wild type and sub variants of Omicron i.e., BA.2.75 and BA.5. (A)** demonstrated the binding mode of the wild type RBD and hACE2, **(B)** shows the binding mode of BA.2.75-RBD with the hACE2 while **(C)** show the binding mode of BA.5-RBD with hACE2.

**Evaluation of the Binding strength through KD (Dissociation constant)**

For convincible understanding of the binding dissimilarities among these complexes the KD (dissociation constant) was computed to rank strengths of biomolecular coupling[35]. The KD kinetics is widely practiced for the antigen antibodies binding, protein-ligand interaction and large biological macromolecule interactions affinity prediction. The lowest KD the value, the stronger the interaction [36]. For the wild type, the KD has been reported previously as 5.20E-10 while for these two sub variants the KD was estimated to be 4.3E-09 for BA.2.75 and 3.8E-10 for BA.5[32]. These findings are consistent with a significantly lower equilibrium dissociation constant (KD) obtained in in vitro binding assays of SARS-CoV-2 compared to SARS-CoV [37, 38]. This shows that these sub variants increase the binding strength of RBD towards hACE2 and enhances the transmission.

**Residues Communication and Pathways signaling**

To understand the inter residues connectivity network, webPSN 2.0 was used. Variations were observed in the hub residues. In the wild type 236 hub residues while in BA.2.75 and BA.5 has 138 and 145 hub residues respectively. The findings strongly align with the previously reported studies where less number of hub residues were reported in different variants [39]. The hub residues in each structure is shown in **Fig.3A-3C**. This shows the subtle conformational changes mediated by these mutations. Further variations in the GlobalMetapath, average path length, hub in the GlobalMetapath and other parameters are given in **Table 2**. This shows the overall changes in the internal residues networking that consequently alter the interaction paradigm and signaling.



**Fig.3. Structural Network analysis of the wild type, BA.2.75 and BA.5-RBD with the hACE2.** **(A)** Shows the hub residues occurrence and distribution in the wild type RBD in complex with hACE2. The green sphere represents the hub residues in the wild type, while **(B-C)** shows the hub residues occurrence and distribution of the BA.2.75 and BA.5-RBD in complex with hACE2.

**Table 1:** Protein Network components and parameters.

|  |  |  |  |
| --- | --- | --- | --- |
| Path Summary | Wild Type | BA.2.75 | BA.5 |
| Number of nodes in path | 86 | 99 | 91 |
| Number of links in path | 85 | 98 | 90 |
| Number of shortest paths | 984220 | 484987 | 594856 |
| Average path length | 30.0851 | 22.9576 | 23.4048 |
| Average path hub % | 46.0049 | 47.6182 | 48.6481 |

**Computation of Dynamic features of the wild type and Mutant Systems**

Assessing the dynamic features of the protein complexes determine the behavior of proteins during the course of simulation. It gives important information regarding the stability, binding/unbinding events, flexibility and interaction paradigm. Herein, the stability was calculated as RMSD using the simulation trajectories. The RMSD comparison revealed significant variations in the wild type, BA.2.75 and BA.5 complexes. For instance, the wild type complex remained the most stable with no significant dynamic perturbation. An average RMSD was calculated to be 2.0 Å. The wild type RMSD graph is reproduced from our unpublished results **(Fig.4A)**. On the other hand, BA.2.75 variant demonstrated significantly instable dynamic behavior. The RMSD of the wild type and both variants converge with each other initially which shows a similar configuration space explored by them. The findings are consistent with the previous results where a gradually increase in the RMSD was observed for the mutant structures [40]. The RMSD of the BA.2.75 given in **Fig.4A** progressively increases over the simulation time. During the first 100ns the RMSD fluctuated between 4-7 Å while then continues to increase gradually until 400ns. Afterwards the graph stabilized by determining a uniform pattern of RMSD till the end of simulation. Interesting the non-stability effects are previously reported to be associated with the radical function of the SARS-CoV-2 proteins [41]. In the case of BA.5 a similar configurational space was followed and after 200ns the RMSD continues to increase gradually. The mean RMSD during the first 1-200ns was calculated to be 3.0 Å, however then increased by demonstrating structural deviations and the mean RMSD for the whole simulation was calculated to be 4.50 Å. Unlike the wild type these two variants reported comparatively instable dynamics particularly by the BA.2.75 variant this opposing the wild type which may functionally cause damage to the host cell. The SARS-CoV-2 variants i.e. Delta plus, Iota, Kappa, Mu, Lambda, and C.1.2 have been previously characterized to report similar instable dynamic in contrast to the wild type[33]. In large scale MD simulations studies the SARS-CoV-2 RBD has been reported to be conformational more stable hence, corroborate with the current findings by demonstrating stable dynamics[42]. The omicron parent strain (B.1.1.529) also demonstrated similar configurational space which shows the agreement between these findings and previous studies[28]. The larger deviation in the BA.2.75 and the wild type may show the functional variation caused by the acquired mutation which has also been demonstrated experimentally that this variant replicates more efficiently than the others[29]. The RMSD graph for the BA.5 variants is given in **Fig.4B**.

For the structural packing which may determine the binding and unbinding events experienced by each structure during simulation we calculated the radius of gyration (Rg) using the simulation time-dependent trajectory. The Rg graphs of the wild type and BA.2.75 converged initially during the first 200ns and then the BA.2.75 demonstrated higher Rg values until 400ns. Afterwards the Rg value decreased back and remained uniform till the end of simulation. On the other hand, the BA.5 demonstrated comparatively higher Rg pattern, but no significant deviation was observed. This shows the compact structure of these complexes and minimal unbinding events determined by these complexes during the simulation thus reveals a binding stability of the RBD during simulation. The Rg graphs for the wild type, BA.2.75 and BA.5 variants are given in **Fig.4C** and **4D**.



**Fig.4: Structural stability and packing analysis of the wild type, BA.2.75 and BA.5 variants.** **(A)** Shows the RMSD of the wild type and BA.2.75 variant in complex with hACE2 while **(B)** Shows the RMSD the wild type and BA.5 variants. **(C-D)** demonstrate the Rg graphs for the wild type, BA.2.75 and BA.5 variants.

**Residues Flexibility Assessment during simulation**

Employing the knowledge proteins flexibility is key in demonstrating the biological functionality of a protein. It benefits to explicate the role of indispensable residues vital for molecular recognition, disease progression, biological engineering and therapeutics designing. Conformational alterations that span a wide variety of amplitude scales are typically linked to protein function. As a result, knowing about protein flexibility is just as important as knowing about protein structure when it comes to understanding protein’s function and improving drug development [43]. Seeing the significant role of residues flexibility herein, we computed the residues flexibility as RMSF for each complex. Given in **Fig.5A**, the wild type complex demonstrated less flexible dynamics than the others. The findings assimilate the previous findings for other variants of SARS-CoV-2 where the higher conformational flexibility was reported and associated with higher binding [28, 32, 33]. In **Fig.5B**, the flexibility of the apo structures of the wild type, BA.2.75 and BA.5 is shown where the minimal fluctuation for the wild type RBD was observed while for the BA.2.75 a higher flexibility was recorded. The flexibility of the three important loops resides between 474-505 was also evaluated and given in **Fig.5C**. It can be seen that loop1 and loop 2 are largely perturbed by the mutations while the loop 3 demonstrated almost alike behavior. The findings strongly agree with the B.1.1.7, B.1.351, P.1, B.1.617, and B.1.618 variants where the flexibility of these three loops was observed to have increased which then causes better conformational optimization and result in efficient binding [5, 28, 31, 32, 34]. Moreover, the lower level of flexibility demonstrated by loop 3 is also well consistent with the previous literature using structural modelling approaches [42]. We also calculated the RMSF of the ACE2 apo state for each complex which revealed more similar pattern of residues flexibility thus shows that the effect of RBD mutation on the over simulation rather than ACE2 alone (**Fig.5D**). A similar pattern of RMSF was also recorded previously [31]. The differential flexibility index demonstrates variations in conformational optimization and binding strength.



**Fig.5: Residues flexibility analysis of the wild type, BA.2.75 and BA.5 variants.** **(A)** Shows the RMSF of the wild type, BA.2.75 and BA.5 variants while **(B)** Shows the RMSF the wild type, BA.2.75 and BA.5 variants apo RBD. **(C)** demonstrate the RMSF graphs for the wild type, BA.2.75 and BA.5 variants RBD loops (474-505). **(D)** show he RMSF the wild type, BA.2.75 and BA.5 variants apo ACE2.

We further examined the dynamics of the three important loops essential for direct contact with the host receptor (ACE2). For this purpose, snapshots at 0ns, 100ns, 200ns, 300ns, 400ns and 500ns from each trajectory was retrieved and compared with the native conformation. The three loops correspond to γ1 (474–485), γ2 (488–490), and γ3 (494–505) from each trajectory revealed differential fluctuation dynamics which yield functionally different output. As given in **Fig.6A**, the wild type demonstrated very conserved flexible movement with less deviation from the mean structure. The three loops almost determined the native flexibility index. On the other hand, the BA.2.75 and BA.5 demonstrated very flexible nature. The γ1 (474–485) in the BA.2.75 variant particularly reported very flexible behavior while the BA.5 was comparatively less than this. Moreover, the beta sheet in the BA.2.75 and BA.5 in the γ2 (488–490) has flipped and thus alter the conformation of the protein which consequently alter the binding paradigm as this beta sheet is present at the interface site. Regarding the loop 3 i.e. γ3 (494–505) the wild type and BA.2.75 reported a similar pattern while the BA.5 deviated significantly. Secondary structure transition can also be witnessed in this loop where the loop structure has adapted the beta sheet structure. The results show that the flexibility of these three loops is increased by the mutations as an allosteric effect and thus enhances the chances of bonding with the nearby residues to connect and form a stable connection. These observations were also reported in previous studies where structural modelling and docking simulation revealed the flexibility of the loops present in the interface [28, 31, 32]. From these findings, it can be observed the S protein is undergoing structural adjustments to bind efficiently to the ACE2 receptor and, in turn increasing entry to the host cells. The loops dynamic at different time interval for each complex is shown in **Fig.6A-6C**.

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**Fig.6: Investigation of the three loops correspond to γ1 (474–485), γ2 (488–490), and γ3 (494–505) in the RBD of the wild type, BA.2.75 and BA.5 variants. (A)** represent the comparatively structural analysis of the three loops in the wild type at 0ns, 100ns, 200ns, 300ns, 400ns and 500ns. (B) represent the comparatively structural analysis of the three loops in the BA.2.75 variant at 0ns, 100ns, 200ns, 300ns, 400ns and 500ns. (C) represent the comparatively structural analysis of the three loops in the BA.5 variants at 0ns, 100ns, 200ns, 300ns, 400ns and 500ns.

**Hydrogen Bonding Analysis**

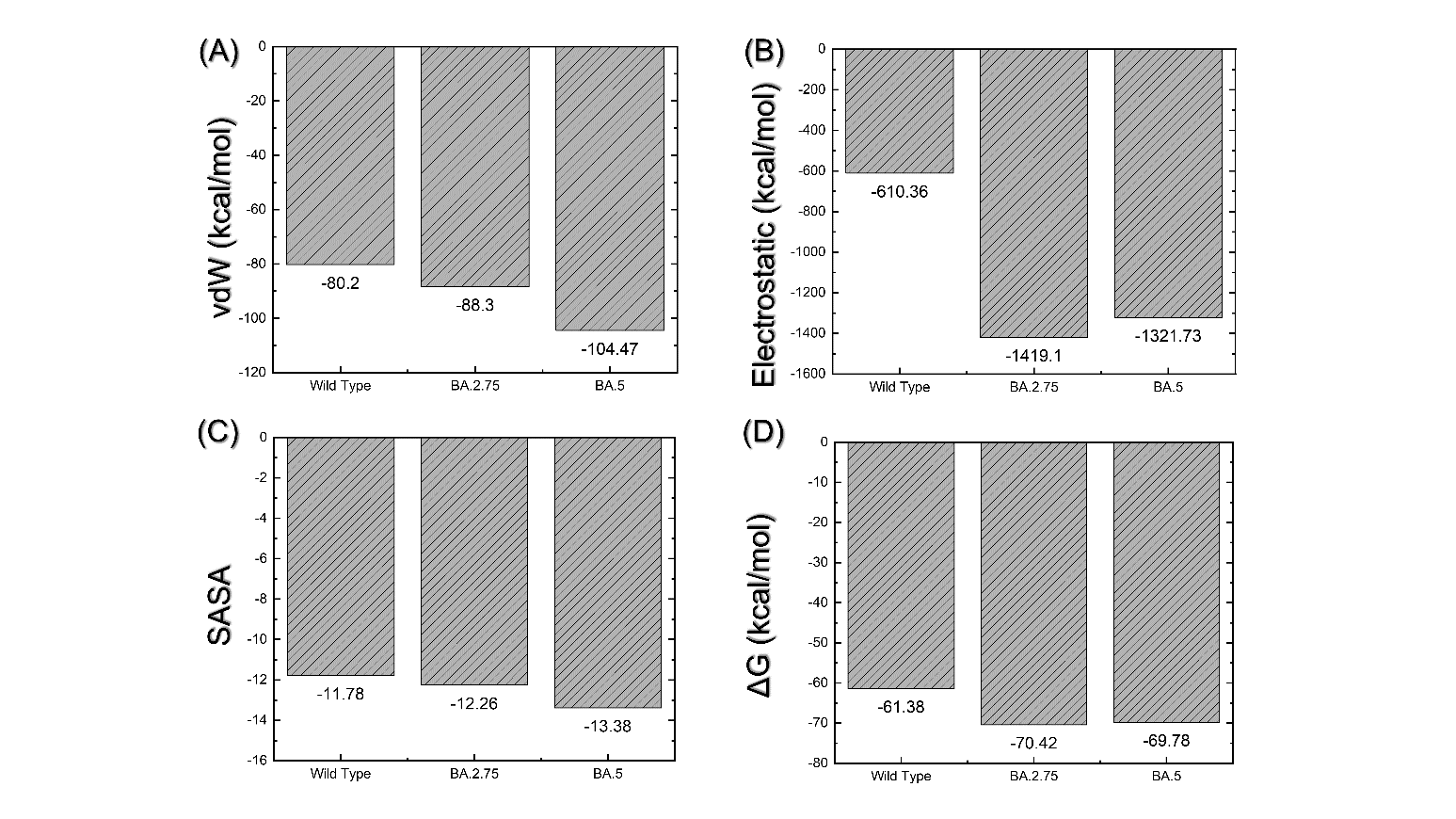
The association of large macromolecules i.e., two or more proteins is essentially driven by plentiful aspects, particularly by hydrogen and hydrophobic contacts at the interface. The environment solvated with water always compete with the residues to form hydrogen bonds [44]. The mechanisms underlying protein-protein interaction, as well as the ramifications for hydrogen bonding, are unclear [45]. Whether hydrogen bonds direct the protein coupling is an enduring apprehension, and the mechanism is not yet clearly determined [46, 47]. Therefore, it is imperative to comprehend the hydrogen bonding landscape in the protein-protein association. For instance, previously, computation of hydrogen bonds for the RBD and hACE2 complexes of different variants such as B.1.1.7 B.1.351, P.1, B.1.617, and B.1.618 has been reported to determine the strength of the association between these two receptors [28, 31, 32]. Hydrogen bonding over the simulation time was calculated and average number of hydrogen bonds in each trajectory were computed. The results shown in **Fig.7A-7B** demonstrated that the wild type had 375 average hydrogen bonds while the BA.2.75 had 376 while the BA.5 complex had 379 average hydrogen bonds during simulation. This shows that the additional hydrogen bonding contacts steer the robust binding of these variants in contrast to the wild type which consequently increase the entry and spread of the virus.

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**Fig.7. Hydrogen bonding analysis of the wild type and variants complexes during the simulation. (A)** represent the bonding paradigm over the simulation time for the wild type and BA.2.75 variant while **(B)** represent the hydrogen bonding pattern for the wild type and BA.5 variant.

**Computing the Bindin free Energy**

Re-evaluation of the docking conformation of the interacting protiens can be achieved by determining the binding free energy which gives more accurate and faster results than the conventional method. The binding estimation for the other variants including the alpha variant, beta, gamma, delta, omicron and others are previosly reported [28, 30-32, 48]. We estimated the binding free energy for all the complexes and presented in **Fig.8A-8D.** Th binding free energy results were obtained from our previously published article which revealed the vdW for the wild type was -80.20 kcal/mol while of the BA.2.75 it was -88.30 kcal/mo. On the other hand, the vdW was calculated to be -104.47 kcal/mo. The results of the vdW strongly align with the interactions in each complex. The highest number of hydrogen bonds observed in BA.5 can be reflected by the vdW energy while BA.2.75 and the wild type also reports the similar results. Moreover, the electrostatic energy for the wild type was estimated to be -610.36 kcal/mol while for the BA.2.75 it was -1419.10 kcal/mol and -1321.73 kcal/mol for the BA.5 variant respectively. This show that the binding of the BA.2.75 is steered by the electrostatic interactions while the BA.5 additional contacts are due to the vdW energy. Moreover, previously the total binding free enrgy for the wild type was reported to be -61.38 kcal/mol, however, the TBE for the BA.2.75 was estimated to be -70.42 kcal/mol and for the BA.5 variant the TBE was 69.78 kcal/mol repectively. These outcomes strappingly agrees the preceding studies where the higher binding by the SARS-CoV-2 variants has been informed due to the assimilated mutations in the RBD [28, 30-32, 48]. Hence, this consquently inform the higher binding affinity of BA.2.75 and BA.5-RBDs for the hACE2 receptor and infectivity.



**Fig.8: Binding free energy analysis of the wild type, BA.2.75 and BA.5 variants.** **(A)** Shows the vdW for the wild type, BA.2.75 and BA.5 variants, **(B)** show electrostatic energy the wild type, BA.2.75 and BA.5 variants. **(C)** demonstrate SASA for the wild type, BA.2.75 and BA.5 variants. **(D)** show he TBE for the wild type, BA.2.75 and BA.5 variants.

**Conclusions**

Using structural modelling and simulation methods, we explored the mechanism of interaction of the RBD from BA.2.75 and BA.5 variants of SARS-CoV-2 with the host receptor (hACE2) and validated by binding free energy calculation. Our analysis revealed significant structural deviation due to the mutations which consequently alter the binding affinity. Secondary structure transition was witnessed in the loops where the loop structure and the flexibility is increased by the mutations as an allosteric effect and thus enhances the chances of bonding with the nearby residues to connect and form a stable connection. The BFE further informed the higher binding affinity of BA.2.75 and BA.5-RBDs for the hACE2 receptor and infectivity. The current study provide invaluable insights into the development of structure-guided therapeutics against the circulating variants.

**Conflict of Interest**

Declared None

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