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# *In silico* Tools and Techniques for Screening and Development of Peptide-Based Spike Protein Inhibitors against Novel Coronavirus (Sars-CoV-2)

Raghunath Satpathy*

*School of Biotechnology, Gangadhar Meher University, Amruta Vihar, Sambalpur Odisha, 768004, India*

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**Abstract**

The recent pandemic situation created by the novel coronavirus (SARS-CoV-2) across the globe is a great concern. So, the discovery of novel antiviral agents is desirable to address this challenge. In this context, the antiviral peptides (AVPs) possess an enormous potential and can be considered to develop novel therapeutic strategies to combat SARS-CoV-2 infection. The anti-viral peptides are mostly preferable over small inhibitor molecules for having high target specificity and lower side effects. The spike protein is an important structural protein of SARS-CoV-2 that binds with the human angiotensin-converting enzyme-2 leading to host entry of the virus. Hence, the activity of the anti-viral peptides will be based on the interference of the peptide inhibitor between the binding site of spike protein, and the ACE2 protein ultimately will prevent the virus invasion process. Several database resources are available that contain many anti-viral peptides from natural sources. However, the experimental basis of establishing the therapeutic importance of every protein from the database is a difficult and time-consuming task. Hence the available bioinformatics tools and techniques can be suitably used to screen, structure prediction, evaluation of ant-viral peptide- SARS-CoV-2 spike protein interaction, toxicity prediction, molecular dynamics simulation, and so on. In this review, the implementation of some of the major computational tools, their availability, and effectiveness in predicting the peptides against the Spike protein have been discussed.

**Keywords:** Antiviral peptides**,** novel coronavirus, Spike Protein, Bioinformatics, Screening Methods, Binding Affinity

## 1. Introduction

The novel coronavirus disease was first identified in China during the last month of 2019. Further, this novel virus (SARS-CoV-2) infection continued to outbreak globally at an alarming rate. The genomic sequence study of the virus has been observed to share some homology with the related virus such as (Middle East respiratory syndrome-CoV) and the coronavirus (SARS-CoV) [1-3]. High infection, as well as the death rate of the novel coronavirus in comparison to other previously known coronaviruses, created the research challenge to discover the potential drug candidates for the pharmacological treatments. From the beginning of the pandemic, several molecules have been repurposed and proposed to prevent the infection of the virus; however, till yet, no such effective drug is available for the treatment [4-6]. Some of the repurposed drugs including remdesivir, favipiravir, lopinavir, ritonavir, ribavirin for the inhibitor of viral RNA- replication have been approved but there is no evidence regarding their clinical efficacy [7-11]. In the host-virus interaction process of SARS-CoV-2, the Spike glycoproteins (molecular weight 180-200 kDa) play a major role. The Spike glycoproteins (S) contain an N-terminus region (extracellular), transmembrane (TM) anchored region, and a short C-terminal region (Figure 1). It was studied that, the *open* conformation of the S-protein facilitates the virus interaction with ACE2 protein of host (human) and further leads to the fusion process with the host cell membrane. The S protein also contains the polysaccharide coating that prevent the virus from the host immune system recognition process [12-14].

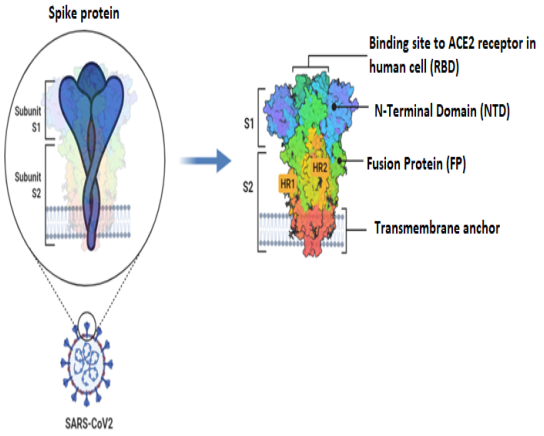


**Figure 1**. Structural components of the spike glycoprotein (SARS-CoV-2)

* Corresponding author. e-mail: rnsatpathy@gmail.com.

**Table 1.** Target regions of the Sars -CoV-2 spike glycoprotein

| Subunit of Spike Protein | S. No | Specific target region of S protein | Sequences regions | Function |
|---|---|---|---|---|
| S1 | 1 | N-terminal domain (NTD) | 13-305 | Helps in attachment and recognition of virus |
| | 2 | Receptor binding domain (RBD) | 319-541 | Binds to the cell receptor hACE2 |
| | 3 | Receptor binding motif (RBM) | 437-508 | Binds to the cell receptor of hACE2 |
| S2 | 1 | Fusion peptide (FP) | 788-806 | Helps in anchoring the target membrane with the S protein, and play important role in mediating membrane fusion |
| | 2 | Heptad repeat 1 (HR1) | 912-984 | Essential for the viral fusion and cell entry mechanism |
| | 3 | Heptad repeat 2 (HR2) | 1163-1213 | Essential for the viral fusion and entry function of the S2 subunit |
| | 4 | Transmembrane domain (TM) | 1213-1237 | The downstream TM domain anchors the S protein to the viral membrane |

The length of the spike protein of the SARS-CoV-2 virus comprises 1273 amino acids long, and it is further divided into two subunits, S1and S2, which are actively involved in the host cell entry process. The spike protein is trimeric in its native form and the S1 and S2 subunits of the virus form a "crown" structure from which the term "corona" is originated in the Latin language translation. The S1 subunit is classified into N-terminal domain (NTD), the receptor-binding domain (RBD), and the carboxy-terminal domain (CTD). The CTD domain also contains two subdomains such as SD1 and SD2. Similarly, the S2 subunit consists of two regions such as heptapeptide repeat 1 (HR1) and heptapeptide repeat 2 (HR2). The HR1 act as a fusion peptide and the HR2 act as the transmembrane domain (TM) (Figure 1). In the viral entry process, the S protein directly interacts with the cell surface receptor, human angiotensin-converting enzyme-2 (hACE2). Particularly, the open conformation state of the RBD domain is accessible for binding with the hACE2. After the binding event to the human ACE2 receptor, S1 and S2 cleavage sites are accessed by the host protease due to the conformational change in the S protein [15-21]. The detailed sequence position and the function of the different components of the spike protein have been shown in Table 1, and the mechanism is shown in Figure 2.

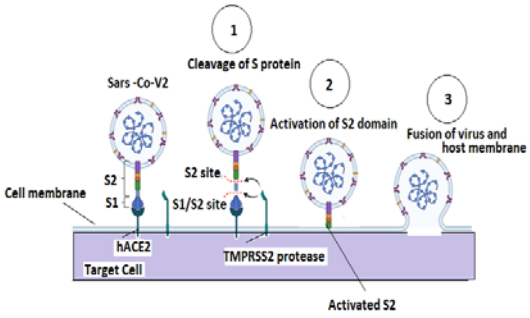


**Figure 2.** Basic entry mechanism of SARS-CoV-2 into the host cell

Soon after the process, the proteolytic cleavage occurs at the position of S1 and S2 subunit junction site as well as at the S2 subunit region with the help of a serine protease enzyme TMPRSS2. Following this cleavage event, HR1 and HR2 regions of the S2 subunit interact to form a structural arrangement known as *fusion core*. The *fusion core* formation further enhances the entry of the virus into the host cell by the cellular endocytic mechanism. Finally, the viral RNA is released into the host cell and the viral replication process is initiated. Interestingly, research has established that the SARS-CoV-2 shows an enhanced affinity towards binding (about 10 to 20-fold) with hACE2, as compared to another related virus such as SARS-CoV [22-23].

Recently, efforts have been given to discover potential antiviral therapeutic molecules. The traditional biochemical approach of small molecule inhibitors is associated with several obstacles like drug resistance, huge cost, time-consuming as well as toxicity [24-25]. In this context, the antiviral peptides (AVPs) are considered as one of the important therapeutic molecules due to their *specificity*, *efficacy, potency,* and desirable *pharmacokinetic properties* [26]. For the last 50 years, Antiviral peptides (AVPs) have shown their effectiveness, hence generating a perspective for treating viral infections such as novel coronavirus. The key feature of antiviral peptides are, they are involved in the specific interaction and inhibition of target protein. Therefore, targeting the AVPs in the form of novel therapeutics against the pathogenic virus-like SARS-CoV-2 might be established as a promising tool to develop an effective treatment process. Prediction and application of effective anti-viral peptides against respiratory disease-causing viruses like SARS-CoV, SARS-CoV2, MERS-CoV have been described and reported by many researchers [27-33].

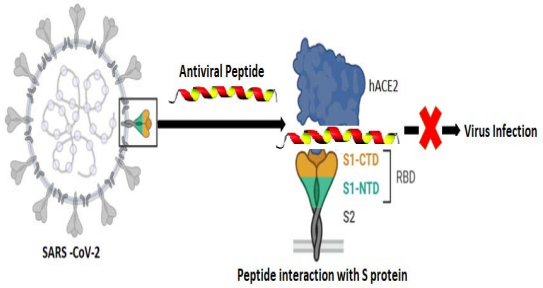


**Figure 3.** Possible mechanism of action of the anti-viral peptides with the spike protein

The potential AVPs are to be identified, hence they can be targeted specifically to the RBD domains in the S protein of novel coronavirus so that, the interference will restrict the host cell receptor-mediated viral entry. The possible mechanism of AVP- based inhibition of spike protein is presented in Figure 3. The binding features of the Spike protein of SARS-CoV-2 with ACE2 have been studied, and it was revealed several charged amino acids of ACE2 are important for its interaction [34]. Li et al. also studied the binding affinity of peptides that binds to the

specific hotspot area of the spike protein by computing the rate of inhibition [35].

The objective of the review is to provide a basic methodology that is involved in the searching and development of an effective spike inhibitor peptide molecule. Additionally, the challenges and scope of the process have been discussed.

## 2. In-silico development process of peptide molecules against the SARS-CoV-2 spike protein

An experimental basis for searching for novel and effective anti-viral peptide molecules that can specifically block the S protein function of the novel coronavirus is a challenging task. However, the computational approach facilitates the process for searching and predicting an effective antiviral peptide. This method will also lead to discovering and understanding the mechanism behind the spike protein-peptide interaction that corresponds to the antiviral effects [36-39]. Several steps are to be followed in the in-silico based novel effective peptide discovery against the spike protein inhibitor are schematically presented in Figure 4 and described in the sections below.

## 3. Retrieval of peptide sequence information

The information about the antiviral peptide sequence information from natural sources can be obtained from several available databases. Searching the literature regarding the specific peptides that can inhibit the spike protein of the virus may also help to explore the potential one. Along with the amino acid sequences, several databases also contain an analytical tool for peptide analysis. Some of the important databases and their availability that contains anti-viral peptides are represented in Table 2.

**Table 2**. Important databases contain information about anti-viral peptides

| S.No | Name of the database | Availability |
|---|---|---|
| 1 | AVPdb Database | https://webs.iiitd.edu.in/raghava/satpdb/catalogs/avpdb |
| 2 | Antimicrobial Peptide Database (APD) | https://aps.unmc.edu/database/anti |
| 3 | Database of Antimicrobial Activity and Structure of Peptides (DBAASP) | https://dbaasp.org/ |
| 4 | LAMP2 | http://biotechlab.fudan.edu.cn/database/lamp/ |
| 5 | CAMPR3 (Collection of Anti-Microbial Peptides) | http://www.camp.bicnirrh.res.in/index.php |
| 6 | DRAMP (Data repository of antimicrobial peptides) | http://dramp.cpu-bioinfor.org/ |

## 4. Screening criteria for the peptides

After retrieval of the anti-viral peptide sequences from the database search, it is essential to screen the spike binding activity of these peptide sequences based on a certain feature. For example, Mustafa et al. studied several features of the probable active peptides against the MERS-CoV spike protein [40]. As the MERS-CoV spike protein resembles the SARS-CoV-2 spike protein, hence similar type of criteria can be used to screen the potential peptides [41-42]. In addition to this, several strategies have been employed by many researchers for screening probable spike peptides having spike binding characteristics [43-46]. Some of the criteria for screening of potential peptide molecules are:

(i) *length of amino acids* should be 20aa to 55aa (length of the peptide sequence is proportional to the antiviral activity)

(ii) *positively charged residues* should be more abundant

(iii) *net charge* of the peptide > 0 as the spike protein of Sars- CoV-2 is neutral and less negatively charged in nature

(iv) the peptide should be *non-toxic* to human cells

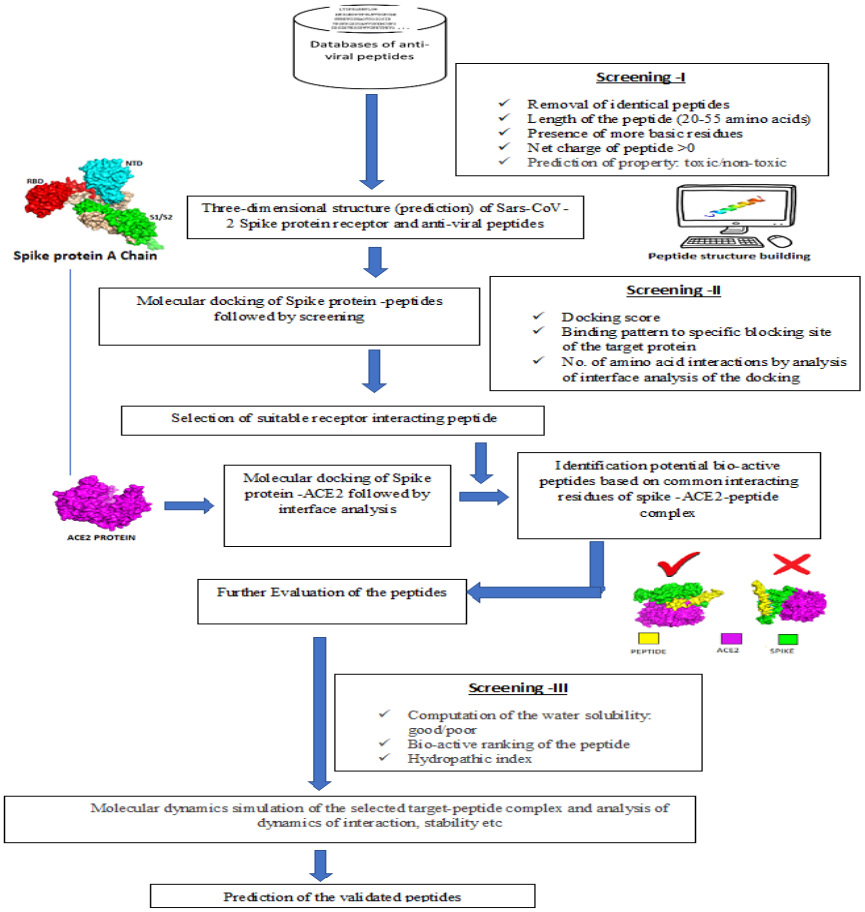


**Figure 4**. steps for *in silico* prediction of novel bioactive peptides against spike proteins

## 5. Prediction of the 3D structure of spike glycoproteins and anti-viral peptides

The protein data bank (PDB) available at https://www.rcsb.org/ may be searched for the availability of the three-dimensional structure of the (SARS-CoV-2) spike proteins as well as for the small peptides. Recently, Gowthaman et al. developed a database Cov 3D (available at https://cov3d.ibbr.umd.edu), which contains coronavirus structural data from the PDB including the spike protein [47]. Also, the 3D structure prediction of the spike protein as a target can be performed from the amino acid sequence followed by validation. UniProt database (https://www.uniprot.org) is usually used to retrieve the amino acid sequence information of the spike protein. Several tools available for the 3D structure prediction of spike protein, are being used by many researchers (Table 3).

**Table 3.** Popular 3D structure prediction tools for peptide molecules

| S. No | Name of the tool | Availability | Recently used by researchers for spike protein structure prediction |
|---|---|---|---|
| 1 | SWISS MODEL | https://swissmodel.expasy.org/ | (Padilla-Sanchez 2020; Allam et al. 2020) |
| 2 | Modeller | https://salilab.org/modeller/ | (Hall et al. 2020; Martin et al. 2020; Hassanzadeh et al. 2020) |
| 3 | Phyre 2 | http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index | (Jaimes et al. 2020) |
| 4 | RaptorX | http://raptorx.uchicago.edu/ | (King et al. 2021; Awadelkareem et al. 2020) |
| 5 | I Tasser | https://zhanggroup.org/I-TASSER/ | (Prashantha et al. 2021; Ibrahim et al. 2020) |

Similarly, for the small peptide sequences, the 3D structure can be predicted by using online server like pepfold (available at https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/). This web-based tool predicts the 3D structure of linear peptides sequences from 5 to 50 amino acid range. Also, other open-source stand-alone programs like open Chimera (https://www.cgl.ucsf.edu/chimera/) can be used to build peptide structure from the sequences. Many of the protein-protein docking programs take the peptide sequence along with the target protein sequence as input and automatically predict the 3D structure thereby preparing the structural

file for the docking simulation. After the prediction of the 3D structure of the peptide, the energy minimization should be performed till it attains negative free energy. Evaluation of the predicted structure can be performed by computing the Ramachandran plot, side-chain placement, and so on. For the structure verification of the predicted structures, the SAVES servers may be used (https://saves.mbi.ucla.edu/).

## 6. Molecular docking and molecular dynamics (MD) simulation study of anti-viral peptides with SARS-CoV-2 spike proteins

To compute the binding affinity as well as the binding pose of the selected potential anti-viral peptide with the virus spike protein, molecular docking is to be performed. Molecular docking is a computational approach in which the affinity, pose of the ligand is evaluated along with the receptors. Mainly two types of algorithms are involved in this process, searching algorithm and scoring algorithms. Based on the implementation of the combinations of algorithms, different types of docking programs are available [48-49].

The protein-peptide docking programs are broadly classified into three categories. The first one is template-based docking (a protein-peptide input structure is searched for a known template structure from the database followed by comparative analysis). The second method is known as local docking (the peptide binding site is searched on the given input receptor by the user given peptides). The third method is global docking in which the peptide behaves as rigid in nature and binding pose and position are evaluated by the exhaustive search on the receptor. Also, in the molecular docking process of protein and peptide, the number of flexible bonds, size, a loop structure, and terminal charges in the peptide structure are the important parameters on which the accuracy of the prediction depends [50-51]. In this process, the Spike protein is behaving as the receptor and the peptide sequence as the ligand and the binding affinity, the binding pose can be computed from the receptor-ligand complex by application of suitable searching and scoring functions in several docking programs [52-60], presented in Table 4.

**Table 4.** Molecular docking programs that are frequently used for protein-peptide interaction study

| S. No | Name of the server | Availability |
|---|---|---|
| 1 | GalaxyPepDock | http://galaxy.seoklab.org/pepdock |
| 2 | Rosetta FlexPepDock | http://flexpepdock.furmanlab.cs.huji.ac.il |
| 3 | PepCrawler | http://bioinfo3d.cs.tau.ac.il/PepCrawler |
| 4 | HADDOCK peptide docking | http://milou.science.uu.nl/services/HADDOCK2.2/haddock.php |
| 5 | GRAMM-X | http://vakser.compbio.ku.edu/resources/gramm/grammx |
| 6 | DINC 2.0 | http://dinc.kavrakilab.org |
| 7 | pepATTRACT | http://bioserv.rpbs.univ-paris-diderot.fr/services/pepATTRACT |
| 8 | CABS-dock | http://biocomp.chem.uw.edu.pl/CABSdock |
| 9 | ClusPro PeptiDock | https://peptidock.cluspro.org |
| 10 | PIPER-FlexPepDock | http://piperfpd.furmanlab.cs.huji.ac.il |
| 11 | HawkDock server | http://cadd.zju.edu.cn/hawkdock |
| 12 | Z dock server | https://zdock.umassmed.edu |
| 13 | SwarmDock server | https://bmm.crick.ac.uk/~svc-bmm-swarmdock |
| 14 | pyDockWEB | https://life.bsc.es/pid/pydockweb |
| 15 | 3D garden | http://www.sbg.bio.ic.ac.uk/3dgarden |
| 16 | Hex server | http://hexserver.loria.fr |
| 17 | DOCKSCORE | http://caps.ncbs.res.in/dockscore |
| 18 | FRODOCK | https://chaconlab.org/modeling/frodock |
| 19 | HPEPDOCK Server | http://huanglab.phys.hust.edu.cn/hpepdock |
| 20 | MDockPeP | https://zougrouptoolkit.missouri.edu/mdockpep |
| 21 | ClusPro 2.0 | https://cluspro.org |

In the computational procedure, after molecular docking simulation, the implementation of a reliable specific scoring function is the basic requirement for the prediction of the correct binding nature of the protein-peptide complex. In this context, theoretically calculation of the end-point binding free energy by the methods such as Molecular mechanics Poisson–Boltzmann surface area (MM/PBSA) is used. By using this method, the binding affinities, as well as the binding conformations, are obtained for protein-peptide complex systems [61]. In the MM-PBSA method, the free energy (ΔG) of receptor-ligand (protein-peptide) binding is estimated by deducting the free energy between the complex and the unbound form. Previously, the MM-PBSA method has been successfully used to analyze and evaluate the protein-protein docking complexes [62-65]. A significant number of specific viral protein-peptide interactions is used for the determination of affinity of the potential AVPs and used for the in-depth understanding of the behaviour towards the target molecule. To investigate the spike protein-peptide interaction, it is essential to study the interface region of the docked complex. The interface analysis results from the numbers of amino acid interactions with the specific binding domains. This also leads to evaluating

the different types of interaction such as cation–π, hydrogen bonding, and electrostatic interaction essential to describe the anti-viral inhibition property of the peptide [66-67].

Molecular dynamics (MD) simulation is a computational method used for understanding and prediction of the structure as well as the function of biological macromolecules. This method is based on the physical principles (Newton's equations of motion). The macromolecular conformation is represented by the dynamic model from which the motion of the individual atoms and subsequent conformational changes are calculated from a trajectory file. Specifically, by the MD simulation, the macroscopic behaviour from various microscopic interactions present in molecular systems is computed. The MD simulation methods are frequently used in the drug discovery process to study the receptors (targets) and their association with ligand (drug) molecules after docking to discover the novel molecules by computing various parameters. MD simulation can also be used to study the configuration change in the protein structure that involves the monomeric or the trimeric form of the spike glycoprotein. In addition to this, a comparative structural analysis of the ligand-bound state of spike protein and native (unbound) state can be performed. Currently, the MD simulation techniques are used in the case of spike protein to study the proper binding of the ligand, total energy variation profile of the complex, dynamicity of protein domain, variation of hydrogen bonds, dynamics of actively interacting residues of the spike protein throughout the simulation period and so on [68-69].

**Table 7**. Molecular dynamics simulation software and their availability

## 7. Identification of bioactive peptide molecules

Along with the docking score and the *pose* of the binding peptide and several physicochemical screenings are also essential to select the potential peptide inhibitor molecules. The major problem is in the therapeutic use of peptides is their toxicity. Hence the peptide features like toxicity, bioavailability, in *vivo* instability, half-life, and ability to cross the membrane are to be evaluated carefully. These factors obtained from the peptide evaluation output can be utilized further in the specific rational design process of novel peptides with enhanced stability and other required physicochemical properties [70-71]. Some of the important bioinformatics tools used to compute such peptide properties are given in Table 6.

**Table 6:** Peptide designing and screening tools and their availability

Several scientific studies on the peptide-based inhibitors against the pathogenic virus have been developed and proposed in the last few years. The finding of small effective peptides was considered to be one of the best molecules that can be targeted to the spike protein [72]. Wong et al., in 2020 conducted *in silico* study to identify potential SARS-CoV-2 cell entry inhibitors from peptides derived from different edible insects. In-silico-based gastrointestinal (GI) digestion of the insect protein was performed to generate peptide molecules. Subsequent molecular docking study with the spike protein resulted that, a tri-peptide generated from the source mealworm can act as the effective inhibitor [73]. Allam et al. analyzed the peptide and polyphenol-based inhibitor that can block the SARS-CoV-2 spike protein by inhibiting the glucose-regulating protein 78 receptors. The in-silico screening process was conducted by taking the available databases of bioactive peptides. Protein-peptide docking analysis resulted in five potential peptides that can inhibit the GRP78 binding site [74]. Salman et al., conducted the computational protein-protein interaction study to obtain the effective peptides from the inhalers that can bind the spike protein. Molecular docking simulation of S protein (receptor) with different compounds such as alpha-1-antitrypsin, dornase-alfa, angiotensin-converting enzyme-2 (ACE-2), human palate were analyzed. The peptide molecules obtained were further predicted as the potential anti- SARS-CoV-2 agents [75]. Protein peptide docking followed by the interface analysis study also showed that the amphibian derived peptides such as Dermaceptin-9 from the amphibian genus *Phyllomedusa* is an effective spike protein inhibitor of Sars- CoV-2 [76-77]. In 2020, Barh et al. implemented three different *in silico* strategies to design the potential novel peptide inhibitors that can interact and inhibit the Sars CoV-RBD - hACE2 interaction. The key binding residues were identified from the interaction study followed by identifying the peptide binders from the bacterial peptide database. Then a chimeric peptide was designed that is capable of binding the key residues of the SARS-CoV-2 -RBD - hACE2 complex. For the screening of the best potential peptides, the parameters like physiochemical properties, numbers, and positions of key residues binding, binding energy, and antiviral properties were considered [78]. Alibakhshi et al. conducted a computational study to design the effective peptides by targeting the RBD of SARS-CoV-2 spike protein and human ACE2 interaction. The analysis was based on taking stretches of peptides from the ACE2 protein. Further, the different mutants were designed by computational method, and interaction of the peptides with spike protein was studied by using the molecular docking simulation method. As a subsequent study, molecular dynamics simulation was carried out to evaluate the best mutant peptide that interacts with the S protein in comparison to the wild peptide [79]. A similar study was conducted by Panda et al., to design a peptide having a resemblance to human ACE-2 protein. The non-interacting residues of ACE2 were mutated to generate a mutant peptide library. The molecular docking by HADDOCK server followed by molecular dynamics simulation (150 nanoseconds) by Gromacs tool was used to identify the novel mutant peptide developed the enhanced binding affinity about three times [80].

## 8. Challenges and Future research scope

There is a continuous increasing trend in the knowledge level in the experimental data related to the structure of SARS-CoV-2 and its entry process to the host cell. Much of the information is available now in the form of published literature and databases. This data provides a solid background to apply the computational techniques such as modeling, identification, and characterization of novel antiviral peptides that would interfere with spike-ACE2 binding and the membrane fusion process. Although

the peptide molecules are advantageous over other small molecules, properties of the peptides like toxicity, immunogenicity, and stability of peptides remain as the major issues and need to be addressed in the development process of peptide-based inhibitors [81-85].

Moreover, the following specific challenges are associated with the discovery process of potential peptide inhibitors against the spike protein of SARS-CoV-2 and need to be addressed.

- S1 RBD domain of Spike glycoprotein is part of a highly mutable region, hence targeting to inhibit the domain is challenging, however, specifically targeting the peptide molecules to the HR region of the S2 subunit, with enhanced affinity might be effective to prevent the SARS-CoV-2 infections.
- Other than binding affinity property of AVPs to S-protein, prediction of several important parameters such as penetration capacity to the tissue, stability in the plasma, protease enzyme degradation potential, immunological interference, and toxicity is necessary.
- The full-length wild-type S protein structure of SARS-CoV-2 is still not available in the database. Hence important missing amino acid residues and loops might affect the in-silico results regarding peptide-protein binding. Hence, the complete form of the crystal structure of the spike protein (both wild-type as well as variants) is to be determined.

## 9. Conclusions

SARS-CoV-2 is evolved as a novel pathogenic virus with a significantly enhanced infection rate. Due to the deadliest nature of this coronavirus, it is essential to search for an effective medication to respond to this infectious disease to avoid pandemic situations. Specifically, the interaction of the spike glycoprotein of the virus with the human ACE2 receptor causes the viral entry into the host cell. So, the use of peptide-based inhibitor molecules against the spike protein will be a promising approach to inhibit the process. Currently, the therapeutic application of peptide molecules is implemented against many diseases. Anti-viral peptides are most often preferred over small molecules due to their specific, few side effects, and no drug resistance activity. So, the study about targeting the spike glycoprotein of the virus with the potential novel anti-viral peptides will create a milestone in this emerging area. However, the mutation acquired by the virus, *in vivo* bioavailability, toxicity, and stability of anti-viral peptides are the major limitations. The available sophisticated bioinformatics tools and databases can be implemented for structure prediction of peptides, binding affinity study, validation, and dynamics analysis, *in silico* toxicity, bioactivity, and stability prediction, and so on. Furthermore, these results obtained from the *in-silico* analysis can be further combined along with *the experimental* results, for the development of proper validated peptide-based inhibitors by targeting the SARS-CoV-2 glycoprotein.

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## Conflict of interest

Nothing

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