In silico studies on milk derived peptides as potential inhibitors against SARS CoV-2 spike protein receptor binding domain

Sutanu Mukhopadhyay (✉ mukhopadhyaysutanu@gmail.com )
Department of Chemistry, Ramakrishna Mission Vivekananda Centenary College, West Bengal 700118, India

Anasua Sarkar
Computer Science and Engineering Department, Jadavpur University, Calcutta, India
https://orcid.org/0000-0001-7365-3924

Research Article

Keywords: SARS-CoV-2, Protein-Protein Interaction, Protein-Peptide Docking, Human ACE2 receptor, Milk Peptide

DOI: https://doi.org/10.21203/rs.3.rs-618799/v1

License: ☺️ ☑️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

COVID-19 (Corona Virus Infected Diseases-19) is caused by a strain of coronavirus called SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2). There's no permanent diagnosis available till date to combat the disease. The viral infection in humans is initiated by binding of RBD (receptor binding domain) of spike protein to human angiotensin-converting enzyme 2 (hACE2) receptor protein. In this computational study, milk-derived peptides are screened against Receptor Binding Domain (RBD) of spike protein of the virus. Milk is considered as one of the most nutrient-rich liquid foods having several antibacterial and antiviral activities. Milk derived peptides including Casein and Whey derived peptides are known to have profound anti-viral and immunomodulatory activities. After extensive literature search, some peptides having anti-viral activities against different viruses, are shortlisted for this study and their three-dimensional structures are modelled for protein-peptide docking against SARS-CoV-2 spike protein RBD. After performing protein-peptide docking and protein-protein docking using different servers such as HPEPDOCK, FIREDOCK, HADDOCK, HDOCK, it has been observed that in presence of the peptides, the interaction between spike RBD and hACE2 has been reasonably decreased, which implies that milk-derived peptides can be potential peptide-inhibitors against the RBD of the virus along with other medications. Further studies on milk-derived peptides should be performed to develop peptide drugs based on milk-derived peptides.

Introduction

A new strain of coronavirus was first detected in December 2019 at Wuhan, China. This virus is designated as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the disease caused by the virus is known as COVID-19 [1]. As per the latest update on March 17, 2021, a total of 121 M people has been affected by the disease and it causes the demises of 2.67 M people worldwide [2]. There's no permanent medication available to combat the virus till date! Keeping in mind the urgent need of a potential anti-viral against SARS-CoV-2, drug repurposing can be a very useful route to fight against the viral infection [3].

SARS-CoV-2 is a type of beta-coronavirus with positive-sense single-stranded RNA [4]. Like other coronaviruses, SARS-CoV-2 also has four structural and various nonstructural proteins. The structural proteins are: spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid (N) protein. Spike, envelope and membrane proteins form the viral envelope together [5]. The spike (S) protein allows the virus to be attached into the host surface by interacting with human angiotensin-converting enzyme-2 (hACE2) receptors which are expressed in many organs including the lung, small intestine, testis, and kidney [6] [7].

The S protein comprises S1 and S2 domains. The S1 domain is responsible for binding to ACE2 receptors via its receptor-binding domain (RBD), whereas the S2 domain performs the fusion, enabling viral genome entry [8]. The viral infection in humans is initiated by binding of RBD (receptor binding domain) of spike protein to human angiotensin-converting enzyme 2 (hACE2) receptor protein [9], which suggests
that if a therapeutic agent can disrupt the protein-protein interaction between RBD and hACE-2, the viral infection might be terminated or reduced at a very early stage of infection. Small molecules or peptide inhibitors can be designed to disrupt the PPI but small molecules are not suitable for targeting large protein-protein interactions; on the other hand, peptides, due to their large surface area can target the protein binding interface more efficiently than that of small molecules [10]. Recently, several works have been done by researchers around the world in this context. A group has proposed human ACE-2 alpha-helix based peptide inhibitors by computational studies [11]. Another work has been done on natural food preservative peptide nisin to interact with the SARS-CoV-2 spike protein receptor human ACE2 [12].

Some experimental studies suggest that milk proteins can efficiently interfere with viral infections [13][14]. Lactoferrin is one of the most studied milk-protein for its antiviral potential [15]. In this in silico study, some milk-derived peptides having immunomodulatory effects and antiviral activities against other viruses have been selected for experimentation [16]. At first, all those peptides, human ACE2 and human ACE2 alpha-helix were computationally docked against SARS-CoV-2 spike protein RBD, then RBD-peptide complexes were again docked against ACE2 to check whether the binding affinity between the RBD and hACE-2 were decreased in the presence of the peptides or not. Peptides with a reasonable binding affinity with the spike protein RBD have been reported as potential peptide inhibitor against SARS-CoV-2 viral infection. Various physicochemical properties of the peptides such as hydrophobicity, IC50, toxicity, instability index, theoretical pI etc. also have been calculated using open source web-servers and software.

Methodology

A. Peptide sequence retrieval:

Various milk derived peptide sequences have been retrieved from literature, having proven anti-viral activities experimentally against various viruses like, HIV, Herpes virus etc. (Table) [16].

B. Bioinformatic analyses of antiviral potential of the peptides:

Using iAMPPred web-server (http://cabgrid.res.in:8080/amppred/index.html) antiviral potential of the peptides have been estimated. iAMPPred server uses Support Vector Machine (SVM) algorithm to predict the antimicrobial, antiviral and antifungal peptides [17].

C. de novo structure determination and validation:

De novo structure predicting PEP-FOLD3 web server (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/) was used to model selected peptides. It’s based on structural alphabet (SA) letters to describe the conformations of four consecutive residues, couples the predicted series of SA letters to a greedy algorithm and a coarse-grained force field [18]. The structures have been further
validated by Ramachandran plot with the help of Ramachandran plot server by zlab (https://zlab.umassmed.edu/bu/rama/) [19][20].

**D. Molecular docking:**

SARS CoV-2 Spike RBD-human ACE2 complex .pdb structure (6M0J) has been downloaded from RCSB PDB website [21]. Firstly, RBD chain was kept and hACE2 chain, water and ions were removed from the system. Then ACE2 and peptides were docked against RBD using Patchdock server (https://bioinfo3d.cs.tau.ac.il/PatchDock/), followed by refinement by Firedock server (http://bioinfo3d.cs.tau.ac.il/FireDock/) [22][23]. To get a more convenient result, docking was also performed using HPEPDOCK (http://huanglab.phys.hust.edu.cn/hpepdock/) and HADDOCK 2.4 (https://wenmr.science.uu.nl/haddock2.4/) web servers. [24] [25]. ACE2 has been docked against both peptide-bound RBD and peptide-free RBD using HDOCK server to check whether the peptides can reduce the interaction between ACE2 and RBD or not [26]. The .pdb files were renumbered using WHAT IF server (https://swift.cmbi.umcn.nl/whatif/) before using Haddock 2.4 [27]. Binding Energy has been calculated using Prodigy server (https://bianca.science.uu.nl/prodigy/) [28]. Pymol was used for visualizing the three-dimensional interactions of the protein-protein complexes. Two-dimensional PPI plot was derived by using the dimplot functionality of the Ligplot + software [29] [30].

**E. Computation of various physical and chemical parameters:**

Protparam (https://web.expasy.org/protparam/) is a tool for computing various physical and chemical parameters for a given protein or peptide. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY). Amino acid sequence of the peptides has been given as an input to determine the physicochemical properties of the peptides [31]. Half maximal inhibitory concentration (IC 50) has been calculated using the AVP-IC50 Pred web-server (http://crdd.osdd.net/servers/ic50avp/) [32]. Hemolytic or hemotoxic or RBC lysing potential of the peptides have been estimated by HemoPI: Hemolytic Peptide Identification Server (https://webs.iiitd.edu.in/raghava/hemopi/) [33]. Toxicity of the peptides was determined by ToxinPred server (http://crdd.osdd.net/raghava/toxinpred/) [34].

**Results And Discussions**

**A. Peptide sequence retrieval:**

From a book chapter named “Milk Derived Peptides with Immune Stimulating Antiviral Properties” By Haiyan Sun and Håvard Jenssen from a book “Milk Protein” edited by Walter Hurley, University of Illinois (https://www.intechopen.com/books/milk-protein/milk-derived-peptides-with-immune-stimulating-antiviral-properties?fbclid=IwAR0WLVwxgv-vfmmw4mk9RZtEWCd-wJrvFw0DTne3XdxODqggOqganNcvyj8), six (6) milk-derived peptides having antiviral and/ or
immunomodulatory effects have been identified and selected for docking against SARS CoV-2 viral protein [16]. A detailed description of the proteins given below:

| Peptides and their Precursor protein | Peptide sequence | Antiviral activity |
|--------------------------------------|-----------------|--------------------|
| Peptide 01 (Bovine lactoferrin)      | FKCRRWQWRMKKLGAPSITCVRAF | Anti-herpes simplex virus activity, ACE-inhibition, immunomodulation activity [35][36] |
| Peptide 02 (Human lactoferrin)       | GRRRRSVQWCAVSQPEATKCFQWQR NMRKVRGP | Anti-herpes simplex virus activity [36] |
| Peptide 03 (Human lactoferrin)       | GRRRRSVQWCAVSQPEATKCFQWQR NMRKVRGP | Anti-human immunodeficiency virus activity [37][38] |
| Peptide 04 (Human Lactoferrin)       | EDLIWK           | Inhibit herpes simplex virus 1 infection [39] |
| Peptide 05 (Bovine Lactoferrin)      | FKCRRWQWRW       | Immunomodulation activity [40] |
| Peptide 06 (Human Lactoferrin)       | KWNLLRQAQEKFGBKDKS | Immunomodulation activity [41] |

Table I: Peptides, their source proteins and antiviral activities

B. Bioinformatic analyses of antiviral potential of the peptides:

According to iAMPPred web-server, a peptide having >= 0.5 antiviral activity can be referred to as antiviral. Among the six peptides, except the Peptide 06, all have satisfied this criterion of being antiviral. However, Peptide 01 has the highest antiviral potential followed by Peptide 04. The antiviral activities of the peptides as predicted by the server given below:
Peptide Sequence | Anti-viral Activity predicted by iAMpred server
--- | ---
1. FKCRRWQWRMKKLGAPSITCVRRAF | 0.9
2. GRRRRSVQWCAVSQPEATKCFQWQRNRKVRGPPVSCIRDSPIQCI | 0.79
3. GRRRRSVQWCAVSQPEATKCFQWQRNRKVRGP | 0.5
4. EDLIWK | 0.89
5. FKCRRWQWRW | 0.79
6. KWNLLRQAQEFKGDKS | 0.42

Table II: Peptides and their antiviral activity predicted by iAMpred server

C. de novo structure determination and validation:

For each case of peptides, the best model predicted by PEP-FOLD 3.5 has been selected for further investigations. Each peptide model then validated by Ramachandran Plot using PROCHECK web-service. From the plots, it is evident that for all the peptide models, almost all the residues are placed in the allowed region or generously allowed region in Ramachandran plot. All the plots are available in Supporting Information.

D. Protein-Peptide Docking:

For cross validation, protein-peptide docking has been performed using three different servers named HPEPDOCK, FIREDOCK and HADDOCK 2.4, followed by Gibbs free energy estimation using Prodigy server (refer Table III, IV).

HPEPDOCK is a web server for protein-peptide blind docking based on a hierarchical algorithm. According to the HPEPDOCK score, Peptide 01 (-241.390) and Peptide 02 (-241.619) have higher docking score compared to other peptides, while ACE2 (-351.74) has the highest docking score with the RBD.

Patchdock is a molecular docking algorithm based on shape complementarity principles and Firedock performs large-scale flexible refinement and scoring of protein-protein docking. After using Patchdock, followed by Firedock, it has been noted that Peptide 05 shows better docking score than that of ACE2 against spike protein RBD, not only that, Peptide 03 and Peptide 04 both show reasonably good docking score which is comparable with ACE2.

Finally, using HADDOCK 2.4 web-server, protein-protein docking has been performed for all the six peptides and ACE2. The model having lowest HADDOCK score has been selected for further studies for each of the peptides.

Three peptides, Peptide 01 (-103.6 +/- 1.3), Peptide 02 (-102.8 +/- 7.2) and Peptide 03 (-109.2 +/- 5.5) are showing relatively better docking score towards SARS-CoV-2 spike protein receptor binding domain.
Among those six peptides, Peptide 03 shows the best docking score with spike protein RBD, i.e., -109.2 +/- 5.5, though ACE2 has a better docking score with RBD compared to Peptide 03, i.e., -134.1 +/- 3.6. From the $\Delta G$ (kcal mol$^{-1}$) calculation using Prodigy server, it is evident that Peptide 02 (-11.4 kcal/mol) and Peptide 03 (-11.2 kcal/mol) have comparable $\Delta G$ values with ACE2 (-11.9 kcal/mol) (refer Table IV). From the protein-peptide docking studies with RBD, it is clear that among all the six peptides, Peptide 03 has the most promising anti-viral activity against SARS-CoV-2 spike protein Receptor Binding Domain (RBD), followed by Peptide 02 and Peptide 01.

**Table III**

Protein-Peptide Docking Scores from different servers

To check whether in presence of Peptide 03, the interaction between ACE2 and RBD will be affected or not, protein-protein docking has been performed with and without Peptide 03 bound with RBD. In absence of Peptide 03, the protein-protein docking score predicted by HDOCK was -310.19. While protein-protein docking between Peptide 03-bound RBD and human ACE2, the interacting residues of ACE2 and RBD (from the 2D Ligplot + figure of the crystal structure 6M0J) are mentioned as binding site residues during docking, it gives a docking score of -247.94; hence, in presence of Peptide 03, the interaction between ACE2 and RBD has been decreased significantly. Apart from that, in the 2D protein-protein interaction map, Peptide 03-bound RBD-human ACE2 complex and Peptide-free spike protein RBD-human ACE2 complex, there are eleven (11) hydrogen bonds present in both the cases.
### Peptides in Protein-peptide complexes

| Peptides in Protein-peptide complexes | ΔG (kcal mol⁻¹) | K_d (M) at 25.0 °C |
|--------------------------------------|----------------|--------------------|
| 1. Peptide 01                        | -10.6          | 1.6E-08            |
| 2. Peptide 02                        | -11.4          | 4.6E-09            |
| 3. Peptide 03                        | -11.2          | 5.8E-09            |
| 4. Peptide 04                        | -7.8           | 1.8E-06            |
| 5. Peptide 05                        | -8.4           | 6.5E-07            |
| 6. Peptide 06                        | -8.9           | 2.8E-07            |
| 7. ACE2                              | -11.9          | 1.9E-09            |

**Table IV: Binding Energy and K_d calculation using Prodigy server**

In the case of peptide 03, there are eleven hydrogen bonds between Arg5-Glu749 (2), Ser6-Glu749, Ser6-Cys753, Cys10-Tyr770, Val12-Asn766, Ser13-Asn766, Lys19-Gly711, Lys19-Tyr714, Arg31-Asn752 (2) (Fig.4). While in case of peptide 02, there are only nine hydrogen bonds present, Arg5-Asn752, Arg5-Tyr754, Val12-Tyr770, Gln14-Thr765, Lys19-Tyr718, Lys19-Ser759, Trp23-Glu749, Arg40-Tyr770, Ser42-Arg668 (Fig.3) and in the case of peptide 01, there are total eight hydrogen bonds present, Lys2-Glu671, Lys2-Tyr760, Trp6-Ser759, Met10-Tyr714, Gly14-Gln763, Cys20-Glu749, Cys20-Gln758, Arg23-Glu749 (Fig.2). On the other hand, considering SARS-CoV-2 spike protein RBD-human ACE2 interactions, there are total eleven hydrogen bonds are found, Ser1-Ala740, Gly6-Asn752, Asp12-Lys682, Lys13-Gln758, Gln17-Gln758, Asp20-Tyr714, Gln24-Gly711, Gln24-Tyr714, Tyr65-Asn752, Lys335-Gly761, Lys335-Gln763. Among them, three residues Gly711, Tyr714 and Asn752 of RBD also interact with the peptide 03, that's the probable reason for decreasing the binding affinity of ACE2 towards RBD in presence of peptide 03.

### C. Computation of Physicochemical Properties:

All the physicochemical properties of the six peptides have been summed up in **Table V**. Among the six peptides, Peptide 02 and Peptide 03 have greater half-life (estimated) than the others. A protein having instability index < 40 can be treated as stable, whereas if instability index ≥ 40, the corresponding protein will be unstable, from that point of view, none of the peptides are stable. As the peptides are unstable, the stability should be enhanced using various biochemical techniques such as cyclization of the peptide, replacement of natural peptide bonds by pseudo-peptide bonds, selective chemical modification etc. before experimental validation against spike protein [42]. Otherwise, peptidomimetic drugs can be produced based on the functionality of the peptides, which are basically small organic molecules mimicking the characteristics of the peptides, but a way more stable than the peptides [43]. In brief, some processing techniques, such as chemical modification or incorporation of synthetic amino acids, can be applied to increase peptide stability and, consequently, lower susceptibility to hydrolysis by proteases.
The aliphatic index of a protein is the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). The GRAVY value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number. The heat stability of peptide was indicated by its aliphatic index. The higher aliphatic index means higher heat stability. The hydrophilicity and hydrophobicity of peptide were predicted by GRAVY. The peptide was hydrophobic when the GRAVY value was plus; otherwise, it was hydrophilic. Interestingly, in the case of Peptide 05, aliphatic index is zero (0), signifies the absence of aliphatic side chains (Ala, Val, Ile, Leu).
| Peptide | MW     | Theo. pl | Estimated half-life                                                                 | Instability index | Aliphatic index | GRAVY |
|---------|--------|----------|-------------------------------------------------------------------------------------|-------------------|-----------------|-------|
| 1.      | 3125.82| 11.84    | 1.1 hours (mammalian reticulocytes, in vitro). 3 min (yeast, in vivo). 2 min (Escherichia coli, in vivo). | 77.92             | 50.80           | -0.576 |
| 2.      | 5544.50| 11.24    | 30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo). | 97.10             | 53.83           | -0.851 |
| 3.      | 4003.65| 11.88    | 30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo). | 88.33             | 32.42           | -1.288 |
| 4.      | 802.93 | 4.37     | 1 hour (mammalian reticulocytes, in vitro). 30 min (yeast, in vivo). >10 hours (Escherichia coli, in vivo). | 40.43             | 130.00          | -0.583 |
| 5.      | 1551.84| 11.71    | 1.1 hours (mammalian reticulocytes, in vitro). 3 min (yeast, in vivo). 2 min (Escherichia coli, in vivo). | 165.81            | 0.00            | -1.830 |
| 6.      | 2076.39| 10.00    | 1.3 hours (mammalian reticulocytes, in vitro). 3 min (yeast, in vivo). 3 min (Escherichia coli, in vivo). | 40.67             | 51.76           | -1.618 |

**Table V:** Physicochemical properties of the peptides

According to the ToxinPred web server, none of the peptides are toxic. For toxicity prediction, SVM (Swiss-Prot) + Motif based method was used and other options were set as default. HemoPI server was used to identify the hemolytic potency of the peptides and hybrid method (SVM + Motif based) was used for
prediction. Hemolytic potency of a peptide is decided by PROB score, which is the normalized SVM score and ranges between 0 and 1, where 1 means the peptide is very likely to be hemolytic and 0 means it is likely to be non-hemolytic. For the aforementioned peptides, the PROB score ranges from 0.46 (peptide 06) to 1.00 (Peptide 01 and 05), which signifies that there is a high probability for them of being hemolytic.

| Peptide   | SVM  | RF   | IBk  |
|-----------|------|------|------|
| Peptide 01 | 22.21| 39.55| 9.71 |
| Peptide 02 | 24.53| 17.85| 9.71 |
| Peptide 03 | 26.99| 3.17 | 9.71 |
| Peptide 04 | 36.67| 60.11| 11.41|
| Peptide 05 | 23.8 | 9.06 | 0.9  |
| Peptide 06 | 30.5 | 36.52| 17.01|

**Table VI**: IC 50 values predicted by different ML algorithms in micromolar unit

According to the FDA, IC50 represents the concentration of a drug that is required for 50% inhibition in vitro experiments. Using AVP-IC$_{50}$Pred server and choosing hybrid model I as prediction model, IC50 values of the peptides have been predicted by three different machine learning algorithms named, Support Vector Machine, Random Forest, IBk (Weka); details of the values are given in **Table VI** (values are in micromolar unit):

**Conclusions**

Peptide therapeutics have various advantages like low toxicity, ease of synthesis, high target specificity etc. In this present computational study, according to the docking score and free energy calculations, Peptide 03 shows comparatively better binding affinity with the spike protein RBD. Presence of Peptide 03 also reduces the interaction between human ACE2 and spike protein RBD. Being a low molecular weight peptide and readily bioavailable, its binding to RBD is expected to interfere the bonding interaction between RBD of spike of SARS-CoV-2 and human ACE2 and could reduce the infection. Molecular dynamics simulation is planned to be conducted to further verify the interactions of these protein-peptide complexes. Further in vitro and in vivo validation should be done to determine its doses and efficacies against SARS-CoV-2 spike proteins.

**Declarations**

**Conflict of Interest:**
Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

**Author contribution:**

S.M.: conceptualization, methodology, investigation, writing—original draft preparation, A.S.: supervision, writing—reviewing and editing.

**References**

1. Lu H, Stratton CW, Tang Y-W. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. J Med Virol. 2020;92(4):401–402.

2. Wikipedia contributors. Template: COVID-19 pandemic data. Wikipedia, The Free Encyclopedia. Accessed March 17, 2021. http://en.wikipedia.org/w/index.php?title=Template:COVID19_pandemic_data&oldid=1024821984

3. Gordon DE, Jang GM, Bouhaddou M, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature. 2020;583(7816):459–468.

4. Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J Med Virol. 2020;92(4):418–423.

5. Yao H, Song Y, Chen Y, et al. Molecular architecture of the SARS-CoV-2 virus. Cell. 2020;183(3):730–738.e13.

6. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270–273.

7. Wang Q, Zhang Y, Wu L, et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell. 2020;181(4):894–904.e9.

8. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020;181(2):281–292.e6.

9. Tai W, He L, Zhang X, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol. 2020;17(6):613–620.

10. Cierny M, Kurcinski M, Kamel K, et al. Protein–peptide docking: opportunities and challenges. Drug Discov Today. Published online 2018. doi:10.1016/j.drudis.2018.05.006

11. Han Y, Král P. Computational design of ACE2-based peptide inhibitors of SARS-CoV-2. ACS Nano. 2020;14(4):5143–5147.

12. Bhattacharya R, Gupta AM, Mitra S, Mandal S, Biswas SR. A natural food preservative peptide nisin can interact with the SARS-CoV-2 spike protein receptor human ACE2. Virology. 2021;552:107–111.

13. Pan Y, Lee A, Wan J, et al. Antiviral properties of milk proteins and peptides. Int Dairy J. 2006;16(11):1252–1261.
14. Swart PJ, Kuipers ME, Smit C, et al. Antiviral effects of milk proteins: acylation results in polyanionic compounds with potent activity against human immunodeficiency virus types 1 and 2 in vitro. AIDS Res Hum Retroviruses. 1996;12(9):769–775.

15. van der Strate BW, Beljaars L, Molema G, Harmsen MC, Meijer DK. Antiviral activities of lactoferrin. Antiviral Res. 2001;52(3):225–239.

16. Sun H, Jensse H. Milk derived peptides with immune stimulating antiviral properties. In: Milk Protein. InTech; 2012.

17. Meher PK, Sahu TK, Saini V, Rao AR. Predicting antimicrobial peptides with improved accuracy by incorporating the compositional, physico-chemical and structural features into Chou’s general PseAAC. Sci Rep. 2017;7:42362.

18. Lamiable A, Thévenet P, Rey J, Vavrusa M, Derreumaux P, Tufféry P. PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. Nucleic Acids Res. 2016;44(W1):W449-54.

19. Ramachandran GN, Ramakrishnan C, Sasisekharan V. Stereochemistry of polypeptide chain configurations. J Mol Biol. 1963;7:95–99.

20. Anderson RJ, Weng Z, Campbell RK, Jiang X. Main-chain conformational tendencies of amino acids. Proteins. 2005;60(4):679–689.

21. Lan J, Ge J, Yu J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020;581(7807):215–220.

22. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res. 2005;33(Web Server issue):W363-7.

23. Andrusier N, Nussinov R, Wolfson HJ. FireDock: fast interaction refinement in molecular docking. Proteins. 2007;69(1):139–159.

24. Zhou P, Jin B, Li H, Huang S-Y. HPEPDOCK: a web server for blind peptide-protein docking based on a hierarchical algorithm. Nucleic Acids Res. 2018;46(W1):W443-W450.

25. van Zundert GCP, Rodrigues JPGLM, Trellet M, et al. The HADDOCK2.2 Web server: User-friendly integrative modeling of biomolecular complexes. J Mol Biol. 2016;428(4):720–725.

26. Yan Y, Tao H, He J, Huang S-Y. The HDOCK server for integrated protein-protein docking. Nat Protoc. 2020;15(5):1829–1852.

27. Umcn.nl. Accessed May 24, 2021. http://swift.cmbi.umcn.nl/servers/html/renumb.html

28. Xue LC, Rodrigues JP, Kastritis PL, Bonvin AM, Vangone A. PRODIGY: a web server for predicting the binding affinity of protein-protein complexes. Bioinformatics. 2016;32(23):3676–3678.

29. The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.

30. Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model. 2011;51(10):2778–2786.

31. Gasteiger E, Hoogland C, Gattiker A, et al. Protein identification and analysis tools on the ExPASy server. In: The Proteomics Protocols Handbook. Humana Press; 2005:571–607.
32. Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K, Tomita M. Identification of the bactericidal domain of lactoferrin. Biochim biophys acta. 1992;1121(1–2):130–136.

33. Jenssen H, Andersen JH, Uhlin-Hansen L, Guteberg TJ, Rekdal Ø. Anti-HSV activity of lactoferricin analogues is only partly related to their affinity for heparan sulfate. Antiviral Res. 2004;61(2):101–109.

34. Saidi H, Eslahpazir J, Carbonneil C, et al. Differential modulation of human lactoferrin activity against both R5 and X4-HIV-1 adsorption on epithelial cells and dendritic cells by natural antibodies. J Immunol. 2006;177(8):5540–5549.

35. Carthagena L, Becquart P, Hocini H, Kazatchkine MD, Bouhlal H, Belec L. Modulation of HIV binding to epithelial cells and HIV transfer from immature dendritic cells to CD4 T lymphocytes by human lactoferrin and its major exposed LF-33 peptide. Open Virol J. 2011;5(1):27–34.

36. Giansanti F, Massucci MT, Giardi MF, et al. Antiviral activity of ovotransferrin derived peptides. Biochem Biophys Res Commun. 2005;331(1):69–73.

37. Ueta E, Tanida T, Osaki T. A novel bovine lactoferrin peptide, FKCRRWQWRM, suppresses Candida cell growth and activates neutrophils: FKCRRWQWRM effect on Candida and neutrophils. J Pept Res. 2001;57(3):240–249.

38. Tang Z, Yin Y, Zhang Y, et al. Effects of dietary supplementation with an expressed fusion peptide bovine lactoferricin-lactoferrampin on performance, immune function and intestinal mucosal morphology in piglets weaned at age 21 d. Br J Nutr. 2009;101(7):998–1005.

39. Siciliano R, Rega B, Marchetti M, Seganti L, Antonini G, Valenti P. Bovine lactoferrin peptidic fragments involved in inhibition of herpes simplex virus type 1 infection. Biochem Biophys Res Commun. 1999;264(1):19–23. doi:10.1006/bbrc.1999.1318

40. Ueta E, Tanida T, Osaki T. A novel bovine lactoferrin peptide, FKCRRWQWRM, suppresses Candida cell growth and activates neutrophils. J Pept Res. 2001;57(3):240–249. doi:10.1111/j.1399-3011.2001.00821.x

41. Tang, Z., Yin, Y., Zhang, Y., Huang, R., Sun, Z., Li, T., ... Tu, Q. (2009). Effects of dietary supplementation with an expressed fusion peptide bovine lactoferricin-lactoferrampin on performance.

42. Peptide Stability. (n.d.). Retrieved June 6, 2021, from Sciencedirect.com website: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/peptide-stability

43. Vagner, J., Qu, H., & Hruby, V. J. (2008). Peptidomimetics, a synthetic tool of drug discovery. Current Opinion in Chemical Biology, 12(3), 292–296.

Figures
Figure 1

3D Representation and 2D Interactions between human ACE2 (A) and SARS-CoV-2 Spike protein RBD (B)

Figure 2

Protein-peptide interactions for spike protein RBD and peptide 01
**Figure 3**

Protein-peptide interactions for spike protein RBD and peptide 02

---

**Figure 4**

Protein-peptide interactions for spike protein RBD and peptide 03

---

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SupportingInformation1.pdf