COVID-19:Attacks the 1-Beta Chain of Hemoglobin and Captures the Porphyrin to Inhibit Human Heme Metabolism

Wenzhong Liu [1,2,*], Hualan Li[2]

[1] School of Computer Science and Engineering, Sichuan University of Science & Engineering, Zigong, 643002, China;
[2] School of Life Science and Food Engineering, Yibin University, Yibin,644000, China;
[*] Correspondence: liuwz@suse.edu.cn;

Abstract

The novel coronavirus pneumonia (COVID-19) is an infectious acute respiratory caused by the novel coronavirus. The virus is a positive-strand RNA virus with high homology to bat coronavirus. In this study, conserved domain analysis, homology modeling, and molecular docking were used to compare the biological roles of specific proteins of the novel coronavirus. The results showed that some viral structural and nonstructural proteins could bind to the porphyrin, respectively. At the same time, orf1ab, ORF10 and ORF3a proteins coordinated to attack heme on the 1-beta chain of hemoglobin, dissociating iron to form porphyrin. Deoxyhemoglobin is more vulnerable to virus attacks than oxidized hemoglobin. The attack will cause less and less hemoglobin that can carry oxygen and carbon dioxide, producing symptoms of respiratory distress. Virus attack damaged many organs and tissues. Lung cells are toxic and inflammatory due to derivatives produced by the attack, which eventually resulted in ground-glass-like lung images. Capillaries easily broken due to inflammation. Proteins such as fibrinogen filled the capillaries' cracks through the coagulation reaction. Therefore, many fibrin and thrombus gathered in the lung tissue of critically ill patients. The mechanism also interfered with the normal heme anabolic pathway of the human body, expecting to result in human disease. This paper is only for academic discussion, the correctness of the theory needs to be confirmed by other experiments. According to the reader's suggestion, the content of the drug-related efficacy analysis has been deleted. Due to the side effects of drugs, please consult a qualified doctor for detailed treatment information, and do not take the drug yourself. We look forward to these discoveries bringing more ideas to people and inspiring people's confidence in defeating the virus.

Keywords: Novel Coronavirus; Respiratory distress; Ground-glass-Like Lung; E2 glycoprotein; ORF8; orf1ab; Chloroquine; Blood; Diabetic**;** Fluorescence resonance energy transfer; Ancient virus; Cytokine Storm; Coagulation; Fibrosis

1 Introduction

The novel coronavirus pneumonia (COVID-19) is a contagious acute respiratory infectious disease. Patients with the coronavirus pneumonia show fever> 37.3 ℃, with symptoms such as dry cough, fatigue, difficulty breathing, and frost-glass-like symptoms in the lungs[1-3]. Some patients also have severe diarrhea[4], such as watery stools. Most mild patients get a better prognosis. Some serious patients quickly develop acute respiratory distress syndrome, shock, acidosis, coagulopathy, and even death. Many mucus, fibrin, and thrombosisare discovered in the dissected lung tissue[5,6]. The disease is highly transmitted. Now the number of infected people has reached millions around the world, and the

infected people are not restricted by race and borders.

Researchers performed virus isolation tests and nucleic acid sequencing to confirm that a novel coronavirus caused the disease[7,8]. It is noted that the nucleic acid of the novel coronavirus is a positive-stranded RNA[8]. Its structural proteins include: Spike Protein (S), envelope protein (E), membrane protein (M), and nucleocapsid phosphoprotein. Transcribed non-structural proteins include: orf1ab, ORF3a, ORF6, ORF7a, ORF10 and ORF8. The novel coronavirus is highly homologous to the coronavirus in bats[9,10], and has significant homology with SARS virus[11,12]. Researchers have studied function of novel coronavirus structural proteins and some non-structural proteins[13,14]. But, the novel coronavirus has potential genomic characteristics, some of which are mainly the cause of human outbreaks[15,16]. For example, CoV EIC (Coronavirus envelope protein ion channel) been implicated in modulating virion release and CoV – host interaction[17]. Spike proteins, ORF8 and ORF3a proteins are significantly different from other known SARS-like coronaviruses, and they may bring about more serious pathogenicity and transmission differences than SARS-CoV[18]. Earlier studies find that the novel coronavirus enters epithelial cells through the spike protein interacting with the human ACE2 receptor protein on the surface, causing human infection. Due to the limitations of existing experimental methods, the specific functions of virual proteins such as ORF8 and ORF3a still unclear. The pathogenicity mechanism of the novel coronavirus remains mysterious[19].

Literature[20] disclosed biochemical examination indexes of 99 patients with novel coronavirus pneumonia, and the report also reflected the abnormal phenomenon of hemoglobin-related biochemical indexes of patients. This report demonstrates that the hemoglobin and neutrophil counts of most patients have decreased, and the index values of serum ferritin, erythrocyte sedimentation rate, C-reactive protein, albumin, and lactate dehydrogenase of many patients increase significantly. This trace implies that patients' hemoglobin is decreasing, and the body will accumulate too many harmful iron ions, which will form inflammation in the body and increase C-reactive protein and albumin. Cells react to stress due to inflammation, producing large amounts of serum ferritin to bind free iron ions to reduce damage. Most carbon dioxide is transported in the form of bicarbonate in plasma. A small portion of carbon dioxide is transported by carbamoyl hemoglobin of erythrocytes.Oxygen is transported in the blood by hemoglobin of red blood cells.Hemoglobin consists of four subunits, 2-α and 2-β, and each subunit has an iron-bound heme[21,22]. Heme is an essential component of hemoglobin. It is a porphyrin containing iron. The structure without iron is called porphyrin. When iron is divalent, hemoglobin can release carbon dioxide and capture oxygen atoms in alveolar cells, and iron is oxidized to trivalent. When hemoglobin is made available to other cells in the body through the blood, it can release oxygen atoms and capture carbon dioxide, and iron is reduced to divalent. Therefore, we think that viral proteins may attack hemoglobin, causing the heme to dissociate into iron and porphyrin, and then the viral proteins capture the porphyrin. This interpretation also was consistent with some existing clinical features.

Both lungs hold the role of exchanging carbon dioxide and oxygen. COVID-19 virus attacked on hemoglobin would yield iron, carbon dioxide, and oxygen, which might put both lung cells in a toxic and inflammatory state. Then, it will form multiple ground glass images and penetration shadows on both sides of the lung[23-25]. Ground glass images are often associated with rapid and noticeable hypoxemia.

In the early and severe stage, patients have varying degrees of respiratory distress symptoms. Many doctors find that ECMO patients have some strange clinical features that are low oxygen, low blood oxygen saturation[26,27] and high dissolved oxygen, which may be a critical reason in the low

success rate of rescuing critical patients[28]. ECMO has cardiopulmonary function in vitro, can help patients exchange oxygen and carbon dioxide in vitro. During this process, drugs such as anticoagulant can be added at the same time.We believed that this phenomenon indicated that hemoglobin, which could carry oxygen in the blood of critically ill patients, became too low. Due to the deep attack of the virus, their normal functioning hemoglobin was few.Hemoglobin could carry oxygen in the blood was little, so patients with ECMO had low oxygenation. If the virus could bind a large amount of porphyrin, heme synthesis was inhibited, so there was less hemoglobin synthesis.Besides, the virus attacked hemoglobin, which reduced the amount of hemoglobin that typically was oxygenated. Many hemoglobins could not carry oxygen after the attack. As a result, blood oxygen saturation decreased. Oxygenated hemoglobin was red blood cell hemoglobin that bound oxygen. After the virus attacked the oxidized hemoglobin, the lost oxygen atoms accumulated in the blood. They hardly entered into the tissue cells, so the dissolved oxygen in the blood got higher.

Virus attacks will result in damage to many organs and tissues. For example, Capillaries easily broken due to inflammation[29]. Proteins such as fibrinogen fill the capillaries' cracks through the coagulation reaction. Therefore, many fibrin and thrombus accumulate in the lung tissue of critically ill patients[30]. If the amount of hemoglobin being attacked is much larger than the synthetic hemoglobin, the patient will be under a certain degree of anemia. Different degrees of systemic coagulation occurred as anemia[31-34], vascular damage, and the body's immune response. Vascular injury may be the principal factor for coagulation in COVID-19 patients. Then, the patient is under a higher D-Dimer content[35,36]. Studies have concluded that the COVID-19 virus also infects T-cells[37], which may make T-cells function abnormally[38]. COVID-19 severe patients often present with mononucleosis[39,40]. Macrophages could engulf free iron, damaged red blood cells and hemoglobin. Of course, macrophages also handled viral inclusions[41], thrombin, fibrin, and foreign matter generated by inflammatory tissues.Thrombin and fibrin may be produced by the coagulation reaction

If the virus could attack hemoglobin in red blood cells, some prerequisites are needed here, such as virus infection of red blood cells or hemolysis of red blood cells. There are now some confusing signs. For example, EMMONS, et al. observe Colorado tick fever virus is in red blood cells through electron microscopy[42], which indicates that red blood cells have a risk of some virus infection. Mitra, Anupam, et al. find thatvirus Leukoerythroblastic reactionin a single patient with COVID-19 infection[43]. Bhardwaj, et al. find that pRb and its interaction with Nsp15 affect coronavirus infection[44]. Recently, it reports that COVID-19 virus infects cells through the Spike-CD147 pathway. CD147 plays an essential role in some systems such as Ok blood group[45], and T lymphocytes[37]. Statistical analysis shows that the susceptibility of COVID-19 has a certain relationship with blood type. The rate of infection of blood type A is high, while the rate of infection of blood type O is minimal. Blood type is an important antigen on the erythrocyte membrane. The latest report is shown that the number of red blood cells in rhesus monkeys infected with the COVID-19 virus is decreasing. However,the mechanism of red blood cell infection by the novel coronavirus remains unclear.

Plasmodium, babesia, and trypanosomes can also infect red blood cells, causing similar symptoms in patients. Trypanosoma extracellular vesicles can also fuse with mammalian red blood cells[46], causing the red blood cells to be readily cleared, leading to anemia. Thrombin sensitization-related adhesion protein and apical membrane antigen are important proteins for babesia invasion of red blood cells[47]. There are numerous types of Plasmodium, which can infect red blood cells. Plasmodium usually infects red blood cells in three steps. First, it sticks to to red blood cells through proteins such as AMA[48]. Then, the Plasmodium ligand and erythrocyte receptor complete a tight connection. For

example, the principal receptor for P. falciparum to invade red blood cells is CD147 protein[49]. Finally, dynein, MTRAP (homologous to thrombin-sensitive protein), and other kinetic system proteins complete the process of rapid slide of Plasmodium into red blood cells[50].If the COVID-19 virus also used thrombin-sensitized protein-mediated sliding into red blood cells, it could account for this phenomenon of systemic coagulation in severe patients to a certain extent. It is useful to that, there may be a coincidence. Patients infected with Plasmodium and babesia are treated with chloroquine and quinine, and patients infected with trypanosomiasis can be healed with anisace (quinoline pyrimidyl sulfate). Chloroquine is a drug with serious side effects and some novel coronavirus pneumonia has been cured by chloroquine. Meanwhile, one detail we can notice is that chloroquine is also a commonly used drug for treating porphyria[51,52].

The main symptoms of porphyria are in the skin and neurovisceral organs. Existing reports show pigmentation in critically ill patients, but this may be a side effect of drugs such as polymyxin B. Some patients with severe skin diseases may also have fever symptoms, which are easily confused with COVID-19 patients[53]. Researchers have diagnosed COVID-19 infection with erythema rash, extensive urticaria and chickenpox-like vesicles[54]. Some patients with novel coronavirus pneumonia have similar neurovisceral symptoms. Of course, in the later stage of severe inflammatory infection, cytokine storm may also induce the failure of many organs. The symptoms are such as diarrhea[55,56], hypotension[57], and electrolyte disturbances[58]. Organs such as heart[59], liver[60], and kidneys[61] are damaged and develop complications. In terms of neurological symptoms, some early stealth infections experienced the loss of taste and smell[62,63]. Patients with severe novel coronavirus pneumonia also suffer from rare neurological diseases such as epilepsy[64,65] and encephalitis[66,67]. Therefore, we believed that combining viral proteins and porphyrins interfered with the normal heme anabolic pathway of the human body, causing the series of human pathological reactions.

In short, COVID-19 viral protein may conduct a series of activities by binding porphyrin. The porphyrin in the human body mainly stores on hemoglobin in the form of heme. Because the virus requires too much porphyrin, it has evolved the function of attacking heme on hemoglobin and dissociating iron to form porphyrin. Because of the severe epidemic, and the existing conditions with limited experimental testing methods for the proteins' functions, it is of high scientific significance to analyze the proteins' function of the novel coronavirus by bioinformatics methods. In this study, conserved domains prediction, homology modeling, and molecular docking techniques were used to analyze the functions of virus-related proteins. This study revealed that some proteins had a function to combine with porphyrin to form a complex, while orf1ab, ORF10, ORF3a coordinately attack the heme on the 1-beta chain of hemoglobin to dissociate the iron to form the porphyrin. This mechanism of the virus inhibited the normal metabolic pathway of heme and made people show symptoms of the disease.

2 Materials and Methods

2.1 Data set

The protein sequences were downloaded from NCBI: All proteins of the novel coronavirus; Heme-binding protein; Heme oxidase; Protein sequences were utilized to analyze conserved domain. Some proteins of novel coronavirus were also used to construct three-dimensional structures by homology modeling.

At the same time, the PDB files were downloaded from the PDB database: *HEM*; Human Oxy-Hemoglobin 6bb5; DEOXY HUMAN HEMOGLOBIN 1a3n. some SARS-COV-2 proteins such

as nsp16-nsp10 6w4h, main protease 6y2e, main protease in apo form 6m03, Nsp9 RNA binding protein 6w4b, Nsp9 RNA-replicase 6w9q, spike closed state 6vxx, spike open state 6vyb. 6bb5 and 1a3n are employed to protein docking. HEM is used to molecular docking. SARS-COV-2 proteins are utilized to molecular docking or protein docking.

2.2 Flow view of bioinformatics analysis

A series of bioinformatics analysis was performed based on published biological protein sequences in this study. The steps are illustrated in Figure 1: 1. Conserved Domains of Viral Proteins are analyzed by MEME[68-70] Online Server. Conserved domains were used to predict function differences of viral proteins and human proteins. 2.The three-dimensional structure of viral proteins was constructed by homology modeling of Swiss-model[71,72], AlphaFold and Robetta. 3.Using molecular docking technology (LibDock tool) of Discovery-Studio 2016[73], the receptor-ligand docking of viral proteins with human heme (or porphyrins) was simulated. Depending on the results of bioinformatics analysis, the related molecular of the disease was proposed.

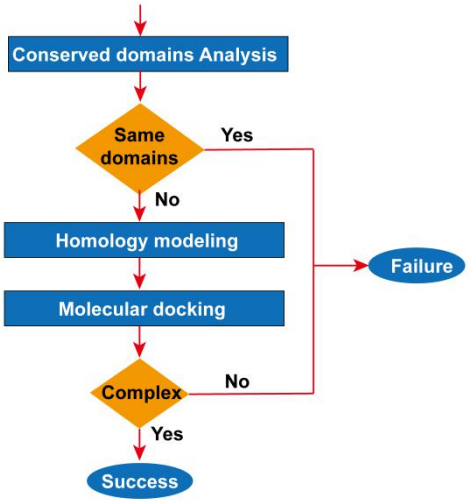


Figure 1. Flow view of Bioinformatics Analysis

2.3 Analysis of conserved domain

MEME Suite is an online website that integrates many tools of predicting and annotation motif. The maximum expectation (EM) algorithm is the basis for MEM's identification of the motif. The motif is a conserved domain of a small sequence in a protein. Motif-based models could assess the reliability of phylogenetic analysis. After opening the online tool MEME, the protein sequences of interest are merged into a text file, and the file format remains fasta. Then select the number of motifs you want to find, and click the "Go" button. At the end of the analysis, the conserved domains are displayed after clicking the link.

In this study, we used the MEME tool to determine that the viral protein does not have conserved domains of heme-binding protein and heme oxidation protein. It represents that the viral protein cannot bind heme and oxidize heme.

2.4 Homology modeling

SWISS-MODEL is a fully automatic homology modeling server for protein structure, which can be accessed through a web server. The first step is to enter the Swiss-model, enter the sequence, and click "Search Template" to perform a simple template search. After the search is completed, you can

choose a template for modeling. A template search will be performed clicking the "Build Model" and a template model is chosen automatically. As can be seen, several templates were searched, and then numerous models were built. Just a model is chosen here. The model in PDB format is downloaded and visualized in VMD. SWISS-MODEL is used to model S, E, N, ORF6, ORF7, and ORF8 proteins.

The quality of ROBETTA modeling is good, there are many reference templates, and it can also model some viral proteins with low similarity. After the user submits the sequence online, the server gives a modeling queue. After successful modeling, the server will send the 3D structure file to the submitter's mailbox. This online server also has restrictions on the sequence length. In this study, we modeled the ORF10 sequence with ROBETTA. The score for ORF7 modeling is 0, so we did not select the ORF7 file from here.

AlphaFold's algorithm significantly outperforms traditional models in the field of protein folding. The AlphaFold project team has constructed 3D files of some viral proteins. We selected the modeling files of ORF3 and M protein for this study.

2.5 Molecular docking technology

The purpose of molecular docking is to show that some viral proteins can bind to porphyrin. Molecular docking is the process of finding the best matching pattern between two or more molecules through geometric matching and energy matching. The steps for using LibDock molecular docking with Discovery-Studio are as follows:

1. Preparation of a ligand model. Open a ligand file such as HEM, and click “Prepare Ligands” in the “Dock Ligands” submenu of the “Receptor-Ligand Interactions” menu to generate a heme ligand model for docking. First delete FE (iron atom) in HEM, and then click the “Prepare Ligands” button, then the porphyrin ligand model will be generated.

2. Prepare a protein receptor model. Open the protein's pdb file (generated by homology modeling), and click “Prepare protein” in the “Dock Ligands” submenu of the “Receptor-Ligand Interactions” menu to generate a protein receptor model for docking.

3. Set docking parameters to achieve docking. Select the generated protein receptor model. From the “Define and Edit Binding Site” submenus in the “Receptor-Ligand Interactions” menu, click “From receptor Cavities”. A red sphere appears on the protein receptor model diagram. After you right-click the red ball, you can modify the radius of the red ball. Then, in the “Receptor-Ligand Interactions” menu, select “Dock Ligands (LibDock)” in the “DockLigands” submenu. In the pop-up box, select the ligand as the newly established ligand model-ALL, and select the receptor as the newly established receptor model-ALL, and the sites sphere as the sphere coordinates just established. Finally click RUN to start docking.

4. Calculate the binding energy and choose the pose with the lowest binding energy. After docking is complete, many locations of ligand will be displayed. Open the docked view, and click the “Caculate Binding Energies” button in the “Dock Ligands” submenu of the “Receptor-Ligand Interactions” menu. In the pop-up box, selectthe receptor as the default value, select ligand as the docked model -ALL, and then start to calculate the binding energy. Finally, compare the binding energy and choose the pose with the lowest binding energy[74-76].

5. Export the joint section view. For the docked view, after setting the display style of the binding area, click the "Show 2D Map" button in the "View Interaction" submenu of the "Receptor-Ligand Interaction" menu to pop up the view of the binding section. This view can be saved as a picture file.

2.6 Protein docking technology

The main purpose of Protein docking technology is to study the attack of viral proteins on hemoglobin. Discovery-Studio's ZDOCK is another molecular docking tool for studying protein interactions. We used it to study the attack of hemoglobin by viral non-structural proteins. The following is the docking of nsp16-nsp10(orf1ab) and hemoglobin, and other docking methods with virus non-structural proteins are the same. After opening the PBD files of Human Oxy-Hemoglobin 6bb5 and orf1ab protein, click the "Dock proteins (ZDOCK)" button of "Dock and Analyze Protein Comlexes" under the "macromolecules" menu. In the pop-up interface, select Human Oxy-Hemoglobin 6bb5 as the receptor, orf1a as the ligand, and then click the "Run" button. After the computer finishes computing, click on the "protein pose" interface and select the pose and cluster with the highest ZDOCK score. It could obtain the position of orf1ab on Human Oxy-Hemoglobin 6bb5. Deloxy HUMAN HEMOGLOBIN 1a3n has a similar docking pattern with nsp16-nsp10 protein.

3 RESULTS

3.1 Viral proteins cannot bind heme and oxidize heme

In humans, hemoglobin can be degraded into globin and heme. Heme is composed of porphyrin and an iron ion. The iron ion is in the middle of the porphyrin. Heme is insoluble in water and can be combined with heme-binding proteins to form a complex and be transported to the liver. The heme is degraded into bilirubin and excreted from the bile duct, and the body can reuse iron in the molecule. The following bioinformatics methods were applied to study virus proteins binding to porphyrin.

To understand whether the structural protein of the virus can bind heme, we first analyze whether the structural protein has a similar domain of heme-binding protein. First, MEME's online server was employed to search for conserved domains in each viral structure protein and human heme-binding protein (ID:NP_057071.2 heme-binding protein 1, ID: EAW47917.1 heme-binding protein 2). Table 1 presents that four viral proteins (surface glycoprotein, envelope protein, membrane glycoprotein, and nucleocapsid phosphoprotein) and heme-binding proteins have conserved domains, E-value values are high than 0.05, there were not statistically significant. These results show that the four viral proteins are not the ability to bind heme. Whether the viral proteins interact with porphyrin is determined by molecular docking.

After that, the following analysis was conducted to find out whether the structural protein can dissociate heme, that is, whether it has the ability of heme oxidase. MEME's online server was manipulated to search for conserved domains of structural proteins and heme oxidase proteins (NP_002124.1: heme oxygenase 1;BAA04789.1: heme oxygenase-2;). The viral proteins have domains, but E-value values are high than 0.05, there were not statistically significant. As a result, conserved domains of structural proteins were not found. Combining the results of the previous analysis, that is, structural proteins may not oxidize the heme. It is worth noting that the E value of ORF3, ORF10, orf1ab, and NP_002124.1 is the smallest among nonstructural proteins, and E value of ORF3, ORF10 is less than zero. Whether these three proteins affect heme of Hemoglobin is determined by molecular docking too.

Table 1. E-value of domains between viral protein and human heme-binding protein

Viral protein	NP_057071.2	EAW47917.1
surface glycoprotein	1.60E-01	3.40E+00
envelope protein	7.00E-01	3.70E+00
membrane glycoprotein	1.10E+01	9.80E+00
nucleocapsid phosphoprotein	2.90E+00	5.20E+00
orf1ab	2.00E+00	2.40E-01
ORF3a	6.00E+00	1.10E+00
ORF6	3.30E+01	1.60E+00
ORF7a	7.20E+00	2.50E+00
ORF8	4.70E+00	1.20E+01
ORF10	1.10E+00	4.70E-01

Table 2. E-value of domains between viral protein and heme oxidase proteins

Viral protein	NP_002124.1	BAA04789
surface glycoprotein	5.4E+000	3.7E-001
envelope protein	3.2E-001	4.4E+000
membrane glycoprotein	6.1E-001	1.3E+000
nucleocapsid phosphoprotein	1.0E+000	1.3E+001
orf1ab	1.2E+001	1.4E+001
ORF3a	1.3E-001	7.0E+000
ORF6	1.4E+000	9.9E+000
ORF7a	2.4E+001	7.1E+000
ORF8	5.9E+000	4.2E+000
ORF10	6.6E-001	2.3E+000

3.2 Virus structural proteins binding porphyrin

We studied the binding of four structural proteins to porphyrin through molecular docking.The 3D-structural file of heme was downloaded from the PDB database.

The Swiss-model online server modeled the surface glycoproteins to produce a three-dimensional structure, and the E2 templates were selected. The three-dimensional structure file of spike protein is downloaded from the PDB database website. Discovery-Studio realized molecular docking of surface glycoproteins and the porphyrin. We used the spike protein to dock with porphyrin. Some sites can dock successfully, but the calculation of binding energy is not successful. E2 glycoprotein is derived from templates 1zva.1.A. The docking of E2 glycoprotein and heme was also fruitless. When the iron ion was removed, the heme became a porphyrin, many kinds of docking were finalized between the E2

glycoprotein and the porphyrin. Calculating the binding energy, the docking pose with the lowest binding energy 8.93 kcal/mol (Table 3) was accepted.

Analysis of envelope protein adopted the same methods. The template 5x29.1.Awas selected as the 3D structure template of envelope protein. Discovery-Studio found several kinds of docking of the envelope protein and the porphyrin, where the docking pose with the lowest binding energy -194.71 kcal/mol (Table 3) was chosen. Figure 2.A-1 shows the docking result, which is the molecular model of envelope protein binding to the porphyrin. Figure 2.A-2 is the two-dimensional view of the binding section, which several amino acids of envelope protein interacting with porphyrin.

The homology modeling file of the membrane protein comes from a part of the project on COVID-19 viral protein modeling published by the AlphaFold group. We used molecular docking technology of Discovery-Studio's libdock to achieve the docking of membrane proteins and porphyrins. The posture with the lowest binding energy -288.46 kcal/mol(Table 3) was selected. Figure 2.B-1 shows the docking results. Figure 2.B-2 is the binding region. There are several amino acids of membrane protein interacting with porphyrin.

Same methods were utilized to analyze the nucleocapsid phosphoprotein. The template of the nucleocapsid phosphoprotein was 1ssk.1.A. Discovery-Studio provides the docking between the nucleocapsid phosphoprotein and the porphyrin with the lowest binding energy -309.546 kcal/mol (Table 3**)**. Figure 2.C-1 shows the docking result, which is the molecular model of the nucleocapsid phosphoprotein bind to the porphyrin. Figure 2.C-2 is the two-dimensional view of the binding section, where several amino acids of thenucleocapsid phosphoprotein are bound to the porphyrin.

As mentioned before, it is impossible to assess whether the surface glycoprotein can bind porphyrin. It was found the binding energy of nucleocapsid phosphoprotein was the lowest, the binding energy of E2 glycoprotein was the highest, and the binding energy of envelope protein and the binding energy of membrane glycoprotein was medium. It means that binding E2 glycoprotein to the porphyrin is the most unstable, the binding of binding envelope protein to the porphyrin is stable, the binding of membrane glycoprotein to the porphyrin is stable too, and nucleocapsid phosphoprotein to the porphyrin is the most stable. However, the specific mechanism of binding of most viral structural proteins to porphyrin is not clear.

Table 3. Binding energy of structure proteins and the porphyrin

Viral structureprotein	Libdockscore	Binding Energy (kcal/mol)
surface glycoprotein (E2) (modeling)	87.94	8.93
envelope protein (modeling)	105.29	-194.71
membrane glycoprotein (modeling)	83.43	-288.46
nucleocapsid phosphoprotein (modeling)	92.29	-309.55

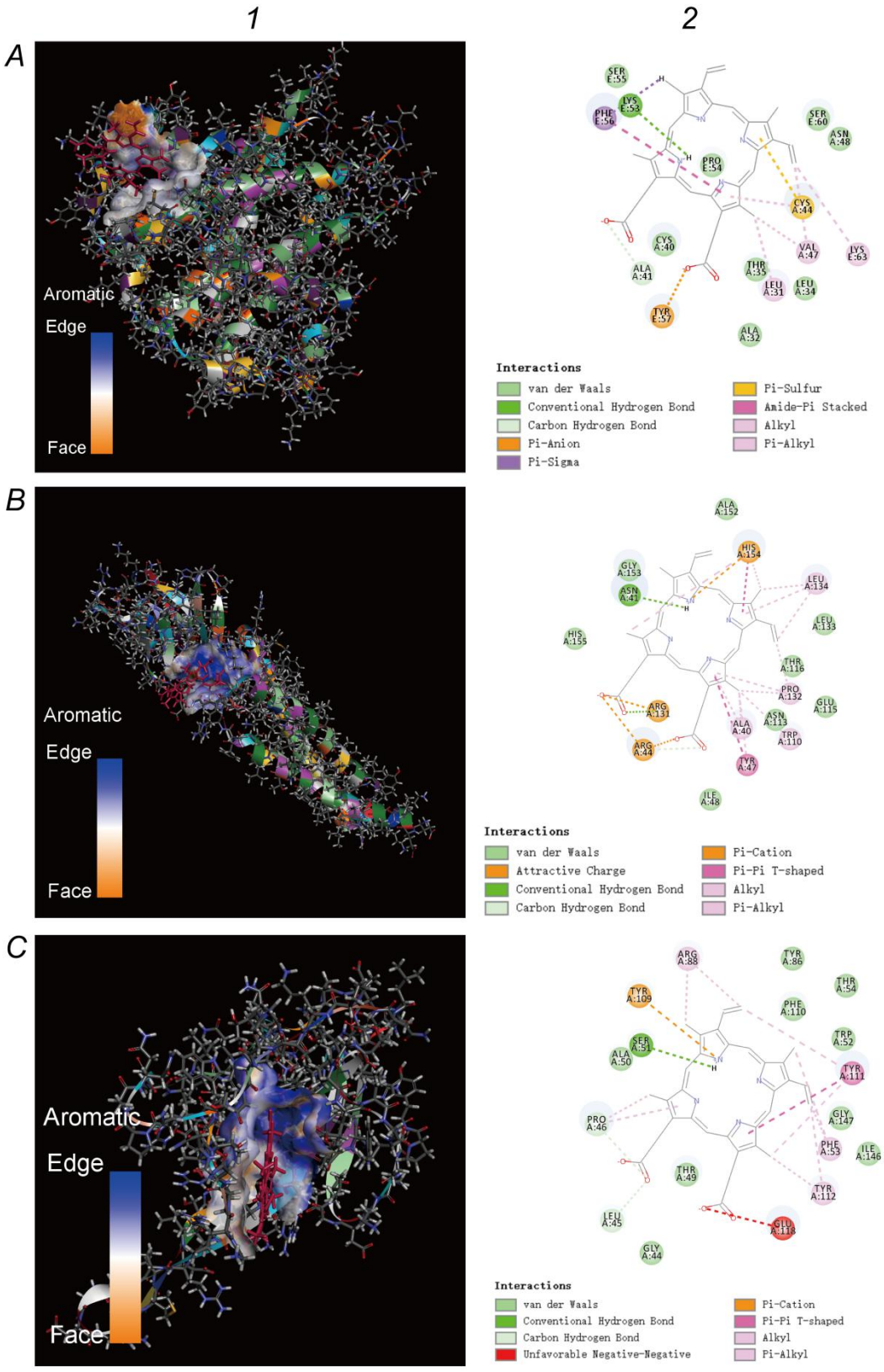


Figure 2. Molecular docking results of viral structure proteins and the porphyrin (red structure). ***A***. Molecular docking results of envelope protein and the porphyrin. ***B.*** Molecular docking results of the membrane glycoprotein and the porphyrin. ***C.*** Molecular docking results of the nucleocapsid phosphoprotein and the porphyrin. ***1.***Viral structure proteins. ***2.*** View of the binding sections

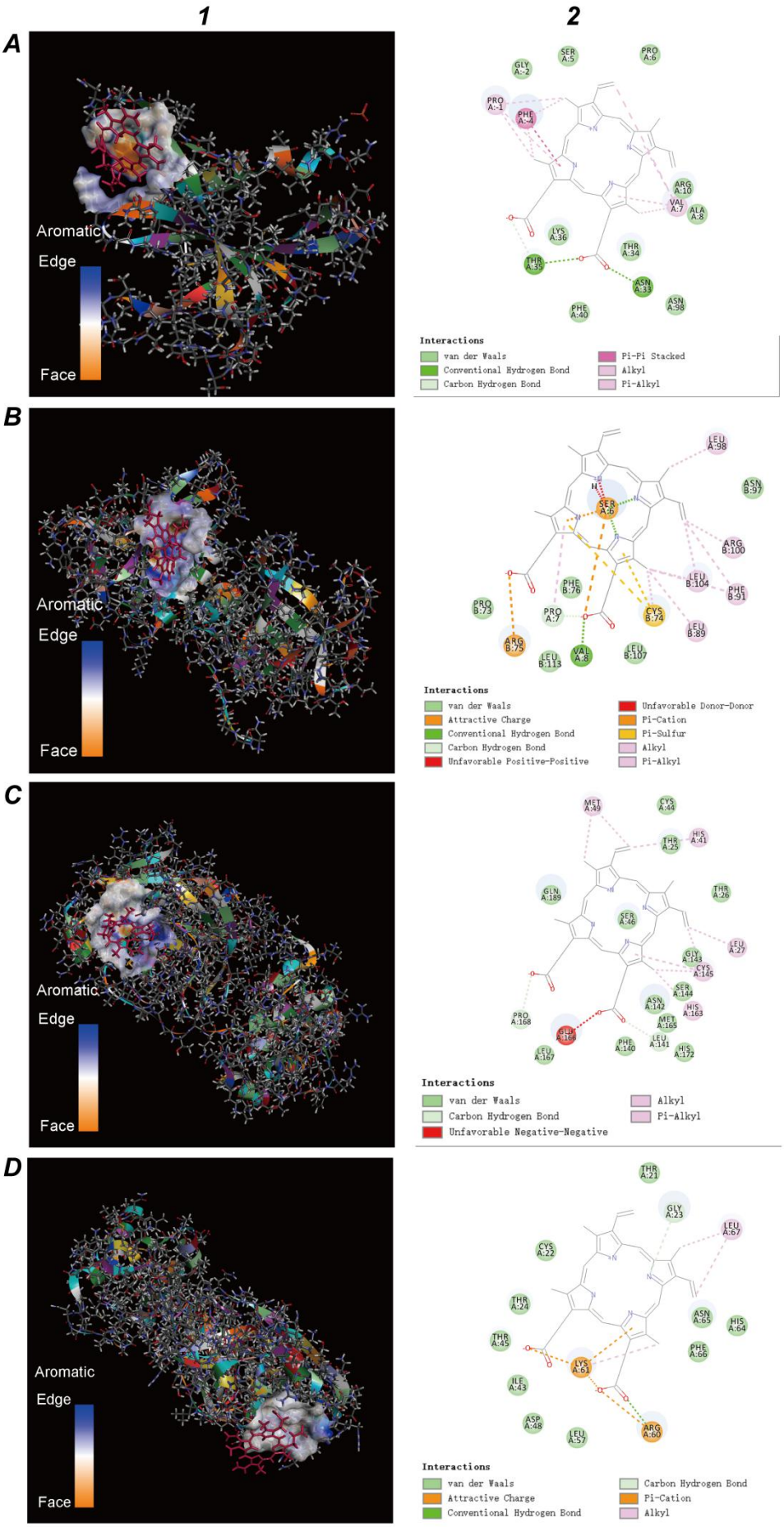
3.3 Virus non-structural proteins bind to the porphyrin

Molecular docking technique used to analyze the binding of non-structural proteins to porphyrin.

orf1ab can be cleaved into many sub-proteins, but the crystal structure of many sub-proteins has not been determined, and the detailed mechanism of action is not precise. The crystal structure of the nsp16-nsp10 complex, nsp9, main protease proteins of orf1ab have been determined. We mainly study the docking of these proteins with porphyrin. Then molecular docking of nsp16-nsp10 protein and porphyrin was finished by Discovery-Studio. nsp16-nsp10 protein and heme could not complete the docking experiment, but by removing iron ions to make heme into a porphyrin, and the radius of action increased, then several types of docking were completed. We calculated the binding energy. Nevertheless, through inspection, it was found that the posture with the lowest binding energy was not in the active pocket. Therefore, it considered that nsp16-nsp10 cannot effectively bind to porphyrin. Similarly, we completed the docking of Nsp9 RNA-replicase, Nsp9 RNA binding, main protease in apo form, main protease free, four forms of protein, and calculated the binding energy. The minimum binding energy of this form of proteins are -189.81kcal/mol, -201.78kcal/mol, -120.35kcal/mol, -120.18 kcal/mol (Table 4). It shows that these four forms of protein could effectively bind to porphyrin (Figure 3.A-D.1). Figure 3.A-D.2 are the two-dimensional views of the binding sections.

Table 4. Binding energy of non-structural proteins and the porphyrin

Viral non-structuralprotein	Libdockscore	Binding Energy (kcal/mol)
nsp16 - nsp10 (orf1ab)	-	-
Nsp9 RNA-replicase (orf1ab)	60.14	-189.81
Nsp9 RNA binding (orf1ab)	75.58	-201.78
main protease in apo form (orf1ab)	120.86	-120.35
main proteasefree (orf1ab)	73.50	-120.18
ORF3 (modeling)	73.74	-234.85
ORF6a (modeling)	-	-
ORF7a (modeling)	94.37	13,667.40
ORF8 (modeling)	100.62	-218.84
ORF10 (modeling)	-	-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PRO A:6
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PRO A:-1
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ALA A:8
LYS A:36
THR A:34
THR A:35
ASN A:33
ASN A:98
PHE A:40
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Pi Stacked
Alkyl
Pi-Alkyl
B
Aromatic
Edge
Face
LEU A:98
ASN B:97
SER A:6
ARG B:100
LEU B:104
PHE B:91
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PRO B:73
PRO A:7
CYS B:74
LEU B:89
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Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Positive-Positive
Unfavorable Donor-Donor
Pi-Cation
Pi-Sulfur
Alkyl
Pi-Alkyl
C
Aromatic
Edge
Face
MET A:49
CYS A:44
HIS A:41
THR A:25
GLN A:189
THR A:26
SER A:46
LEU A:27
GLY A:143
CYS A:145
SER A:144
ASN A:142
HIS A:163
PRO A:168
MET A:165
GLU A:166
PHE A:140
LEU A:141
LEU A:167
HIS A:172
Interactions
van der Waals
Carbon Hydrogen Bond
Unfavorable Negative-Negative
Alkyl
Pi-Alkyl
D
Aromatic
Edge
Face
THR A:21
GLY A:23
LEU A:67
CYS A:22
THR A:24
ASN A:65
HIS A:64
THR A:45
PHE A:66
LYS A:61
ILE A:43
ASP A:48
LEU A:57
ARG A:60
Interactions
van der Waals
Attractive Charge
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Cation
Alkyl

Figure 3. Molecular docking results of some sub-proteins of orf1ab and the porphyrin (red). ***A***. Molecular docking results of the Nsp9 RNA-replicase proteinand the porphyrin. ***B.*** Molecular docking results of the Nsp9 RNA binding proteinand the porphyrin. ***C.***Molecular docking results of the main protease protein(in apo form) and the porphyrin. ***D.*** Molecular docking results of the main protease protein(free) and the porphyrin. ***1***.*S*ub-proteins of orf1ab.***2***. View of the binding sections

The same analysis steps were used to the binding of ORF3 protein to porphyrin. The structure file of ORF3 was downloaded from the AlphaFold project. The docking result (Figure 4.A-1) represents the molecular model of ORF3 protein binding to the porphyrin, where the docking poses with the lowest binding energy -234.85 kcal/mol(Table 4). Figure 4.A-2 represents the two-dimensional view of the binding section.

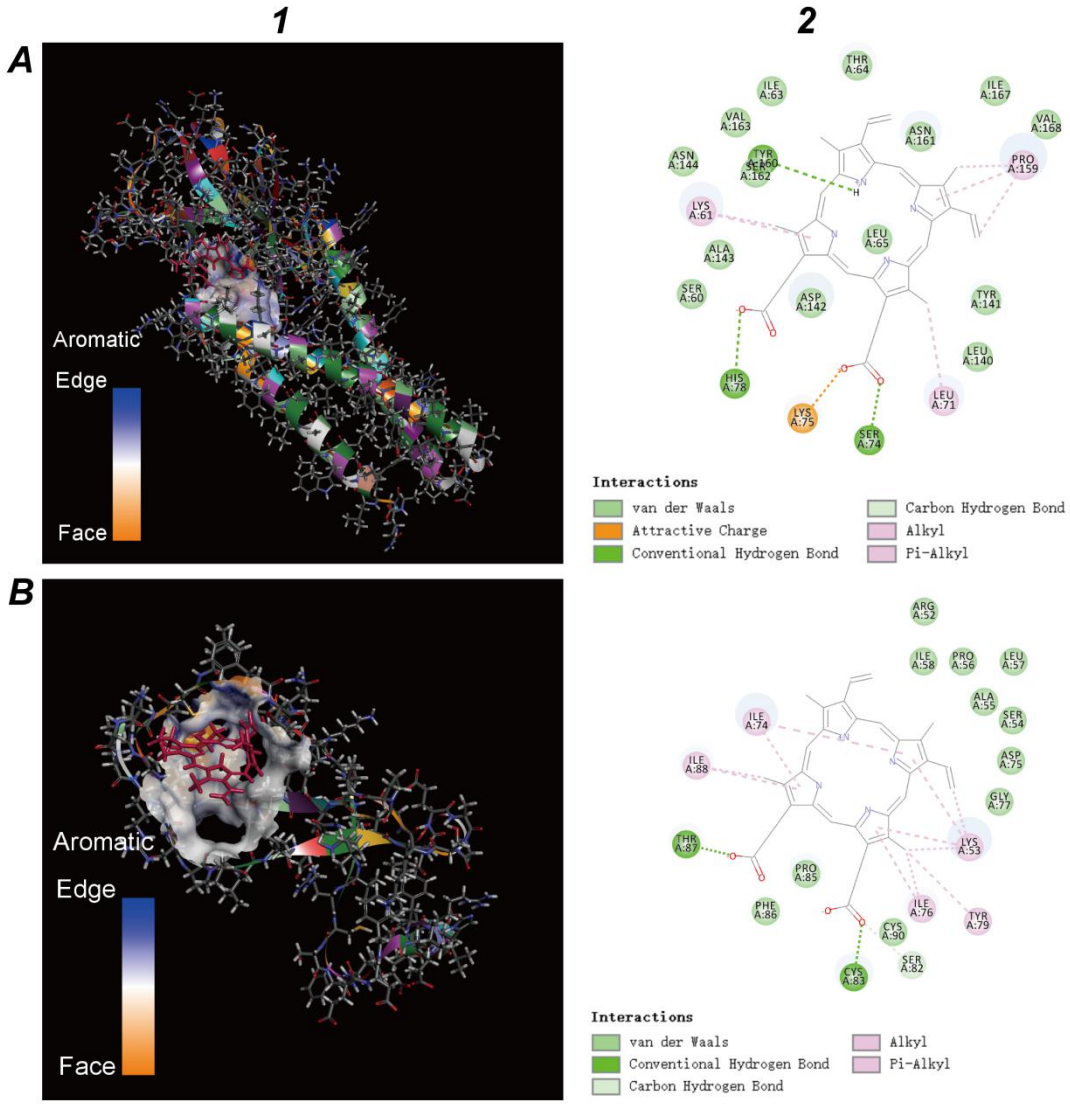

Figure 4. Molecular docking results of ORF3, ORF8 proteins and the porphyrin (red). ***A***. Molecular docking results of the ORF3proteinand the porphyrin. ***B.*** Molecular docking results of the ORF8 proteinand the porphyrin. ***1***.ORF3, ORF8 proteins. ***2***. View of the binding sections

To study the binding properties of ORF8 protein to porphyrin, the same analysis steps as the structural protein method were used. The structure file was created based on the ORF7 template. Several kinds of docking of the ORF8 protein and the porphyrin, where the docking pose with the lowest binding energy -218.84 kcal/mol(Table 4)was selected. The docking result (Figure 4.B-1)

represents the molecular model of ORF8 protein binding to the porphyrin. Figure 4.B-2 is the two-dimensional view of the binding section, where some amino acids of the ORF8 are bound to the porphyrin.

Same methods of ORF8 protein were used to analyze the ORF7a protein. The ORF7a's template is 1yo4.1.A. The ORF7a protein and the porphyrin had the lowest binding energy 13667.4kcal/mol(Table 4). ORF6a is derived from templates 3h08.1.A, but the docking of ORF6a with porphyrin failed.The docking of ORF10 with porphyrin also failed. It means that ORF10, ORF6 and ORF7a cannot bind porphyrin effectively.

The results show that orf1ab, ORF3a, and ORF8 can bind porphyrin, while ORF10, ORF7a, and ORF6 cannot bind porphyrin. The binding energies of orf1ab, ORF3a, ORF8, and porphyrin were compared respectively. The orf1ab protein has many roles, and the detailed mechanism of its binding to porphyrin is unclear. The sequences of ORF10 and ORF6 are very short, so they should be short signal peptides. ORF3 and ORF8 had lower binding energy, which means that ORF3 and ORF8 had the most stable binding to porphyrin. ORF7 cannot bind porphyrin and may help ORF8 capture porphyrin.

3.4 Viral non-structural protein attacks the heme on the beta chain of the hemoglobin

The porphyrin in the human body is mainly iron porphyrin, namely heme. Many hemes are not free but bound to hemoglobin. Viruses have a massive demand for porphyrins. Therefore, the novel coronavirus may target hemoglobin, attack heme, and acquire porphyrin. In order to study the attack behavior of nsp16-nsp10 (orf1ab), ORF3a, and ORF10 proteins, we used ZDOCK molecular docking technology to examine these three proteins. ZDOCK molecular docking technology can analyze protein interactions and find the approximate location of these three proteins on hemoglobin.

For oxidized hemoglobin, nsp16-nsp10 acted on the middle bottom of the alpha and beta chain and closed to the alpha chain (Figure 4.A). ORF3a acted at the bottom of the beta chain (Figure 4.C). ORF10 acted below the alpha chain (Figure 4.E). The possible mechanism was that nsp16-nsp10 first attacked the alpha chain, and then, ORF3 and ORF10 successively attack the beta chain.

For deoxyhemoglobin, nsp16-nsp10 acted on the top of the 1-beta (Figure 4.B). ORF3a acted at the bottom of the 1-beta (Figure 4.D). ORF10 acted on the top of 1-beta (Figure 4.F). ORF3 and ORF10 have embedded in deoxyhemoglobin and directly docked to the heme of the beta chain. It indicates that the viral protein can attack heme on hemoglobin. The possible mechanism was that nsp16-nsp10 first attacked the 1-beta chain, and then, ORF3 and ORF10 successively attack the 1-beta chain.

It is challenging to perform molecular simulations. Due to the close distance of the attack postures of some proteins, so it is unclear the order of three proteins attacked. The nsp16-nsp10 molecule may be an essential protein, playing a vital role throughout the attack. It is worth noting that the above simulation shows that the deoxyhemoglobin is more vulnerable than oxidized hemoglobin. Attack of oxidized hemoglobin by viral proteins will lead to less and less hemoglobin that can carry oxygen. The invasion of viral proteins on deoxyhemoglobin will make less and less hemoglobin that can carry carbon dioxide and blood sugar. People with diabetes can have unstable blood sugar. Body cells have extreme inflammation due to excess iron, carbon dioxide and oxygen. Patients with respiratory distress will be made worse, organs and tissues of the whole body have different degrees of damage.

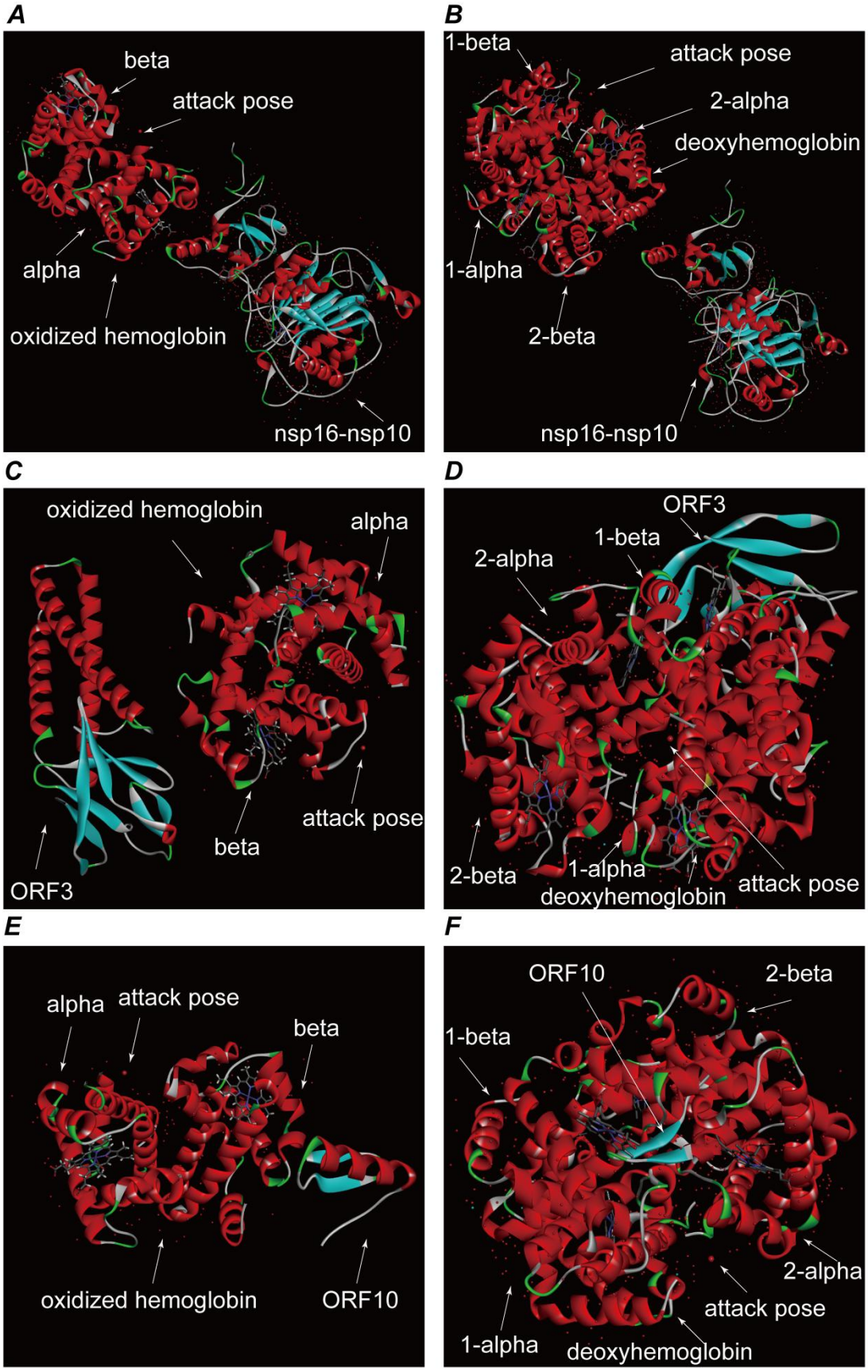

Figure 4. Viral non-structural protein attackhemoglobin. ***A***. nsp16-nsp10 attacks the oxidized hemoglobin. ***B***.nsp16-nsp10 attacks the deoxyhemoglobin. ***C.*** ORF3a attacks the oxidized hemoglobin.

D.ORF3a attacks the deoxyhemoglobin. ***E***. ORF10 attacks the oxidized hemoglobin. ***F***.ORF10 attacks the deoxyhemoglobin.

4 Discussion

4.1 The novel coronavirus originated from an ancient virus

For the most primitive-life viruses, it is not very easy to see their role in binding the porphyrin. The porphyrin compounds are widely present in photosynthetic or non-photosynthetic organisms, and they are associated with critical physiological processes such as catalysis, oxygen transfer, and energy transfer. The porphyrin is also an ancient compound widely present on the earth. The porphyrin was first found in crude oil and asphalt rock in 1934. The porphyrin has unique photoelectronic properties and excellent thermal stability and has broad application prospects in materials chemistry, medicine, biochemistry, and analytical chemistry. It is excellent performance in two-photon absorption, fluorescence effect, energy transfer, and other aspects. Fluorescence resonance energy transfer (FRET) is a non-radiative process in which a donor in an excited state transfers energy to a receptor in the ground state through a long-range dipole effect. The FRET characteristics of the porphyrin may be the primary survival mode on which the original virus relied.

There are numerous theories about the origin of viruses, one of which is called co-evolution theory, which viruses can evolve from complexes of the protein and the nucleic acid. Various methods do not explain that a virus survived independently of non-appearing cells at the beginning of life, so the origin of a virus remains a mystery. This paper proposes that a virus could be bind to the porphyrin, which could explain the survival problem of an original virus. Because the porphyrin has the energy transfer characteristic of fluorescence resonance, viruses that bind to porphyrins could obtain energy through this light-induced method. A virus that gained power could achieve minimal displacement movements. Depending on the research results in this study, the novel coronavirus was a life form dependent on the porphyrin. Therefore, we could believe that the novel coronavirus originated from an ancient virus that may have evolved over countless generations in bats.

4.2 Higher permeability of porphyrins into cell membranes leads to high infections

Highly evolving of the novel coronavirus also displays some paradoxical characteristics. The current theory finds that the novel coronavirus binds to the human ACE2 receptor through a spike protein. The novel coronavirus enters human cells in the form of phagocytosis. The novel coronavirus pneumonia is highly contagious. What causes the high infectivity of the novel coronavirus? We believe that in addition to the invasive method of spike-ACE2, it should maintain the original invasive pattern.

Medical workers have detected the novel coronavirus from urine, saliva, feces, and blood. The virus can also live in body fluids. In such media, porphyrin is a prevalent substance. Porphyrin compounds are a class of nitrogen-containing polymers, and existing studies have found that they have a strong ability to locate and penetrate cell membranes. At the beginning of life, virus molecules with porphyrins directly moved into the original membrane structure by porphyrin permeability. This study showed that the structural protein of the novel coronavirus could bind to porphyrins. Therefore, the coronavirus may also directly penetrate the human cell membrane through porphyrin, so the infection is robust.

4.3 The complexity of individual immunity

Some theories suggest that an immune response occurs in the body after a patient becomes ill. Some patients develop immune antibodies after recovery. According to this study, the virus protein could bind to porphyrin. However, from the current research, it is unclear which immune antibodies have been raised against viral proteins. Besides, some patients may be killed by their cytokine storm. Comparing to patients with SARs, the anatomical characteristics of the dead are different. The complex of virus proteins and the porphyrin may be little soluble. Too much mucus in the tissues of the deceased patients was the factor of too much mucin protein. Mucin could turn loosely connected cells into tightly adhered cells and increases lubrication between cells. It suggests the compound leads to reduced cell connectivity, and cells need mucin to consolidate tissue-cell connectivity and lubricity. Also, when a patient enters a severe infection period, viral structural proteins were mainly used for virus assembly. Therefore, we cannot find noticeable virus inclusions in tissue cells of the dissected patient.

4.4 Immune cells are infected and secrete antibodies and viral proteins

Immune cells, such as plasma cells, are also known as effector B cells. Plasma cells are mostly observed in the connective tissue of the intrinsic membrane both in the digestive tract and the respiratory tract. They are antibody-secreting cells. Plasma cells have the function of synthesizing and storing antibodies, namely immunoglobulins, and participate in humoral immune responses. Depending on the source of antibody production, antibodies include natural antibodies, such as anti-A and anti-B antibodies in blood group ABO. According to the agglutination state of antigen reaction, antibodies are divided into complete antibody IgM and incomplete antibody IgG. The detection of IgM and IgG in the blood can determine whether the human body is infected with the virus. There is a great amount of IgM in the blood of patients with suspected the novel coronavirus pneumonia. Through treatment, the amount of IgM in the patient is reduced, and the amount of IgG is raised, indicating that his body has resistance and immunity. There are reports that plasma cells also have ACE2 receptor; that is, it could be a Spike-ACE2 infection pathway. Because of reports showing that the spleen, bone marrow, and lymph nodes of severe patients are also significantly damaged, we speculate that plasma cells are also closely related to the infection and recovery of patients with the coronavirus.

Plasma cells can secrete various antibodies, which also explain the release of viral proteins in the body. Viral proteins orf1ab, ORF3a, and ORF10 were synthesized in cells and attacked hemoglobin and heme outside the cells. Viral proteins were possible outside the cell through secreted protein pathways. Secreted proteins mainly include digestive enzymes, antibodies, and some hormones. Based on the above viewpoint that disease infection was linked to plasma cells, we believed that viral proteins were secreted mainly from the inside to the outside of the cell through the secretory pathway of antibodies. One possible mechanism was that after the plasma cell was infected, the viral transcription and translation processes were launched, and then viral proteins such as orf1ab, ORF3a, and ORF10 were secreted out of the cell. However, it was not clear whether the viral proteins were secreted outside the cell by binding to blood group antibodies.

We planed to simulate this mechanism, but the amount of calculation was too big. After we input "blood antibody" in the search box of the PDB database, the web page showed nearly 160,000 records and nearly 47,000 records associated with humans. Besides, the molecular docking simulation of antibodies and proteins such as orf1ab is the docking of proteins and proteins, and the calculation process is very complicated. Therefore, we cannot simulate this mechanism. We suggest that other laboratories use supercomputers to simulate this mechanism. .

4.5 Viral proteins attach hemoglobin through the immune hemolysis or infection

of the red blood cell

Red blood cells mainly contain hemoglobin. During hemolysis, hemoglobin escapes from the cells and dissolves in the plasma. At this time, the ability of hemoglobin to carry oxygen is lost. Hemolysis occurs due to the rupture of red blood cell membranes and the dissolution of the matrix. Alternatively, the expansion of the red cell membrane pores allows hemoglobin to escape, leaving behind a double concave disc-shaped cell membrane --- "hematocyte". Immune hemolysis is specific hemolysis brought about by the antigen-antibody reaction. Physical, chemical or biological factors generate non-specific hemolysis. After hemolysis of red blood cells, viral proteins may attach hemoglobin. Considering that some researchers have calculated that people with some O types of blood are not easily infected with COVID-19, we speculate that immunohemolysis may be the first method for viral proteins to attach to hemoglobin. The virus has a Spike-CD147 pathway, which may be one way for the virus to infect red blood cells. The virus or virus protein complex entered into the red blood cell by thrombin-sensitive protein and dynein system protein. This may be the second method for viral proteins to attach to hemoglobin. Resulting from limited computational tools, we cannot simulate whether viral proteins attack hemoglobin outside or inside red blood cells.

4.6 Higher hemoglobin caused higher morbidity

The novel coronavirus pneumonia might be closely related to abnormal hemoglobin metabolism in humans. The number of hemoglobin is a significant blood biochemical indicator, and the content is different in different genders. The number of normal men is significantly higher than that of normal women, which might also be a reason why men are more likely to be infected with the novel coronavirus pneumonia than women. Besides, patients of novel coronavirus pneumonia are most of the middle-aged and older adults. Many of these patients have underlying diseases such as diabetes. Diabetic patients have higher glycated hemoglobin. Glycated hemoglobin is deoxyhemoglobin. Glycated hemoglobin is a combination of hemoglobin and blood glucose, which is another reason for the high infection rate for older people.

This study has confirmed that orf1ab, ORF3a, and ORF10 could coordinately attack heme on the beta chain of hemoglobin. Both oxygenated and deoxygenated hemoglobin are attacked. Deoxygenated hemoglobin is more attacked by the virus. During the attack, the positions of orf1ab, ORF3, and ORF10 are slightly different. It showed that the higher the hemoglobin content, the higher the risk of disease. However, it is not sure that the disease rate incited by abnormal hemoglobin (structural) is relatively low. The hemoglobin of patients and recovers could be detected for further research and treatment.

4.7 Inhibiting the heme anabolic pathway and causing the disease

This article considered the virus directly interfered with the assembly of human hemoglobin. The main reason was that the normal heme was too low. Heme joins in critical biological activities such as regulation of gene expression and protein translation. Porphyrin is an essential material for the synthesis of heme. Because the existing traces show there is too much free iron in the body of critically ill, it could be that virus-producing molecule competes with iron for the porphyrin, inhibiting the heme anabolic pathway and causing symptoms in humans.

It is not clear whether the spatial molecular structure of heme and porphyrin in patients with porphyria is the same as that in healthy people. If there is an abnormal structure, it is not clear whether this porphyrin can bind to a viral protein to form a complex, or if a viral protein can attack this heme. It

could be proved by clinical and experimental research.

5 Conclusions

Since the emergency epidemic, it is of high scientific significance to use bioinformatics to analyze the roles of novel coronavirus proteins. In this study, domain prediction methods were applied to search for conserved domains. The structure of protein molecules was obtained using homology modeling methods. Molecular docking technology was used to analyze the binding part of viral proteins to the porphyrin. The study results show that some viral proteins could combine to the porphyrin to form a complex, respectively. At the same time, orf1ab, ORF3a, and ORF10 proteins could coordinate attack the heme on hemoglobin.Deoxyhemoglobin is more vulnerable to virus attacks than oxidized hemoglobin.The attack will lead to less hemoglobin to carry oxygen and carbon dioxide. Patients with respiratory distress will be made worse. Lung cells are toxic and inflammatory due to derivatives produced by the attack, which eventually resulted in ground-glass-like lung images. Capillaries easily were broken due to inflammation. Proteins such as fibrinogen fill the capillaries' cracks through the coagulation reaction. Therefore, many fibrin accumulates in the alveolar tissue of the patient. As the porphyrin complexes of the virus produced in the human body, the mechanism also interfered with the normal heme anabolic pathway of the human body, they made a wide range of infection and disease.

Depending on the computational simulation and discussion analysis of this study, we speculated the primary pathogenic mechanism of this virus. The virus may first infect cells with ACE2 receptors, including immune cells. Immune cells produced antibodies and viral proteins. Antibodies and red blood cells generated immune hemolysis, or red blood cells were infected by Spike-CD147 pathway. Hemoglobin was attached and then attacked. The toxic and inflammatory due to derivatives produced by the attack. The virus also captured porphyrin and inhibited heme metabolism,then organs have complications. Cytokine storm causes multiple organs failure.

This paper is only for academic discussion. Due to the side effects and allergic reactions of drugs, please consult a qualified doctor for treatment details, and do not take medicine yourself.

We look forward to these discoveries bringing more ideas to people and inspiring people's confidence in defeating the virus.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets and results supporting the conclusions of this article are available at https://pan.baidu.com/s/1WQkmvLJlwmGt6l-__r65cA, code: 9os7.
Or : https://mega.nz/folder/kmQDmKJb#co7GKeL3Jucs5VPxNCtAvQ

Competing interests

The authors declare that they have no competing interests.

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Author’s contribution

Funding was obtained by WZL. Design, analysis, writing: WZL. Data curation, check manuscript: HLL All authors have read and agreed to the published version of the manuscript.

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Author details

[1] School of Computer Science and Engineering, Sichuan University of Science & Engineering, Zigong, 643002, China. [2] School of Life Science and Food Engineering, Yibin University, Yibin, 644000, China.

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