

# Structural Insights Into the Design of Peptide-based Drug or Diagnostic Reagent Antagonizing SARS-COV-2

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## Research article

**Keywords:** SARS-CoV-2, 2019-nCoV, COVID-19, ACE2

**Posted Date:** August 19th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-45305/v1>

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# Abstract

## Background

Very limited drug and diagnostic reagents are currently available to tackle the emergence of SARS-CoV-2. However, recent findings about the structure of the complex about PD of ACE2 and RBD of SARS-CoV-2 spike protein hold great promise for the design of novel vaccines and peptides. To provide some suggestions for the design of peptide-based drug or diagnostic reagents antagonizing SARS-CoV-2, and describe the interactions between the receptor-binding domain of SARS-CoV-2 and PD domain of its receptor, ACE2.

## Methods

Based on the PD-RBD crystal structure, the molecular interaction details of PD-RBD was contrasted.

## Results

Amino acid mutations located in RBM of SARS-CoV result in the formation of new interactions between SARS-CoV-2 and  $\alpha$ -helix 1, which can increase the binding affinity of SARS-CoV-2 to ACE2. It is confirmed that the  $\alpha$ -helix 1 on ACE2 is the most important domain for binding spike glycoprotein of SARS-CoV-2, which can be used as a leading peptide for drug and diagnostic reagents development.

## Conclusion

Based on the molecular-level characterization analysis between the PD and RBD, severe important amino acid residues (Q24, T27, K31, and H34) on  $\alpha$ -helix 1 are proposed to mutate into increasing the binding affinity. Although the information provided in this study is predictive and based on no experimental evidence, it may provide useful suggestions for the experimental scientists to synthesize the proposed peptide and test their binding affinity and blocking capacity, and may be helpful for the understanding of SARS-CoV-2 entry.

## Background

The coronavirus disease (COVID-19) is a newly emerged disease caused by a novel coronavirus designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously provisionally named 2019-nCoV.[1] COVID-19 has been declared as the sixth public health emergency of international concern on January 30, 2020, by WHO, and caused a major public health issue in China. As of April 1, 2020, more than 900,000 confirmed cases have been identified. As a typical RNA virus, the genome sequences of SARS-CoV-2 are more closely related (with 88% identity) to bat-SL-CoVZC45 and bat-SL-CoVZXC21 than SARS-CoV (about 79%) and MERS-CoV (about 50%).[2, 3] More, structural analysis

reveals that the receptor-binding domain (RBD) structure of spike glycoprotein in SARS-CoV-2 is similar to that of SARS-CoV.[4] The RBD of SARS-CoV is known to be essential for the binding of the virus to the human angiotensin-converting enzyme 2 (ACE2) receptor at the advent of the infection processes.[5] Recent studies confirm that SARS-CoV-2 also can infect the human respiratory epithelial cells using the same cell entry receptor, ACE2, as SARS-CoV.[6, 7]

ACE2 is a zinc metalloproteinase and one of the key regulators of the renin-angiotensin system (RAS).[8] ACE2 plays a counter-regulatory manner to ACE, which can modulate the balance between vasoconstrictors and vasodilators within the heart and kidney, and play an important role in regulating cardiovascular and renal function.[9] Except for the presence in vascular endothelium, ACE2 is a type I membrane protein expressed in the epithelia of the lung, kidney and small intestine, which might provide possible routes of entry into the SARS-CoV and SARS-CoV-2.[10–12] ACE2 is a type I integral membrane glycoprotein orientated and consists of a C-terminal collectrin-like domain (CLD) and an N-terminal peptidase domain (PD).[13] Besides, to provide the catalytic site facing the extracellular space, the PD of ACE2 also provides the direct binding site for the spike proteins of SARS-CoV and SARS-CoV-2.

The entry of SARS-CoV-2 into host cells is mediated by spike glycoprotein, which makes it an attractive target for the design of antiviral agents and vaccines (entry inhibitors). The crystal structure of the ACE2 and co-crystallization of the PD-RBD complex has revealed the molecular details of the interaction between the PD of ACE2 and RBD of S protein of SARS-CoV.[14–18] Notably, most-recent *bioRxiv* preprints report the structure of dimeric full-length human ACE2 and structures of the complex about PD of ACE2 and S protein of SARS-CoV-2.[19–21] Another significant progress also has been made in the structure determination of the SARS-CoV-2 spike in the prefusion conformation.[22] More importantly, the structure of SARS-CoV-2 chimeric receptor-binding domain complexed with its receptor human ACE2 was released (PDB ID: 6VW1). The co-crystallization of the PD-RBD complex has been resolved, which is facilitating SARS-CoV-2 research.

In a previous study, several peptides representing various regions of ACE2 critical to virus infection are chemically synthesized. The  $IC_{50}$  of three peptides (a.a. 22–44, 22–57, 22–44 and 351–357 linked together by glycine) for antiviral activity of SARS-CoV are 50  $\mu$ M, 6  $\mu$ M and 0.1  $\mu$ M, respectively.[23] Besides, several peptides targeting the main protease of SARS coronavirus are designed by computer-aided molecular modeling.[24–26] These studies suggest that the peptide may be a promising candidate as a therapeutic agent against SARS-CoV-2.

The affinity with ACE2 to the RBD of 2019-nCoV is 10–20 times higher than that of the RBD of SARS-CoV.[21] Interestingly, CR3014 and m396, the SARS-CoV-specific human monoclonal antibody targeting the ACE2 binding site of SARS-CoV, failed to bind SARS-CoV-2 spike protein.[27] There are differences in the molecular details of the interaction between SARS-CoV and SARS-CoV-2. It is still necessary to develop a novel peptide against SARS-CoV-2.

Based on co-crystallization structure analysis, the interaction region of PD was narrowed down to three amino acid fragments (the  $\alpha$ -helix 1, loop 2 and  $\beta$ -sheet 5), which binds the RBD of SARS-CoV-2 (residues 399–521) with greater affinity. The results of alanine scanning mutagenesis analyses of ACE2 revealed that several amino acid residues in the  $\alpha$ -helix 1 are important to virus infection. Through the modification of  $\alpha$ -helix 1, novel peptides with higher affinity may be discovered. In this study, we applied the computer-aided molecular simulation methods to analyze the molecular-level characterization of protein-protein association for the design of peptide-based entry inhibitors. We hope that this work will be helpful for the understanding of SARS-CoV-2 entry and the discovery of effective antiviral agents against the virus to address the ongoing public health crisis.

## Materials And Methods

The X-ray structures (PDB ID: ZAJF) of the SARS-CoV spike receptor-binding domain complexed with ACE2 receptor deposited in the Protein Data Bank (PDB) was used for comparative analysis[18]. Important progress also has been made in the research about the crystal structure of the SARS-CoV-2 spike RBD bound to the ACE2 receptor, and those crystal structure data have been disclosed on the Internet (<https://www.jianguoyun.com/p/DTJ03HoQ3MX2BhjiwN8C>; <http://nmdec.cn/?from=groupmessage#/resource/detail?no=NMDCS0000001>; <https://www.jianguoyun.com/p/DVLSdMkQiJ2OCBjbwt8C>). These structural data were used for the preliminary analysis of this study. The recently published crystal structure (PDB ID: 6VW1) was selected for the formal study. Those proteins were subsequently prepared by the Prepare Protein protocol using the Discovery Studio 2017R2 software package (DS; <https://www.3ds.com/>).

The  $\alpha$ -helix 1, loop 2 and  $\beta$ -sheet 5 with the conformation in the crystal structure were defined as a single ligand fixed at the binding interface, respectively. To understand the interactions between them and RBD, the View Interactions tools in DS was used to analyze the interactions between protein residues. Favorable interactions, such as hydrogen bonds, hydrophobic interactions, and electrostatic attractions, were analyzed. Receptor surfaces (interpolated charge surface, hydrophobic surface, and hydrogen bond donor/acceptor surface), which provided a unique insight into the inner workings of a receptor, were created on a receptor close to the current ligand in DS.

The PyMol,[28] a molecular visualization system on an open-source foundation, was used for preparing structural figures to illustrate the details of the interaction of spike protein complexed with ACE2.

## Results And Discussion

### Molecular-level characterization of protein-protein association for the PD and RBD

The in-depth genome annotation has revealed differences between SARS-CoV-2 and SARS-CoV, which may have caused more than 300 amino acid substitutions between these coronaviruses.[29] In total,

there were 28 amino acid substitutions between the amino acid sequences of SARS-CoV-2 (residues 336–516) and the corresponding consensus sequences of SARS-CoV (residues 323–502). Notably, no acid substitutions occurred in the region away from the binding surface, which may have little or no effect on the binding affinity. Respectively, 28 amino acid substitutions were all located in the receptor-binding motifs (RBM, residues 438–506) that directly interact with human receptor ACE2 protein, accounting for 41% of the total amino acids in this region (Fig. 1). These mutations could cause great changes in the binding affinity between the PD of ACE2 and the RBD of spike protein, which may cause pathogenic divergence of SARS-CoV-2 and results in the failure of the antibody targeting the binding surface (e.g. CR3014 and m396).

Close to half of the amino acid mutations in the RBM domain result in a significant increase in the affinity of SARS-CoV-2 to ACE2 compared with SARS-CoV, which also leads to greater infectivity in SARS-CoV-2. The molecular details of the interaction between the PD of ACE2 and RBD of S protein show that there are more interactions between SARS-CoV-2 and ACE2 (Fig. 2 and Fig. 3). The number of hydrogen bonds between SARS-CoV-2 and ACE2 is 9 (Fig. 2), which is close to twice the number between SARS-CoV and ACE2 (5). Similar to SARS-CoV, the  $\alpha$ -helix 1 (residues 19 and 20 are defined as components of the area), loop 2 and  $\beta$ -sheet 5 on ACE2 have been identified to be important for binding spike glycoprotein of SARS-CoV-2.[30] No changes in interaction occurred in loop 2, although the residues F486 in SARS-CoV was substituted by L472 in SARS-CoV-2 (Fig. 2C and Fig. 4B). Besides, no significant change in the interaction model was found in  $\beta$ -sheet 5 (Fig. 2D and Fig. 4C). Notably, amino acid substitutions located in RBM of SARS-CoV result in the formation of five hydrogen bonds, one electrostatic attraction and one hydrophobic interaction between SARS-CoV-2 and  $\alpha$ -helix 1 in ACE2, which does not exist in the interaction between SARS-CoV and ACE2.

In general, the substitution of amino acids leads to the increase of infectivity of SARS-CoV-2 by increasing the interaction between the RBM of spike protein and  $\alpha$ -helix 1 on the PD of ACE2. The peptide-based on the structure of  $\alpha$ -helix 1 may be a promising candidate as a diagnostic reagent or therapeutic drug against COVID-19.

## Characterization of interaction surface between the $\alpha$ -helix and RBD

The electrostatic attraction is one of the predominant forces in protein-protein interactions. Interestingly, the number of charged amino acids accounts for 26% of the  $\alpha$ -helix 1. The basic amino acid residues and acid amino acid residues formed obvious positive and negative charge areas on the surface of the intercalated charge of  $\alpha$ -helix 1, respectively (Fig. 5A). In contrast, there are only two charged amino acids (K403, E484) of RBD in the interaction surface (Fig. 5B). A weak attractive charge interaction existed between K31 and E484 with a 5.33 Å charge-charge distance. Besides, no static attraction was formed between K403 and the residues on  $\alpha$ -helix 1.

As a protein surface, most of the RBM region was hydrophilic. Two amino acids (F456 and Y489) in the hydrophobic region participate in the hydrophobic interaction with K31 on  $\alpha$ -helix 1, respectively. The  $\alpha$ -

helix 1, a component of ACE2 protein, was located on the protein surface. The binding surface of  $\alpha$ -helix 1 (Fig. 6, underside) located on the protein surface, which is hydrophobic. On the contrary, the upper surface is hydrophobicity, which may affect the stability of the polypeptide. Therefore, the hydrophilicity of the upper surface may be considered in the process of peptide design and development. It is worth noting that T27 and D30 located upper the hydrophobic region did not form hydrophobic interaction with the  $\alpha$ -helix 1.

## Peptide design suggestion based on the structure of $\alpha$ -helix 1

There are 34 residues on the  $\alpha$ -helix 1 (residues 19–52). Interestingly,  $\alpha$ -helix 1, like a centipede, holds the RBD tightly with eleven feet (S19, Q24, T27, D30, K31, H34, E35, E37, D38, Y41 and Q42) colored by element (Fig. 7). Based on the analysis of the interaction mechanism, the following aspects of structural transformation on the “feet residues” may be effective. (1) Because of the synthesis difficulty of length peptide, the residues (residues 45–52) located outside the binding region with RBD (Fig. 7) could be deleted. Besides, the effect of amino acid deletion on the stability of helix needs further study. (2) D30 is close to the hydrophobic region of RBD; it can be replaced by the nonpolar amino acids (e.g. Met, Val, Ile, Leu) to increase hydrophobic interaction. (3) T27 is located in a hydrophobic pocket which can be replaced by the nonpolar amino acids, such as Phe, Met, Val, Ile, Leu. (4) To increase the electrostatic attractions, K31 may be replaced by the basic amino acids (Arg or His) to reduce the distance between positive and negative charges. (5) To form the new electrostatic attractions, H34 may be replaced by another acid amino acid (Asp or Glu). Furthermore, to balance the hydrophilicity of peptides, polar amino acids were used instead of some residues which do not interact with RBD (Fig. 7, colored in red).

## Conclusions

Both SARS-CoV-2 and SARS-CoV are attached to the ACE2 cell receptor by spike protein, which is mediated by RBD of the spike protein. Recently, the structure of the complex between the PD of ACE2 and RBD of SARS-CoV-2 spike protein was reported. Based on the PD-RBD crystal structure, this article contrasts the molecular interaction details of PD-RBD, aiming at identifying the affinity difference between SARS-CoV-2 and SARS-CoV. Notably, close to 50% of amino acid residues located in the RBM is mutated (compared with SARS), which cause pathogenic divergence of SARS-CoV-2. Similar to SARS-CoV, the  $\alpha$ -helix 1, loop 2 and  $\beta$ -sheet 5 on ACE2 have been identified to be important for binding spiker glycoprotein of SARS-CoV-2. Amino acid substitutions located in RBM of SARS-CoV result in the formation of new interactions between SARS-CoV-2 and  $\alpha$ -helix 1 in ACE2, which does not exist in the interaction between SARS-CoV and ACE2. The  $\alpha$ -helix 1 on ACE2 is a critical domain for virus infection.

Peptides representing various regions of ACE2 critical for virus infection are chemically synthesized, and showed moderate antiviral activity of SARS-CoV. Based on the molecular-level characterization analysis between the PD and RBD, our study gives some suggestions for peptide design. Peptides representing the  $\alpha$ -helix 1 may have a better binding affinity to SARS-CoV-2 than that of SARS-CoV. The severe amino acid

on  $\alpha$ -helix 1 may be proposed to mutate to increase the affinity. Q24 may be replaced by the nonpolar amino acids to increase hydrophobic interaction. T27 located in a hydrophobic pocket may be replaced by the nonpolar amino acids. To increase the electrostatic attractions, K31 may be replaced by the basic amino acids with longer side chains to reduce the distance between positive and negative charges. To form the new electrostatic attractions, H34 may be replaced by another acidic amino acid. Besides, to balance the hydrophilicity of peptides, polar amino acids may be used instead of some residues, which do not interact with RBD.

The chemical properties and stability, biological activity and reactivity, as well as the practical aspects of synthesis, are challenges in the development of peptide drugs. In addition, the variation of the virus may lead to the structure change of RBD, and then affect the binding of peptide targeting the RBD. We only provide some suggestions on the design of peptide-based drug or diagnostic reagents antagonizing SARS-CoV-2, hoping to be helpful for the understanding of SARS-CoV-2 entry and discovery of effective antiviral agents against the virus to address the ongoing public health crisis.

## Abbreviations

COVID-19

Coronavirus disease; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; RBD: Receptor Binding Domain; ACE2: Angiotensin Converting Enzyme 2; RAS: Renin Angiotensin System; CLD: Collectrin Like Domain; PD: Peptidase Domain; PDB: Protein Data Bank; DS: Discovery Studio; RBM: Receptor Binding Motifs; VDW: Van Der Waals

## Declarations

### Acknowledgements

Not applicable.

### Authors' contributions

JHJ drafted the manuscript. PD revised the manuscript. All authors read and approved the final manuscript.

### Funding

Research reported in this paper was supported by grant ZHYX2019022 awarded by the Chongqing Medical University (to PD).

### Availability of data and materials

All data are available from the corresponding author by request.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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Received: 13 July 2020; Accepted:

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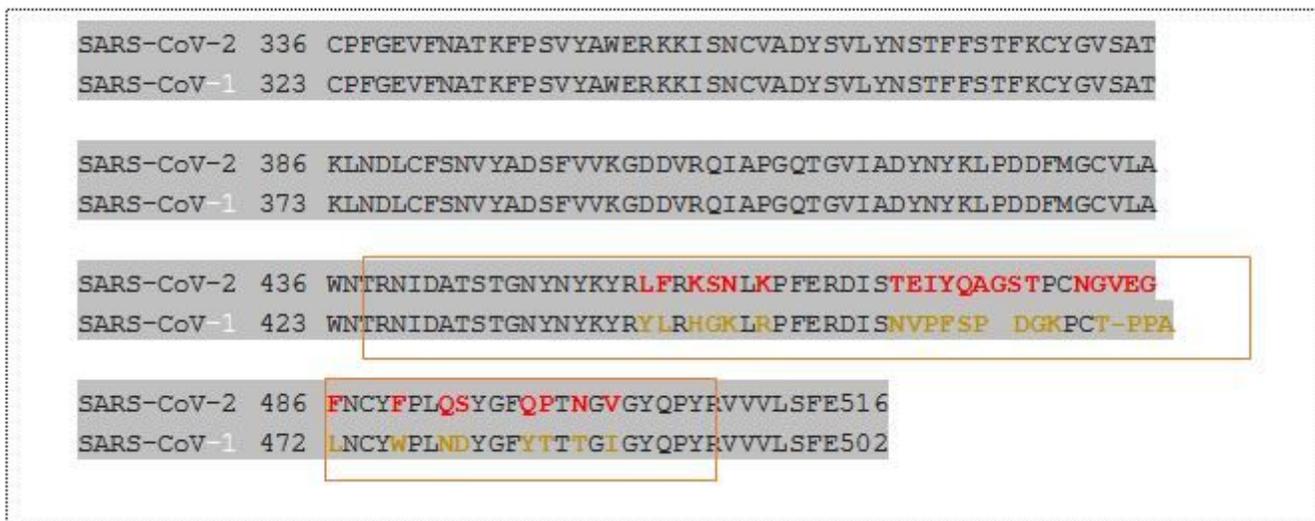
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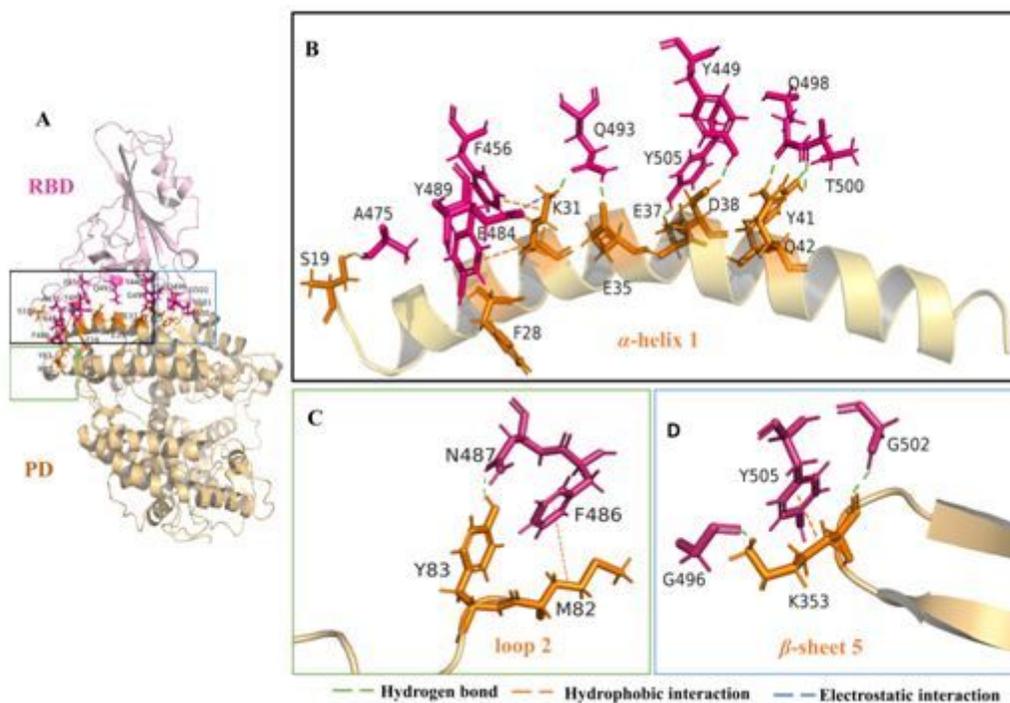
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## Figures



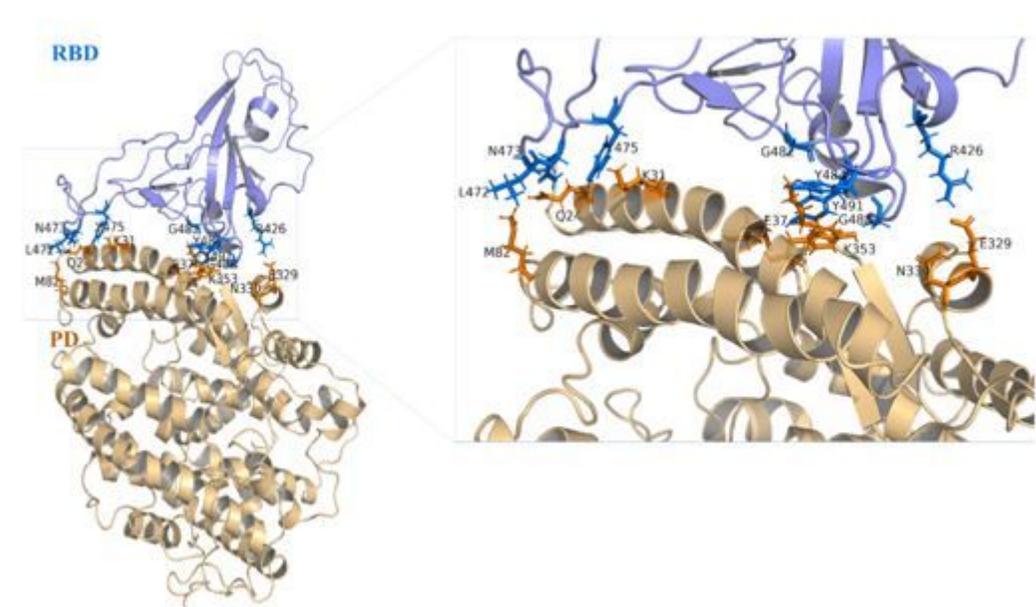
**Figure 1**

Amino acid sequence alignment of SARS-CoV-2 (PDB ID: 6VW1) and SARS-CoV (PDB ID: 2AJF). The background of the identical residues is shown in blue. Inconsistent residues are colored with red (SARS-CoV-2) and yellow (SARS-CoV), respectively. The residues in the orange frame are located in the receptor-binding motifs (RBM) domain.



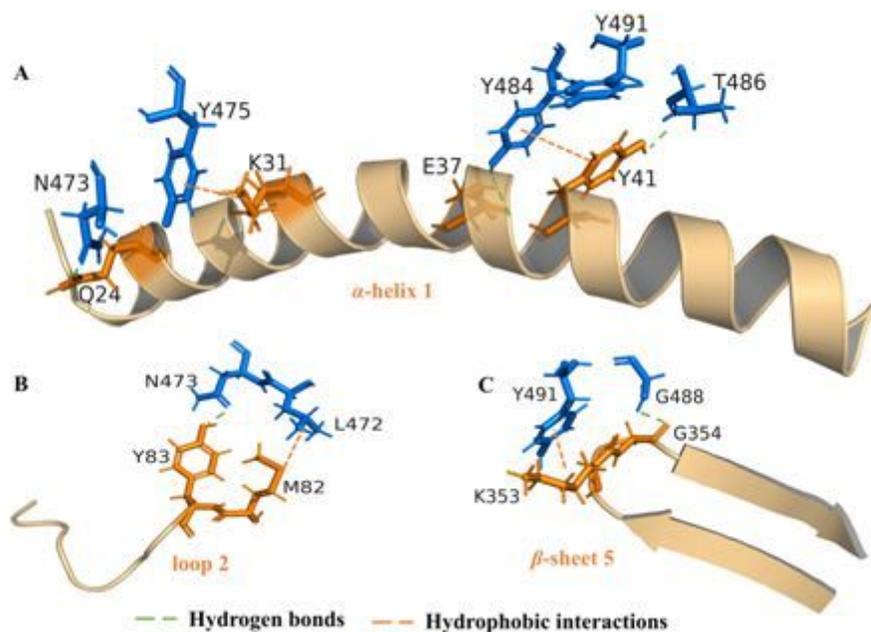
**Figure 2**

(A) The molecular details of the interaction between the PD of ACE2 and RBD of S protein of SARS-CoV-2 (PDB ID: 6VW1). RBD, the receptor-binding domain of S proteins of SARS-CoV-2, colored in red. PD, the peptidase domain at N-terminal of ACE2, colored in orange. (B) Hydrogen bonds (S19-A475, K31-Q493, E35-Q493, D38-Y449, E37-Y505, Y41-T500, and Q42-Q498), hydrophobic interactions (K31-Y489 and K31-F456) and electrostatic attractions (K31-E484) between  $\alpha$ -helix 1 and the residues on RBD of SARS-CoV-2, which is significantly stronger than that of SARS-CoV (Figure 4A). (C) Only one hydrogen bond (Y83-N487) and one hydrophobic interactions (M82-F486) are formed between loop 2 and the residues on RBM of SARS-CoV-2, which are the same as that in SARS-CoV (Figure 4B). (D) Hydrogen bonds (K353-G496, K353-G502) and hydrophobic interactions (K353-Y505) between  $\beta$ -sheet 5 and the residues on RBM of SARS-CoV-2, which is similar to that in SARS-CoV (Figure 4C).



**Figure 3**

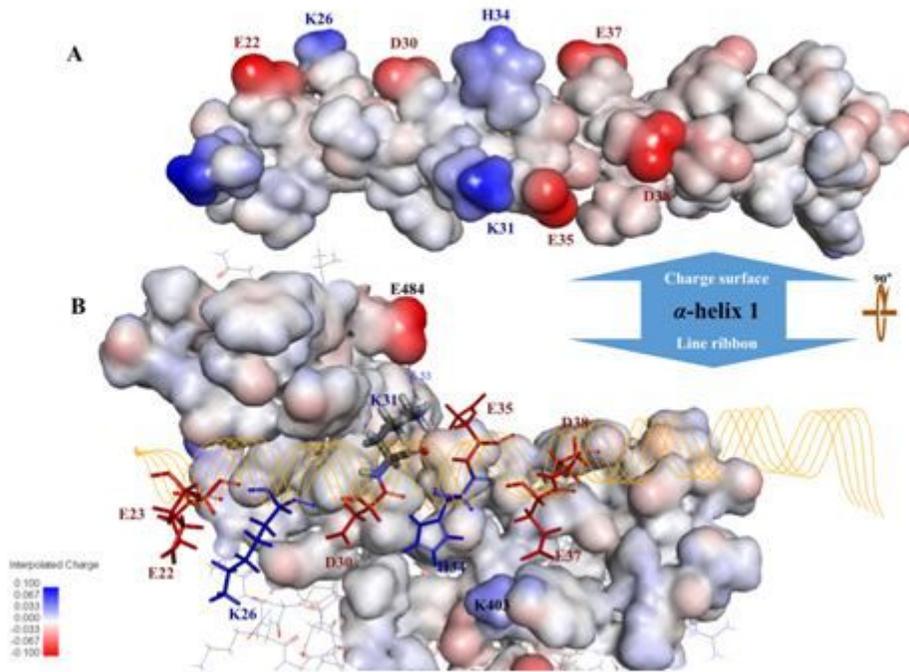
The interaction between the PD of ACE2 and RBD of S protein of SARS-CoV (PDB ID: 2AJF). RBD, the receptor-binding domain of S proteins of SARS-CoV-2. PD, the peptidase domain at N-terminal of ACE2.



**Figure 4**

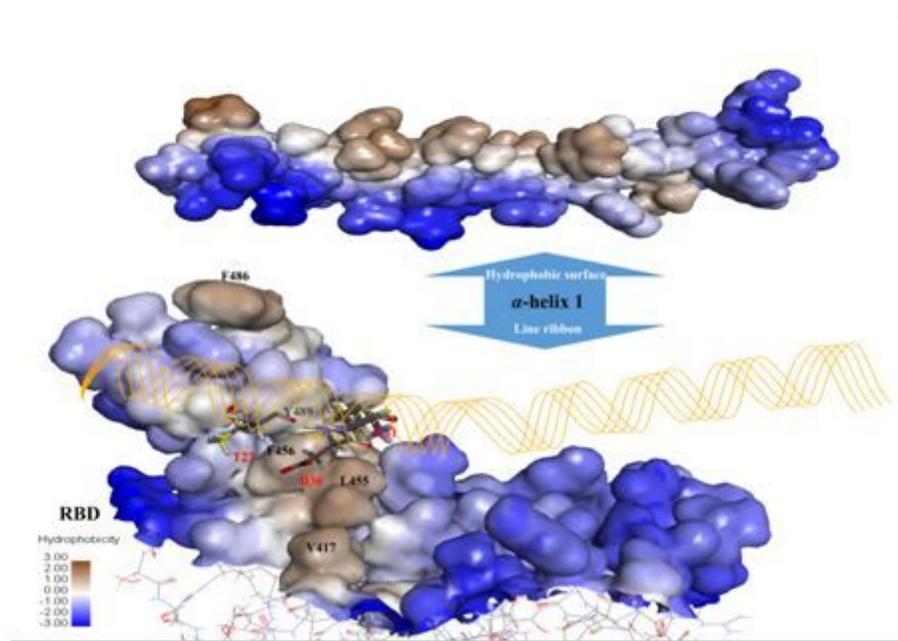
The molecular details between the PD and RBD (PDB ID: 2AJF). (A) Hydrogen bonds (Q24-N473, E37-Y484, and Y41-T486) and hydrophobic interactions (K31-Y475 and Y41-Y484) between  $\alpha$ -helix 1 and the residues on RBM of SARS-CoV. (B) One hydrogen bond (Y83-N473) and one hydrophobic interaction

(M82-L472) are formed between loop 2 and the residues on RBM. (C) One hydrogen bond (G354-G488) and one hydrophobic interaction (K35382-Y491) are formed between  $\beta$ -sheet 5 and the residues on RBM.



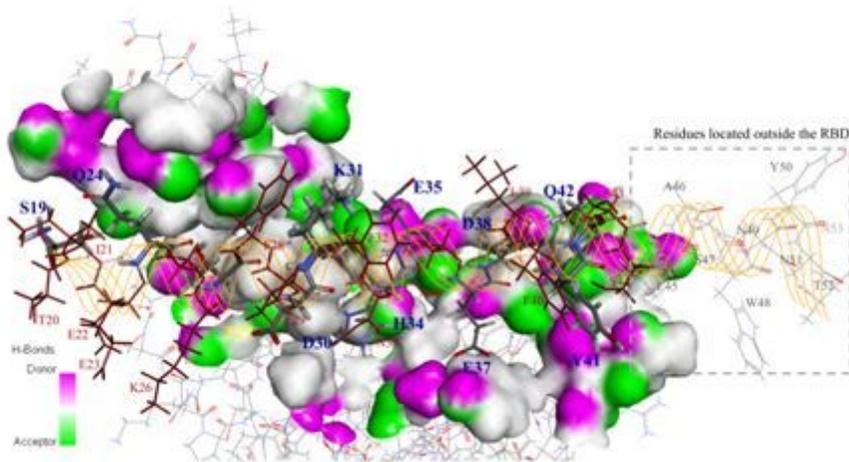
**Figure 5**

(A) The VDW (the surface is based on the van der Waals radius of each atom in the molecule) interpolated charge surface of  $\alpha$ -helix 1 on PD of ACE2; blue represents positive charge and red refers to negative charge. (B) The VDM interpolated charge surface of RBD of SARS-CoV-2; Nine charged residues on  $\alpha$ -helix 1 (residues E22, E23, K26, D30, K31, H34, E35, E37, and D38) were situated above the RBD; blue represents positive charge and red refers to negative charge. Basic and acidic amino acid residues are shown by blue and red colors, respectively. The  $\alpha$ -helix 1 is displayed with an orange line ribbon.



**Figure 6**

The VDM hydrophobic surface of  $\alpha$ -helix 1 and RBD of S protein of SARS-CoV-2; blue represents positive charge and red refers to negative charge. The surface colored by the hydrophobicity of the receptor residues, from blue for hydrophilic to brown for hydrophobic. The yellow line ribbon presents the  $\alpha$ -helix 1.



**Figure 7**

Residues on  $\alpha$ -helix 1 and its relative position in the binding region. RBD is shown with the VDM hydrogen bond donor/acceptor surface, which is colored by hydrogen bond type, with receptor donors colored in magenta and receptor acceptors in green. The orange line ribbon presents the  $\alpha$ -helix 1.