De novo design of ACE2 based peptide; a potential candidate for COVID-19 therapeutics

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Research Article

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Abstract

To date, the current Covid-19 pandemic caused by SARS-CoV-2 has infected 99.2 million while killed 2.2 million people throughout the world and is still spreading widely. The unavailability of potential therapeutics against this virus urges to search and development of new drugs. It enters human cells by interacting with ACE2 and Glucose Regulated Protein 78 (GRP78) receptors expressed on human cell surface through utilizing receptor binding domain (RBD) of its Spike glycoprotein. The RBD domain is highly conserved and is also a potential target for blocking its interaction with human cell surface receptors. We extended our previously proposed tACE2 inhibitory peptide approach (that inhibits RBD and ACE2 interaction) to *de novo* design of shorter peptides. These peptides can selectively bind to RBD with much higher affinities than the cell surface receptors. Thus, these can block all the binding residues required for binding to cell surface receptors. We used selected amino acid regions (21–40 and 65–75) of ACE2 as scaffold for peptide design using EvoDesign. The method mutates the scaffold residues keeping Cα-backbone of the designed mutant fix, while favorable positioning of mutant R group results in increased binding affinity towards its partner protein. Our designed peptides Pep1 showed interactions with RBD covering almost all of its binding residues with significantly higher binding affinity (-13.2 kcal mol^{-1}) than the wild type receptors. The MD simulation results showed that designed peptides form a stabilized complex with RBD. We suggest that blocking the RBD through *de novo* designed peptides can serve as a potential candidate for COVID-19 treatment after been experimentally validated.

Introduction

SARS-CoV-2 belongs to Coronaviruses (CoVs) group which are enveloped positive-stranded RNA viruses [1]. SARS-CoV-2 was emerged and started causing coronavirus disease at Wuhan china during December 2019 (Covid-19). Hence, it is the utmost public health emergency at present with no treatment available so far, with an urgent need of potent drug against Covid-19 [1]. Currently, SARS-CoV-2 has affected the whole world and possibly it can re-emerge in the future with some virus beneficial mutations which might lead to more-worst outcome. Coronaviruses use spike glycoprotein to attach and fuse with host cells, followed by entry into the cell. The receptor binding domain (RBD) of S protein mainly interacts with the human cell surface receptors, such as angiotensin-converting enzyme II (ACE2) [2] and GRP78 [3], followed by entry into the cell. A recent study has diagnosed SARS-CoV-2 in serum, urine and fecal samples with a low detection rate [4,5], which suggests that SARS-CoV-2 utilize different types of cell surface receptors. Although it is challenging to determine whether the urinary tract, bladder or blood cells are also infected by SARS-CoV-2, but virtual screening of RBD with different cell surface receptors can raise the possibility of fecal/urine-respiratory infection.

Interestingly, the SARS-CoV-1 and -2 bind with cell surface receptors through RBD (a highly conserved region of S Protein) [24], which suggests this domain a suitable target for drug designing [6]. The structural insights of SARS-CoV-2 and ACE2 interactions have been extensively studied [7,8]. The RBD residues critical for interaction with ACE2 are located at position 417, 458, 493-498 and 500-502 [9,10,7,8], while, the residues of RBD at position 417 and 480-490 and 505 are mainly involved in binding with

GRP78 receptor [3]. This suggests that almost similar binding residues of RBD are used to interact with different cell surface receptors. The overall sequence of RBD is highly conserved with more than 99.9% homology with worldwide sequences of RBD reported [10]. Structural elucidation has also confirmed the highly conserved nature of RBD [8]. Blocking the binding residues of RBD can impede the SARS-CoV-2 to infect the human cells [11]. The interactions between RBD and cell surface receptors have been extensively elucidated [12,9,7], which can be exploited to design peptide based inhibitors targeting binding residues of RBD. Several studies have reported peptides for blocking the fusion of SARS-CoV-2 RBD with human cell surface receptors and have shown successful inhibitory effects [13-15]. Previous studies have shown that the residue of ACE2 at position 21-40 and 76 are optimal for binding with RBD [11,10]. Another natural peptide (SBP1) derived from ACE2 (amino acid position 21-43) showed successful inhibition of RBD with a disassociation constant (K_d) of 47 nM [15]. There are several other peptides reported for blocking RBD of SARS-CoV-2 and SARS-CoV-1 [16,9,14]. However, these peptides may not cover all the binding residues of RBD. Engineering the optimal regions of ACE2 and expand their binding interaction network can significantly block the infection of SARS-CoV-2 into human cells. *De novo* protein design is a novel approach used to optimize the binding interface of protein-protein interactions by mutating the residues into favorable mutants which provide new binding interactions with increased binding affinity and preserved secondary structure [17]. Recently, Huang et al., [11] redesigned the previously reported two natural peptides from hACE2 through Evo Design [18] and produced a hybrid peptide with improved binding affinity for RBD and showed interactions with residues Y453, F456, Y473, A475, N487 and Y489 of RBD.

In the current study, we aimed to design peptides on the basis of our previously reported truncated ACE2 (tACE2) [10] by using EvoDesign, a *de novo* peptide design approach, to increase not only the binding affinity but also extend the binding interaction network with RBD. We have selected two regions of ACE2 (21-40 and 65-75) as a template for *de novo* peptide design [12]. We designed two peptides, Pep1 and Pep2 for binding with RBD and determined their binding affinity and complex stability through protein-protein docking and molecular dynamics (MD) simulations. The present study will open a new path for designing therapeutic peptides against Covid-19.

Materials And Methods

2.1 Designing Covid-19 therapeutic peptides

The PDB structure of RBD of SARS-CoV-2 S glycoprotein (PDB ID: 6m17) was obtained from PDB database. Two peptides (Pep1 & Pep2) were deigned against the binding residues at position 417, 453, 458, 493-498 and 500-505 of RBD [7]. The amino acid position 21-40 of tACE2 binds with the binding residues 493-498 and 501-505 of RBD[10], while 65-75 amino acid region of tACE2 interacts with binding residues 417, 453 and 458 of RBD [6]. Therefore, we selected these two fragments of ACE2 from amino acid position 21-40 and 65-75 as scaffold1 and 2, respectively, for *de novo* peptide design to further enhance their binding affinity for RBD. The 3D structure of the scaffold peptides were produced through I-TASSER [19] and optimized for energy minimization through FoldX [20]. The optimized scaffold

structures were submitted as template to EvoDesign server using interface design. TM-score >0.7 was set as the fold cutoff value and redesign the entire sequence of the scaffold. The TM-score >0.5 indicates that the designed peptide have similar fold to that of scaffold [21]. EvoDesign outputs the top 10 sequences selected from the largest clusters. The top ten designed sequences obtained for each peptide was sorted based on TM-score, sequence identity and lowest free energy. The sequence with the lowest free energy was considered as favorable design. However, we selected Pep1 and Pep2 from their corresponding top 10 sequences based on their Z score and haddock docking score. The 3D models of the designed peptides were produced through I-TASSER [19]. The selected designed peptides were analyzed for their fold similarity through Tm-align [22].

2.2 Docking of RBD with designed peptides

Protein-protein docking of the designed peptides with RBD was performed through HADDOCK, a flexible protein-protein docking tool [23]. The structures of designed peptides were optimized before docking for amino acid side chain clashes and energy minimization by using FoldX [20]. HADDOCK performs protein-protein docking by retrieving information from experimentally determined protein-protein complexes. The energy function used by HADDOCK consists on combination of interaction energies and HADDOCK score, which is a combination of non-bonded intermolecular interactions [24]. All the generated docking poses were analyzed through PyMOl [25]. The best docked complex of RBD with designed peptides were selected on the basis of haddock score and were further analyzed for binding affinity ΔG (kcal mol^{-1}) and complex stability by using an online protein binding energy prediction server, PRODIGY [26]. The peptides-RBD docked complexes with higher binding affinity were subjected to Molecular Dynamic simulation to further confirm complex stability.

2.3 Determination of RBD-peptides complex stability through MD simulation

MD simulation of RBD in complex with designed peptides, Pep1 and Pep2 were performed through GROMACS 5.0.4 [27] using CHARM 36.0 force field. The protein complex was solvated in TIP3P cube box water model to provide an aqueous environment. To neutralize the total charge of the system, three Na ions were added to the box followed by energy minimization to remove conflict between the atoms. The system was then equilibrated through NVT and NPT at constant temperature (300 K) and pressure (1 bar), respectively. MD simulation was then run for 100 ns at 300K. Root mean square deviation (RMSD) and Root mean square fluctuation (RMSF) plots were produced through gnuplot.

Results And Discussion

3.1 *De novo* design of inhibitory peptides against RBD

RBD domain of spike glycoprotein mediates the entry of SARS-CoV-2 into the human respiratory cells by interacting with cell surface receptor ACE2 [8]. Therefore, blocking the interaction residues of RBD might block its interaction with ACE2, hence making it unable to infect human cells. The RBD of SARS-CoV-2 and SARS-CoV-1 is highly conserved [28] and mainly uses residues 417, 453, 458, 490, 493-495, 498, 501

and 502 for binding to ACE2 [29,7] [8]. Another study also reported same residues of SARS-CoV-2 RBD involved in binding with another cell-surface receptor (Glucose Regulated Protein 78 (GRP78) for entry into the cells [3], which indicates that SARS-CoV-2 interacts with different receptors located on human cell surface, using almost same binding residues of RBD. Therefore, blocking the binding residues of RBD through inhibitory peptides can potentially block entry of SARS-CoV-2 into the human cells and can also be useful against future pandemic if caused by newly emerged coronaviruses due to the conserved nature of RBD [8].Thereby, targeting the RBD to block its interaction with ACE2 is ideal choice for SARS-CoV-2 drug discovery.

Previously, we targeted these nine residues of RBD to be blocked through tACE2 [10]. However, the current study involved re-designing the binding interface of tACE2 to produce shorter peptide with more binding affinity and covering all the binding residues of RBD [30]. Short therapeutic peptides have gain interest due to its specificity and low immunogenicity response. The RBD binding residues 490, 493-495, 498, 501, 502 are clustered at one region (region1) while 417 and 458 are clustered slightly distal from the later (region2).Therefore either two peptides can block these two regions or single peptide with extended binding network can hinder interaction between RBD and cell surface receptors.

The residues of ACE2 at amino acid position 21-40 (scafold1) and 65-76 (scafold2) were re-designed and produced 10 *de novo* sequences for each scaffold. Two best sequences, Pep1 and Pep2 were selected from top-10 *de novo* sequences produced by EvoDesign from scaffold1 and 2, respectively. The TM score 0.61 of Pep1 indicate its similar fold to that of scaffold1, while Pep2 Tm-score was 0.16 indicating its different fold than the scaffold2 structure. The Lower RMSD of Pep1 (0.58 A˚) are in agreement with its Tm score, while Pep2 showed RMSD 2.12, which indicate slight deviation of secondary structure from their scaffold (Fig 1). Similarly, the amino acid sequence of Pep1 showed 30% similarity while Pep2 showed 20% similarity with their corresponding native sequences (Table 1). The designed peptides with high similarity to their native sequence usually exhibit higher binding affinity towards its partner protein [11]. We further investigated the binding pattern and affinity of the designed peptides for RBD through protein-protein docking.

3.2 Protein-protein docking

To test the binding properties, protein-protein docking of the designed peptides with RBD was performed through HADDOCK. The HADDOCK-Score of Pep1 and Pep2 was -119 and -111, respectively, when docked with RBD (The more negative the better binding affinity). The haddock score of Pep1 is greater than that of the intact ACE2 (-111) docked with RBD [10]. The docking RMSD of Pep1 and Pep2 in complex with RBD were 0.6 and 0.8, respectively, showing the high likelihood of the docked complexes with native one [24].

Our docking results showed that nine residues Ala2, Lys7, Ans10-Asp14, Ser16 and Phe20 of Pep1 interact with Arg403, Lys417, Tyr453, Lys458, Gln493-Gly496, Gln498, Thr500, Asn501 and Tyr505 residues of RBD (Fig.2 A-C), while Leu67-Asp69, Thr72 and Glu75 of Pep2 interact with Arg404, Lys417, Tyr495 and Tyr 505 of RBD (Fig 2D-E). These results confirm that Pep1 cover almost all the binding

residues of RBD involved in interaction with human ACE2 and GRP78, making this peptide ideal for further investigation for its therapeutic potential.

Previous studies has shown that binding residues of RBD are located at two distinct position, region 1 (490, 493-495, 498, 501, 502) and region 2 (417 and 458) [8,10], which demands two different peptides to be block. Interestingly, our *de novo* designed peptide Pep1 showed binding with region 1 as well as region 2 residues (Fig. 2C). The superimposition of docked Pep1 with its scaffold showed that redesigning changed the Phe20 into Arg with favorable side chain positioning for interaction with Lys458 of RBD, while mutation Ala16Ser results in interaction with Tyr417 of RBD (Fig. 1). Both of these residues are located at region 2 and reported to be critical for interaction with RBD [8]. The *de novo* design approach created optimum mutation which increased binding network of the designed peptide Pep1, resulting in successful blocking of the RBD binding residues required for interaction with human cell surface receptors.

3.3 Binding affinity of designed peptides for ACE2

We further determined the binding affinity of the designed peptides for RBD and complex stability. The binding affinity showed by Pep1 for RBD was -13.2 kcal mol^{-1} at 36 ºC as optimum temperature which is greater than the binding affinity of intact ACE2 (-10.7 kcal mol^{-}) [10] and GRP78 (-9.8 kcal mol^{-1}) for RBD [3]. The binding affinity of Pep1 for RBD was also higher than the binding affinity (-11kcal mol^{-1}) of therapeutic peptides proposed previously for blocking RBD of SARS-CoV-2 [14]. It seems that the favorable mutations and side chain rearrangement resulted in dramatic increase in binding affinity of Pep1 for RBD. We further determined the dissociation constant K_d values of peptide-receptor complexes. The Pep1-RBD complex showed K_d value 3.9×10^{-10} M, which is lower than the previously reported K_d values of inhibitory peptides proposed for S protein of SARS-CoV-2 [31] and intact ACE2-RBD complex [10]. The smaller K_d value indicates high stability and strong binding affinity between protein-protein complex [32].The lower K_d value of Pep1-RBD complex suggest that the designed peptide Pep1 are tightly bound to the corresponding region of RBD. Binding affinity and K_d values of Pep2-RBD complex was found lower than the Pep1-RBD complex. This indicates that region 21-40 has important role in binding with RBD.

3.4 MD simulation showed stability of designed peptides-RBD complex

To investigate the structural stability and dynamic behavior of the designed peptides in complex with RBD, we performed MD simulation of the RBD in complex with Pep1 and Pep2. The docking conformation with lowest energy was subjected to MD simulation. To investigate structural stability of the complex, RMSD plot of the complex backbone was produced. The RMSD values of Pep1-RBD complex remained 0.2-0.25 nm initially for 40ns and then increased up to 0.4-0.5 nm for 60-100 ns of MD run. Similarly, the RMSD values of Pep2-RBD complex remained 0.3-0.4 nm during initial 90 ns while slightly increased up to 0.95 nm during 90-100 ns (Figure 3A). In general, the RMSD $\leq$0.3 nm during a 20 ns MD run indicate strong complex stability [33,34]. Overall, a uniform lower RMSD of Pep1-RBD complex

indicates that Pep1 bind more tightly to RBD than the Pep2. The RMSD value of Pep1-RBD complex is also lower than the previously reported therapeutic peptides for SARS-CoV-2 treatment [31,10].

Root mean square fluctuation (RMSF) determined in the docked complexes shows residues flexibility with high RMSF values indicate the mobility of residue side chains in relation to their average position [1]. The RMSF plot of Pep1-RBD complex shows that the residues of RBD at position 358, 417 and 490-500 showing lower fluctuation (nm) than the Pep2-RBD complex. The overall RMSF value of Pep1-RBD complex is less than 0.2 nm in region 1 & II window, which is lower than the RMSF value (0.35nm) of RBD when bound to intact ACE2. The residues involved in binding interaction with lower RMSF values indicates the most stable region of the complex [35]. The lower RMSF values of RBD binding residues indicate that Pep1 form a stable complex with RBD, as RMSF value <0.4 nm reveals complex stability [36].

The Radius of gyration (Rg) was determined to describe the structural integrity and folding behavior of the designed peptides in complex with RBD. A low Rg value reveals better structural integrity and folding behavior [37,38]. Pep1-RBD complex showed a uniform and stable Rg value between 1.80-1.84 nm throughout a 100 ns MD run, while the Rg value of Pep2-RBD complex increased to 2.23 nm during 90-100 ns. Overall the Rg values for both peptides remained ≥1.84, which is lower than the Rg value (2.2nm) showed by intact ACE2-RBD complex [10], which indicates structural integrity of Pep1- and Pep2-RBD complex (Figure 3C). Overall, the MD simulation results suggests that the *de novo* designed peptides form a stabilized complex with RBD and propose their potential to block the SARS-CoV-2 Spike glycoprotein for interaction with human cell surface receptors.

Conclusion

SARS-CoV-2 infects human cells through their receptor binding domain of its spike glycoprotein by interacting with different cell surface receptors, ACE2 and GRP78. The *de novo* peptide design opens a new path for producing more potential therapeutic peptides that can mask the RBD critical residues required for interaction with human cell surface receptors, making the SARS-CoV-2 unable to infect human cells. Our *de novo* designed peptides covering almost all the binding residues of RBD with increased binding affinity and complex stability. A stabilized interactions network was shown by Pep1and Pep2. The designed peptides can be tested experimentally for their binding affinity towards spike glycoprotein, followed by analyzing their potential to inhibit the targeted human cell line from SARS-CoV-2 pseudoparticles infection, live virus infection inhibition in cell culture, followed by assessment of its potential inhibitory activity in animal model of infection.

Declarations

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Code availability: N/A

Authors' contributions: A.B has performed the *In silico* designing, MD simulation and manuscript preparation. M.A and S.R have contributed in manuscript writing. All the authors has reviewed the final version of the manuscript

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Tables

Table 1: Summary of the *de novo* designed peptides produced by using ACE2 as scaffold.

Designed Peptide	Sequence	Tm score[1]	Sequence identity	RMSD[2]
Pep1	EAAAKAKLSNENHDNSTVSF	0.6	30%	0.58
Pep2	PCLGDQATVAE	0.25	20%	2.12

[1] Tm score indicate the fold similarity between two structures. A Tm scores >0.5 correspond to almost similar fold while value <0.2 indicate randomly chosen unrelated proteins.

[2] RMSD calculated by TM-align shows the structural variations of two superimposed structures.

Figures

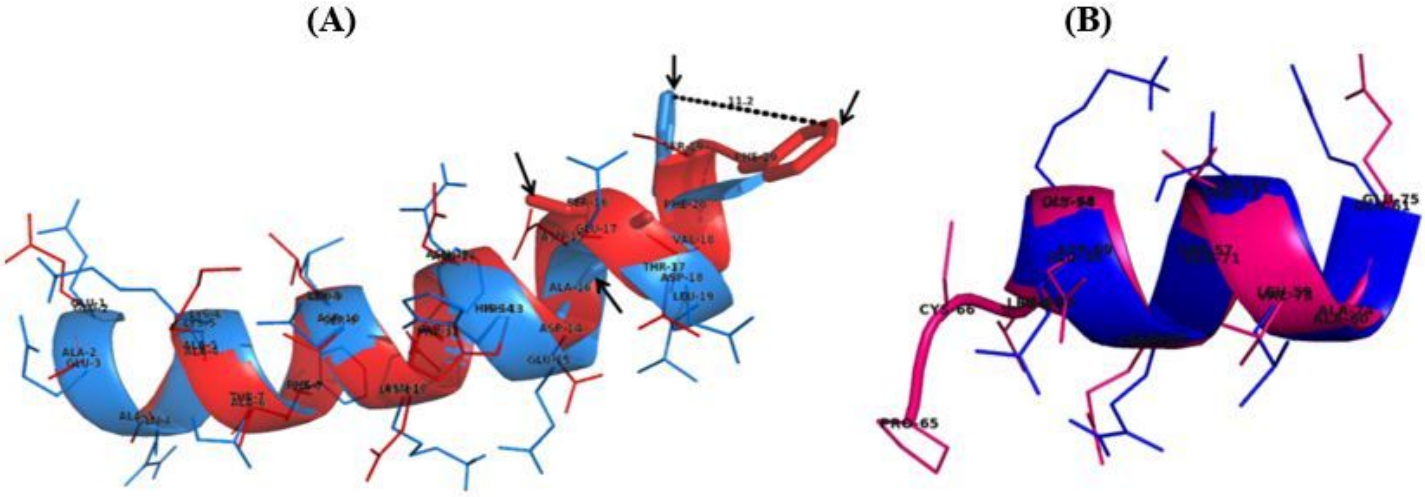

Figure 1

Superimposed models of de novo designed peptides showing comparison of their secondary to that of scaffold structure. (A) The Pep1 (red) showed almost similar secondary structure with C-α backbone rmsd 0.58 A˚. However, changed in positions of Pep2 residues R groups with respect to scaffold (blue) was observed, as shown the Phe20 side chain is moved 11.2 A˚ away from their native position in Pep1, which provide a favorable position for binding with Lys458 of RBD. (B) The Pep2 (pink) showed notable different secondary structure composition from scaffold (dark blue) with C-α backbone rmsd 2.12 A˚.

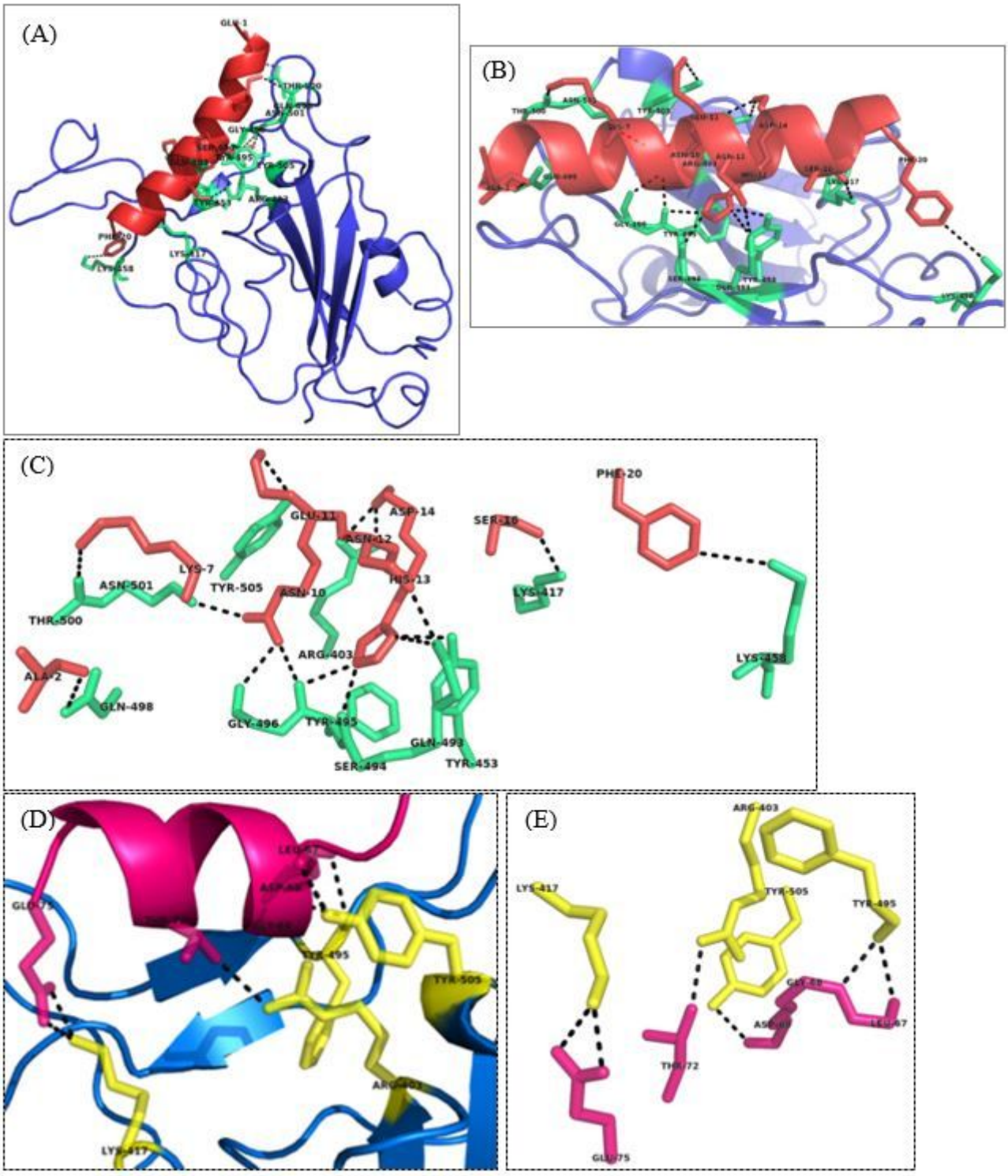

Figure 2

Structural analysis of the designed peptides in complex with RBD. (A) Pep1-RBD complex showing positioning of designed peptide Pep1 (red) in the binding interface of RBD (blue). (B) Pep1 comprises on a single helical structure (red) showing their interactions with the RBD binding residues shown in green. (C) The residues of pep1involve in binding interactions with RBD residues. (D) Pep2-RBD complex showing positioning of Pep2 (pink) at the binding interface of RBD (blue). (E) The residues of Pep2 involve in binding interaction with RBD residues (yellow) are denoted by black lines.

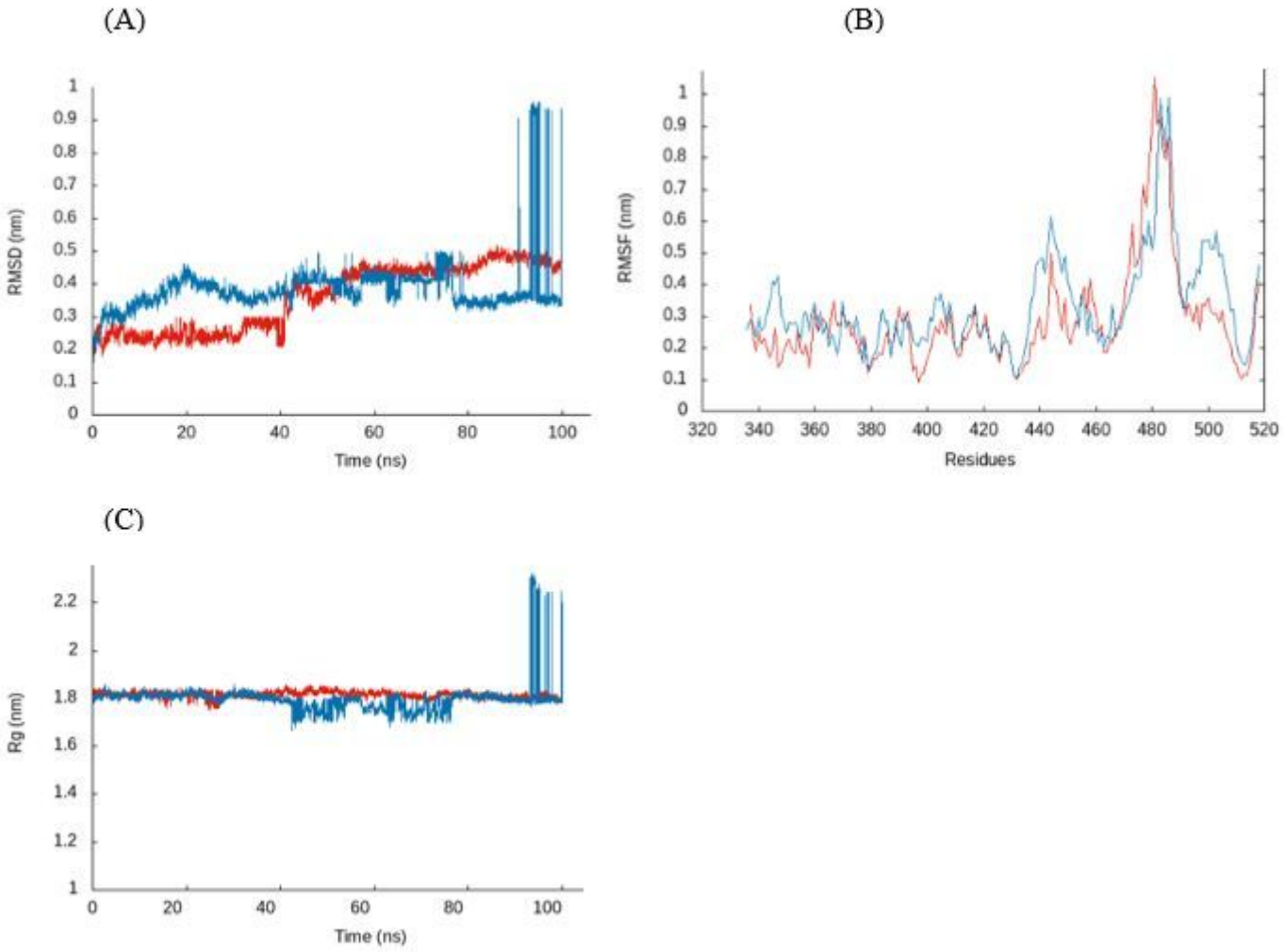

Figure 3

(A) RMSD plot the backbone atoms of Pep1-RBD complex (red) and Pep2-RBD complex (blue). (B) RMSF plot showing fluctuation of residues side chains of RBD in complex with Pep1 (red) and Pep2 (blue). (C) Radius of gyration (Rg) plot of Pep1-RBD (red) and Pep2-RBD complex (blue).

Supplementary Files

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