SARS-CoV-2 infection of cats and dogs?

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Abstract

SARS-CoV-2 causes severe pneumonia epidemics and probably originated in horseshoe bats, but the intermediate host is unknown. The interaction of SARS-CoV-2 spike protein and its acceptor protein ACE2 is an important issue in determining viral host range and cross-species infection, while the binding capacity of Spike protein to ACE2 of different species is unknown. Here, we used the atomic structure model of SARS-CoV-2 and human ACE2 to assess the receptor utilization capacity of ACE2s from different species including cats, chimpanzees, dogs, cattles. Our results show, domestic cats (*Felis catusc*) and dogs (*Canis lupus familiaris*) are more susceptible to infection by SARS-CoV-2 and that they can efficiently transmit the virus to previously uninfected animals that are housed with them. Especially, cats could be a choice of animal model for screening antiviral drugs or vaccine candidates against SARS-CoV-2.

Introduction

The severe respiratory disease COVID-19 caused by a novel and highly pathogenic coronavirus (SARS-CoV-2) is spreading all over the world, and COVID-19 has posed a serious global public health emergency[1]. As of April 2, there are more than 1,010,000 confirmed cases globally, leading to at least 53,000 deaths. On one hand, the homology of SARS-CoV-2 and bat SARS-like coronavirus (bat-sl-covzc45) is higher than 85%[2, 3]. On the other hand, the virus is unlikely to spread directly from bats to humans due to the lack of direct contact, meaning there is an unknown intermediate host. Although bats are likely reservoir hosts for SARS-CoV-2, the identity of any intermediate host that might have facilitated transfer to humans is unknown.

Recently, pet dogs and cats have been tested to be positive for SARS-CoV-2 infection[4, 5]. On March 5, 2020, samples from the mouth and nose of a pet dog in Hong Kong showed SARS-CoV-2 positive but no symptom of COVID-19. The dog's owner is a COVID-19 patient who was diagnosed in Hong Kong on Feb. 25, 2020. Experts believe the dog was probably infected by humans, and the dog's low level of infection with a novel coronavirus could be the first case in the world[4]. On March 27, 2020, Belgian public health authorities announced a pet cat in liege had been diagnosed with COVID-19. Researchers found SARS-CoV-2 in the feces of the cat, which was suffering from breathing difficulties. Belgian health officials stated subsequently the cat was infected with a novel coronavirus from its owner, and this is the first confirmed case of a pet infection in Europe[5]. Back to 2003, studies about SARS have shown that ferrets (*Mustela furo*) and domestic cats (*Felis domesticus*) were susceptible to infection by SARS-CoV and that they can efficiently transmit the virus to previously uninfected animals that are housed with them[6].

The specificity of the interaction between virus and its receptor is an important issue in regulating both the cross-species and human-to-human transmissions infection. SARS-CoV-2 invades cells mainly through Spike protein recognition of host cell receptor angiotensin-converting enzyme 2 (ACE2), such binding triggers a

cascade events leading to the fusion between cell and viral membranes for cell entry[7, 8]. ACE2 is expressed in most mammals, but not all ACE2 can be utilized by SARS-CoV-2 as the receptor[3]. The utilization of ACE2 by SARS-CoV-2 can rapidly screen and narrow the range of intermediate hosts of SARS-CoV-2. Currently, it is not clear which mammals are involved in the evolution of SARS-CoV-2 and which animals are infected by SARS-CoV-2.

In this study, we use the reported crystal structure of Spike protein binding to human ACE2 to construct 3D models of 10 kinds of animals by homologous modeling, among which 7 kinds of animals are easy to contact with humans in daily life, and the other 3 kinds of animals are commonly used model animals in the laboratory. According to the algorithm, the amino acid positions, types and sizes of the interactions between proteins were calculated, and the binding free energy was finally calculated. Our results may help to screen out SARS-CoV-2 intermediate hosts and to figure out the transmission model of SARS-CoV-2 and finally to control the COVID-19 disease.

Materials and methods

Sequence alignment

The amino acid sequences of the extracellular domains of these eleven ACE2 (residues Ser19-Asp615): *Homo sapiens* (human), *Pan troglodytes* (chimpanze), *Macaca mulatta* (Rhesus monkey), *Felis catusc* (domestic cat), *Equus caballus* (horse), *Oryctolagus cuniculusc* (rabbit), *Canis lupus familiaris* (dog), *Sus scrofa* (pig), *Ovis ariesc* (sheep), *Bos Taurus* (cattle), *Mus musculus* (house mouse) were downloaded from GenBank (File S1). Protein sequence alignments were done using Clustal Omega.

Protein-protein docking

Homology model of the target ACE2 protein was built by modeller9.18 using crystal structure of human ACE2 as template. One hundred independent structures were constructed and the one with best DOPE score was chosen for further energy minimization in Amber18 using ff14SB force field. Rosetta3.7 was used to perform

protein-protein docking to get the ACE2-SARS-CoV-2-RBD complex (PDB:6VSZ)[9].

Molecular dynamics simulation

The ACE2-SARS-CoV-2-RBD complex was immersed in an octahedron box of TIP3P water that was extended by 10 Å from the solute, with a rational number of counter ions of Na+ to neutralize the system. G Amber ff14SB force field was used to parameterize the protein. 10,000 steps of minimization with constraints (10 kcal/mol/Å2) on heavy atoms of complex, including 5,000 steps of steepest descent minimization and 5,000 steps of conjugate gradient minimization, was used to optimize each system. Then each system was heated to 300 K within 50 ps followed by 50 ps equilibration in NPT ensemble. Finally, 100 ns MD simulation on each system at 300 K was performed. The minimization, heating and equilibrium are performed with sander program in Amber18. The 100 ns production run was performed with pmemd. cuda. Based on the 100 ns MD simulation trajectory, binding free energy (ΔG) was calculated with MM/GBSA method according to the following equation：

$$\Delta G = \Delta H - T\Delta S = \Delta E_{ele} + \Delta E_{VDW} + \Delta G_{gb} + \Delta G_{np} - T\Delta S$$

where ΔEele and ΔEVDW refer to electrostatic and van der Waals energy terms, respectively. ΔGgb and ΔGnp refer to polar and non-polar solvation free energies, respectively. Conformational entropy (TΔS) was not calculated for saving time. Besides, the ligands were compared based on the same target, so it is reasonable to ignore the entropy.

Results and discussion

In order to analyze the possibility of SARS-CoV-2 infection on mammals that humans may come into contact with in daily life, we collected the amino acid sequence of ACE2 of pet cats, dogs, livestock cattles, horses, sheeps and pigs, as well as laboratory animal monkeys, chimpanzees, mice and rabbits in Genbank. We aligned the amino acid sequence of the extracellular domains of these eleven ACE2, the results are shown in Table 1. Compared with the ACE2 of human, the homology of amino acid sequence was between 95.0%~67.4%. It's worth noting that, as a

commonly used model animal in the laboratory, mice had the lowest homology. That is to say, the N-terminal peptidase domain of ACE2 in these animals is very similar to that of the known human hosts of infection. Chimpanzees and monkeys seem to be the most susceptible to SARS-CoV-2, while mice is the least sensitive.

To further analyze the possibility of infection in these animals，we determined the crystal structure of the SARS-CoV-2 spike receptor-binding domain (RBD) bound with the cell receptor ACE2, calculated the binding free energy. The predicted results are shown in Table 2, chimpanzees has the highest binding affinity, even higher than human, while gradually decreases in order of cats, cattles, monkeys, dogs, pigs, horses, sheeps, mice, rabbits. Although they belong to different species, they all have the binding affinity of the interaction. The binding affinity of cats and chimpanzees are very similar to human, while rabbits and mice are the lowest. Higher affinity values might be related to the dynamic of infection and the rapid spread observed for this virus. These data suggested that the higher binding affinity of RBD of coronavirus to ACE2 will confer the virus higher infectivity and pathogenicity[7, 10].

Computer modeling of interaction between SARS-CoV-2 RBD and ACE2 has identified some residues potentially involved in the actual interaction. Spike protein contacts with the helical structure of 19-83aa of human ACE2 and the folding of 347-358aa, producing intermolecular interactions. The results are shown in Table 3, Figure1. Structural analysis revealed a total of 11 residues of the SARS-CoV-2 RBD contact 13 residues of the human ACE2, and there are 15 hydrogen bonds at the SARS-CoV-2 RBD/ACE2 interface. Cats are particularly similar to humans, a total of 11 residues of the RBD contact 12 residues of the ACE2, 14 hydrogen bonds at the SARS-CoV-2 RBD/ACE2 interface. Structural analysis identified in ACE2-binding residues critical to cats in SARS-CoV-2 RBD, most of which were either highly conserved or had side chain properties similar to human (Table3 and Figure2). Taken together, these results show that the SARS-CoV-2 RBD/ACE2 of cats interfaces share substantial similarity in the number of interacting residues, and hydrophilic interaction networks. In the study of SARS, the infection experiments on many kinds of animals show that ferrets and domestic cats can be used as potential animal

infection models such as vaccine and drug screening[6]. This is consistent with the results of recent virus infection experiments: ferrets and cats have effective replication ability. Virus RNA was found in the nose, soft jaw, tonsil and small intestine of ferrets, but no virus was detected in other organs, which proved that SARS-CoV-2 was only replicated in the upper respiratory tract[11]. A research in human body have been reported that: ACE2 receptor is highly expressed in human nasal cells, but not detected in lung cells[12]. Meanwhile, the expression of ACE2 in the small intestine is high, which is consistent with the recently reported gastrointestinal tract as a potential route of SARS-CoV-2 infection [2, 13]. Compared with human, ACE2 and SARS-CoV-2 binding affinity of mice are very low, similar interacting residuals are less, and hydrophobic interaction networks are weaker. Therefore, SARS-CoV-2 may replicate inefficiently in mice and rats, ruling them out as animal models to test vaccine or antiviral drugs candidates against SARS-CoV-2.

Some experts point out it is a protracted battle, that is, the control of the source of the virus. Most of the answers given are that the carrier of the virus will be controlled in poor countries until the end. We can also consider the common contact of human animals, which is also a hidden carrier of the virus. Bird flu, for example, fails because it is impossible to kill all birds, and the virus mutates too quickly for vaccine development. So do coronaviruses have this trend?

Based on the potential interaction between S protein and mammalian ACE2, it was speculated that SARS-CoV-2 preserved the ability to infect many mammals including chimpanzees, cats, cattles, monkeys, dogs. Cats' binding free energy, as well as key amino acids, are highly similar to humans, meaning they could serve as model animals for developing vaccines and drugs, implicating these animal species as possible intermediate hosts or animal models for SARS-CoV-2 infections. About 60 percent of people infected with the virus are asymptomatic carriers and bats are a special case of SARS asymptomatic carriers, so these animals can carry the virus and infect people[14, 15]. Modeling with genetic method, according to the HcoV-OC43 and MERS-CoV the evolutionary rate of SARS-CoV evolution are analyzed, the results showed that SARS-CoV may spread in the bats is transmitted to other hosts such as

pangolins, dozens of years later it was the third time in the 17 years of coronary virus outbreak, will probably have a virus across species boundaries[3, 16, 17].Considering the widespread of stray cats, wildlife markets and stock farms in Wuhan, it was not strange that these animals could serve as potential intermediate hosts of SARS-CoV-2. Therefore, pets exposed to the patient should be screened for SARS-CoV-2. During the process of epidemic prevention, we should prevent the predict possible of zoonosis event or cross-infection in the future. However, these are still preliminary results predicted by sequence analysis, and more laboratory and epidemiological investigation are required to uncover the true intermediate hosts of SARS-CoV-2.

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Declaration of interest statement

The authors declare no competing interests.

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Figure legends

Figure 1 Comparison of amino acids homology of ACE2 in the helical structure of 19-83aa and the folding of 347-358aa between human and other organisms

Figure 2 Structure simulation of SARS-CoV-2 RBD with ACE2 from human, dogs, cats, chimpanzees, cattles, monkeys

Table 1 Comparison of amino acids homology of ACE2 between human and other organisms

Table 2 Binding affinity (ΔG) values of the interaction between spike and viral ACE2 receptor

Table 3 Potential interaction between the S protein receptor binding domain (RBD) and ACE2 of a variety of animals

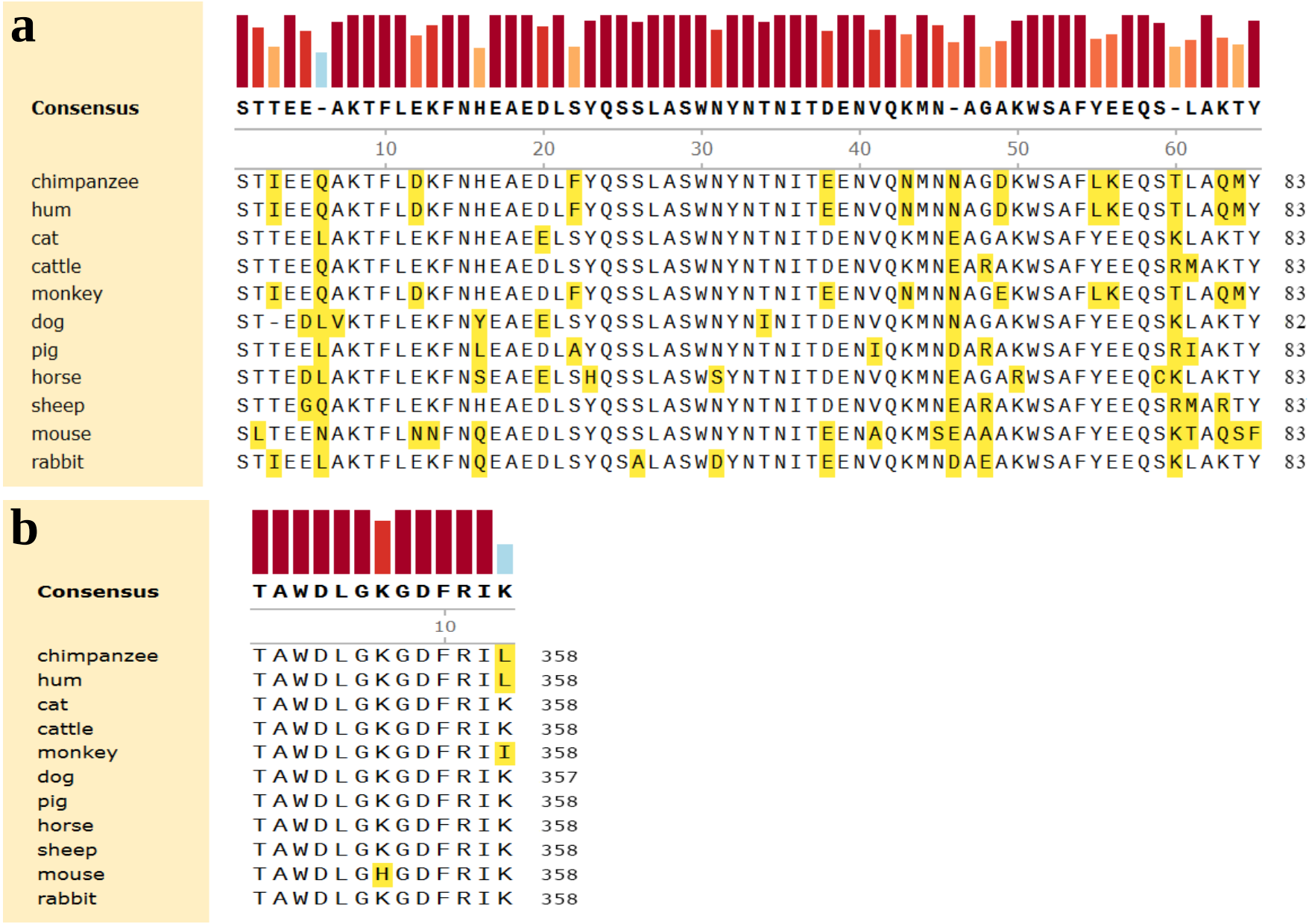
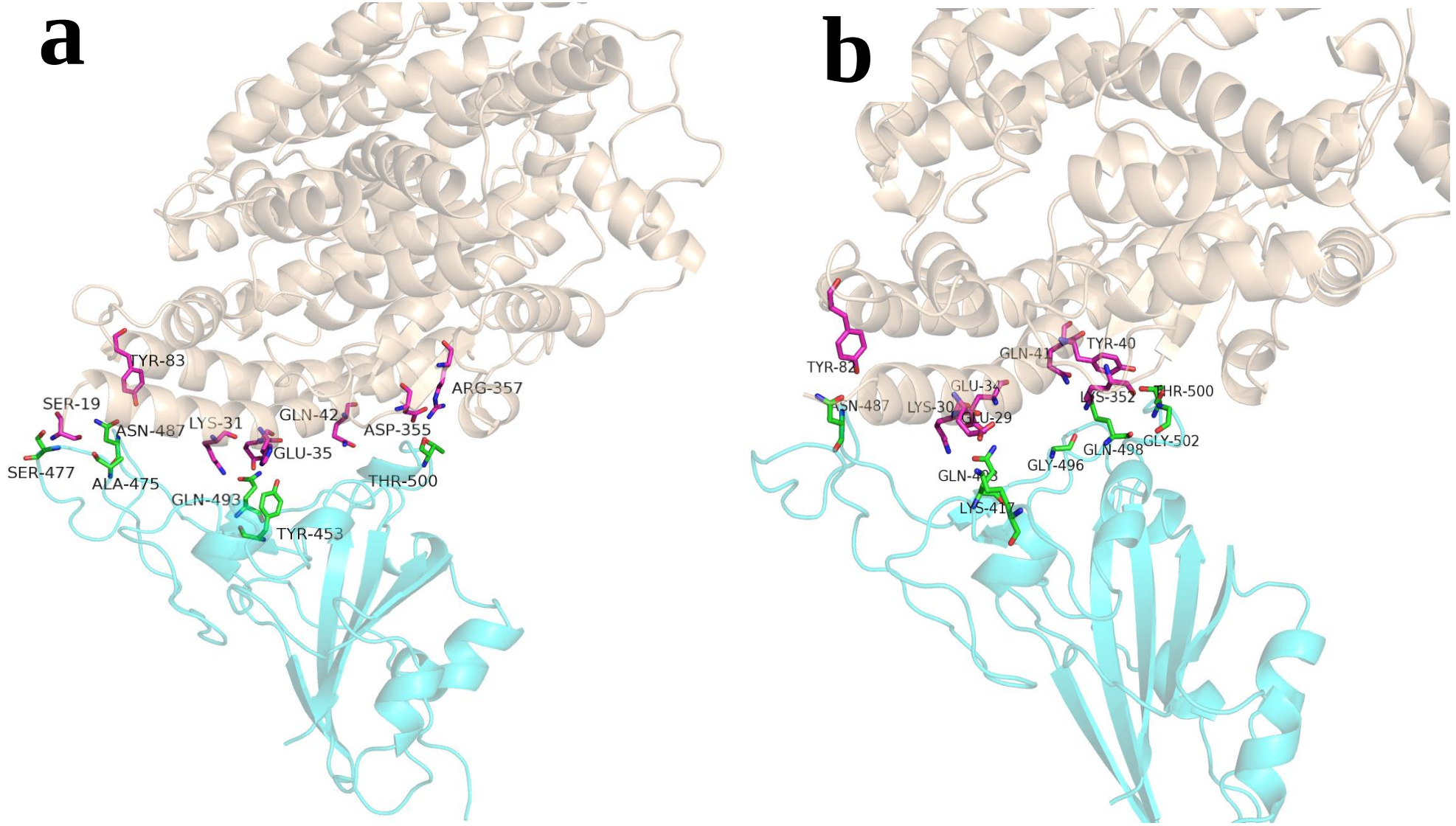
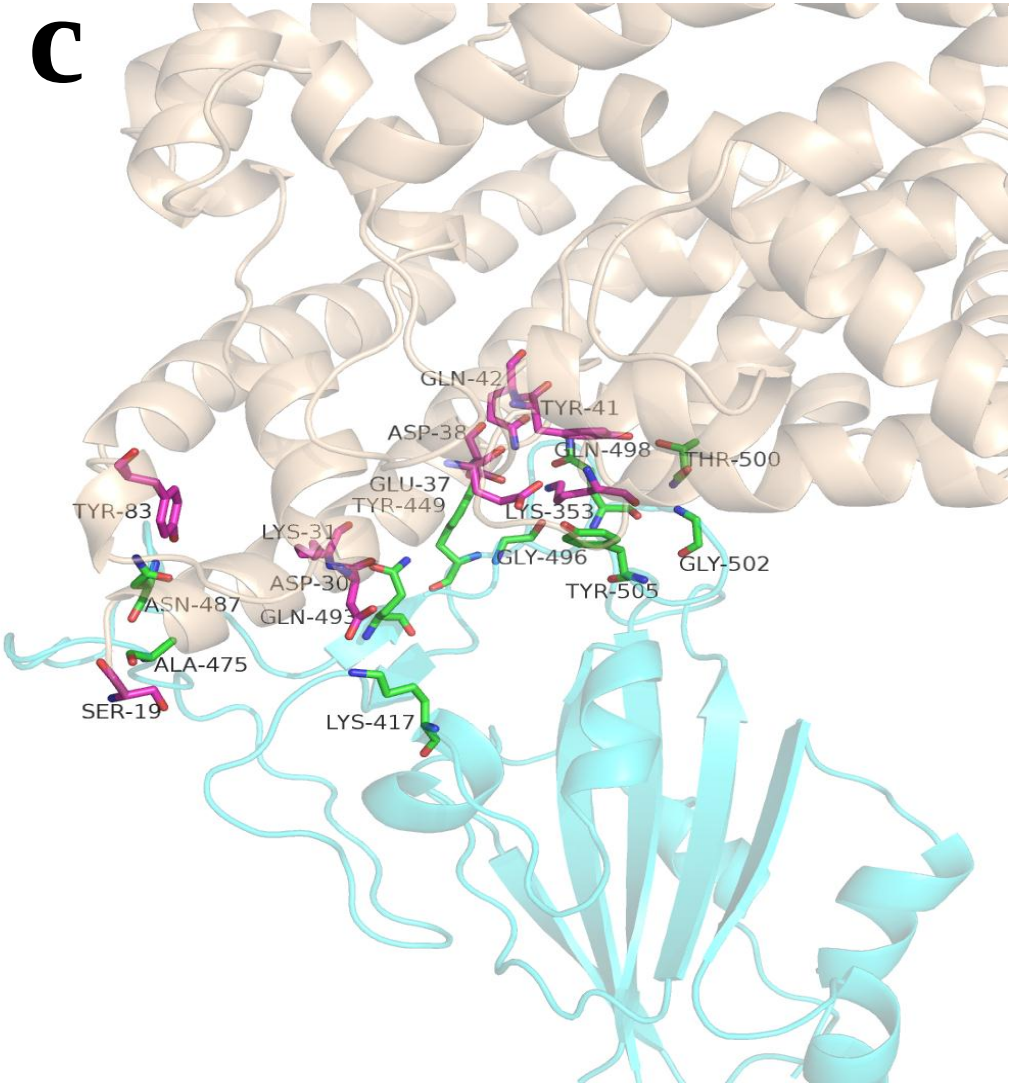
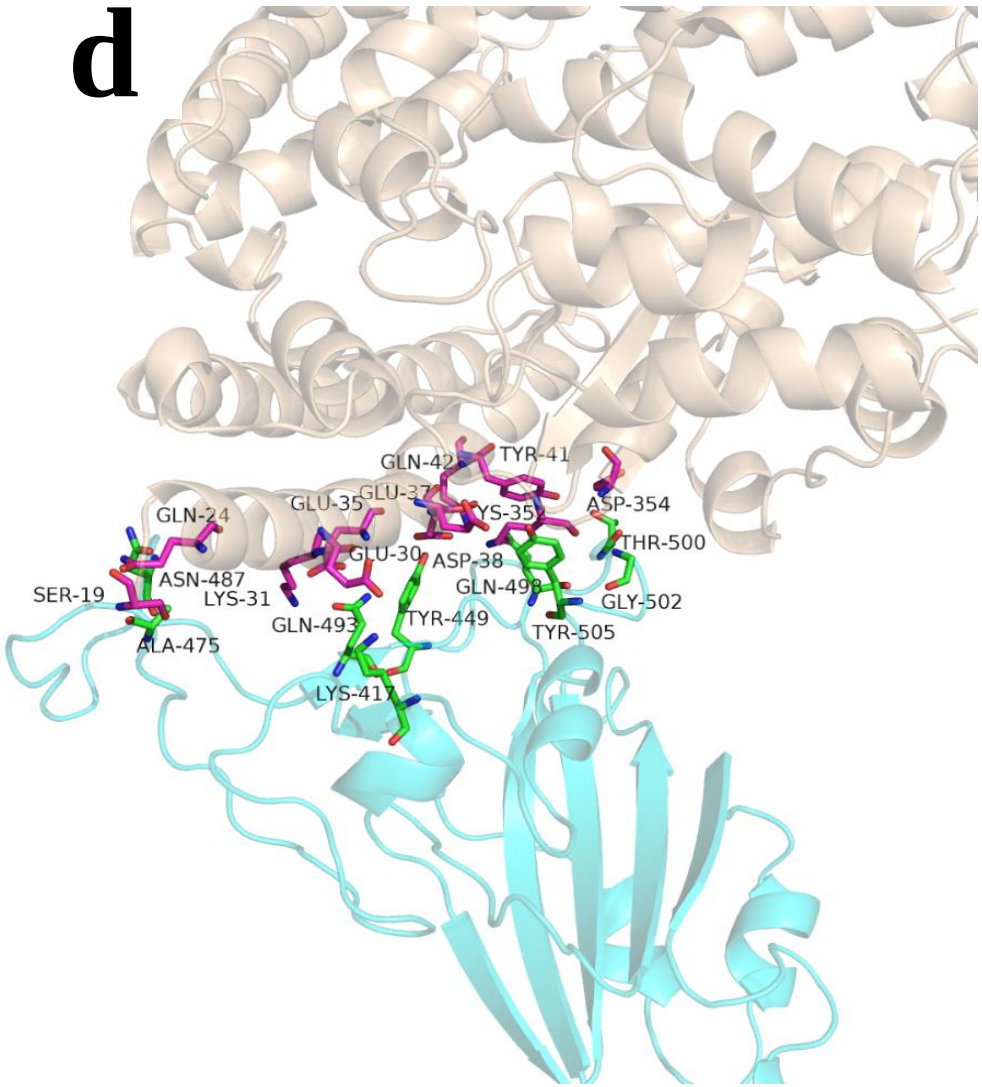


Figure 1. Comparison of amino acids homology of ACE2 in the helical structure and the folding between human and other organisms. (a) The helical structure of 19-83aa. (b) The folding structure of 347-358aa. The different amino acids are shown in yellow.







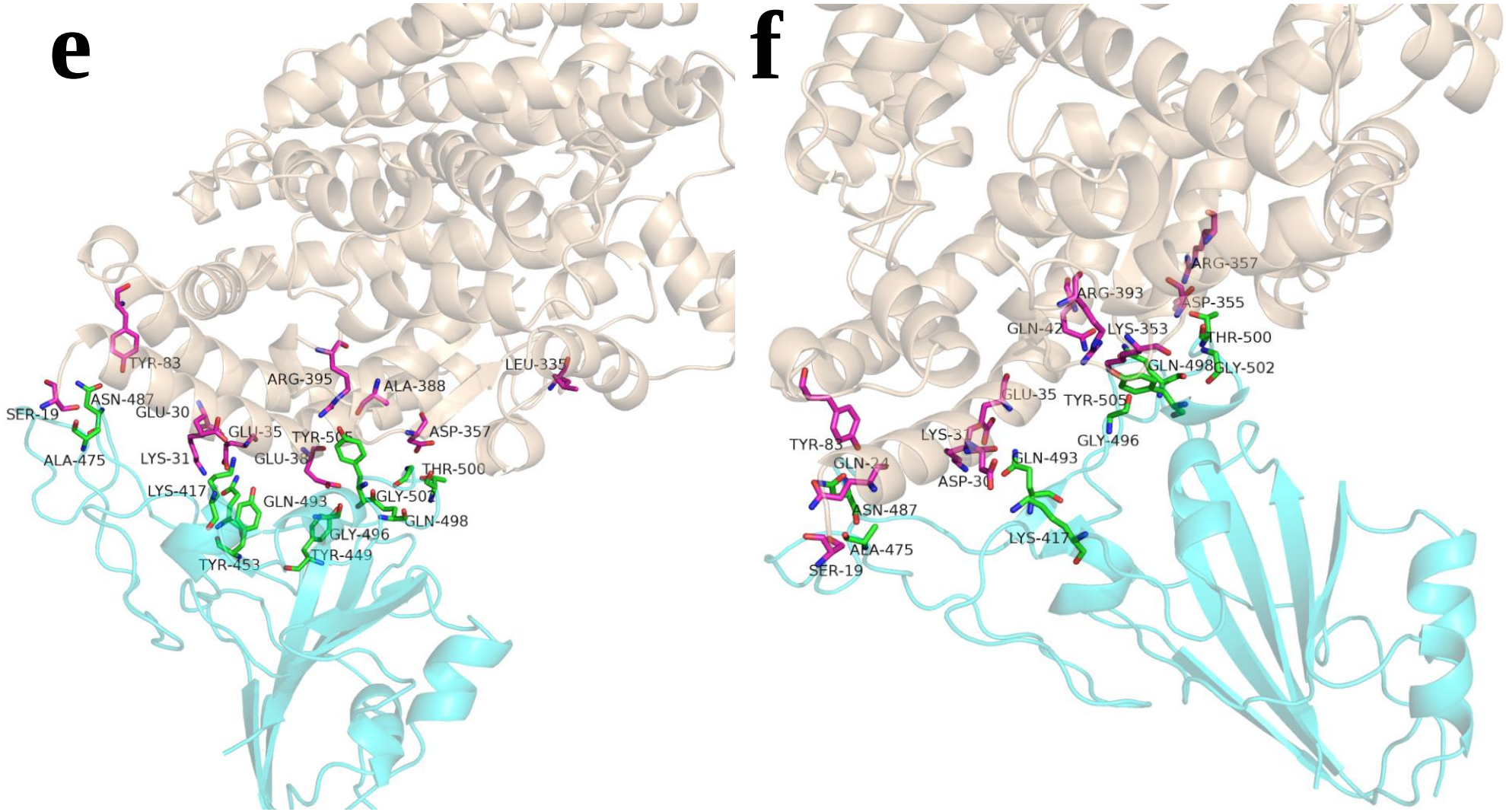


Figure 2. Overall structure of SARS-CoV-2 RBD bound with ACE2. (a)-(f) are respectively human, dogs, chimpanzees, cattles, cats, and monkeys. ACE2 is colored light coffee. SARS-CoV-2 RBD core is colored cyan. Amino acid interaction sites are also shown.

Table 1. Comparison of amino acids homology of ACE2 between human and other organisms

Species	Protein-ID	Identity %
Homo sapiens (human)	XP_011543853.1	100
Pan troglodytes (chimpanzee)	XP_016798468.1	95.0
Macaca mulatta (Rhesus monkey)	XP_028697658.1	91.2
Equus caballus (horse)	XP_001490241.1	83.1
Oryctolagus cuniculus (rabbit)	XP_002719891.1	82.5
Felis catus (domestic cat)	XP_023104564.1	81.2
Canis lupus familiaris (dog)	NP_001158732.1	80.1
Sus scrofa (pig)	XP_020935033.1	78.8
Ovis aries (sheep)	XP_011961657.1	78.4
Bos taurus (cattle)	XP_024843618.1	77.3
Mus musculus (mouse)	NP_001123985.1	67.4

Table 2. Binding affinity (ΔG) predicted values for the interaction between spike and viral ACE2 receptor

Protein-protein complex (spike/ Viral ACE2)	ΔG (kcal/mol)
chimpanzee	-50.7659
human	-46.7995
cat	-44.8766
cattle	-40.281
monkey	-39.326
dog	-37.7814
pig	-30.719
horse	-28.4732
sheep	-26.3634
mouse	-21.9914
rabbit	-16.749

Table 3. Potential interaction between the S protein receptor binding domain (RBD) and ACE2 of a variety of animals

RBD	human	chimpanzee	cat	cattle	monkey	dog	pig	horse	sheep	mouse	rabbit
405D	-	-	-	-	-	-	-	-	-	387R	-
417K	30D	30D	30E	30E	30D	29E	30E	30E	30E	-	Q30
449Y	38D	38D	38E	38D	-	-	-	38E	38D	38D	-
453Y	34H	-	34H	-	-	-	-	-	-	-	-
475A	19S	19S	19S	19S	19S	-	19S	19S	19S	-	-
477S	19S	-	-	-	-	-	-	-	-	-	-
487N	24Q	-	-	24Q	24Q	-	-	-	-	24N	-
487N	83Y	83Y	83Y	-	83Y	82Y	83Y	83Y	-	-	83Y
493Q	31K	31K	31K	31K	31K	30K	31K	31K	-	-	31K
493Q	35E	-	35E	35E	-	34E	35E	35E	-	34Q	35E
495Q	-	-	-	-	-	-	-	-	31K	-	-
496G	-	353K	355K	-	353K	352K	-	353K	352K	-	-
498Q	42Q	42Q	38E	-	42Q	41Q	-	-	-	-	-
498Q	353K	353K	355K	352K	353K	352K	-	353K	-	-	-
500T	-	41Y	-	41Y	-	40Y	41Y	-	-	-	-
500T	355D	-	-	354D	355D	-	355D	355D	-	355D	-
500T	357R	-	357D	-	357R	-	-	-	-	-	-
502G	353K	353K	355K	352K	353K	352K	353K	353K	352K	353H	353K
505Y	-	-	395R	-	-	-	-	-	-	-	-
505Y	37E	37E	388A	37E	393R	-	-	386A	--	386A	-