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

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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 designed 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 PyMOI [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 $\Delta G$ (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 (scaffold1) and 65-76 (scaffold2) 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 Å) 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. 2A-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 have shown that binding residues of RBD are located at two distinct positions, region 1 (490, 493-495, 498, 501, 502) and region 2 (417 and 458) [8,10], which demands two different peptides to be blocking. 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 °C as optimum temperature which is greater than the binding affinity of intact ACE2 (-10.7 kcal mol$^{-1}$) [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 (-11 kcal 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 ≤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 Pep1 and 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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Availability of data and material: N/A

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

References

1. Muralidharan N, Sakthivel R, Velmurugan D, Gromiha MM (2020) Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 Protease against COVID-19. Journal of Biomolecular Structure and Dynamics:1-6

2. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS (2020) Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive care medicine:1-5

3. Ibrahim IM, Abdelmalek DH, Elshahat ME, Elfiky AA (2020) COVID-19 spike-host cell receptor GRP78 binding site prediction. Journal of Infection

4. Kim J-M, Kim HM, Lee EJ, Jo HJ, Yoon Y, Lee N-J, Son J, Lee Y-J, Kim MS, Lee Y-P, Chae S-J, Park KR, Cho S-R, Park S, Kim SJ, Wang E, Woo S, Lim A, Park S-J, Jang J, Chung Y-S, Chin BS, Lee J-S, Lim D, Han M-G, Yoo CK (2020) Detection and Isolation of SARS-CoV-2 in Serum, Urine, and Stool Specimens of COVID-19 Patients from the Republic of Korea. Osong Public Health Res Perspect 11 (3):112-117. doi:10.24171/j.phrp.2020.11.3.02

5. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W (2020) Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA 323 (18):1843-1844. doi:10.1001/jama.2020.3786

6. Lizbeth R-SG, Jazmín G-M, José C-B, Marlet M-A (2020) Immunoinformatics study to search epitopes of spike glycoprotein from SARS-CoV-2 as potential vaccine. Journal of Biomolecular Structure and Dynamics:1-15. doi:10.1080/07391102.2020.1780944

7. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q (2020) Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 367 (6485):1444-1448

8. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L (2020) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581 (7807):215-220

9. Procko E (2020) The sequence of human ACE2 is suboptimal for binding the S spike protein of SARS coronavirus 2. bioRxiv

10. Basit A, Ali T, Rehman SU (2020) Truncated human angiotensin converting enzyme 2; a potential inhibitor of SARS-CoV-2 spike glycoprotein and potent COVID-19 therapeutic agent. Journal of Biomolecular Structure and Dynamics:1-10. doi:10.1080/07391102.2020.1768150

11. Huang X, Pearce R, Zhang Y De novo Design of Protein Peptides to Block Association of the SARS-CoV-2 Spike Protein With Human ACE2. Aging
12. Wan Y, Shang J, Graham R, Baric RS, Li F (2020) Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. Journal of virology 94 (7)

13. Du L, He Y, Zhou Y, Liu S, Zheng B-J, Jiang S (2009) The spike protein of SARS-CoV—a target for vaccine and therapeutic development. Nature Reviews Microbiology 7 (3):226-236

14. Kit O, Kit Y (2020) NATURAL PEPTIDES VERSUS COVID-19: INFORMATION FOR CONSIDERATION. doi:10.13140/rg.2.2.17806.92481

15. Zhang G, Pomplun S, Loftis AR, Loas A, Pentelute BL (2020) The first-in-class peptide binder to the SARS-CoV-2 spike protein. bioRxiv

16. Han DP, Penn-Nicholson A, Cho MW (2006) Identification of critical determinants on ACE2 for SARS-CoV entry and development of a potent entry inhibitor. Virology 350 (1):15-25. doi:https://doi.org/10.1016/j.virol.2006.01.029

17. Chevalier A, Silva D-A, Rocklin GJ, Hicks DR, Vergara R, Murapa P, Bernard SM, Zhang L, Lam K-H, Yao G (2017) Massively parallel de novo protein design for targeted therapeutics. Nature 550 (7674):74-79

18. Pearce R, Huang X, Setiawan D, Zhang Y (2019) EvoDesign: designing protein–protein binding interactions using evolutionary interface profiles in conjunction with an optimized physical energy function. Journal of molecular biology 431 (13):2467-2476

19. Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y (2015) The I-TASSER Suite: protein structure and function prediction. Nature methods 12 (1):7-8

20. Schymkowitz J, Borg J, Stricher F, Nys R, Rousseau F, Serrano L (2005) The FoldX web server: an online force field. Nucleic acids research 33 (suppl_2):W382-W388

21. Pierce BG, Wiehe K, Hwang H, Kim B-H, Vreven T, Weng Z (2014) ZDOCK server: interactive docking prediction of protein–protein complexes and symmetric multimers. Bioinformatics 30 (12):1771-1773

22. Zhang Y, Skolnick J (2005) TM-align: a protein structure alignment algorithm based on the TM-score. Nucleic acids research 33 (7):2302-2309

23. Van Zundert G, Rodrigues J, Trellet M, Schmitz C, Kastritis P, Karaca E, Melquiond A, van Dijk M, De Vries S, Bonvin A (2016) The HADDOCK2. 2 web server: user-friendly integrative modeling of biomolecular complexes. Journal of molecular biology 428 (4):720-725

24. Vangone A, Rodrigues J, Xue L, van Zundert G, Geng C, Kurkuoglu Z, Nellen M, Narasimhan S, Karaca E, van Dijk M (2017) Sense and simplicity in HADDOCK scoring: Lessons from CASP-CAPRI round 1. Proteins: Structure, Function, and Bioinformatics 85 (3):417-423

25. Schrodinger L (2010) The PyMOL molecular graphics system. Version 1 (5):0

26. Xue LC, Rodrigues JP, Kastritis PL, Bonvin AM, Vangone A (2016) PRODIGY: a web server for predicting the binding affinity of protein–protein complexes. Bioinformatics 32 (23):3676-3678
27. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, Lindahl E (2015) GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX 1:19-25

28. Li Y, Wang H, Tang X, Ma D, Du C, Wang Y, Pan H, Zou Q, Zheng J, Xu L (2020) Potential host range of multiple SARS-like coronaviruses and an improved ACE2-Fc variant that is potent against both SARS-CoV-2 and SARS-CoV-1. bioRxiv

29. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L (2020) Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature:1-9

30. Fosgerau K, Hoffmann T (2015) Peptide therapeutics: current status and future directions. Drug discovery today 20 (1):122-128

31. Zhang G, Pomplun S, Loftis AR, Loas A, Pentelute BL (2020) The first-in-class peptide binder to the SARS-CoV-2 spike protein. bioRxiv:2020.2003.2019.999318. doi:10.1101/2020.03.19.999318

32. Johnson RJ, McCoy JG, Bingman CA, Phillips Jr GN, Raines RT (2007) Inhibition of human pancreatic ribonuclease by the human ribonuclease inhibitor protein. Journal of molecular biology 368 (2):434-449

33. Rao SN, Head MS, Kulkarni A, LaLonde JM (2007) Validation studies of the site-directed docking program LibDock. Journal of chemical information and modeling 47 (6):2159-2171

34. Rani N, Vijayakumar S, PTV L, Arunachalam A (2016) Allosteric site-mediated active site inhibition of PBP2a using Quercetin 3-O-rutinoside and its combination. Journal of Biomolecular Structure and Dynamics 34 (8):1778-1796

35. Ardalan N, Mirzaie S, Sepahi AA, Khavari-Nejad RA (2018) Novel mutant of Escherichia coli asparaginase II to reduction of the glutaminase activity in treatment of acute lymphocytic leukemia by molecular dynamics simulations and QM-MM studies. Medical hypotheses 112:7-17

36. Maqsood B, Basit A, Khurshid M, Bashir Q (2020) Characterization of a thermostable, allosteric L-asparaginase from Anoxybacillus flavithermus. International Journal of Biological Macromolecules 152:584-592. doi:https://doi.org/10.1016/j.ijbiomac.2020.02.246

37. Bhowmick S, ALFaris NA, ALTamimi JZ, ALOthman ZA, Aldayel TS, Wabaidur SM, Islam MA (2020) Screening and analysis of bioactive food compounds for modulating the CDK2 protein for cell cycle arrest: Multi-cheminformatics approaches for anticancer therapeutics. Journal of Molecular Structure:128316

38. Chatterjee S, Maity A, Chowdhury S, Islam MA, Muttinini RK, Sen D (2020) In silico analysis and identification of promising hits against 2019 novel coronavirus 3C-like main protease enzyme. Journal of Biomolecular Structure and Dynamics:1-14. doi:10.1080/07391102.2020.1787228

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.