Identification of Potential ACE2-Derived Peptide Mimetics in SARS-CoV-2 Omicron Variant Therapeutics using Computational Approaches

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ABSTRACT: The COVID-19 pandemic has become a global health challenge because of the emergence of distinct variants. Omicron, a new variant, is recognized as a variant of concern (VOC) by the World Health Organization (WHO) because of its higher mutations and accelerated human infection. The infection rate is strongly dependent on the binding rate of the receptor binding domain (RBD) against human angiotensin converting enzyme-2 (ACE2human) receptor. Inhibition of protein–protein (RBDhuman−SARS-CoV-2/omicron−ACE2human) interaction has been already proven to inhibit viral infection. We have systematically designed ACE2human-derived peptides and peptide mimetics that have high binding affinity toward RBDhuman. Our peptide mutational analysis indicated the influence of canonical amino acids on the peptide binding process. Herein, efforts have been made to explore the atomistic details and events of RBDhuman−SARS-CoV-2/omicron−ACE2human interactions by using molecular dynamics simulation. Our studies pave a path for developing therapeutic peptidomimetics against omicron.

Worldwide, SARS-CoV-2 and its variants have had an unprecedented effect on various aspects of society, including public health and the global economy. At the outset of the COVID-19 pandemic, the WHO requested global scientists to focus on the discovery and development of safe and effective drugs against SARS-CoV-2. Even though the original goal of the discovery and development of vaccines against SARS-CoV-2 and some of its variants was successfully fulfilled, the emergence of new variants of SARS-CoV-2 which are resistant to antibodies continues to present new challenges to the scientific community.

Numerous SARS-CoV-2 strains are being monitored by the WHO. These are broadly classified as variants of concern (VOC), variants of interest (VOI) and variants under monitoring (VUM) based on strain infection rate, severity, and future risk. Recently, the WHO added the new variant omicron (B.1.1.529) as a VOC because of its higher infectivity rate. In the last quarter of 2021, the very first SARS-CoV-2 omicron variant cases were identified in South Africa. As of June 21, 2022, the SARS-CoV-2 variant has led to largest global surge of confirmed cases (537 591 764) with cumulative mortality rate (6 319 395). Phylogenetic analysis on the whole genome has revealed its closer sequence identity with the alpha (B.1.1.7) variant, which suggests that omicron’s primary emergence in the population is way beyond its expectation even in the vaccinated population.

Omicron is considered to be a highly mutated variant of SARS-CoV-2 that has emerged to date, with enhanced transmissibility and partial resistance to vaccine-induced immunity. The large number of mutations on the surface of the spike protein, including the immunodominant RBDomicron, has facilitated omicron evasion of numerous antibodies. The receptor binding domain (RBD) of spike protein that makes direct interaction with ACE2human has shown unique mutated residues in most of the variants of concern. Omicron is a highly mutated variant that consists of >60 mutations, out of which 15 mutations (G449D, S477L, S494P, S477N, K457N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, and Y501H) occurred in the RBDhuman region.

In particular, the RBDomicron−ACE2human core interacting region contains 11 mutated amino acids (K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, and Y501H), whereas the hairpin loop consists of three key mutations (S477L, S477P, and S475F). The three mutations Q493R, G496S, and Q498R have played a key role in the increased binding affinity of the omicron variant. These additional mutations played a major role in the omicron variant outracing other prominent variants. These unique mutations at the binding region are mainly involved in the conformational fingerprint of the interaction site of RBDhuman. Moreover, they are
involved in causing distinct changes in charges on the RBDomicron surface.

Previous studies on SARS-CoV-2 indicated significant amino acids on the binding surface in both RBD (Y_{505}, T_{500}, Q_{498}, Q_{497}, Y_{489}, N_{487}, F_{486}, G_{476}, F_{466}, Y_{449}, and K_{447}) and ACE2human (G_{354}, K_{353}, Y_{383}, Q_{262}, Y_{431}, D_{430}, H_{344}, K_{325}, D_{320}, and Q_{24}). Inhibiting RBDs(SARS-CoV-2/omicron)-ACE-2 interactions by using ACE2human-derived peptide (I_{1}−S_{42}) and peptidomimetics offers various advantages like high specificity and affinity. On the basis of ACE2human interacting residues, potential peptides that would act against omicron were designed. In order to estimate the atomistic interaction difference between the complexes of RBDhuman-SARS-CoV-2 and RBDomicron-ACE2human, all-atom MD simulations on crystal structure complexes of both RBDhuman-SARS-CoV-2-Ace2human (wild type) (PDB code: 6LZG) and RBDomicron-ACE2human (PDB code: 7T9L) were performed.

Our systematic ACE2human peptide-directed mutational approach demonstrates the influence of canonical amino acids in the binding region of RBDomicron. Maintaining the secondary structure of the α-helical peptide is essential to produce the desired clinical response. Mutating amino acids (M, A, L, E, K) which have high α-helix inducing properties will significantly contribute to maintain the α-helical secondary structure of the peptides, whereas mutating to low α-helix propensity amino acids such as (P, G) may disrupt peptide α-helicity, thereby depleting its bioactivity. Our mutational analysis of the total α-amino acids spectrum helps researchers to understand the influence of each amino acid, thereby helping to design and develop the peptides with high α-helix propensity. The designed peptidomimetics specifically bind to the RBDomicron with high affinity. In conclusion, we have identified and designed peptides that are potent against RBDomicron.

In order to compare the ACE2human-derived protein–peptide/peptidomimetic interactions on RBDs of SARS-CoV-2 and omicron, the multichain 3D structures of the RBDs(SARS-CoV-2/omicron)-ACE2human (PDB Code: 6LZG/7T9L) complex were collected from the RCSB database. Superposition of RBDhuman-SARS-CoV-2 and RBDomicron binding sites revealed that mutated residues (Q_{809}R and Q_{810}R) in omicron are of noticeable longer side chains (Figure 1A–C). A high initial structure similarity was observed between RBDomicron and RBDhuman-SARS-CoV-2 with the RMSD of 0.336 Å.

Initial protein interaction analysis was performed using Maestro (Schrödinger), which provided residues involved in the interaction site.

Over all, 17 RBDhuman residues (Y_{449}, Y_{453}, F_{450}, A_{475}, G_{479}, N_{477}, F_{466}, N_{467}, Y_{459}, R_{492}, S_{494}, S_{499}, R_{488}, T_{500}, Y_{501}, G_{502}, and H_{503}) are within the contact distance of 4.0 Å with 17 residues (S_{19}, Q_{36}, T_{27}, F_{29}, D_{30}, K_{31}, H_{34}, E_{35}, K_{353}, G_{354}, D_{355}, R_{357}, D_{38}, Y_{41}, Q_{262}, M_{262}, and Y_{83}) of ACE2human. Whereas in SARS-CoV-2, 19 RBD residues (K_{447}, G_{466}, Y_{449}, Y_{553}, L_{555}, F_{556}, A_{575}, G_{479}, F_{466}, N_{467}, Y_{459}, R_{492}, Q_{262}, G_{490}, Q_{498}, T_{500}, N_{501}, Q_{502}, and Y_{503}) are in close contact with 20 ACE2human residues (S_{19}, Q_{36}, T_{27}, F_{29}, D_{30}, K_{31}, N_{320}, H_{34}, E_{35}, K_{353}, G_{354}, D_{355}, R_{357}, E_{377}, D_{38}, Y_{41}, Q_{262}, M_{262}, and Y_{83}). Both RBD(SARS-CoV-2/omicron) share 10 common residues (Y_{449}, Y_{453}, F_{450}, A_{475}, G_{479}, N_{477}, F_{466}, N_{467}, Y_{459}, R_{492}, S_{494}, S_{499}, R_{488}, T_{500}, Y_{501}, G_{502}, and H_{503}).
Figure 2. Red (negative) and blue (positive) colors denote charge potential. (A) Gaussian-based smooth electrostatic potential of RBD$^\text{SARS-CoV-2}$. (B) Gaussian-based smooth electrostatic potential of RBD$^\text{omicron}$. (C) Charge distribution on RBD$^\text{SARS-CoV-2}$ residues (red (negative charge) and blue (positive charge)). (D) Charge distribution on RBD$^\text{omicron}$ residues (red (negative charge) and blue (positive charge)). (E) Theoretical titration curves of RBD$^\text{SARS-CoV-2}$ (pH vs charge) (charge at pH 7.4 ($z = +1.226$) and isoelectric point (pl = 7.776)). (F) Theoretical titration curves of RBD$^\text{omicron}$ (pH vs charge) (charge at pH 7.4 ($z = +4.267$) and isoelectric point (pl = 8.340)).

Figure 3. (A–H) ACE$^\text{-human}$ α-1 helix residues interacting with RBD$^\text{SARS-CoV-2}$ (hydrogen bonds in red dotted line). (I–P) ACE$^\text{human}$ α-1 helix residues interacting with RBD$^\text{omicron}$ (hydrogen bonds in red dotted line).
The overall binding affinity was observed (Table S1). These mutations played a major role in the omicron variant outacing the other prominent variants.\(^{17}\)

The net charge on RBD\(_{\text{omicron}}\) (\(z = +4.267\)) (Figure 2C,E) was slightly higher than that of the net charge on RBD\(_{\text{SARS-CoV-2}}\) (\(z = +1.26\)) (Figure 2D,F).

Q\(_{493}R\), Q\(_{499}R\), and Y\(_{505}H\) mutations at the binding site of RBD\(_{\text{omicron}}\) imposed higher positive charges than that of RBD\(_{\text{SARS-CoV-2}}\) (Figure 2C,F), which promotes the viral infusion into the host alveolar cells.\(^{30,31}\)

Comparative analysis of the electrostatic surface of RBD\(_{\text{omicron}}\) with RBD\(_{\text{SARS-CoV-2}}\) by using Gaussian-based dielectric function\(^{32,33}\) gave insights into the surface properties of RBD\(_{\text{SARS-CoV-2}}\)/RBD\(_{\text{omicron}}\) (Figure 2A,B). The mutated residues on RBD\(_{\text{omicron}}\) showed moderate effect on the overall positive charge of the protein. Even though the positively charged residue, mutated in the place of negatively charged residue (K\(_{41}^\alpha\)-N) in RBD\(_{\text{omicron}}\), may deplete the binding affinity over RBD\(_{\text{omicron}}\), the overall net charge seems to be dependent on other mutations (Figure 2E,F). The overall binding affinity is increased because of mutations (Q\(_{493}R\), Q\(_{499}R\)) that are responsible for additional \(\pi-\pi\) interactions in addition to the hydrogen bond interactions (Figure 3L,N,O).

**Comparative Electrostatic Analysis.** The comparative electrostatic potential analysis showed variations in the charge potential of RBD\(_{\text{omicron}}\) (Figure 2A,B). This elucidates the significance of mutations in the tight-binding process of RBD\(_{\text{omicron}}\) toward the negatively charged ACE\(_2\) human. The RBD\(_{\text{SARS-CoV-2}}\) in complex with ACE\(_2\) human was subjected to further molecular dynamics simulations to get atomistic details of protein–protein interactions.

**Molecular Dynamics Simulation Analysis.** Time-dependent all-atom molecular dynamics simulations has been proven to be an efficient tool to analyze the folding behavior of proteins in biological conditions. During the whole production run, Ca-RMSD and Cα-RMSF were noted to investigate the structural changes and to assess the stability of the protein complex (Figure S1A,B). In this study, initially the RMSD of the Ca-backbone of RBD\(_{\text{omicron}}\) was raised from 0 to 2.7 Å over the time period of 5 ns, whereas in both PDB crystal structures of ACE\(_2\) human (PDB Code: 6LZG/7T9L) Ca-RMSD raised from 0 to 2.7 Å over the time period of 5 ns. Stable Ca-RMSD (within the 1 Å window frame) was observed in both complexes (RBD\(_{\text{SARS-CoV-2}}\)/ACE\(_2\) human) between 200 and 300 ns MD simulations (Figure S1A).

RMSF analysis showed that minor fluctuations were observed in the binding site hairpin loop regions especially between K\(_{493}\) and G\(_{485}\) (Figure S1B). The presence of disulfide bonds between (C\(_{480}\) and C\(_{485}\)) was greatly responsible for RBD\(_{\text{omicron}}\) folding and stability (Figure 1F).

Secondary structure elements (SSE) analysis showed that RBD\(_{\text{omicron}}\) consisted of 6.50% helix and 22.74% strand with total SSE of 29.25% (Figure S5B), whereas RBD\(_{\text{SARS-CoV-2}}\) consisted of 4.80% helix and 22.97% strand with 27.77% as total SSE (Figure S5A). There was no significant (\(p\)-value < 0.05) difference observed. SSE components on the whole trajectory showed a unique \(\alpha\) helical region, which appeared between S\(_{483}\) and D\(_{489}\) during the evolution of the RBD\(_{\text{omicron}}\). ACE\(_2\) human complex (Figures S5B and S4).

The hydrogen bond formation between RBD\(_{\text{SARS-CoV-2}}\)/omicron and ACE\(_2\) human defines the binding affinity of the protein complex. Hydrogen bond occupancy analysis performed using the VMD H-bond tool has provided information on the residues involved in RBD\(_{\text{SARS-CoV-2}}\)/omicron-ACE\(_2\) human binding interactions. In the analysis, the interacting residues and their hydrogen bond occupancies involved in the binding site of RBD\(_{\text{SARS-CoV-2}}\)/omicron-ACE\(_2\) human are considered (Figure S2). It is observed that there is a significant difference (\(p\)-value 2.49 \(\times\) 10\(^{-38}\)) in the occurrences of hydrogen bonds between RBD\(_{\text{SARS-CoV-2}}\)/omicron-ACE\(_2\) human, where RBD\(_{\text{omicron}}\), ACE\(_2\) human exhibited a higher number of hydrogen bonds (2166) in comparison to RBD\(_{\text{SARS-CoV-2}}\)-ACE\(_2\) human (1160).

The analysis also revealed that 10 ACE\(_2\) human residues (S\(_{19}\), Y\(_{34}\), H\(_{446}\), L\(_{450}\), T\(_{488}\), Y\(_{489}\), Y\(_{493}\), K\(_{493}\), and D\(_{495}\)) are primarily involved in hydrogen bonding in the interaction site, where 9 ACE\(_2\) human residues (S\(_{19}\), Q\(_{24}\), H\(_{446}\), E\(_{453}\), D\(_{458}\), Y\(_{489}\), Y\(_{493}\), K\(_{493}\), and D\(_{495}\)) have common hydrogen bond interactions in both omicron/SARS-CoV-2. Acidic D\(_{458}\) of ACE\(_2\) human has a hydrogen bond interaction with R\(_{498}\) of RBD\(_{\text{omicron}}\). A high percentage of hydrogen bond occupancy of 28.34% and 15.17% is observed between ACE\(_2\) human residues (Y\(_{483}\), Y\(_{489}\)) and the polar hydroxyl group of N\(_{487}\), T\(_{500}\) of RBD\(_{\text{omicron}}\), respectively (Figures 3P and S2). Second, D\(_{355}\)-T\(_{500}\) (18.06%) residue combinations (RBD\(_{\text{omicron}}\)/ACE\(_2\) human) are primarily occupying the hydrogen bonds in the total production run of the RBD\(_{\text{omicron}}\) and ACE\(_2\) human complex. Acidic and negatively charged amino acids (Q\(_{493}\) and Q\(_{499}\)), mutated to respective basic and positively charged amino acids (R\(_{493}\) and R\(_{499}\)) in omicron, also contributed strongly to the hydrogen bonding with ACE\(_2\) human residues (E\(_{453}\), D\(_{489}\)). Notably, uncharged RBD\(_{\text{omicron}}\) residues (N\(_{487}\)) made special hydrogen bond interactions with ACE\(_2\) human residues (Y\(_{505}\)). Numerous hydrophobic and nonpolar aromatic amino acids (Y\(_{489}\), Y\(_{453}\), Y\(_{483}\), and Y\(_{505}\)) of RBD\(_{\text{SARS-CoV-2}}\)/omicron are involved in the network of hydrogen bonds with ACE\(_2\) human residues (D\(_{355}\), H\(_{350}\), Y\(_{489}\), and E\(_{453}\)) (Figure S2). Aromatic tyrosine residues such as Y\(_{489}\) of RBD\(_{\text{omicron}}\) and Y\(_{83}\) of RBD\(_{\text{SARS-CoV-2}}\) make strong hydrogen bond (OH–O) interactions (Figure S6F). However, in the case of RBD\(_{\text{SARS-CoV-2}}\)–2, 4 tyrosine residues (Y\(_{453}\), Y\(_{449}\), Y\(_{489}\), and Y\(_{505}\)) primarily contributed for the hydrogen bonds with ACE\(_2\) human residues, wherein (Y\(_{453}\), Y\(_{505}\)) residues interacted with positively charged side-chain ACE\(_2\) human residues (H\(_{447}\), K\(_{493}\)) respectively. Weakly acidic tyrosine residues (Y\(_{489}\), Y\(_{505}\)) make hydrogen bonds with polar uncharged ACE\(_2\) human residues (Y\(_{34}\), E\(_{453}\)), respectively (Table S1). RBD\(_{\text{omicron}}\)/ACE\(_2\) human molecular interaction and hydrogen bond analysis over 30 ns simulations has provided ACE\(_2\) human key residues residing in the α-1 helix (S\(_{19}\), T\(_{230}\), Q\(_{84}\), T\(_{277}\), F\(_{280}\), D\(_{300}\), K\(_{311}\), H\(_{346}\), E\(_{355}\), D\(_{389}\), Y\(_{411}\), and Q\(_{414}\)), where the α-2
Molecular Docking and Interaction Analysis. Comparative analysis of the generated mutated peptides and protein–peptide molecular docking studies provided important information on the role of ACE2<sub>human</sub> peptide point mutations in both omicron/SARS-CoV-2. The docking of the control peptide on RBD<sub>50</sub>SARS-CoV-2/omicron has not shown any significant difference (Table S2) in the docking score (HADDOCK scores of (omicron/SARS-CoV-2) (~109.1/~101.0), respectively); however, the overall docking scores of omicron/SARS-CoV-2 have demonstrated that numerous peptide mimetics also bind with high affinity with RBD<sub>50</sub>SARS-CoV-2/omicron rather than RBD<sub>50</sub>SARS-CoV-2 (p-value = 2.82 × 10<sup>-79</sup>, significant) (Figure 4).

In proteins, aromatic amino acids (F, Y, and W) usually make strong aromatic–aromatic interactions because of perpendicular/coogwheel or parallel interactions between benzene rings. The presence of numerous tyrosine (Y) and phenylalanine (F) amino acids in the RBDs<sub>SARS-CoV-2/omicron</sub> have clear impact on the binding affinity of peptides. Mutating positively charged amino acids (D<sub>330</sub>, D<sub>335</sub>) to aromatic phenylalanine (F) gave top HADDOCK scores in both

Figure 4. Heatmap depicting the docking scores of peptide and peptide mimetics in both (A) RBD<sub>SARS-CoV-2/ACE2<sub>human</sub></sub> and (B) RBD<sub>omicron/ACE2<sub>human</sub></sub>. The y-axis denotes the canonical amino acids except proline and glycine segregated according to their properties (nonpolar and aliphatic amino acids (A, V, L, M, I), aromatic (F, Y, W), polar and uncharged (S, T, C, N, E), positively charged (K, R, H), and negatively charged (D, E)). Control Peptide (Control). "*" Denotes the high α-helix propensity inducing amino acids (M, A, L, E, K). (C) Standard deviation was obtained by using 24 replicates (each canonical amino acid mutated at 24 amino acid length peptide). Canonical amino acids showed significant variation (p-value = 5.78 × 10<sup>-11</sup>, significant) in the docking scores (peptide/peptide mimetics, RBD<sub>SARS-CoV-2/omicron</sub>). (D) Standard deviation was obtained by using 24 replicates (each canonical amino acid mutated at 24 amino acid length peptide). Canonical amino acids showed significant variation (p-value = 0.00031, significant) in the docking scores (peptide/peptide mimetics)-RBD<sub>omicron</sub>. (E) Standard deviation was obtained by using 456 replicates. Significant variation (p-value = 2.82 × 10<sup>-79</sup>, significant) in the docking scores was observed while docking peptide and peptide mimetics over the binding sites of both RBDs<sub>SARS-CoV-2/omicron</sub>

A detailed analysis of hydrogen bond interactions between ACE2<sub>human</sub> α-1 helix on both RBDs<sub>SARS-CoV-2</sub> (Figure 3A–H) and RBD<sub>omicron</sub> (Figure 3I–P) can be explored further to design high-affinity peptide/peptide mutants against RBDs<sub>SARS-CoV-2/omicron</sub>. Moreover, α-2 helix residues of ACE2<sub>human</sub> make hydrogen bond interactions with RBDs<sub>SARS-CoV-2/omicron</sub> (Figure S6A,B (SARS-CoV-2) and Figure S6E,F (omicron)). Additionally, the linker region (β3–β4) residues of ACE2<sub>human</sub> interacting with RBDs<sub>SARS-CoV-2/omicron</sub> (Figure S6C,D (SARS-CoV-2) and Figure S6G,H (omicron)) also play a pivotal role in the binding of ACE2<sub>human</sub> toward RBDs.

Designing a Peptide Mimetic Library. Most of the crucial interactions between RBDs<sub>SARS-CoV-2/omicron</sub>-ACE2<sub>human</sub> was found to be from the α-1 helix of ACE2<sub>human</sub>. The 24 amino acid peptide derived from the α-1 helix region ranging between I<sub>21</sub> and S<sub>34</sub> was used for generating a library with mutated residues. Systematic mutation of each position of the 24 amino acid ACE2<sub>human</sub> peptide (IEEQAKTFLDKFNHEADELFY-QSS) with 18 canonical amino acids except proline (P) and glycine (G) was carried out, and a 414-membered peptide mimetic library was created. The generated peptidomimetic library was systematically divided based on the properties shown on the helix wheel (Figure 1G). Hence, the 414-membered peptide mimetic library was further subjected to peptide docking over the binding sites of both RBDs<sub>SARS-CoV-2/omicron</sub>.

In proteins, aromatic amino acids (F, Y, and W) usually make strong aromatic–aromatic interactions because of perpendicular/coogwheel or parallel interactions between benzene rings. The presence of numerous tyrosine (Y) and phenylalanine (F) amino acids in the RBDs<sub>SARS-CoV-2/omicron</sub> have clear impact on the binding affinity of peptides. Mutating positively charged amino acids (D<sub>330</sub>, D<sub>335</sub>) to aromatic phenylalanine (F) gave top HADDOCK scores in both
phenylalanine (F) peptidic mutation improved the hydrophobic characteristics of the peptide. Moreover, interactions in between benzene rings with RBDs aromatic amino acids (F456 and Y491) also improved the overall binding affinity. Y41 was observed to make a π−π interaction with RBD\textsuperscript{omicron} specific Y501. Disrupting the aromatic−aromatic interaction between ACE\textsubscript{2}human peptide Y41 and omicron Y501 drastically decreased the binding affinity (Figure 4A,B).

Overall, the analysis made by MD and single mutant peptide docking proved that the presence of aromatic and high α-helix propensity inducing amino acids are essential to maintain the structural integrity and bioactivity of the peptides. It is interesting to note that while designing peptide mimetics, the amino acids (E\textsubscript{227}, E\textsubscript{233}, A\textsubscript{239}, K\textsubscript{260}, T\textsubscript{278}, F\textsubscript{289}, L\textsubscript{291}, F\textsubscript{311}, E\textsubscript{312}, E\textsubscript{313}, A\textsubscript{360}, E\textsubscript{374}, L\textsubscript{389}, F\textsubscript{401} and Y\textsubscript{41}) necessary for aromaticity and α-helicity were untouched. Moreover, it was ensured that the salt bridge (Q\textsubscript{993}−E\textsubscript{171}) and π stacking interactions (Y\textsubscript{501}−Y\textsubscript{41}) were maintained constantly. The amino acids (I\textsubscript{13}, Q\textsubscript{24}, D\textsubscript{90}, N\textsubscript{133}, H\textsubscript{156}, D\textsubscript{308}, Q\textsubscript{432}, S\textsubscript{456} and S\textsubscript{46}) that are nonaromatic and low α-helix propensity were carefully mutated. While designing peptide-1, these 9 amino acids were mutated with high α helix propensity inducing amino acids (M, A, L, E, K), whereas in peptide-2 the 9 amino acids on control ACE\textsubscript{2}human peptide were mutated with high α-helix propensity inducing and aromatic amino acids (M, A, L, E, K, F, Y, W). However, in peptides-3 to 5, the canonical amino acids excluding proline and glycine were randomly mutated (Table 1).

Out of the plethora of peptides in the library, four peptides showed higher binding affinity when compared with the control α-helix. Docking these mutated peptides on RBD\textsuperscript{omicron} showed that peptides mutated with high α-helix propensity inducing and aromatic amino acids (peptide-2) display significant improvement in the HADDOCK score (−154.5 kcal/mol) rather than the peptide (peptide-1 (−109.1 kcal/mol)) that was mutated with high alpha propensity inducing amino acids. Freely mutated peptides (peptides 3, 4, and 5) showed top binding scores of −162.7, −162.1, and −158.3 kcal/mol, respectively (Table 1).

In conclusion, host receptor recognition and attachment by virion is facilitated by the interface of RBD\textsuperscript{omicron} with ACE\textsubscript{2}human. Thus, design of peptides that have a resemblance with ACE\textsubscript{2}human α-1 helix can inhibit the protein−protein interaction and be a potential therapeutic strategy in order to combat the virus. The crystal structure complex (RBD\textsuperscript{omicron}−ACE\textsubscript{2}human) was subjected to molecular dynamic simulation for insights into the atomistic details of protein−protein interactions. I\textsubscript{2} to S\textsubscript{44} residues of ACE-2 are considered as key residues that interact with the RBD\textsuperscript{omicron} domain, and these were mutated to generate a library of 432 novel peptides. In this study, four peptides were proposed that exhibited stronger binding interaction with RBD\textsuperscript{omicron} in comparison to that of RBD\textsuperscript{SARS-CoV-2}, which are considered to be worthy of further investigation (Table 1). In this work we demonstrated a robust strategy to design novel antiviral peptides that act specifically against upcoming SARS-CoV-2 variants.

### METHODS

For this study, the raw state RBD\textsuperscript{SARS-CoV-2/omicron}−ACE\textsubscript{2}human complex crystal structures (PDB Codes: 6LZG/7T9L) were obtained from the RCSB database\textsuperscript{27} and imported into Maestro, Schrödinger.\textsuperscript{29} Initially, the protein structures were prepared by subjecting the crystal structures (PDB Codes: 6LZG/7T9L) to protein preparation wizard of Maestro, Schrödinger,\textsuperscript{29} where water and ligand molecules were removed and missing hydrogens were added to the crystal structures (PDB Codes: 6LZG/7T9L). The prime module was used to incorporate the missing side chains and loops to the protein structures. We carefully unaltered the crystal symmetry of the proteins by subjecting restrained minimization only on hydrogens. Default PROPKA pH of 7.0 was used during the preparation process.

### Comparative Electrostatic Models

Electrostatic potential analysis provides guided parameters to design specific inhibitors against any biomolecule. Binding affinities during the protein−protein interaction is mainly dependent on the electrostatic energy. Protein complexes RBD\textsuperscript{SARS-CoV-2/omicron}−ACE\textsubscript{2}human (PDB codes 6LZG/7T9L) were prepared by using Maestro Schrödinger,\textsuperscript{29} and 3D models of RBD\textsuperscript{SARS-CoV-2} and RBD\textsuperscript{omicron} were extracted. These models were used to calculate the electrostatic potential by using a linear solver to solve the Poisson−Boltzmann equation.\textsuperscript{35} Protonation of the RBD\textsuperscript{SARS-CoV-2} and RBD\textsuperscript{omicron} models were made through Amber force field parameters. 70% of the box was filled with protein RBD\textsuperscript{SARS-CoV-2/omicron} and the remaining 30% contained solvent and ions. Periodic boundary conditions (bndcon = 2) were added to the models. Internal and external dielectric constants were set at 4.0 and 80.0 respectively. Default salt concentration (salt = 0.15) was maintained to mimic the physiological solutions. A maximum of 80 linear iterations during the calculation process were allowed.

| Mutation Type                        | Name                 | Peptide Sequence                                  | Docking Score (kcal/mol) |
|--------------------------------------|----------------------|--------------------------------------------------|--------------------------|
|                                      |                      | SARS-CoV-2                                      | Omicron                  |
| Control                              | NH\textsubscript{2}−IEEQAKTLDFKNHEADLFLYQSS−COO | -101.1                                          | -109.1                   |
| High α-helix propensity              | Peptide-1            | NH\textsubscript{2}−KEEMAKTLFLEKMLEAEALFYMLK−COO | -101.4                   | -109.1                   |
| Aromatic and high alpha propensity   | Peptide-2            | NH\textsubscript{2}−KEEFAKTLFKFMFEAELFYFTW−COO  | -153.8                   | -154.5                   |
| inducing amino acids                 |                      |                                                  |                          |
| 20 amino acids except Proline and    | Peptide-3            | NH\textsubscript{2}−KEEQVSWFLYKNEAWFLYYSW−COO  | -149.7                   | -162.7                   |
| Glycine                              |                      |                                                  |                          |
|                                      | Peptide-4            | NH\textsubscript{2}−KEEQASWFLYKNEAWFLYYSW−COO  | -143.6                   | -162.1                   |
|                                      | Peptide-5            | NH\textsubscript{2}−KEEQASWFLYFNHEAWFLYYSW−COO  | -142.8                   | -158.3                   |

Table 1. Binding Scores of Multimutated Peptide Mimics Interacting with RBDs (SARS-CoV-2/omicron)
Gaussian-based smooth dielectric function considers the continuum solvent that has calculated accurate electrostatic potentials. The variance of Gaussian distribution was set at (sigma = 2.0), and default Gaussian-based smooth dielectric constant was set to (srfcut = 20).

**MD Simulations.** Both RBDs$_{\text{SARS-CoV-2/omicron}}$ and ACE2$^\text{human}$ were subjected to 300 ns of molecular dynamics simulation study to check key amino acids involved in the interaction process. 36 Desmond-Maestro was used to prepare the RBDs$_{\text{SARS-CoV-2/omicron}}$-ACE2$^\text{human}$ complexes by adding SPC water, 37 and neutralize the complexes by adding counterions. Periodic boundary conditions (PBC) were established by using an orthorhombic box, where the box size calculation was performed by using a buffer method with (10.0 Å × 10.0 Å × 10.0 Å) dimensions. OPLS4 force fields 38,39 were applied on the system before subjecting it the 100 ps minimization process. Hence, we used the Desmond standard ensemble, where the Martyna–Tobias klenbarostat with constant pressure of (1.01325 Pa bar) and Nose–Hoover thermostat 40 with constant temperature (310 K) was maintained. Electrostatic forces were considered within the 9.0 Å cutoff distance by the particle mesh Ewald (PME) 41,42 method used in this MD run. The MD equilibration primarily contained 6 major steps: In step 1, Brownian dynamics NVT was used by maintaining constant temperature ($T=10$ K), restraints were kept on solute heavy atoms, and a 12 ps simulation was used. For step 2, NPT with constant step 3, NPT was used by keeping constant temperature ($T=10$ K), where a 12 ps simulation was used. In step 4, NPT with restraints on solute heavy atoms, and a 12 ps simulation were used, whereas for step 5, NPT without restraints for a 24 ps simulation was performed. Finally, both RBDs$_{\text{SARS-CoV-2/omicron}}$ along with ACE2$^\text{human}$ systems were subjected to a 300 ns restrained MD run. Trajectory analysis was performed by using VMD and Desmond.

**Molecular Docking of Peptides.** High ambiguity driven protein–protein docking (HADDOCK) has been proven to be the best docking server for protein–protein and protein–peptide docking. 43 One feature that distinguishes it from other accessible software is the use of experimentally derived data from NMR and mutagenesis, and the provided solution was found to be identical with the experimentally derived structure. Both the interacting residues in peptide and protein are given as input along with the active site residues of the receptor. By defining ambiguous interacting residues (AIRs), the main advantage is that only the poses in which the ligand interacts with the active residues are sampled rather than selection based on conformational energies.44 A three-step protocol is used for both the protein–protein and protein–peptide docking, in which step 1 contains the rigid docking process, step 2 contains semi-flexible docking, and step 3 includes refining structures in the presence of water. The docking score is mainly dependent on the summation of reports obtained through these three steps.45

$$\text{Docking score} = 1.0E_{\text{vdW}} + 0.2E_{\text{elec}} + 1.0E_{\text{desol}} + 0.1E_{\text{AIR}}$$

(1)

The input 3d structure of peptide/peptide mutations were derived from the α1-helix of ACE2$^\text{human}$ crystal structures. Peptide mutations were made by considering steric clashes in the peptide structure. Active site residues derived from the MD studies and protein–peptide interaction analysis were given as inputs. Nonpolar hydrogens were removed in the initial steps. Residues were considered as accessible if they fulfilled the minimum 15% of relative solvent accessibility. All surface residues were passively selected within the 6.50 Å radius of active site residues. A cutoff distance of 2.5 and 3.9 Å was set to define proton-acceptor (hydrogen bonds) and carbon–carbon (hydrophobic contacts), respectively. The nonbonded parameters such as OPLS force fields and electrostatic energy terms were included in the docking process. Missing atoms were rebuilt in the context of the molecule, and initial rigid bond minimization was performed by keeping the alpha backbone dihedral restraints. Detailed docking cluster reports were generated by the HADDOCK server. 46 A default cutoff of RMSD (2 Å) was set, and acceptable structures were ranked among the clusters. Screening of peptide mimetics on RBD$_{\text{SARS-CoV-2}}$ and RBD$_{\text{omicron}}$ provided key information on binding residues. Overall, the studies allowed the design of novel peptide and peptidomimetic inhibitors that competitively bind to RBD$_{\text{SARS-CoV-2}}$ and RBD$_{\text{omicron}}$.

**Statistical Analysis.** R statistical software was used to perform numerous statistical tests (Pearson $\chi$ test, ANOVA, chi-square test, and Tukey’s test) to statistically verify the significant difference across docking and MD studies.

### ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpclett.2c01155.

**Table of molecular interactions between RBD$_{\text{SARS-CoV-2/omicron}}$-ACE2$^\text{human}$ and mutant complexes.**

| Residue | Interaction | Value |
|---------|-------------|-------|
| Cα-RMSD | 0.32 Å |
| Cα-RMSD | 0.34 Å |

The table of Tukey’s test comparison (Tables S1 and S2) is available (PDF).

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### Notes

The authors declare no competing financial interest.

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**ABBREVIATIONS**

RBD, receptor binding domain; VOC, variants of concern; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; ACE-2, human angiotensin-converting enzyme-2; MD, molecular dynamics; HADDOCK, high ambiguity driven protein–protein docking; WHO, World Health Organization; SSE, secondary structure elements.

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