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Insights into the structural peculiarities of the N-terminal and receptor binding domains of the spike protein from the SARS-CoV-2 Omicron variant

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Abstract

Since the new variant of SARS-CoV-2, Omicron (BA.1) has raised serious concerns, it is important to investigate the effects of mutations in the NTD and RBD domains of the spike protein for the development of COVID-19 vaccines. In this study, computational analysis of the Wuhan and Omicron NTDs and RBDs in their unbound and bound states to mAb 4A8 and ACE2 were performed. In addition, the interaction of NTD with antibody and RBD with ACE2 were evaluated in the presence of long glycans. The results show that long glycans at the surface of NTDs can reduce the accessibility of protein epitopes, thereby reducing binding efficiency and neutralizing potency of specific antibodies. Also, our findings indicate that the existence of the long glycans result in increased stability and enhanced affinity of the RBD to ACE2 in the Wuhan and Omicron variant. Key residues that play an important role in increasing the structural stability of the protein were identified using RIN analysis and in the state of interaction with mAb 4A8 and ACE2 through per-residue decomposition analysis. Further, the results of the free energy binding calculation using MM/GBSA method show that the Omicron variant has a higher infectivity than the Wuhan. This study provides a better understanding of the structural changes in the spike protein and can be useful for the development of novel therapeutics.

1. Introduction

Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to global disruptions in everyday life, and millions of people have died as a result of infection by this virus. SARS-CoV-2 is a single-stranded RNA-enveloped virus enclosed with 5′-cap and 3′-poly(A) tail and containing four structural proteins, spike (S), envelope (E), membrane (M), nucleocapsid (N), and 16 non-structural proteins [1–3]. S protein is homotrimeric, consisting of two subunits, S1 and S2. The spike protein is a critical component for binding of the virus to host cells, owing to specific interactions via its receptor binding domain (RBD) with the angiotensin-converting enzyme 2 (ACE2) receptor, thereby mediating virus attachment to host cells. Recent computational results, demonstrated that single-wall carbon nanotube had an affinity to the S1 subunit in the spike glycoprotein [3]. The RBD contains a receptor-binding motif (RBM), which is the most important functional region of the spike protein because of its direct role in the interaction with ACE2 [2]. After two years of global spread of SARS-CoV-2 around the globe, the virus continues to evolve into new variants of concerns (VOCs) with improved transmissibility and resistance to antibodies, especially in vulnerable populations. In November 2021, Botswana, South Africa (SA), and a number of other countries have reported a novel variant of SARS-CoV-2 (lineage B.1.1.529), named Omicron [4]. To date, several Omicron

Abbreviations: ACE2, Angiotensin Converting Enzyme 2; COVID-19, Coronavirus disease 2019; SARS-CoV-2, severe acute syndrome coronavirus; RBD, receptor-binding domain; NTD, N-terminal domain; BFE, binding free energy; ESURF, surface area; vdW, Van der Waal; GB, generalized born; MMGBSA, the molecular mechanics, the generalized Born model and solvent accessibility; MD, Molecular-dynamics; VOC, Variant of Concern; mAb, monoclonal antibodies.

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sub-lineages including BA.1, BA.1.1, BA.2, BA.3, BA.4, BA.5 and re-combinant forms of BA.1/BA.2 such as XE have been reported [5]. Evidence suggests that Omicron (BA.1) with a large number of mutations, especially in the spike protein (26–32 mutations), containing two deletions and seven substitutions mutations found in the N-terminal domain (NTD) and fifteen substitutions occurred in the RBD, has the feature of escaping the immune system and higher transmissibility [6]. The urgent need for an effective therapeutic approach against this variant has attracted the attention of researchers in the design of novel drugs and the study of databases such as STRING, VARIDT, SYNBIP [7–9]. Computational approaches have a significant role in performing new research studies to recognition and treatment of wide range of diseases like neuroscience diseases and viral diseases including COVID-19 and other diseases [10]. Great efforts by have been paid to disrupt the replication of the virus including drug design techniques, vaccine design, discovering inhibitors of key proteins and design of Nabs and SBPs [8,11,12]. In addition, to facilitate drug discovery efforts, internet resources such as Target Therapy Database (TTD) and Drug Bank provide freely accessible and comprehensive information on drug pathways and targets in different development and clinical stages [13–15].

In this study, we used all-atom molecular dynamic simulations to understand the structural dynamics of the N-terminal domain (NTD), the receptor binding domain (RBD), and to characterize peculiarities of the interactions between the ACE2 and RBD from the Wuhan and Omicron variants. In addition, since virus glycosylation is a successful strategy in escaping the immune system, we evaluated the effect of glycosylation on receptor binding domain (RBD), and to characterize peculiarities of the interactions between residue side chains, respectively, is built and validated using ProSA [19] and SAVES v6.0 (https://saves.mbi.ucla.edu/). Notably, there were no Ramachandran outliers in the NTDs and RBDs [8,11,12]. In addition, to facilitate drug discovery efforts, internet resources such as Target Therapy Database (TTD) and Drug Bank provide freely accessible and comprehensive information on drug pathways and targets in different development and clinical stages [13–15].

2. Computational methods

2.1. Sequence and structure alignment

The sequences of NTD and RBD of Wuhan and Omicron (BA.1) variant of the SARS-CoV-2 spike proteins were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/) entries with the accession numbers YP_009724390.1 and UFO69279.1, respectively. The Wuhan and Omicron NTDs contain 292 (14–305) and 289 (14–302) residues, whereas Wuhan (residues 319–541) and Omicron RBDs (residues 316–538) consist of 223 residues. 3D model of the Wuhan NTD and RBD were made by CHARMM-GUI PDB Reader and (PDB ID: 6VSB) was used as a template. 1-TASSER server (https://zhanglab.ccb.med.umich.edu/1-TASSER/) was utilized for prediction of the 3-dimensional structures of corresponding Omicron NTD and RBD. The quality assessment of the predicted structures were done using PROCHECK [2,18], followed by validation using ProSA [19] and SAVES v6.0 (https://saves.mbi.ucla.edu/). Notably, there were no Ramachandran outliers in the NTDs and RBDs (Table S1, Figs. S1 and S2). The structure and sequence alignment were performed using the EMBOSS Needle server [20] and the results were visualized by the ESpript3 software [21].

2.2. System preparation

As the reference structure, the experimental crystal structure of the RBD-ACE2 complexes, PDB ID: 6MOJ (residue 333–526 of the spike protein from the Wuhan) were used in our study [21]. Missing protein residues and mutations were then introduced to the Wuhan structure using CHARMM-GUI Solution Builder tool using GalaxyFill [22] and PyMOL program [23,24]. The NTD-4A8 with the PDB code 7C2L was retrieved from the protein data bank [25]. We prepared fourteen different systems that include the spike NTD-4A8 and RBD-ACE2 complexes as well as the NTD and RBD only systems for each of the Wuhan and Omicron variant. CHARMM-GUI was used to attach the N-glycan chains at the glycosylation sites of the six systems including NTD only systems, NTD-4A8 and RBD-ACE2 complexes (Table S2) [26].

2.3. Molecular dynamics simulations

Topologies of all systems were generated by CHARMM-GUI solution builder module using the CHARMM36(m) all-atom force-field with cut off distances 12 Å for van der Waals interactions, and were neutralized with 0.15 M KCl and solvated using the TIP3P water model. The systems were embedded in a rectangular water box with an edge distance of 10 Å [27–29]. Molecular dynamics simulations carried out using the GROMACS 2020.6 with the Charmm36 m force field, WYF parameter [30] and Hydrogen mass repartitioning (HMR). Since cation-π interactions play an important role in biomolecular identification processes and stability of protein structure, WYF parameter was used to improve the MD simulation, which contains free energies, of cation-Indole-choline and indole-trimethylated lysines interactions related to protein-protein interfaces [31]. Hydrogen mass repartitioning (HMR) technique permits all-atom MD simulation to employ time steps of up to 4 fs by devoting a heavy atom mass to hydrogen atom(s) linked to the heavy atom [32]. The linear-constraint-solving (LINCOS) algorithms was used to limit bonds involving hydrogen atoms [33,34]. The systems were minimized in 5000 steps, and equilibration was performed for 125 ps with constant volume and temperature (NVT). Next, the production step was carried out under a constant pressure and temperature of 310 K (NPT). The single protein systems were run for 100 ns each, and NTD-4A8 and RBD-ACE2 systems production simulations were performed for 50 and 85 ns, respectively. The trajectory analysis was performed using GROMACS tools, the parameters RMSD, RMSF, radius of gyration (Rg), SASA, and the amount of H-bonds were all included, finally trajectories were visualized by PyMol and UCSF Chimera 61 [35].

2.4. RINs analysis

RING 2.0 (http://old.protein.bio.unipd.it/ ring/) [36,37] was used to generate RINs for the free forms of the NTDs and RBDs of Wuhan and Omicron variant. Cytoscape was then used to visualize the RING output. The MCODE plugin in Cytoscape was utilized to find clusters of the resulting networks.

2.4.1. Key residues in the NTD and RBD of Wuhan and Omicron variant

The Network Analyzer program [38] in Cytoscape was used to calculate the local metrics betweenness and stress, and key residues were defined using these two parameters. In order to find key residues, the Z-score of each residue was calculated, and if the Z-score absolute value was more than two, the residue was considered as a key residue.

2.5. Free energy landscape

The free energy landscape of the NTDs and RBDs of the Wuhan and Omicron variant were measured using geo_measures v 0.8 [39]. Geo measures contains a powerful library of g sham and form the MD trajectory against RMSD and Radius of gyration (Rg) energy profile of folding recorded in a 3D plot using the python package matplotlib. The energy is calculated as Gibb’s free energy derived from protein enthalpy and entropy.

2.6. Principal component analysis

Principal component analysis (PCA), a statistical method based on covariance analysis, was performed using GROMACS. PCA was used to analyze trajectory data of the MD simulations to recognize the
configurational space of a harmonic motion with just a few degrees of freedom [40].

2.7. Free energy binding and \( \Delta G \) (destabilizing)

DynaMut2 server was used to evaluate the effect of some important mutations of the Omicron variant on the folding free energy (\( \Delta G_{\text{destabilizing}} \)) RBD-ACE2 complexes [41]. Overall, if the \( \Delta G \) value is below zero, it indicates that the mutation has caused destabilization of the protein; otherwise, it has induced protein stabilization.

2.8. Binding free energy (BFE) calculation

End-state free energy calculations was carried out by gmx_MMPBSA, which is under MMPBSA.py calculation engine and written with Python 3.8 [42]. gmx_MMPBSA_ana tool, graphical user interface, generates different plots from gmx_MMPBSA output files to visualize and analyze the results [42].

The free binding energy for a complex was calculated by the following equation:

\[ \Delta G_{\text{bind}} = \Delta G_{\text{complex}} - \Delta G_{\text{receptor}} - \Delta G_{\text{ligand}} \]

Per-residue free energy decomposition analysis was performed using MM/GBSA method in order to investigate the key residues involved in protein-protein interaction.

3. Results

3.1. Sequence and structure alignment

The pairwise amino acid sequence alignment revealed that the Wuhan NTD shares 95.24% identity and 97.96% similarity with the Omicron NTD (Fig. 1A). Between the anti-parallel \( \beta \)-strands, the glycan-free surface in the NTD includes several loops. According to previous studies [43], this region is very electropositive and is targeted by many antibodies. Based on the analysis of the antibody interaction with NTD [44], it’s called 5 loops region, as it contains loops N1 (residues 14–26), N2 (residues 67–79), N3 (residues 141–156), N4 (residues 177–186), and N5 (residues 246–260), among which loops 3 and 5 play the most...
important part in the interaction with antibodies. As it follows from our 3D-structural superimposition (Fig. S3A), Omicron mutations in the NTD have altered the structure of this domains compared to the Wuhan NTD. For example, deletion of residues 143–145 and the G142D mutation led to the N3 loop deformation and in the glycan movement. It is probably one of the viral strategies to escape the antibodies. Moreover, careful evaluation of the N3 and N5 loop regions, which have the most fluctuations, can be deduced that substitution mutations in the Omicron RBD increase the stability of the RBD-ACE2 complex.

### 3.2. Molecular dynamics study

#### 3.2.1. The root-mean-square deviations (RMSDs)

The root-mean-square deviations of the backbone atoms of all systems were analyzed to determine structural stability and convergence. The results of the comparative stability analysis during the 100 ns MD simulation time indicated that the RMSD values of the Wuhan NTD show high fluctuations. It is noticeable that unlike the Wuhan NTD, the RMSD values for the Omicron NTD show less variability during 40–100 ns of simulation. In addition, the analysis of the effect of N-glycosylation with the long glycans on protein dynamics clearly shows a decrease in the flexibility during 40–100 ns of the MD simulation time (Fig. 2A). RMSD values were also evaluated for the complexes of the Wuhan and Omicron NTDs with mAb 4A8. According to (Fig. 2B), the glycosylated Wuhan NTD-4A8 complex fluctuates more than the glycosylated Omicron NTD-4A8 complex. Presumably one of the reasons for the higher stability of the antibody-Omicron NTD complex is the presence of glycans at this position, which restricts the antibody tendency to move and force it to stay in a more fixed position. Because the results of the Omicron NTD-4A8 complex clearly show more deviation than Wuhan NTD-4A8.

The average values of the RMSDs for the Wuhan RBD and Omicron RBD were found to be ~0.46 and ~0.51 nm, respectively. The plot indicates that the highest value of RMSD (~0.82 nm) was found around 18 ns and 64 ns of simulation. In the last 30 ns of simulation, both systems became stable. Based on the RMSD plot, the Wuhan RBD is more stable than the Omicron RBD (Fig. 2C). As (Fig. 2D) shows, in the last 15 ns of the simulation time both Wuhan and Omicron glycosylated complexes showed less deviations than their counterparts. In addition, based on the RMSD plot both Omicron RBD-ACE2 complexes show stable dynamic and it can be deduced that glycosylation in the Omicron RBD increase the stability of the RBD-ACE2 complex.

#### 3.2.2. Root mean square fluctuations (RMSFs)

Residue-based backbone fluctuations of the Wuhan and Omicron NTDs in glycosylated and non-glycosylated states were evaluated by plotting their RMSF values (Fig. 3A). Three major fluctuation peaks were observed in the Wuhan NTD, residues 62–83, 140–158, 177–189, and 239–260 corresponding to the loop regions N2, N3, N4 and N5, respectively. Fluctuations in these regions, except for the N5 loop, were significantly reduced in the glycosylated state. Comparison of the RMSF profiles of the Wuhan and Omicron NTDs revealed that the flexibility of these mentioned loops, except for the residues 240–256, was decreased in the Omicron NTD. By comparing the plots in the glycosylated state, the effect of the mutations, especially in the N5 loop region, can be clearly seen in the stability of the protein relative to Wuhan. The RMSF analysis also revealed higher flexibility in the glycosylated Wuhan NTD-4A8 complex compare to the glycosylated Omicron NTD-4A8 complex. Careful evaluation of the N3 and N5 loop regions, which have the most interaction with the antibody, illustrated less fluctuations in the Omicron structure. This is probably due to the deletion mutation causing removal of the residues 143–145 and the displacement of long glycans, resulting in the contacts of glycans with neighboring residues, as more deviations in the Omicron structure are clearly observed in the non-glycosylated state (Figs. 3B and 5).

The RMSF for all RBD-ACE2 complexes were also plotted, the average values of RMSF for the Wuhan and Omicron non-glycosylated RBD-ACE2 complexes are ~0.14 and ~0.12 nm, respectively, indicating that the Omicron RBD is more rigid than the Wuhan. In addition, the fluctuations of each residues of the glycosylated RBD-ACE2 complexes were evaluated in order to investigate the effect of the glycosylation on protein dynamics. The average values of RMSF for the glycosylated Wuhan and Omicron complexes were 0.10 and 0.08 respectively. Fig. 3C showed that the both non-glycosylated RBD-ACE2 complexes were more dynamic than glycosylated complexes which indicates that dynamic fluctuations of the protein complexes were reduced by the glycosylation. Interestingly, based on the RMSF plots of the both
glycosylated RBD-ACE2 complexes, it could be deduced that the
decrease of the fluctuations caused by the glycosylation were seen not
only near the glycosylated sites but also in the other regions of the
glycosylated RBD-ACE2 protein complexes. The Wuhan and Omicron
RBD-ACE2 complexes showed similar fluctuations. As Fig. 3C shows,
Three loops in the RBDs, (474–485), (488–490), and (494–505), which
are critical for binding with ACE2, have slightly less fluctuation in the
Wuhan RBD than in the Wuhan and Also, in the glycosylated com-
plexes, these regions have less fluctuations. According to Fig. 3C, the
hydrogen bonds were 5.93, 6.46, 6.82, 7.38 for the states Wuhan RBD-
ACE2, Omicron RBD-ACE2, glycosylated Wuhan RBD-ACE2, glyco-
sylated Omicron RBD-ACE2 complexes, respectively. The higher number
of hydrogen bonds in the Omicron RBD-ACE2 complex (Fig. 4D) sug-
gests higher stability of the interaction between the RBD of Omicron
Variant and ACE2 receptor. As Fig. 4D shows, more hydrogen bonds
were observed in the glycosylated complexes than in the non-
glycosylated counterparts indicating the stronger binding of the RBD
to ACE2 in glycosylated forms.

3.2.4. Solvent accessible surface area (SASA)
Solvent accessible surface area (SASA) is one of the key factors in the
evaluation of stability and folding of protein structures. The SASA
analysis of MD simulation trajectory indicated that Wuhan NTD has a
higher SASA with average value (~154.4 nm²) than the Omicron NTD
(~ 150.8 nm²), suggesting higher compactness of the Omicron NTD. In
addition, by comparison of the plots, it can be inferred that glycosylation
reduces SASA indicating more stable and compact structure of the gly-
cosylated form than the non-glycosylated state (Fig. 6A). Also, by
comparison of the plots, it can be inferred that glycosylation
reduces SASA indicating more stable and compact structure of the gly-

Conformational changes due to the Omicron RBD mutations caused
SASA alterations. The average value of the SASA for the Wuhan and
Omicron RBDs are 138.86 nm² and 140.38 nm², respectively. The result
indicate that Omicron RBD has higher SASA than the Wuhan (Fig. 6C).
Also, the SASA for the RBD-ACE2 complexes was calculated as a function
of time, and the results obviously showed that the Wuhan RBD-ACE2
complex has a greater SASA value (~364.14 nm²) than the Omicron
RBD-ACE2 complex (~363.95 nm²). The SASA analysis for the RBD-
In these plots, the glycosylation effect on the efficiency of protein compactness of this protein in comparison with the Omicron NTD. Also, timecourse were detected in the Wuhan NTD, due to the lesser exposed in the binding interface therefore less stable than glycosylated ACE2 complexes showed that the non-glycosylated complexes are more by a decrease to ~3.75 nm in 32 ns and remained consistent until 50 ns. Further, in the glycosylated Omicron NTD-4A8 complex, there was a gradual increase in the Rg values from ~3.68 nm to ~3.95 nm during the interval from 5 ns to 30 ns followed by a slight decrease to ~3.84 nm in 40 ns and then reached a stable state. Indeed, fewer deviations in the Omicron compared to Wuhan indicate more involvement of glycan interactions with mAb 4A8, and escape of protein from antibody binding (Fig. 7B).

Rg was calculated from the 100 ns trajectory to gain insight into the compactness and rigidity of the RBDs. The average Rg value for the Wuhan and Omicron RBDs were ~2.28 and ~2.15 nm, respectively. In the last 30 ns of the simulation time both RBD systems became stable. The Rg plot of the RBDs showed the general compactness of Wuhan and Omicron RBDs (Fig. 7C). Also, the Rg for the non-glycosylated Wuhan and Omicron RBD-ACE2 complexes fluctuates between ~3.07 and ~3.24 nm, and ~3.05--~3.21 nm, respectively. Based on the comparison between the Rg plots of the Wuhan and Omicron RBD-ACE2 complexes, no significant deviation was observed. In addition, the Rg values of the glycosylated RBD-ACE2 complexes have less deviations than that of the glycosylated complexes. This reflects the impact of the glycosylation on the increase in the protein complex compactness (Fig. 7D).

3.2.6. Free energy landscape

The analysis of free energy landscape (FEL) has been used to identify lower-energy basins (minima) during NTDs and RBDs unbound MD simulations. The plots in (Fig. 8) indicated a comparative view of the free energy landscape as a function of RMSD and Rg for NTDs and RBDs of the Wuhan and Omicron variant. According to Fig. 8, the energy minima basins were shown from red to blue, and the blue zone denotes more stable conformation with minimal energy. As shown in Fig. 8A and B, Omicron NTD has more blue and dark blue zones than Wuhan NTD and also by comparing the distribution of orange and red zones, it can be deduced that Omicron NTD has higher stability than Wuhan. Moreover, the effect of glycosylation NTD on the reduction of Gibbs free energy compare to non-glycosylated state is clearly observed and it is illustrated that glycosylation results in higher stability of protein structures (Fig. 8C and D). Based on Fig. 8E and F, the more blue and dark blue zones in the Wuhan RBD and the different distribution of orange and red zones, Wuhan RBD has a lower Gibbs free energy level than that of Omicron, which shows higher stability of the Wuhan RBD compared with Omicron RBD.

3.2.7. Principal component analysis (PCA)

The overall motion of the proteins were analyzed by PCA using construction of the eigenvectors. Figs. S4A and B, exhibited that the Wuhan NTD has more structural motions than the Omicron NTD and is occupying a larger space. The effect of glycosylation can also be inferred from the PCA, and the corresponding analysis revealed that the motions and degrees of freedom within proteins have become more limited and proteins became more stable due to glycosylation (Figs. S4C and D). The comparison of PCA for RBDs has shown that the motion properties described by the first two eigenvectors are different in the Wuhan RBD as compared to the Omicron (Figs. S4E and F).

3.3. Free energy binding and ΔΔG (destabilizing)

Among the Omicron RBD substitution mutations, residues including K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y and Y505H are located at the interface of RBD and ACE2 receptor. Substitution mutations including G339D, T478K, Y505H, S373P, N440K, E484A, Y505H have a value of predicted stability change (ΔΔG\text{Stability}) above zero and S371L, S375F, Q493R, Q496S, Q498R, N501Y and Y505H have a value of predicted stability change below zero (ΔΔG\text{Stability}). By the Omicron substitutions, the hydrogen bonding between GLU493 of RBD and ACE2 residue 35 was enhanced and, hydrogen bonds such as interactions HI505-LYS353 and LYS417-
Fig. 6. SASA analysis of (A) NTD-Wuhan, NTD-Omicron and evaluation of these two systems in the glycosylated state, (B) NTD-4A8 (Wuhan), NTD-4A8 (Omicron) and evaluation of these two systems in the glycosylated state, (C) RBD-Wuhan and RBD-Omicron. (D) RBD-ACE2 (Wuhan), RBD-ACE2 (Omicron) and evaluation of these two systems in glycosylated state. (A', B', C' and D') Density Function of SASA sampled over the simulations are shown in histograms.

Fig. 7. Radius of gyration (Rg), (A) NTD-Wuhan, NTD-Omicron and evaluation of these two systems in the glycosylated state, (B) NTD-4A8 (Wuhan), NTD-4A8 (Omicron) and evaluation of these two systems in the glycosylated state. (C) RBD-Wuhan and RBD-Omicron. (D) RBD-ACE2 (Wuhan), RBD-ACE2 (Omicron) and evaluation of these two systems in glycosylated state. (A', B', C' and D') Density Function of Rg sampled over the simulations are shown in histograms.
ASP30 are shown in Fig. 9.

3.4. Binding free energy calculation

The MM/GBSA method was used in order to investigate how the Omicron variant mutations affect on the binding of NTD and RBD with mAb 4A8 and ACE2, respectively. The binding free energy of the NTD to mAb 4A8 within the Wuhan and Omicron NTD complexes were calculated from the last 10 ns of trajectories and also evaluation of these two complexes in glycosylated state. The binding free energy of the Wuhan and Omicron NTDs to mAb 4A8 were $-44.90$ kcal/mol and $-25$ kcal/mol, respectively. Furthermore, the binding free energy for the glycosylated Wuhan NTD-4A8 complex was $-34.27$ kcal/mol and $-21.41$ kcal/mol for the glycosylated Omicron NTD-4A8 complex (Table 1). Overall, these results especially in the glycosylated state show the effect of mutations on the increase of the Omicron variant escape from the host’s immune response.

The binding free energies of the four RBD-ACE2 complexes were calculated from the last 10 ns of the trajectories. The energy values for the Omicron RBD-ACE2 complex were $-80.22$ kcal/mol (vdW), $-1366.81$ kcal/mol (electrostatic), and $-37.20$ kcal/mol (total binding energy). In addition, the binding free energy of the RBD to ACE2 in the Wuhan and Omicron glycosylated complexes were $-33.29$ kcal/mol and $-42.91$ kcal/mol, respectively. Importantly, the results indicate that due to existence of the long glycans, the binding free energies are higher for the glycosylated RBD-ACE2 complexes than for the non-glycosylated counterparts. The results demonstrated that the RBD of Omicron variant interacts more effectively with ACE2 than the Wuhan, likely...
contributing to the faster spread of the Omicron variant (Table 1).

### 3.5. Per-residue free energy decomposition analysis

The key binding residues of NTDs and RBDs were identified with MM/GBSA method. As given in Fig. 10A and Table S3, there are thirteen key residues (energy contribution $< -1.00$ kcal/mol) in the NTD for all four systems. The results of the analysis, especially in the residues TYR145, ARG246, and TYR248, emphasize the escape of the Omicron from the antibody. The impact of long glycans on each residue, indicated a decrease in the binding energy of TYR145, ARG246, TYR248, in the Wuhan and ASN148 (→ASN143), ASN149 (→ASN 144), LYS150 (→LYS145) in the Omicron. The per-residue energy decomposition results of the RBD-ACE2 complexes showed that S477 N, Q493R, G496S, Q498R and N501Y with (energy contribution $< -1.00$ kcal/mol) are identified as key residues (Fig. 10B and Table S4) which have a positive effect on the more binding affinity between the Omicron RBD to ACE2. Also, the impact of long glycans on the key residue, indicated a decrease in the binding energy.

### 3.6. RINs analysis of key residues in the NTDs and RBDs of the Wuhan and Omicron variants

In this study, key residues of the NTD and RBD domains of the Wuhan and Omicron variant were identified by RIN betweenness and stress metrics (Fig. 11, Tables S5 and S6). Stress is defined as an indicator of the centrality of a node, which indicates the number of shortest paths that pass through a node. Also, to identify the key structural residue as a mediator of interaction with other nodes, betweenness was used, the high values of which indicate the presence of a node in the shortest path between any two other nodes [46]. The key residue agreement between the two structures of Wuhan and Omicron NTDs is TRP104 (→TRP102), ILE119 (→ILE117), PHE192 (→PHE187), PHE194 (→PHE189), and PHE201 (→PHE196) and for RBDs is PRO384, LEU387, ILE410, ILE418, ASN422. The results indicate that the number of clusters and correspondingly MCODE score for Wuhan NTD are higher than that of the Omicron NTD. MCODE scores for clusters with four or more residues and cluster members for NTD structures are presented in Table S5, and cluster members for RBD structures are presented in Table S6. The highest overlap was observed in the cluster members of both types of NTD structures for ILE105 (→ILE103), PHE135 (→PHE133) and TYR160 (→TYR155) and for RBD structures is PRO384, LEU387, ILE410, ILE418, ASN422. The small difference in key residues identified in the Wuhan and Omicron NTD and RBD structures is due to the effect of the mutations that have occurred in the structures.

### 4. Discussion

This study has focused on the effects of mutations in the Omicron variant on the structure and function of two important SARS-CoV-2
Wuhan for RBD binding to the ACE2 receptor [48], since they are structurally
epitopes against antibody neutralization. Previous studies have compared the loop regions of the Omicron NTD with that of the Wuhan NTD. In this study, analysis of the structural role of glycosylation sites of SARS-CoV-2 VOCs have been observed during viral evolution [47]. In this study, analysis of the structural role of N-glycans showed an increase in the stability of glycosylated proteins compared to the non-glycosylated state. It is interesting to note that glycans attached to the loop regions are of the complex types for protection of immunogenic protein epitopes, no mutation in the NTD. In sum, the results of this study could be useful for the future studies in this field.

5. Conclusions

This computational study analyzed structural peculiarities of the Wuhan and Omicron RBDs and revealed structural changes caused by the mutations found in the Omicron variant. Analysis of the effect of mutations through MD simulation shows the stability of the NTD in the Omicron variant relative to the Wuhan, and probably mutations lead to an increase in the binding affinity of the binding of the Wuhan RBD is more stable than Omicron RBD in unbound form. We further elucidated the effect of different mutations on the interaction of RBD with ACE2. N440K mutation had a significant effect on the increased stability of the Omicron complex. Similar to a previous study [54], Following the results mentioned above, per-residue energy decomposition for the four RBD-ACE2 complexes revealed that positively charged residues including S477 N, G496S, Q498R and N501Y mutations lead to an increase in the binding affinity of the binding of the Omicron RBD to ACE2 [55, 56], which are seen in both glycosylated and non-glycosylated forms in our study. The K417 N mutation which is also found in Beta, Gamma, and in some Delta lineage RBDS [57] was the cause of the absence of a salt bridge leading to a decrease in the binding affinity to ACE2 [58]. Importantly the results of the interaction analysis of the Wuhan and Omicron RBD-ACE2 complexes revealed some key interactions found only in the Omicron RBD-ACE2 complex and also, more number of salt-bridge were found in the Omicron complex. The Q493R substitution in the Omicron RBD caused in new formed salt-bridge analysis and the free energy landscape plots of the Wuhan and Omicron RBDS only systems, it is revealed that Omicron substitution mutations affect the structure of the RBD and protein behavior in the course of 100 MD simulation. Overall, the result demonstrated that the Wuhan RBD is more stable than Omicron RBD in unbound form. We further elucidated the effect of different mutations on the interaction of RBD with ACE2. N440K mutation had a significant effect on the increased stability of the Omicron complex. Similar to a previous study [54], Following the results mentioned above, per-residue energy decomposition for the four RBD-ACE2 complexes revealed that positively charged residues including S477 N, G496S, Q498R and N501Y mutations lead to an increase in the binding affinity of the binding of the Omicron RBD to ACE2 [55, 56], which are seen in both glycosylated and non-glycosylated forms in our study. The K417 N mutation which is also found in Beta, Gamma, and in some Delta lineage RBDS [57] was the cause of the absence of a salt bridge leading to a decrease in the binding affinity to ACE2 [58]. Importantly the results of the interaction analysis of the Wuhan and Omicron RBD-ACE2 complexes revealed some key interactions found only in the Omicron RBD-ACE2 complex and also, more number of salt-bridge were found in the Omicron complex. The Q493R substitution in the Omicron RBD caused in new formed salt-bridge with GLU35 residue of the ACE2. The mutated residues in the RBD indicates strong interactions with some of the residues of the ACE2, including hydrogen bonding that were observed between the ARG493 and ARG498 residues of the Omicron RBD with GLU35 and ASP38 residues of the ACE2 respectively (Table S7). The results of binding free energies calculation of the Wuhan and Omicron RBD-ACE2, indicated the lower total free energy in the Omicron RBD-ACE2 complex than that of the Wuhan. In sum, the results of this study could be useful for the future studies in this field.

5. Conclusions

This computational study analyzed structural peculiarities of the Wuhan and Omicron RBDS and revealed structural changes caused by the mutations found in the Omicron variant. Analysis of the effect of mutations through MD simulation shows the stability of the NTD structure in the Omicron variant relative to the Wuhan, and probably provides a basis for the accumulation of dangerous mutations in the
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