# A natural food preservative peptide Nisin can interact with the 2019-nCoV spike protein receptor ACE2

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**Short Report**

**Keywords:** Nisin, 2019-nCoV, ACE2 receptor, Molecular interaction

**Posted Date:** July 24th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-48319/v1

# Abstract

**Objective:** Food grade antibacterial peptides like nisin remain a primary focus of research for decades for their multi-faceted therapeutic and industrial applications. Nisin, a food grade peptide synthesized by lactic acid bacteria has been examined for its probable interaction with the human ACE2 (hACE2) receptor, the site where spike protein of 2019-nCoV binds.

**Methods:** To address the issue, molecular interaction of eight variants of nisin peptides with hACE2 was investigated by *in-silico* homology modeling and docking.

**Results:** Docking analyses revealed that among all the nisin variants, nisin Z and nisin H showed a significant binding affinity towards hACE2. Furthermore, the affinity is even higher than that of the RBD (receptor binding domain) of the 2019-nCoV spike protein.

**Conclusion:** The study unravels for the first time that nisin, an antibacterial peptide used globally as a safe natural food preservative, has the potential to bind to hACE2, the target site for the new corona virus 2019-nCoV. As nisin being used globally as safer preservative in the food industry, it could be a potential alternative to control the ongoing pandemic COVID-19 by the impairment of interaction of spike protein along with the hACE2 receptor. However, further experimental validation is necessary to determine its doses and mechanistic application to check the competition of nisin and spike protein of 2019-nCoV for accessing the human.


# Introduction

The ongoing outbreak of COVID-19, a severe life-threatening infectious respiratory disease caused by a recently discovered novel coronavirus 2019-nCoV has drastically affected human life with over five millions of cases of infection globally. Till now, there is no specific antiviral medication available for COVID-19, but extensive efforts are underway worldwide. Although vaccines are thought to be the most powerful weapon to fight against virus invasion, it may take quite a long for successful applications in humans. Considering the acute crisis of COVID-19 pandemic, there is an urgent need for developing effective antiviral therapeutics for the prevention and treatment of COVID-19. It is well accepted that the spike protein on the outer surface of 2019-nCoV is a crucial recognition factor for its attachment and entry to the host cells[1]. The viral infection in humans is initiated by binding of RBD (receptor binding domain) of spike protein to the host cell receptor hACE2 (angiotensin-converting enzyme 2).

Therefore, any natural therapeutic agent that blocks hACE2 might prevent the spike protein of 2019-nCoV to bind with and thereby could reduce the incidence of infection. Although small molecules are commonly preferred as therapeutics, they are not effective in blocking protein- protein interactions (PPI) where a deep binding pocket may be missing at the interface[2]. On the contrary, peptides are more suitable for disrupting PPIs by specifically interacting with the interfaces. More importantly, small peptides have reduced immunogenicity. Hence, peptides are ideal candidates for novel therapeutics. The currently used peptides are all synthetic and costly, and have not produced promising results when applied to COVID-19

disease. This study attempts to investigate the ability of food-grade nisin A and its natural variants to block the interaction between hACE2 with spike protein of 2019-nCoV, a key step of COVID-19 disease. Nisin, a pentacylic antibacterial peptide of 34 residues, is produced by certain strains of food- grade *Lactococcus lactis*, a species widely used for cheese manufacture[3–5]. Nisin belongs to a group of cationic peptide antimicrobials collectively called Type A (I) lantibiotics[6]. It was first identified in fermented milk cultures and is now globally used as a natural and safe food preservative in a variety of food products around the world, such as processed cheese, dairy desserts, milk, fermented beverages, meat and canned foods[7–9]. It was approved by the European Union (E234), World Health Organization (WHO) as well as by the US Food and Drug Administration (FDA). Currently, nisin is licensed in over 50 countries[10]. Because of the high safety profile over the past 40 years of usage and its strong antibacterial action against a wide range of food spoilage and pathogenic bacteria, nisin has been extensively studied. It also has multiple applications in biomedicine including bacterial infections, cancer, oral diseases and other veterinary and research filed[10].

Since the discovery of nisin A, eight natural variants of nisin have been discovered which include nisin A, Z, F, Q, H, U, U2 and P[11]. Nisin Z producing organisms are very common in nature[9,12]. We computationally modeled eight variants of nisin for further analysis. All nisin peptides were aligned to show their identity and modeled on SWISS-Model web server. hACE2 and RBD domain of 2019-CoV2 were also modeled on the same platform to increase the acceptability of the experiments. All the peptides and RBD were docked with hACE2 using HADDOCK server. A higher binding affinity of the peptides was examined by docking analysis based on Z-score, binding affinity and buried surface area.

Nisin is a unique molecule containing unusual amino acids including dehydroalanine, dehydrobutyrine, formed by dehydration of serine and threonine residues, respectively. lanthionine and β–methyl lanthionine that is introduced enzymatically at posttranslational level[13.] The thioether bridges of the lanthionines form five thioether rings in the molecule. The crystal structure of nisin has not been developed. The peptide molecule adopts different conformations depending on the environment. The structure of nisin cannot be described in terms of regular secondary-structure elements, due to the presence of the ring systems in which 65 % of the residues are incorporated. However, the NMR structure is available in PDB database, which was used in this study as template to generate the model structures of the nisin variants. The NMR structure of nisin has determined two structured domains: an N-terminal domain (residues 3-19) containing three lanthionine rings, A, B and C; and a C-terminal domain (residues 22-28) containing two intertwined lanthionine rings numbered D and E[14] . These domains are flanked by regions showing structural flexibility. The four-residue rings B, D and E of nisin all show a β- turn structure, which is closed by the thioether linkage. The backbones of the rings B and D form type I1 β-turns. The C-terminal domain consists of three consecutive β-turns. The NMR data will help us to locate residues in nisin interacting with hACE2. The present study evaluates the potential of nisin variants to interact with hACE2 by predicting nisin binding site using nisin- hACE2 docking computation with the NMR structure of nisin in the PDB database. This is the first report on the potential of widely used food-grade antibacterial

peptides nisin to bind with hACE2 and predicting the possibility of nisin as therapeutic against COVID-19.The work is significant in finding a solution to prevent the infection by novel coronavirus 2019-nCoV.

# Materials And Method

### Data mining and alignment

Amino acids sequences of eight nisin variants: nisin Z (accession No: ABV64387.1), nisin A (accession No: AAA26948.1), nisin F (accession No: ABU45463.1), nisin Q (accession No: ADB43136.1), nisin H (accession No: AKB95119.1), nisin U (accession No: Q2QBT0.1), nisin U2 (accession No: ABO32538.1), nisin P (accession No: WP_105156946.1) were retrieved from Genbank database (https://www.ncbi.nlm.nih.gov/protein/). Full length amino acid sequence of ACE-2 of Homo sapience (accession No: NP_001358344.1) and Spike protein of 2019-nCoV (accession No: YP_009724390.1) were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/protein/). Multiple Sequence alignment (MSA) of the nisin variants was performed using the ClustalW of Clustal Omega web server of the Euoropean Bioinformatics Institute (EMBL-EBI) [15]. Esprit 3 software[16] was used to represent the MSA using BLOSUM 62 algorithm.

### Homology modeling

Homology models of all nisin variants were done using the SWISS-MODEL web server[17] and nisin Z (SMTL ID:1wco.1) was used as template. The steriochemical property of each of the models was evaluated by Ramachandran plot using Volume, Area, Dihedral Angle Reporter (VADAR) server[18]. Similarly, the RBD (receptor binding domain) of spike protein of 2019- nCoV and hACE2 receptor was modeled using SMTL ID: 6lzg.1 and SMTL ID: 6m18.1, respectively. All the models of nisin variants were superimposed together to determine the 3-D structural differences at domain label using read scoring matrix in PyMOLsoftware[19].

### Molecular Docking

Molecular Docking was performed to test the binding affinity of all nisin variants towards hACE2. The solvated docking software, HADDOCK[20] was used for this study. The easy interface was utilized since no restraints were defined. Critical (active) residues of ACE2 (K31, E35, D38, M82) were selected for docking. The residues surrounding the active loci were selected as passive in HADDOCK. Active residues are the amino acid residues of the two given interacting protein binding sites that take part in direct interaction, while passive residues are the residues that can interact indirectly. Prodigy@Bonvin lab web server[21] was used to calculate $\Delta G$ to predict the affinity of nisin for hACE2 at 25°C with other parameters remained under default condition. Grand Average of Hydropathy score of hACE2 was calculated on Exappsy Protparam web server[22].

# Results And Discussion

## Sequence and structural alignment

In multiple sequence alignment (Fig.1) of amino acid residues of eight nisin variants (nisin A, Z, Q, H, P, U, U2 and F), Nisin A was found to be closely related to nisin Z, with only a single amino acid difference, His27Asn, showing 97.06% identity. In contrast, nisin P, U, U2, H, Q and F shared only 67.74%, 67.74%, 67.74%, 82.35%, 91.18% and 97.06% amino acid sequence identity, respectively with nisin Z (Supplementary File-1.percentage Matrix). Nisin P is shorter than nisin A (34 residues) by three residues from the C-terminus. Nisin F differs from nisin A by 2 residues: His27Asn and Ile30Val. Nisin Q is different from nisin A due to the presence of valine, leucine, asparagine and valine at positio ns 15, 21, 27 and 30, respectively. Nisin U and U2 differed from nisin A by nine and ten amino acids, respectively. Nisin H by five different amino acids at positions 1, 6, 18, 21 and 31. The residual surface accessibility is present at the bottom of the alignment (Fig.1). The residues that are surface accessible are in blue, while buried residues are in white at the bottom of the MSA. Identical residues are highlighted in red.

## Homology modeling

The model structures of all nisin variants, human ACE2, RBD of spike protein built on using SWISS-MODEL Web Server were validated for steriochemical properties using Ramachondron plot (Supplementary File-2.Ramachondron Plot). We considered the number of amino acids in the disallowed regions except for glycine and proline because of their chirality and imino group, respectively. Homology model of nisin P and U2 had no disallowed amino acids. Nisin H and U had only one residue in disallowed region, whereas two residues were found in the disallowed region for nisin A, F, Q and Z. So all these structures were considered structurally workable for further docking experiments. When all models were superimposed, C-alpha RMSD value was 0.191 as determined using PyMOL softwere. The result indicates that all the nisin models were structurally similar. These models were used to study protein-peptide interaction to determine the binding efficiency of nisin to hACE2 compared to the binding efficiency of RBD of spike protein to hACE2.

## Molecular docking

Most reliable model was selected by lowest HADDOCK score value. The score is calculated as HADDOCKscore =1.0 * Evdw + 0.2 * Eelec + 1.0 * Edesol +0.1 *Eair,

Where Evdw is the intermolecular van der waals energy, Eelec the intermolecular electrostatic energy, Edesol represents an empirical desolvationenergy. Best HADDOCK model of nisin variants in complex with hACE2 was analyzed for three parameters *viz*. Z-score, Buried surface area, and binding affinity. The Z-score indicates how many standard deviations from the average of the cluster is located in terms of score (the more negative the better). Z-score of hACE2-2019- nCoVRBD, hACE2-nisinA, hACE2-nisinZ, hACE2-nisinH, hACE2-nisinQ, hACE2-nisinU, hACE2-nisinU2, hACE2-nisinF, and hACE2-nisinP was predicted as -1.5,-1.6,-1.9,-2.1,-1.4,- 1.7,-0.8,-1.4, and -1.5. Hence, both nisin H and nisin Z were more negative than rest of the nisin variants as well as RBD of spike protein. Burried surface area of nisin Z

and nisin H was calculated as 2332.4 and 2395.1, respectively in contrast to 2092 for the RBD. This suggests that nisin H and nisin Z had better binding efficiency.

The binding affinity of docked structures of all eight variants of nisin in complex with hACE2 was calculated as ΔG derived from analysis with Prodigy for each complex in comparison with the RBD of spike protein of 2019-nCoV. The result revealed ΔG of hACE2-2019-nCoV, hACE2-nisinA, hACE2-nisinZ, hACE2-nisinH, hACE2-nisinQ, hACE2-nisinU, hACEII- nisinU2, hACE2-nisinF, and hACE2-nisinP was -11Kcal/mol, -10.6Kcal/mol, -10.8 Kcal/mol,-113 Kcal/mol, -10.5 Kcal/mol, -10.5Kcal/mol, -12.3Kcal/mol, -12.5 Kcal/mol, and -11.4 Kcal/mol, respectively. The data suggest that nisin Z and nisin H can bind to hACE2 strongly as that of RBD.

GRAVY score of nisin A, Z, H, Q, U, U2, F, P and RBD-2019-nCoV was calculated as 0.415, 0.406, 0.185, 0.524, 0.542, 0.439, 0.171, 0.185, -0.258, respectively (Table1). From the GRAVY score of all nisin variants, Nisin H turned out to be more hydrophilic than nisin A and nisin Z and will thus interact to the hydrophobic groove of hACE2 more efficiently than others variants of nisin.

Based on docking scores, it is evident that nisin Z and nisin H interacts to hACE2 very efficiently. We further analyzed the doc structures on discovery studio to explain the interaction at residue level (supplementary table 1). We have found hydrogen bond (of K31:Y453, K31:E93, E35:G496, E35:Q498, D38:T500, M82:Y489) and hydrophobic bonds (of M82:F456, M82:Y489) as major interacting force for nisinZ-ACE2 interaction. It was found that nisin Z and nisin H recognized four common residues (K31, E35, D38, M82) in hACE2 that were also recognized by RBD of spike. The residues in nisin H interacting with the hACE2 include hydrogen bond of K12:E35, K22:D38, N20:E35, C26:D38, H27:D38, T13:K31, C19:K31, K12:K31, T8:k31, P9:K31 (Fig 2) and hydrophobic bond of C7:M82, C19:K31 and Y21:K31. Among all these interacting residues, T8, P9, C11, K12, T13, C19, K22, C26 were highly conserved among all the nisin variants. Like RBD, surface accessible hydrophilic residues, T8, P9, C11, K12, T13, K22, C26 were found to be involved in binding to hydrophobic groove of hACE2.

Interacting residues of nisin Z were formed hydrogen bond of K12:E35, K22:D38, C7:M82, C19:E35, N20:E35, C27:D38, C19:K31, K12:K31, T13:K31 hydrophobic bond of I4:M82, C7:M82, P9:K31, C19:K31 with hACE2. Interacting residues of nisin Z was predicted as I4, C7, K12, T13, C19, N20, K22, C27. All interacting residues of nisin Z were hydrophilic in nature. Based on this study, we hypothesize that nisin H and Z could be the potential hACE2 blocker to compete RBD of 2019-nCoV for the same site. However, further experimental validation is required to confirm nisin binding to hACE2.

# Conclusion

Among all analyzed nisin variants, nisin Z and nisin H were most effective in interacting with human endothelial cell surface-receptor hACE2, the site where RBD of spike of 2019-nCoV binds to initiate infection. Compared to the RBD of viral spike protein, nisin binding to the hACE2 receptor was much prominent. Nisin being a low molecular weight peptide, its binding to hACE2 with high bioavailability is expected to exclude the virus from binding to the same site. Since nisin is a heat stable natural food

grade peptide, can be produced cost effectively, even in large quantity through microbial fermentation, the present work will create greater interest among researchers to develop a new nisin-based treatment strategy for COVID-19, either through oral or nasal application.

# Declarations

### Conflict of interest:

All authors declare no conflict of interest.


### Acknowledgement:

We most sincerely acknowledge the research grant (BT/PR16246/GET/119/73/2016) received from DBT ( Department of Biotechnology) Govt. of India, New Delhi.


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# Tables

**Table1:** Comparative affinity of interaction between nisin-variants and human-ACE2.

| Name of the sample to interact with ACEII | Binding Affinity(ΔG Kcal/mol) | GRAVY | Z score | Burried surface area |
|---|---|---|---|---|
| RBD 2019-nCoV | -11.0 | -0.258 | -1.5 | 2092.0 |
| Nisin H | -11.3 | 0.185 | -2.1 | 2395.1 |
| Nisin Z | -10.8 | 0.406 | -1.9 | 2332.4 |
| Nisin A | -10.6 | 0.415 | -1.6 | 2311.8 |
| Nisin U | -12.3 | 0.542 | -1.7 | 2347.5 |
| Nisin U2 | -12.5 | 0.439 | -0.8 | 2192.8 |
| Nisin F | -11.4 | 0.171 | -1.4 | 2377.8 |
| Nisin Q | -10.5 | 0.524 | -1.4 | 2297.7 |
| Nisin P | -12.6 | 0.185 | -1.5 | 2190.3 |

# Figures

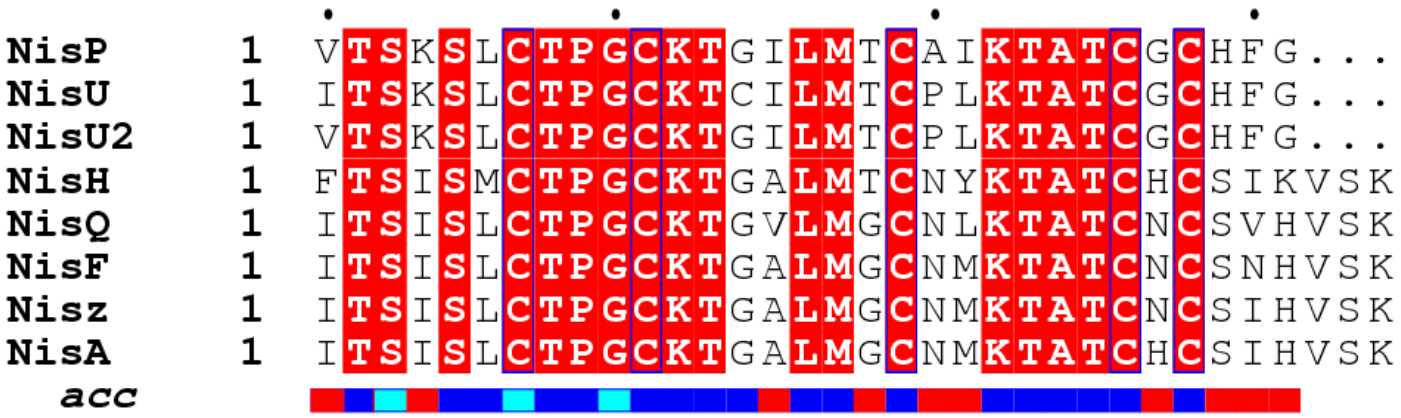


**Figure 1**

Multiple sequence alignment of all nisin variants (nisin P, nisin U, nisin U2, nisin H, nisin Q, nisin F, nisin Z, nisin A) is made using Clustal Omega web server and is displayed by ESpript 3 software. The red highligheted residues are conserved among the eight nisin variants. Surface accessibility is shown at the bottom ( blue surface accessible, white buried residues).

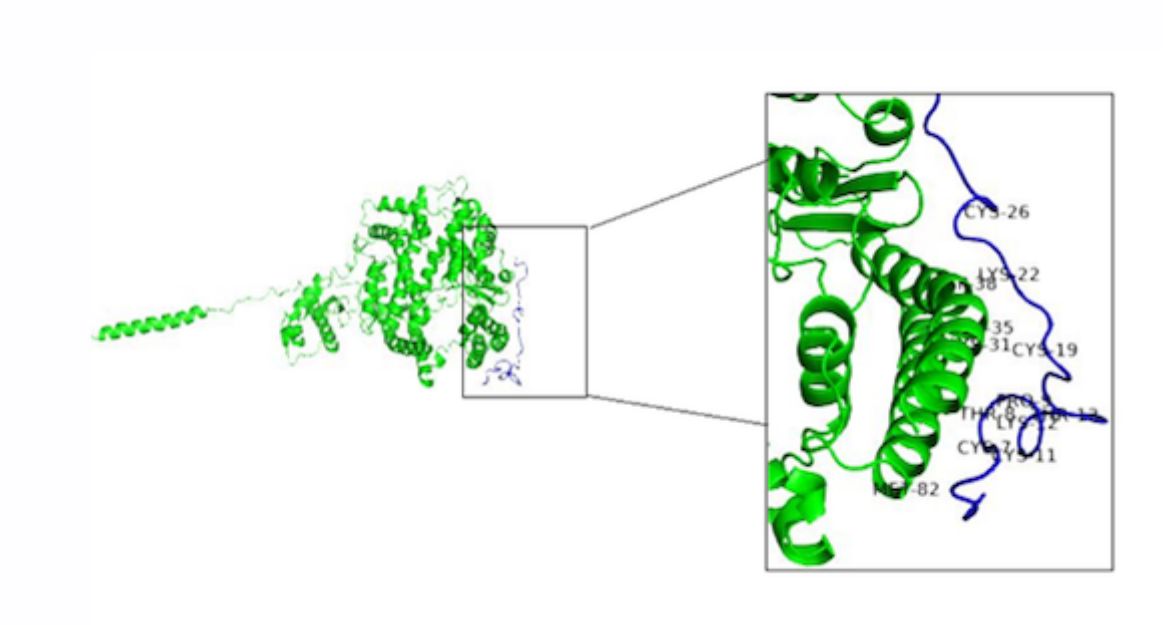


**Figure 2**

Docked structure of humanACE2 and NisH ; Binding interface with interacting residues is indicated in the box region. NisH and humanACE2 are highlighted with blue and green color respectively.

## Supplementary Files

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

- SupplementaryFile1.percentageMatrix.pdf

- supplimentary2.pdf
- Supplementarytable1.pdf