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ORIGINAL RESEARCH

In-silico study of peptide-protein interaction of antimicrobial peptides potentially targeting SARS and SARS-CoV-2 nucleocapsid protein

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Abstract

This study is an attempt to find a suitable therapy using antimicrobial peptides (AMPs) by identifying peptide-protein interaction of AMPs and nucleocapsid protein of SARS and SARS-CoV- 2. The AMPs were shortlisted from the APD3 database (Antimicrobial peptide database) based on various physicochemical parameters. The binding efficacy of AMPs was measured using the lowest energy score of the docked complexes with 10 selected AMPs. For SARS-CoV, AP00180 showed the best pose with a binding affinity value of − 6.4 kcal/mol. Prominent hydrogen bonding interactions were observed between Lys85 (nucleocapsid receptor) and Arg13 (antimicrobial peptide ligand) having the least intermolecular distance of 1.759 Å. For SARS-CoV-2, AP00549 was docked with a binding affinity value of − 3.4 kcal/mol and Arg119 and Glu14 of receptor nucleocapsid protein and ligand AMP having the least intermolecular distance of 2.104 The dynamic simulation was performed at 50 ns to check the stability of the final docked complexes, one with each protein. The two best AMPs were AP00180 (Human Defensin-5) for SARS and AP00549 (Plectasin) for SARS-CoV-2. From positive results of dynamic simulation and previously known knowledge that some AMPs interact with the nucleocapsid of coronaviruses, these AMPs might be used as a potential therapeutic agent for the treatment regime of SARS-CoV-2 and SARS infection.

Keywords Pandemic · Capsid protein · Simulation studies · Molecular dynamics · Binding energy · Favorability percentage

Introduction

The current global coronavirus pandemic has now affected more than 213 countries and territories across the globe (Countries where Coronavirus has spread-Worldometer 2021). SARS-CoV-2 is said to have originated in Wuhan, China in December 2019 and gradually spread across the world affecting more than 24 million people (WHO Coronavirus Disease (COVID-19) 2021). The past coronavirus infections namely SARS (Severe Acute Respiratory Syndrome) and MERS (Middle Eastern Respiratory Syndrome) affected many parts of the world. This called for scientists to find various measures of treatments for viral diseases, even though no effective drug or vaccine accounts for full recovery in all patients (Lai et al. 2020). In the current study, some antimicrobial peptides (AMPs) are suggested with proper In-Silico studies as effective therapeutic agents against the SARS and SARS-CoV-2 infection. There are various classes of AMPs out of which the Human defensins which are produced and secreted by different mucosal and epithelial cells show antimicrobial properties against viral diseases (Amerikova et al. 2019). AMPs are now widely being studied as an alternative to antibiotics for rapid response. It is one of the first pathways to get upregulated during the defence action (Ahmed et al. 2019). The role of AMP with influenza A has also been extensively studied (Hsieh and Hartshorn 2016). The study of structural and non-structural proteins is important to understand how these viruses interact with the human body. In this paper, we targeted the structural

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nucleocapsid protein of SARS and SARS-CoV-2. The protein, coded by the 9th ORF of SARS, is helical in shape and plays a major role in pathogenesis, RNA packaging, and replication of the virus. It is a 46 kDa protein with 422 amino acids (Surjit and Lal 2008). It induces an antigenic and immunogenetic response in the human body (Peng et al. 2006). Along with the envelope M protein, the nucleocapsid protein aids in genomic condensation and packaging. It is also expressed in abundance in case of infection thus making it a crucial target to study (Surjit and Lal 2008; Verheije et al. 2010; Ahlquist et al. 2003). We chose nucleocapsid (N gene) over other SARS structural proteins because it is more stable and conserved. It has the fewer mutation and over 90% amino acid homology. In general, the nucleocapsid protein/gene of coronavirus families are expressed in abundance and highly immunogenic when an infection occurs (Chang et al. 2014; Chen et al. 2020; Pang et al. 2020; Drosten et al. 2003; Marra et al. 2003). The nucleocapsid protein also shows an antigenic response for the T-cell receptors in terms of proliferation and cytotoxic activity in term of vaccine (Grifoni 2020; Cong, et al. 2019; Dutta et al. 2008; Kang, et al. 2020; Lee et al. 2010). There are around 186 AMPs which are antiviral in nature and a total of more than 3000 AMPs present in the antimicrobial peptide database (APD3) (Elnagdy and AlKhazindar 2020). Viral entry into the cell is blocked by human defensin 5 (HD5), which is a potential blocker for SARS-Cov-2 (Maiti 2020).In many kinds of infections by various pathogens, AMPs are one of the first immune pathways which are upregulated in vivo. Of these various classes, the two best AMPs studied till now are the defensins and human cathelicidin LL-37. Antimicrobial peptides like defensins have shown response against blocking cell receptor by binding to it in the case of HIV, inhibiting the protein kinase C signalling pathway in case of HSV, Transferrin has shown their activity by inhibiting cellular trafficking in case of Hantavirus, Rotaviruses etc. (Ahmed et al. 2019; Agier et al. 2015). Although AMPs are usually studied against bacterial infections, we aim to find AMPs for viruses as well for a specific target site namely the nucleocapsid protein of SARS and SARS-CoV-2. Studies have shown that antimicrobial peptides usually affect viral entry into a cell, although its effects have been seen at other sites as well. They generate an innate immune response which is crucial to keeping infections at bay. Such studies were conducted using AMPs in response to the dengue virus using the human neutrophils and further examination by qRT-PCR (Ahmed et al. 2019; Castañeda-Sánchez et al. 2016).The activity of AMPs is decided by their amino acid composition, net charge, size, hydrophobicity and hydrophilicity etc. which have been considered in our study as well which are important from the structural and conformational point of view. It is desired for the selected AMP to be stable (Cunha et al. 2017). Our work focuses on the binding efficacy of AMPs which hinders the activity of nucleocapsid protein of the viruses. Using the docking and dynamic simulation techniques, we aim to find the two most suitable and probable AMPs which will best block the pathway for the viruses.

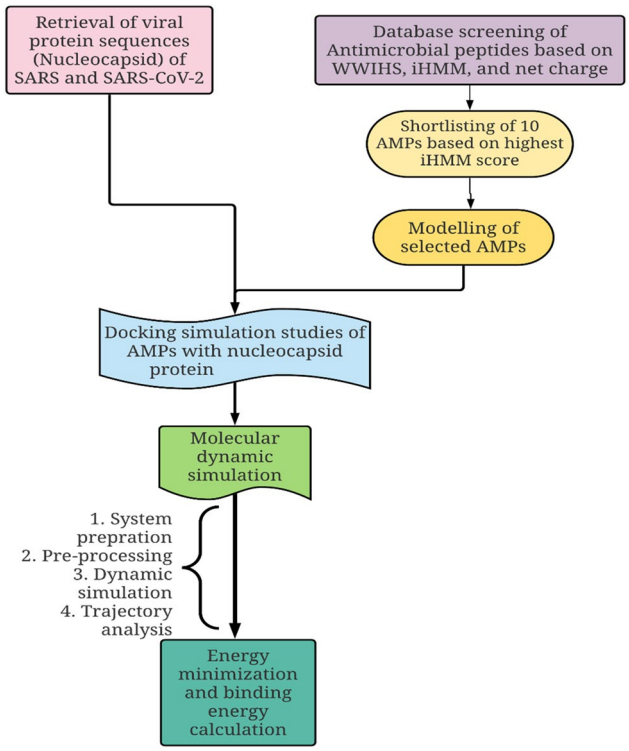
Materials and methodology

Retrieval of the viral protein sequences (Nucleocapsid) of SARS and SARS-COV-2-CoV

The amino acid sequences of viral nucleocapsid strains (SARS, SARS-CoV-2) were retrieved from UniProtserver (Bateman 2019) (Scheme 1). Their PDB structures were selected from the RCSB PDB database (Burley et al. 2019).

Database screening of antimicrobial peptides

A cluster of AMP's was retrieved from the APD3 server (Antimicrobial Peptide Database) (Wang et al. 2016). The screening of the database was made based on certain criteria such as –length of the peptide should be between 20 to 55 AA(The length of peptide correlated with inhibitory action. The longer the length of peptide, better antiviral activity has been observed as compared to sequences of shorter length (Du et al. 2017), an abundance of basic residue in the



Scheme 1 Schematic flowchart depicting overall methodology of the workflow

peptide, the net charge of the peptide should be more than or equal to zero, as the viral membrane is negatively charged, non-toxicity concerning mammalian cells and interfacial activity of peptide including-

a) Wimley-White interfacial hydrophobicity scale (WWIHS > 0)

It is an experimentally calculated free energy scale which helps to determine the propensity of individual amino acids in peptide sequences to partition from water into a phosphatidylcholine interface.

b) Interfacial helical hydrophobic moment (iHHM > 0)

It describes the degree to which a peptide can be segregated into hydrophilic and hydrophobic phases when folded in an alpha- helix.

A peptide with a large iHHM can interact strongly with membranes as a helix due to partitioning–folding coupling even with a WWIHS score that is not positive overall (Galdiero et al. 2015).

Structure prediction of the selected AMPs

The tertiary structure of the selected AMPs was generated by MODELLER and PEPFOLD server depending upon peptide length (Šali and Blundell 1993; Camproux et al. 2004). If the peptide lengths greater than 30AA, MODELLER was used and for peptide, less than 30AA in length, a PEPFOLD peptide structure prediction server was employed.

The generated models were then evaluated based on the Ramachandran Plot generated by ProCheck (Laskowski et al. 1993).

Docking simulation studies

To check the binding affinity between the selected AMP's and the nucleocapsid protein receptor of SARS and SARS-COV-2-CoV (2CJR and 6M3M respectively), docking simulation was performed using the ClusPro v2.0 server. This server assesses massive datasets of putative complexes and gives a pre-set number of favourable docked poses.

For peptide-protein docking, the selected AMPs were set as ligands and docked with both receptors 2CJR and 6M3M. This server rotates the ligand with 70,000 rotations and for each rotation, it translates the ligand in *x*, *y*, and *z* axis relative to the receptor on a grid (Vajda et al. 2016).

Further, Autodock Vina (Trott et al. 2009) was used for docking simulation analysis on the best-docked poses that were obtained from the ClusPro v2.0 server to obtain the accurate interactions between the receptor nucleocapsid proteins and ligand anti-microbial peptides.

Molecular dynamic simulation

Molecular dynamic simulation and energy minimization was done for the AMPs and the nucleocapsid receptor complex using GROMACS. All simulated structures were centered typically in a dodecahedron box with a minimum distance of 2.0 nm between each atom of the protein and the box to reduce and the SPC216 water model was used to solvate the system (Abraham et al. 2015). The solvated protein was subsequently neutralized by Cl^-ions, where 58.21 ions were used for SARS and SARS—2, respectively. Covalent bonds involving hydrogen atoms were constrained using the LINCS algorithm, and long-range electrostatic interactions were treated with the Particle-Mesh Ewald (PME) method employing a real-space cutoff of 10 Å. Energy minimization was executed by the steepest descent method and the conjugate gradient method for the subsequent 50,000 steps (NIH Lincs Program 2021). The system was initially temporarily minimized with backbone atoms confined to the previous coordinates to remove close contacts, and the restrained system was gradually heated to 300 K under constant volume conditions at 100 ps. Each system was equilibrated for 50 ns using the constant isothermal-isobaric (NPT) ensemble at 1 bar and 300 K without any restraints. The Parrinello-Rahman barostat and a V-rescale thermostat were used with an integration time step of 2 fs. Lennard–Jones interactions were calculated using a cutoff of 1 Å. The pair lists were updated every 400 steps. All simulations were performed using GROMACS 2019.1 along with the OPLS– AA force field. To assess the stability of the selected model, a 50 ns MD simulation was performed using the GROMACS software version 5.0. Before the MD simulation, it must be ensured that the system has no steric clashes or inappropriate geometry. The structure is relaxed through a process called energy minimization. Energy minimization of the system is an important step to relax the structure. The complex system energy minimization was done for 100 ps till the force reaches < 1000 kJ/mol. Equilibrating the solvent and ions around the protein is also an important step before the dynamic simulation calculation.

Results and discussion

Filtration of antimicrobial peptides and modelling

Based upon the criteria for database screening, a total of 37 peptides were filtered out of which, ultimately ten antimicrobial peptides were selected based on the iHHM score. The AMPs were modelled using the PEPFOLD server and MODELLER. The validity of each of those models was checked using the Ramachandran plot generated by the Procheck program. The shortlisted AMPs consisted of the

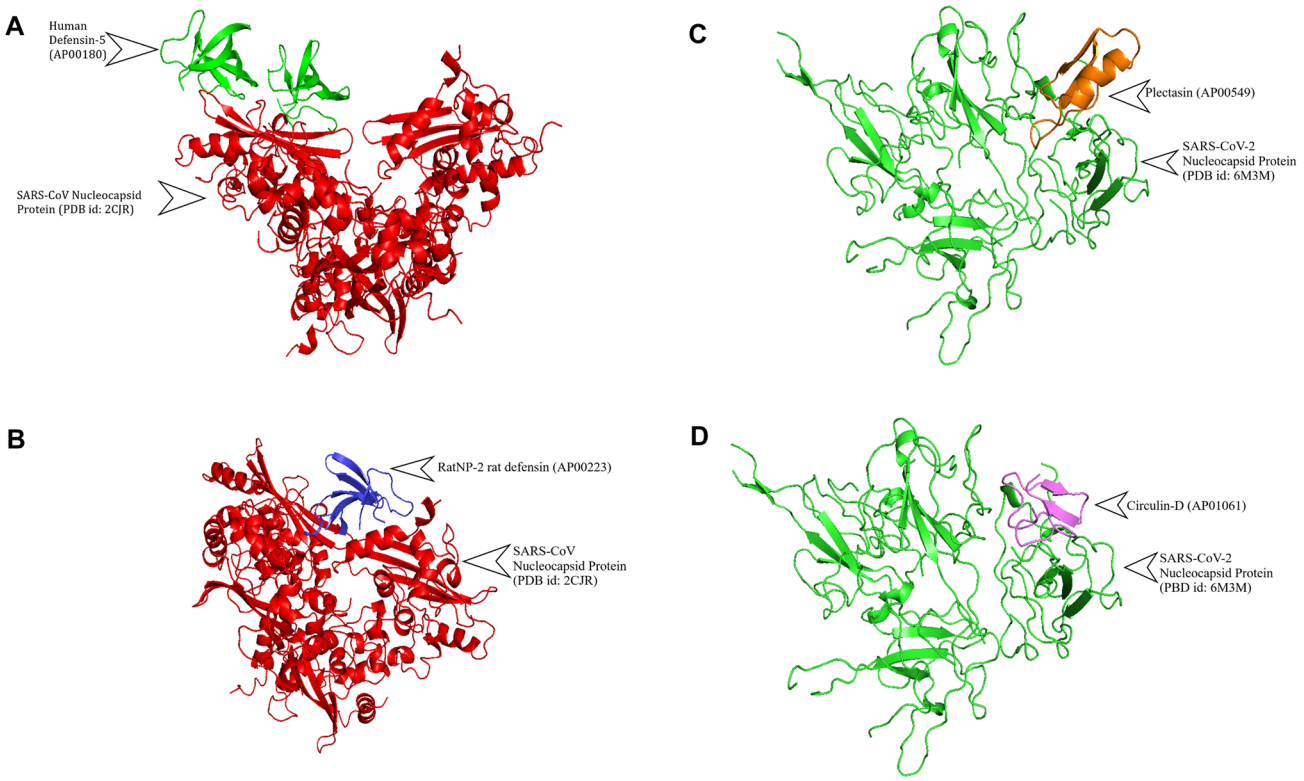


Fig. 1 Molecular docking simulation using ClusPro v2.0 **A** SARS nucleocapsid strain docked with AP00180 **B** SARS nucleocapsid strain docked with AP00223 **C** SARS- CoV- 2 nucleocapsid strain docked with AP01061 **D** SARS- CoV-2 nucleocapsid strain docked with AP00549

majority of residues in the favoured region, with the peptide ATCYCRTGRCATRESLSGVCEISGRLYRLCCR (Human defensin-5) possessing the highest favourability percentage. The Ramachandran plots can be seen in Fig. 1 of supplementary material.

Docking simulation and evaluation of the best complexes

Molecular docking simulation between nucleocapsid protein of SARS (PDB ID:2CJR) and SARS-CoV-2 (PDB ID: 6M3M) and the ten antimicrobial peptides with the help of the ClusPro server was implemented. For SARS, the top two least energy weighted scores were that of AP00180 and AP00223 and for SARS-COV-2, the best-weighted scores were that of AP01061 as well as AP00549 (Table 1). Further docking was implemented on Autodock Vina, where the poses obtained from the ClusPro v2.0 server analysed for their receptor binding domain as well as the interacting bonds between the receptor and the ligand.

Peptide-protein complex interaction studies

After the selection of the top two lowest weighted score AMPs, the docked structures were analysed for the type

Table 1 Lowest energy weighed score of docked complexes using ClusPro

Viral strain [receptor PDB ID]	APD3 ID	Lowest energy weighted score (Kcal/mol)
SARS [2CJR]	AP00160	− 619.6
	AP00692	− 838.4
	AP01061	− 862.8
	AP00180	− 1063.7
	AP00222	− 973.3
	AP00223	− 1013
	AP00036	− 931.9
	AP00549	− 830.1
	AP02148	− 906
	AP02733	− 689.2
SARS-CoV-2 [6M3M]	AP00160	− 645.6
	AP00692	− 826.6
	AP01061	− 1111.0
	AP00180	− 1032.4
	AP00222	− 1067.6
	AP00223	− 1107
	AP00036	− 1037
	AP00549	− 1183
	AP02148	− 1030
	AP02733	− 766.4

Table 2 Shortlisted antimicrobial peptides and their Ramachandran Plot statistics

Peptide	APD3 ID	Definition	WWIHS	NET CHARGE	iHHM	Residues in most favoured regions (%)	Residues in additional allowed regions (%)	Residues in generously allowed regions (%)
ALWMTLLKKVLKAAAKAALNAVLVGANA	AP00160	Dermaseptin-S4	0.92	4	4.71	63.6	27.3	9.1
GWFKKAWRKVKNAGRRVLKGVGIHYGVGLI	AP00692	Hagfish cathelicidin	0.24	8	6.41	61.9	28.6	9.5
KIPCGESCVWIPCVTSIFNCKCKENKVCYHD	AP01061	Circulin D	1.27	1	5.43	63.6	27.3	9.1
ATCYCRTGRCATRESLSGVCEISGRLYRLCCR	AP00180	Human defensin 5	1.39	4	3.83	92.9	7.1	0.0
VTCYCRRTRCGFRERLSGACGYRGRIYRLCCR	AP00222	RatNP-1 rat defensin	1.02	8	4.29	90.4	9.6	0.0
VTCYCRSTRCGFRERLSGACGYRGRIYRLCCR	AP00223	RatNP-2 rat defensin	1.7	7	4.01	90.0	10.0	0.0
DFASCHTNGGICLPNRCPGHMIQIGICFRPRVKCCRSW	AP00036	Bovine beta-defensin 1	0.22	4	4.72	80.8	11.5	3.8
GFGCNGPWDEDDMQCHNHCKSIKGYKGGYCAKGGFVCKCY	AP00549	Plectasin	2.11	1	3.67	76.7	23.3	0.0
FFLLFLQGAAGNSVLCRIRGGRCHVGSCHFPERHIGRCSGFQACCIRTWG	AP02148	Apl-AvBD16	3.26	5	5.26	75.9	24.1	0.0
LFGSVKAWFKGAKKGFQDYRYQKDMAKMNKRYGPNWQQRGGQEPPADAQANDQPP	AP02733	Piscidin 6	2.93	5	6.41	89.3	3.6	3.6

of interaction that exists between the nucleocapsid protein receptor and the antimicrobial protein–ligand. The table provides information regarding the specific interaction between the peptide-protein complex as well as the ΔG score between the interacting chains of nucleocapsid and AMP. Interacting bonds that were observed between the receptors and their specific ligands were hydrogen bonds, alkyl bond, salt bridges and pi-sigma bonds. For SARS, human defensin 5 (AP00180) chains B, C and D were in close interaction with the chains E and F of the nucleocapsid protein. The chains A and B of RatNP-2 rat defensin (AP00223) were in close interaction with A, B, C, E and F chains of SARS nucleocapsid. In both cases, conventional hydrogen bonds were in the predominant number. Circulin D (AP01061) was interacting with the SARS-COV-2 nucleocapsid chains A as well as C and varying bond types between individual amino acids. And lastly, Plectasin (AP00549) was interacting with the chains A and C of SARS-COV-2 nucleocapsid and classical hydrogen bonds being in the highest number as seen in Fig. 1. The interaction between the receptor amino acid molecules and ligand AMP molecules is shown in Table 2 of supplementary material along with their respective bond category.

Molecular docking simulation using Autodock Vina provided the amino acids of nucleocapsid proteins of SARS and SARS-CoV-2 which were directly interacting

with the amino acids of their respective antimicrobial peptides. A total of 6 electrostatic bonds, 11 conventional hydrogen bonds and 8 carbon-hydrogen bonds were found to be interacting between SARS nucleocapsid and AP00180. For SARS and AP00223, 2 electrostatic bonds, 6 conventional hydrogen bonds and 2 carbon-hydrogen bonds were observed. For SARS-CoV-2 and AP00549, 6 conventional hydrogen bonds and 3 hydrophobic bonds were observed and finally, for SARS-CoV-2 and AP01061, 4 conventional hydrogen bonds and 1 hydrophobic bond were observed.

As per Table 3 we can observe that the binding affinity score of SARS-CoV with AP00549 and AP01061 and of SARS-CoV-2 with AP00180 and AP00223 are favourable. It was decided to proceed with only those binding affinity scores which showed high favourability in both-ClusPro as well as Autodock Vina.

Autodock Vina employs Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm that uses local optimization methods for identifying active sites in the target protein. The default size of the grid box wherein the docking would take place is calculated using protein–ligand complex structures. A docking box is initially constructed which encloses the particular ligand and then the box size increases in random direction that ensures that the minimum length in any direction is atleast 22.5 Å. The specific active site of the nucleocapsid protein was generated by the algorithm to provide best suitable interaction (Trott et al. 2009).

Thus, the type of interaction that was predominant between the receptor proteins and antimicrobial peptide ligands were conventional hydrogen bonds, as shown in Table 4 and the most frequent interactions as shown in Fig. 2. The highest interacting bond predominant between the receptor molecule and the AMPs was conventional hydrogen bond. The most prominent interacting amino acids as per the distance given in the table between the receptors and the ligand include Lys85 and Arg13 (Conventional Hydrogen Bond), Ala110 and Glu21(hydrogen and electrostatic interaction) and Lys85 and Cys30 (carbon-hydrogen bond) between AP00180 and SARS nucleocapsid protein. For SARS nucleocapsid and AP00223, Arg13 and Glu111 (hydrogen and electrostatic interaction), Lys85 and Thr8 (Conventional Hydrogen Bond) and Lys 114 and Phe12 (Carbon Hydrogen Bonds) were the most prominent amino acid interactions. For SARS-CoV-2 nucleocapsid protein and AP00549 Asn196 and Asn513 (Conventional Hydrogen Bond) was the most promising hydrogen bonding interaction. For AP01061, Tyr29 and Asn91, as well as Thr256 and Gly5 (Conventional Hydrogen Bond), showed the most promising hydrogen bond interaction as in Fig. 3 and results after molecular docking are seen in Fig. 4

Molecular dynamic simulation analysis

Taking the ClusPro weighted scores as well as Prodigy ΔG values of the four shortlisted AMPs, the two AMPs- one for SARS and one for SARS-COV-2 were further filtered and analysed for molecular dynamic simulation. MD simulation is an appropriate step to ensure the stability of the nucleocapsid protein-AMP complex. SARS -AP00180 complex achieved the force and potential energy in 1840 steps with the energy drift of − 469,527 kJ/mol, whereas SARS -AP00549 achieved the same in 1758 steps with the energy drift of − 254,944 kJ/mol. Potential energies are given in Table 5 and Fig. 5. Constant temperature, pressure and density were maintained for 100 ps (Fig. 6). The final MD simulation was done for 50 ns.

The stability of the molecules was examined based on the RMSD, RMSF and Rg values. The average, maximum and minimum RMSD, RMSF, and Rg are presented in Table 6. The RMSD values of each frame corresponding to time are given in Fig. 4b. RMSD values indicated that both the systems achieved equilibrium in an early phase of a 50 ns trajectory. The difference between the highest and lowest RMSD values of SARS -AP00180 was 1.424 nm, which was quite close to the average value (2.273 nm), demonstrating that the backbone of the SARS -AP00180 complex did not fluctuate much. On the other hand, the SARS-CoV-2 -AP00549 complex system showed a similar rate of RMSD. The difference of the highest and lowest RMSD values of

Table 3 Grid-box dimensions and binding affinity values of Autodock Vina

S. No	Viral Strain	APD3 ID	Gridbox dimension (x)	Gridbox dimension (y)	Gridbox dimension (z)	Binding Affinity scores (kcal/mol)
1	SARS-CoV	AP00180	− 38.480	− 0.019	− 1.677	− 6.4
2	SARS-CoV	AP00223	− 39.313	0.206	− 1.009	− 6.1
3	SARS-CoV-2	AP00549	12.760	− 12.033	− 24.877	− 3.4
4	SARS-CoV-2	AP01061	12.760	− 12.033	− 24.877	− 3.0
5	SARS-CoV	AP00549	− 39.313	0.206	− 1.009	− 6.9
6	SARS-CoV	AP01061	− 39.313	0.206	− 1.009	− 6.2
7	SARS-CoV-2	AP00180	12.760	− 12.033	− 24.877	− 3.7
8	SARS-CoV-2	AP00223	12.760	− 12.033	− 24.877	− 4.1

Table 4 Amino acid interactions of receptor nucleocapsid protein and their respective antimicrobial peptides

Viral strain	APD3 ID	Binding affinity (kcal/mol)	Receptor amino acids	AMP ligand amino acids	Bond category	Distance (Å)
SARS-CoV	AP00180	− 6.4	ALA110	GLU21	Hydrogen bond; Electrostatic	2.233
			LYS85	ARG31	Electrostatic	4.476
			ARG241	GLU21	Electrostatic	4.803
			ALA1	ASP87	Electrostatic	4.792
			ALA1	ASP834	Electrostatic	4.788
			ARG31	GLU14	Electrostatic	3.488
			LYS85	ARG13	Conventional hydrogen bond	1.759
			LYS88	TYR4	Conventional hydrogen bond	2.270
			GLN129	GLU21	Conventional hydrogen bond	2.177
			GLY132	SER17	Conventional hydrogen bond	2.796
			ARG227	THR7	Conventional hydrogen bond	3.086
			THR246	GLY24	Conventional hydrogen bond	2.504
			GLN247	GLY24	Conventional hydrogen bond	2.478
			LYS833	CYS3	Conventional hydrogen bond	2.889
			LYS833	CYS3	Conventional hydrogen bond	2.786
			ARG9	CYS10	Conventional hydrogen bond	3.398
			ARG13	ASP87	Conventional hydrogen bond	3.369
			LYS85	CYS30	Carbon hydrogen bond	3.500
			LYS85	ARG31	Carbon hydrogen bond	3.237
			ASP87	ARG31	Carbon hydrogen bond	3.658
			PRO115	THR2	Carbon hydrogen bond	3.468
			SER612	THR12	Carbon hydrogen bond	3.393
			THR12	SER612	Carbon hydrogen bond	3.005
			GLY18	THR128	Carbon hydrogen bond	3.604
			GLY18	GLN129	Carbon hydrogen bond	3.073
SARS-CoV	AP00223	− 6.1	ARG13	GLU111	Hydrogen Bond; Electrostatic	3.467
			LYS833	GLU14	Electrostatic	4.564
			LYS85	THR8	Conventional hydrogen bond	2.411
			ARG119	GLU14	Conventional hydrogen bond	2.104
			ARG227	VAL1	Conventional hydrogen bond	2.395
			GLN129	ARG15	Conventional hydrogen bond	3.182
			TYR22	GLU219	Conventional hydrogen bond	3.244
			ARG25	PRO611	Conventional hydrogen bond	3.263
			LYS114	PHE12	Carbon hydrogen bond	2.862
			ARG9	CYS10	Carbon hydrogen bond	3.552
SARS-CoV-2	AP00549	− 3.4	ASN94	GLN13	Conventional hydrogen bond	2.656
			ARG132	GLY1	Conventional hydrogen bond	2.637
			ARG132	PHE2	Conventional hydrogen bond	2.177
			ARG132	ASN5	Conventional hydrogen bond	2.891
			ASN196	ASN5	Conventional hydrogen bond	2.082
			GLN13	ASN371	Conventional hydrogen bond	2.870
			THR197	PRO198	Hydrophobic	5.198
			TRP8	PRO179	Hydrophobic	5.431
			TRP8	LEU170	Hydrophobic	5.338

Table 4 (continued)

Viral strain	APD3 ID	Binding affinity (kcal/mol)	Receptor amino acids	AMP ligand amino acids	Bond category	Distance (Å)
SARS-CoV-2	AP01061	− 3	LYS1	ASN1	Conventional hydrogen bond	2.945
			TYR29	ASN91	Conventional hydrogen bond	2.803
			ARG132	PRO3	Conventional hydrogen bond	2.855
			THR256	GLY5	Conventional hydrogen bond	2.852
			THR370	GLU24	Carbon Hydrogen bond	3.474
			ALA93	VAL27	Hydrophobic	4.214

SARS-CoV-2 -AP00549 was 1.473 nm, which was close to the average value (2.887 nm), demonstrating the stability of the SARS-CoV-2 -AP00549 complex throughout the 50 ns MD simulation.

RMSF determine the flexibility of any residue in the SARS -AP00180 and SARS-CoV-2 -AP00549 complex system. The fluctuation of the individual atom explained based on the RMSF values obtained from the 50 ns MD simulation of both the complex systems. The maximum, minimum, and average RMSF values for SARS -AP00180 were 1.354, 0.069 and 0.362 nm, respectively whereas, the maximum, minimum, and average RMSF values for SARS-CoV-2 -AP00549 were 3.08,0.069 and 0.386 nm, respectively can be seen in Table 5. The low average RMSF value suggested that individual atom exhibited stability in the dynamic state of both the systems throughout the MD simulation. The plot of the RMSF values vs. the atom number is shown in Fig. 5. The figures indicate that atoms of the SARS -AP00180 complex system at the positions of about 1500 ~ 3000 and 6500–7000 and, atoms of SARS-CoV-2 -AP00549 complex system at the positions of about 7500 ~ 9000 fluctuated somewhat relative to the others. The remaining atoms in both the complex systems were found to be quite stable during the MD simulation.

Rg for the SARS -AP00180 complex and SARS-CoV-2 -AP00549 complex were 2.634 nm and 1.744 nm, respectively. Rg values showed no change from the beginning of the simulation to the end of the simulation, demonstrating

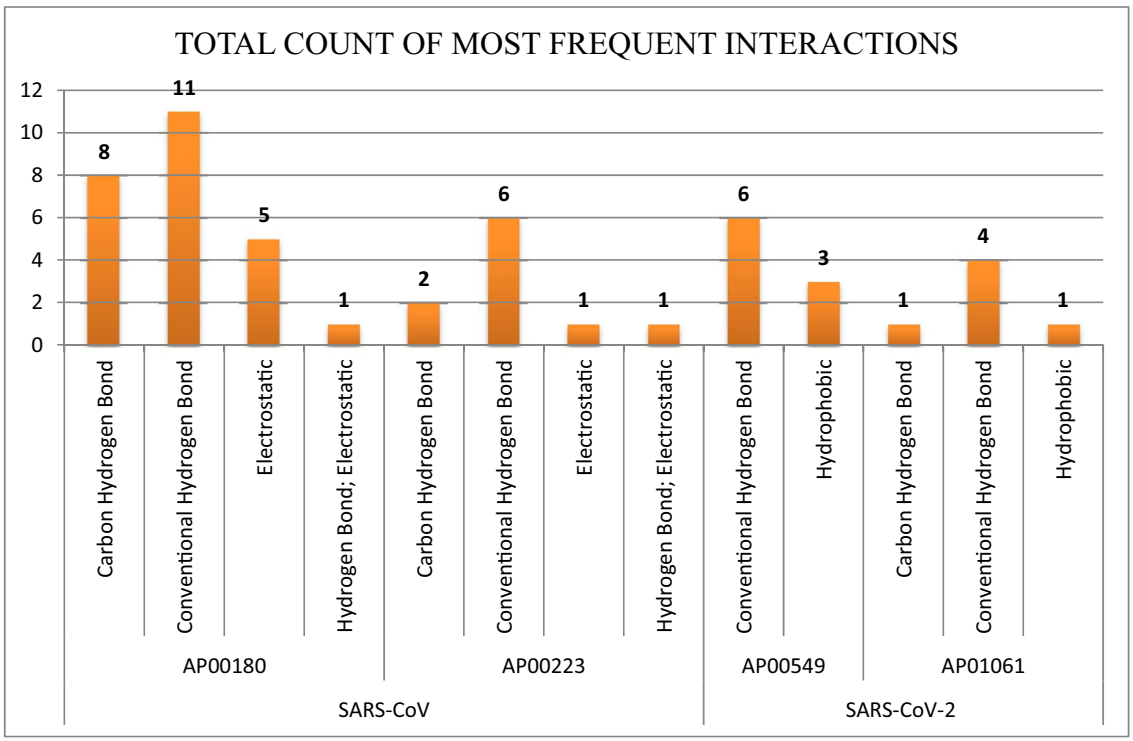


Fig. 2 Total count of most frequent interactions of SARS-CoV and SARS-CoV-2 with their respective AMPs

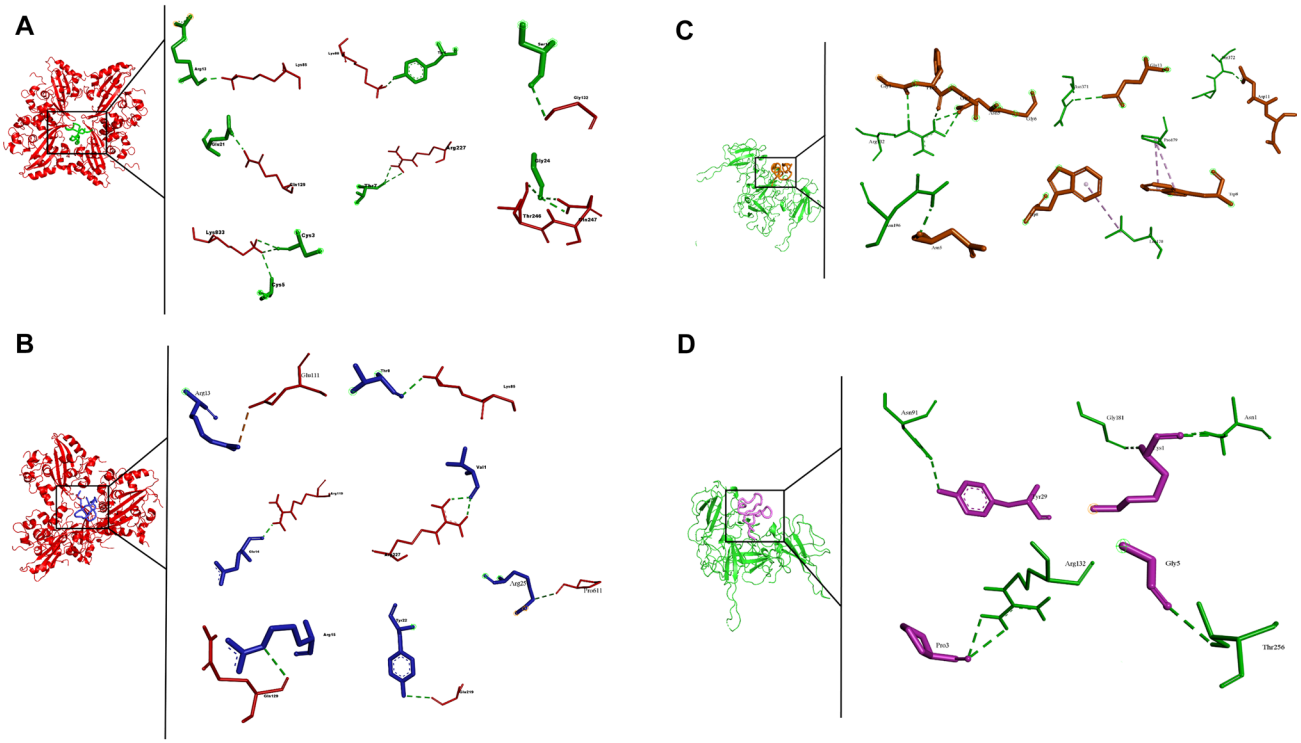


Fig. 3 Molecular docking simulation using Autodock Vina and interacting bonds (conventional hydrogen bonds: green; hydrophobic interactions: pink). **A** SARS nucleocapsid strain docked with AP00180, **B** SARS nucleocapsid strain docked with AP00223, **C** SARS-CoV-2 nucleocapsid strain docked with AP01061, **D** SARS-CoV-2 nucleocapsid strain docked with AP00549

that both the complexes remain stable throughout the 50 ns trajectory. The Rg values corresponding to the simulation shown in Fig. 5 were used to facilitate the interpretation of the secondary structure.

The hydrogen bonds were stable during the 15 ns run as seen in Fig. 7.

Conclusion

In the current work, we have employed a docking, scoring algorithm and dynamic simulation for combined peptide-protein binding search using an AMPs database. Our computational study confirms to provide two most suitable antimicrobial peptides which show positive interaction with nucleocapsid protein and may potentially hinder the activity of the viral protein. This nucleocapsid protein plays a major role in infecting a cell by RNA packaging and replication of the virus. Plectasin (AP00549) showed the lowest energy score (− 1183 kcal/mol) and highest favourability percentage (76.7%) making it efficient for SARS-CoV-2 viral intervention. Human Defensin-5 (AP00180) showed the lowest energy score (− 1063.7) and highest favourability percentage (92.9%) making it efficient for SARS. From our results, it may confirm that these AMPs may be suitable for inhibiting SARS-CoV-2, and SARS virus infection. With the simulation studies, we tried to create a natural environment for the peptide to interact with the protein. The stability of the molecules was examined based on the RMSD, RMSF and radius of gyration values. The difference between the highest and lowest RMSD values of the SARS-CoV-2-AP00549 complex and SARS-AP00180 complex determined that the backbone did not fluctuate throughout the 50 ns MD simulation. For RMSF, the maximum, minimum and average

Table 5 Energy minimization values of the final two docked complexes

Viral strain	APD3 ID	Steepest Descents converged to Fmax < 1000	Potential Energy (kJ/mol)	Maximum force	Norm of force
SARS	AP00180	1840 steps	− 4.4924025e + 06	9.9176465e + 02 on atom 9544	1.1767879e + 01
SARS-CoV-2	AP00549	1758 steps	− 2.7867188e + 06	9.3732727e + 02 on atom 1880	1.1762753e + 01

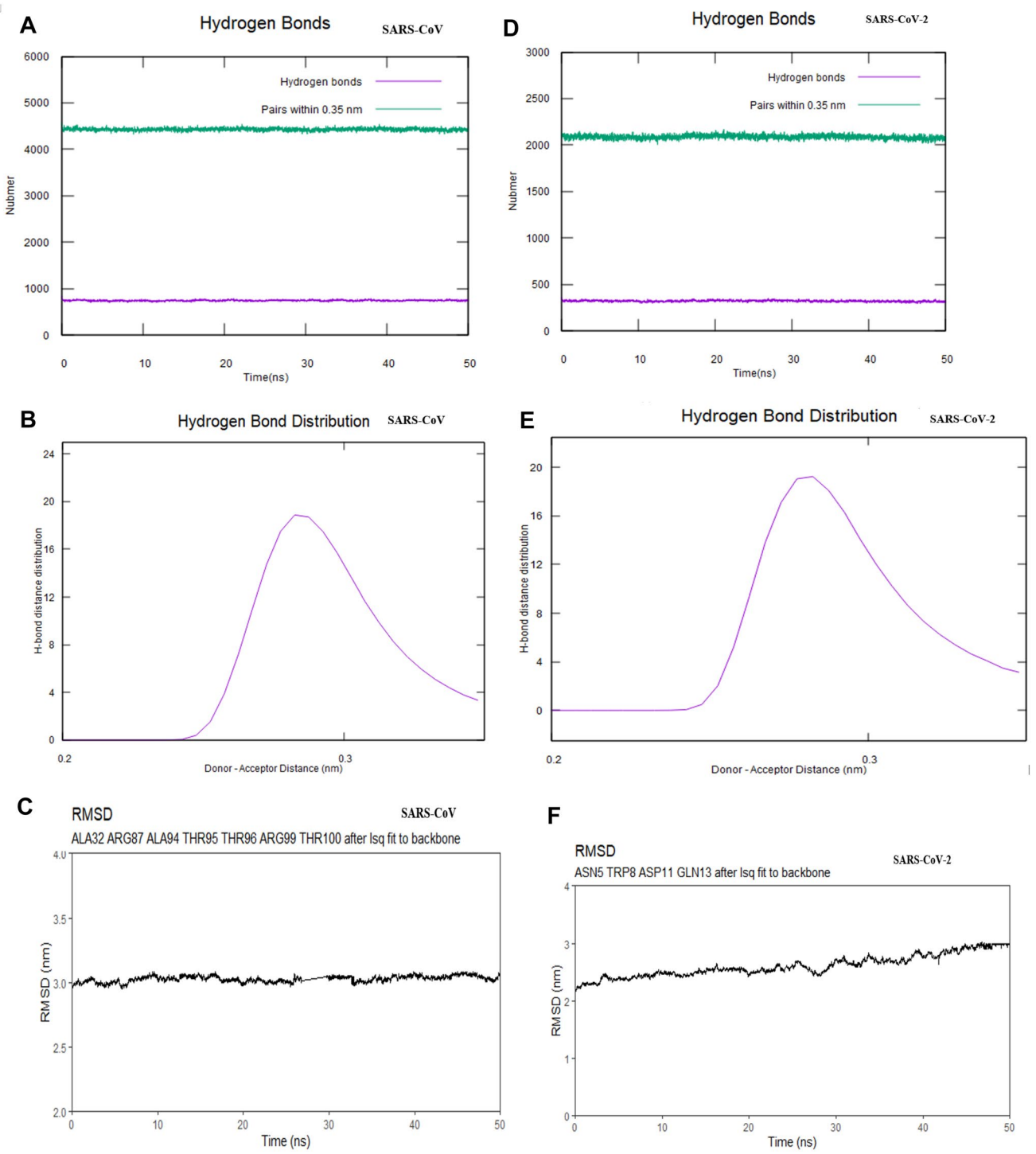


Fig. 4 Molecular dynamic simulation of SARS with AP00180and SARS- CoV-2 with AP00549, (**A**, **D**) number of hydrogen bonds and pairs within 0.35 nm, (**B**, **E**) Distribution of H bonds across the interaction, (**C**, **F**) RMSD of the docked complex

values were used to determine the overall stability of the docked structure and as for Rg values, the complexes exhibited no change from the beginning of the simulation to the end of the simulation, demonstrating the stability of both the complexes throughout the 50 ns trajectory. In-silico studies suggest a potent and successful interaction with the active site of nucleocapsid with the antimicrobial peptide. These molecules can be further taken for the next step of research and all in vitro experiments will be carried out at room temperature that is the reason for conducting MD simulations

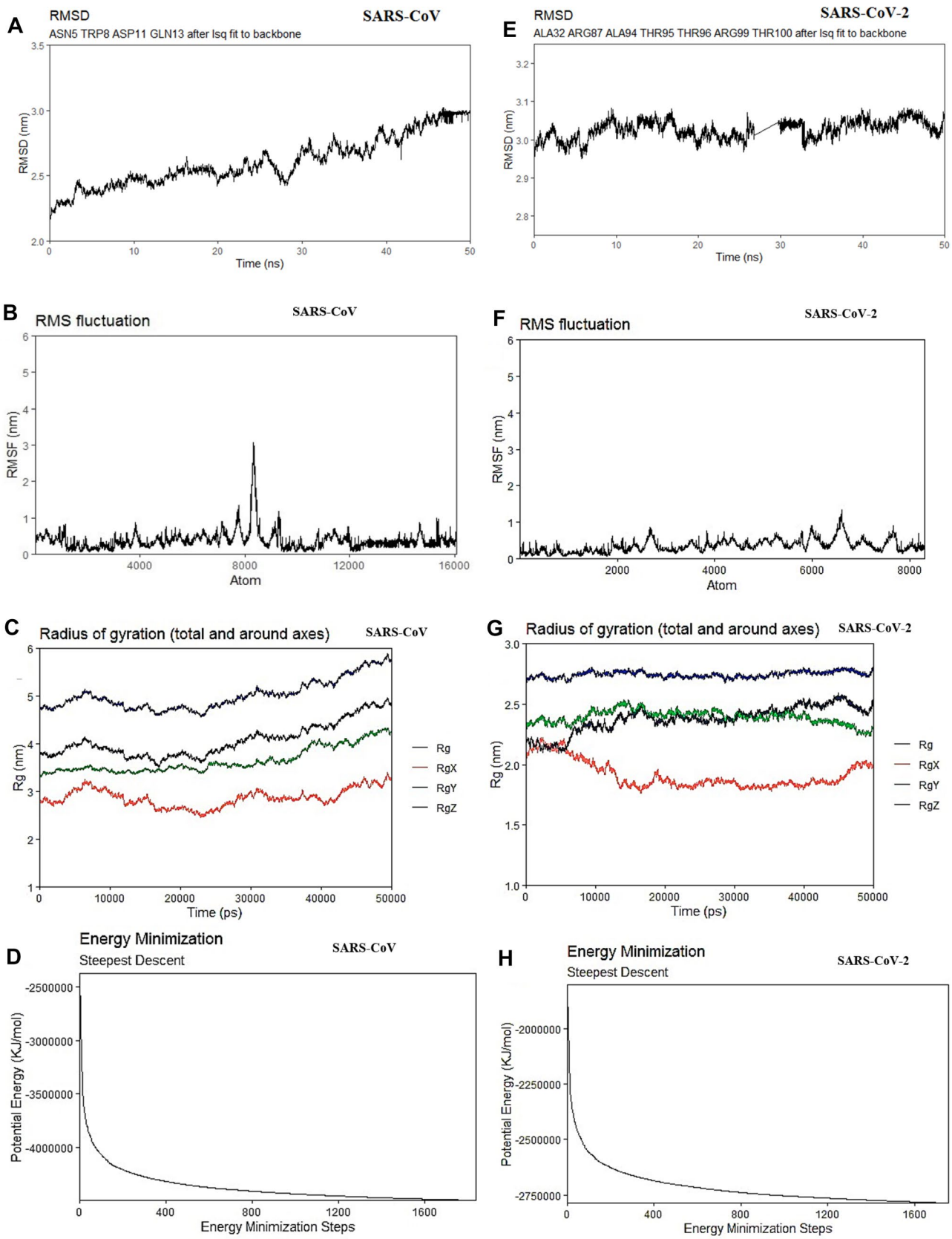


Fig. 5 Molecular dynamic simulation plots by GROMACS. RMSD, RMSF, Radius of gyration and Energy Minimization for SARS-AP00180 (**A**–**D**) and SARS-CoV-2-AP00549 (**E**–**H**) respectively

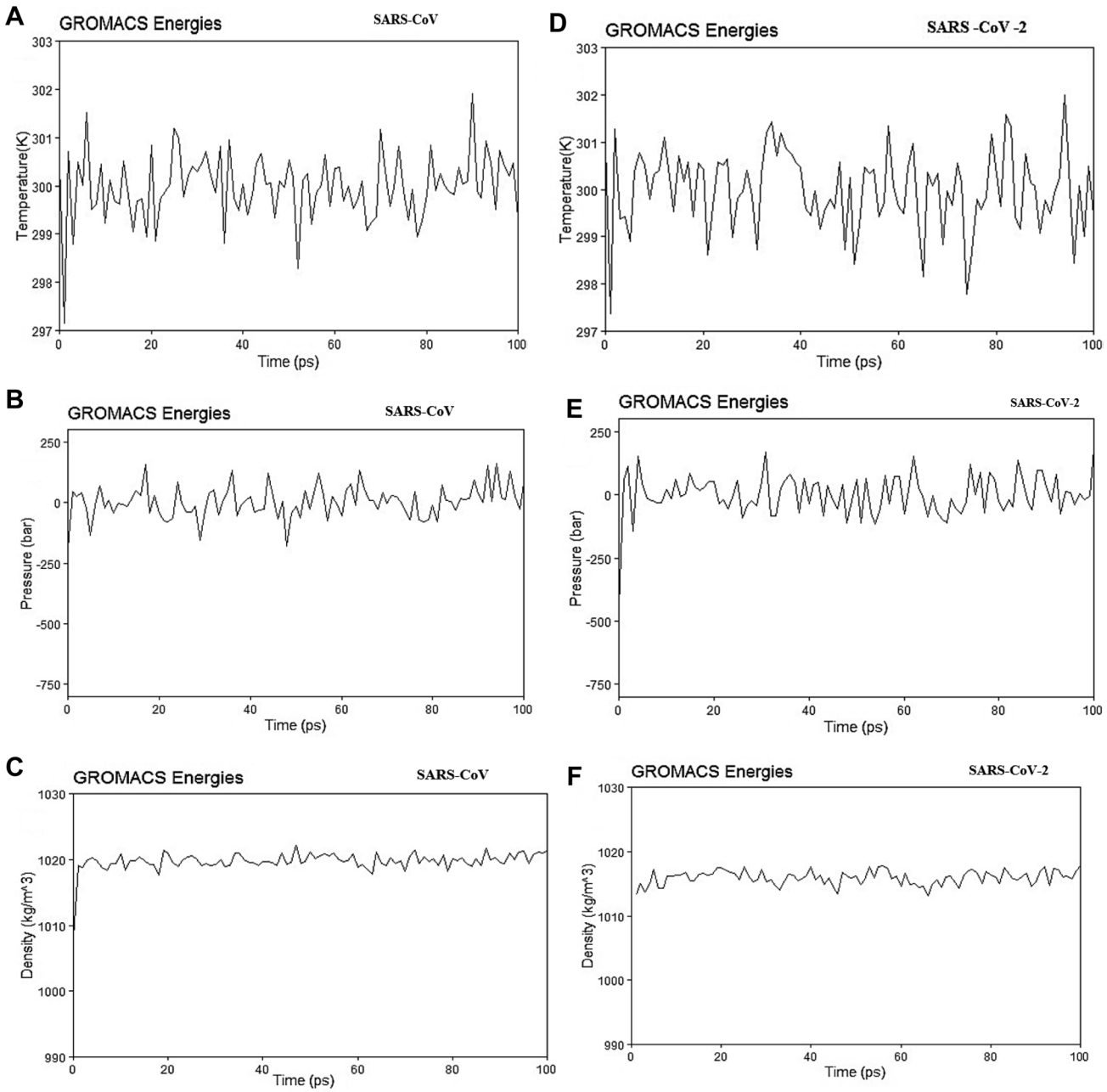


Fig. 6 Molecular dynamic simulation plots by GROMACS. Temperature, pressure, and density for SARS-AP00180 (**A**–**C**), SARS-CoV-2-AP00549 (**D**–**F**) respectively

Table 6 Maximum, minimum, and average values of SARS and SARS-CoV-2

		RMSD (nm)	RMSF (nm)	Rg (nm)
SARS-CoV-2	Maximum	3.499	3.082	1.805
	Minimum	2.026	0.069	1.646
	Average	2.887	0.386	1.744
SARS	Maximum	2.781	1.354	2.693
	Minimum	1.357	0.069	2.527
	Average	2.273	0.362	2.634

at room temperature. Other complementary experiments also carried out at the same temperature so that the structures and energies from different MD simulations can be collected into a single ensemble for analysis. The structure and interaction insights with the AMPs provided in this study may provide a deep understanding for developing anti-SARS-CoV-2 inhibitors. However, the results are preliminary and certainly need experimental validation using in vitro and in vivo studies. Specific assays are needed to confirm the mechanism of action.

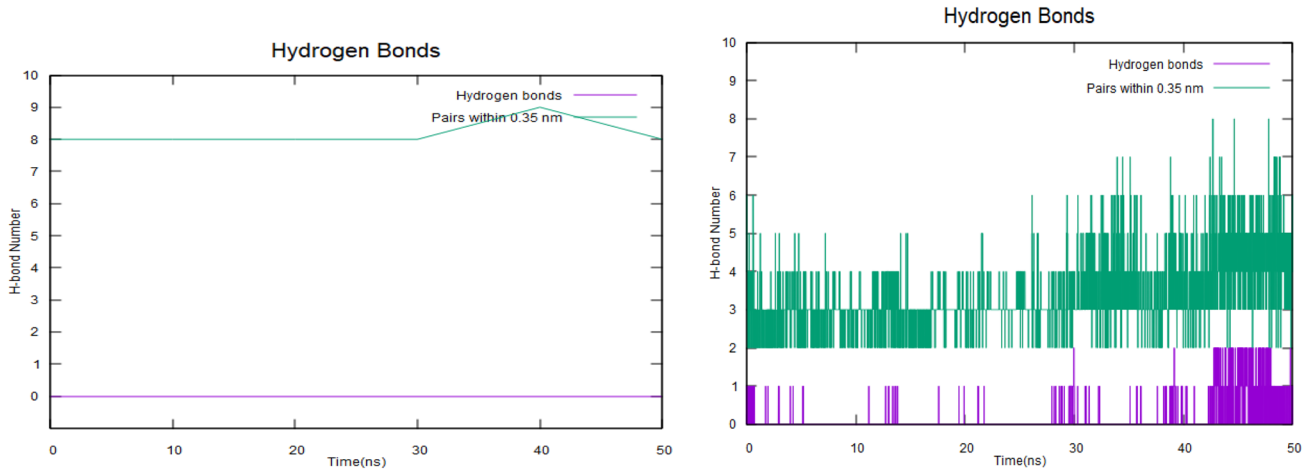


Fig. 7 Molecular Dynamic simulation of SARS-CoV and SARS-CoV-2: Hydrogen bonds of the docked complexes represent the number of hydrogen bonds as a function of time. Pairs within 0.35 nm represent—the number of atom pairs that satisfy the distance criterion but not the angle criterion for hydrogen bonding

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Declarations

Conflict of interest The authors declare that they have no conflict of interests.

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