ACS Chemical Neuroscience

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ACS Chem. Neurosci., **Just Accepted Manuscript** • DOI: 10.1021/acschemneuro.0c00373 • Publication Date (Web): 06 Jul 2020

Considerations around the SARS-CoV-2 Spike Protein with particular attention to COVID-19 brain infection and neurological symptoms

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Running Title: SARS-CoV-2 Spike Protein and brain infection

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Abstract

Spike protein (S protein) is the virus 'key' to infect cells being able to strongly bind to the human angiotensin-converting enzyme2 (ACE2), as it has been reported. In fact, Spike structure and function is known to be highly important for cell infection as well as entering the brain. Growing evidence indicates that different types of coronaviruses not only affect the respiratory system, but they might also invade the central nervous system (CNS). However, very few evidence have been so far reported on the presence of COVID-19 in the brain and the potential exploitation, by this virus, of lung to brain axis to reach neurons has not completely understood. In this article we assessed the SARS-CoV and SARS-CoV-2 Spike protein sequence, structure and electrostatic potential using computational approaches. Our results showed that the S proteins of SARS-CoV-2 and SARS-CoV are highly similar, sharing a sequence identity of 77%. In addition, we found that the SARS-CoV-2 S protein is slightly more positively charged than that of SARS-CoV since it contains four more positively charged residues and five less negatively charged residues which may lead to an increased affinity to bind to negatively charged regions of other molecules through non-specific and specific interactions. Analyzing of the S protein binds to the host ACE2 receptor showed a 30% higher binding energy for SARS-CoV-2 than the SARS-CoV S protein. These results might be useful for understanding the mechanism of cell entry, blood brain barrier crossing and clinical features related to the CNS infection by SARS-CoV-2.

Key Words: ACE2, Brain, COVID-19, Spike Protein

Introduction

The crucial step in the viral infection is the process of viral entry into the host cells and understanding this mechanism is important for exploring the effective therapeutic agents in the treatment of viral infection. Endocytic pathway including endosome and lysosome and the autophagy process in viral entry has attracted considerable attention as therapeutic targets in combating diseases caused by virus in the last decade[1].

The clathrin-dependent endocytotic/exocytotic has been reported the main pathway for some viruses enter host cells such as Hepatitis C virus, Tick-borne encephalitis virus and Zika virus which enter the astrocytes and induce neuro-infection by endocytosis[2,3], Whether SARS-Co-V2 infects neuronal system by this mechanism has yet to be elucidated. Having in mind that other type of coronavirus, swine hemagglutinating encephalomyelitis virus (HEV), employs endocytosis for its trans-synaptic transfer[4].

From a molecular point of view, computational modeling studies highlighted the huge similarity between SARS-CoV-2 with the original SARS-CoV especially in the 3-D structures of the receptor-binding domain of the Spike proteins (S). Several lines of evidence focused on Spike protein as a main tool of the virus to infect cells being able to strongly bind to the Angiotensin converting enzyme 2 (ACE2)[5,6].

The Spike protein is a homotrimer that protrudes from the viral membrane and contains, in each of its monomers, a Receptor Binding Domain (RBD) through which this viral protein directly interacts with the ACE2 receptor located on the surface of many host cells[7–10].

ACE2 is an enzyme attached to the outer surface (cell membranes) of cells in the lungs, arteries, heart, kidney, intestines and brain[11]. The ACE2 which is expressed in the brain,

mainly existing in the brain stem and in the regions involved in cardiovascular function and central regulation of blood pressure including subfornical organ, nucleus of the tractus solitarius, paraventricular nucleus, and rostral ventrolateral medulla[12,13].

In a previous study, Wrapp et al. reported that SARS-CoV-2 S protein exhibits higher binding affinity to the ACE2 receptor than that of the SARS-CoV[14].

SARS-CoV and SARS-CoV-2 share about 96% nucleotide sequence identities, suggesting that SARS-CoV- 2 might have emerged from a bat SARS-like coronavirus. Therefore, in this study we investigated the differences between the sequence, structure and electrostatic potential of SARS-CoV-2 and SARS-CoV Spike proteins both in their open and close conformations using computational approaches and discuss how these divergences may make this new virus highly infectious to the human cells and organs with particular attention to brain infection and neurologic symptoms in patients with COVID-19.

In fact, although the most prevalent symptom that leads COVID-19 patients to the intensive care units, is the heavy respiratory complications, some patients also showed neurologic signs which have been described in three categories: central nervous system (CNS) symptoms or diseases (headache, dizziness, impaired consciousness, ataxia, acute cerebrovascular disease, and epilepsy), peripheral nervous system (PNS) symptoms (hypogeusia, hyposmia and neuralgia), and skeletal muscular symptoms [15,16]. Recently, more serious complications including, acute encephalopathy[17] and acute hemorrhagic necrotizing encephalopathy (ANE)[18] have been reported in case report studies. ANE is a rare complication of viral infections such as influenza and has been related to remarkable increase in intracranial cytokine, which leads to BBB breakdown[19].

There is no evidence regarding the entry of SARS-CoV-2 to the brain to date neither in animal nor human studies. Indeed, several papers reported the presence of SARS-CoV in the central nervous system (CNS) especially found in CSF like in the report in which the status epilepticus of a patient was associated with SARS[20] and others reports in which demyelinating brain pathology have been associated to coronaviruses infection [21]. Besides, some clinical studies performed on patients affected by SARS-CoV have identified the presence of virus particles in the brain, mainly localized in the neurons [22–24].

Therefore, in this article we also discuss the possible pathological interaction between brain and lung, CNS infection and relevant clinical futures in patients with COVID-19 based on our current knowledge.

Results and discussion

The results of sequence alignment, shows that the sequences of the S proteins of SARS-CoV-2 and SARS-CoV are highly similar, sharing a sequence identity of 77%. Nonetheless, some divergences can be observed in the sequence (Figure 1 and supplementary data). These divergences have been examined in a previous study by Jaimes et al. who represented in the 3-dimesional (3D) structures of the proteins[25]. Moreover, Baig et al. suggest that these differences may be related to the higher binding affinity of SARS-CoV-2 S protein to the host ACE2 receptor[26].

More recently, Robson indicated that all human SARS coronaviruses (and indeed the spike proteins of many other related coronaviruses) seem similar in general conformation, and the variations observed in experimental structures are probably more to do with crystallization or other preparation methods[27].

Our findings reveal that, the SARS-CoV-2 S protein is slightly more positively charged than that of SARS-CoV since it contains four more positively charged residues and five less negatively charged residues (Table 1). Even if the difference in charge between SARS-CoV-2 and SARS-CoV S proteins is rather small, this effect can be amplified by the high number of S proteins that are present on a virus particle. This difference in charge between SARS-CoV-2 and SARS-CoV S proteins can have a significant impact in cell adhesion and crossing the blood brain barrier [28,29] which will be discussed more in detail, later in this article.

A two-step process takes place when the S proteins interact with other proteins, such as when the S protein binds to the human ACE2 receptor, to establish a final protein-protein association. (1) The first step is dominated by electrostatic forces that lead the formation of an ensemble of transient and non-specific encounter complexes[30]. In this step, the S protein would be found in the closed conformation. (2) A structural rearrangement takes place in the protein and the three S protein RBDs open up to expose their binding interface to form a well-defined complex, which is stabilized not only by electrostatic forces, but also by polar (salt-bridge and hydrogen bond) and non-polar interactions (π-stack, π-anion, and short-range hydrophobic interactions)[7],[30]. Taking this into account, the electrostatic potential of both SARS-CoV-2 and SARS-CoV S protein surfaces, both in the open and close conformations, has been calculated in this study (see Figure 2), also focusing in their RBDs, in order to analyze the differences in the ability of SARS-CoV-2 and SARS-CoV to bind to other molecules within the human body according to their electrostatic properties, and thus, their capacity to enter human cells.

Several structures of S proteins could be found in the Protein Data Bank (PDB), but in all of them were present not resolved segments. In order to calculate and map a protein electrostatic potential a complete structure is needed, therefore complete 3D structures of SARS-CoV-2 and SARS-CoV protein S, both in the open and close conformation, were modelled using homology modelling techniques.

Having modelled SARS-CoV-2 and SARS-CoV S protein structures, both structures in the close state conformation were superimposed with 1.236 Å Root-Mean-Square Deviation (RMSD) over 427 aligned Cα positions. In this way, the structure of both proteins was compared showing a high structure similarity.

Afterwards, macromolecular electrostatic calculations of the models were performed. In other studies, differences in the RBD:ACE2 interfaces between SARS-CoV-2 and SARS-CoV S protein at a structural level have already been described in detail and have been linked to SARS-CoV-2 higher binding affinity. Herein, these interfaces have been analyzed at the electrostatic potential level (see Figure 3, Figure 4).

Recently, in a report published in Nature, Lan et al., identified residues in the SARS-CoV-2 RBD that are essential for ACE2 binding, the majority of which either are highly conserved or share similar side chain properties with those in the SARS-CoV RBD. They believe that similarity in structure and sequence strongly indicate convergent evolution between the SARS-CoV-2 and SARS-CoV RBDs for improved binding to ACE2[31].

In Figure 2, the electrostatic potentials of SARS-CoV-2 and SARS-CoV S protein (top side) are compared, showing that the SARS-CoV-2 S protein surface exhibits a more positive electrostatic potential than that of SARS-CoV. This same electrostatic potential difference can also be seen in the binding interface of their RBDs (Figure 3). Thus, despite presenting

a high sequence and structural similarity, SARS-CoV-2 and SARS-CoV S proteins have different electrostatic properties. This difference can have an effect on the capacity of the virus to adhere to other molecules. On the other side, human ACE2 binding interface tends to have a predominantly negative electrostatic potential (Figure 4) and, therefore, will interact more strongly with the SARS-CoV-2 S protein both in the open and close conformations.

Comparing SARS-CoV-2 and SARS-CoV S protein sequences, 3D structures and electrostatic potentials, reveals that both proteins have a conserved sequence and structural features, but different electrostatic characteristics in both their external surface and their host-interaction interfaces. As previously described, the SARS-CoV-2 S protein is slightly more positively charged in these regions than that of SARS-CoV, which will lead to an increased affinity to bind to negatively charged regions of other molecules through non-specific and specific interactions.

Moreover, some differences in the amino acidic content of the S protein in the RBD-ACE2 interface can lead to the establishment of more specific interactions with the host receptors. Hence, SARS-CoV-2 is more likely to establish interactions with different targets across the human body than SARS-CoV both through non-specific and specific interactions. All this, ultimately, can increase the capacity of SARS-CoV-2 to enter human cells and binding to the negatives charge barriers such as BBB[32] with respect to SARS-CoV.

In the last months, S protein structure and electrostatic properties have been the object of much investigation. Previous computer-based experiments have also noted that the SARS-CoV-2 RBD exhibits a more positive electrostatic potential than the SARS-CoV RBD[31,33–35] and that the electrostatic potential has a particularly important role in the high infection rate

of SARS-CoV-2. In agreement with our results, it has previously been observed that SARS-CoV-2 binds with a higher affinity to the human ACE2 receptor than SARS-CoV[33]. This was also attributed to the enhanced electrostatic interactions between SARS-CoV-2 and ACE2 due to the SARS-CoV-2 RBD having greater electrostatic complementarity with the binding domain of ACE2 than the SARS-CoV RBD[33]. In particular, it has been reported that the increased positive electrostatic potential of the SARS-CoV-2 binding surface is mainly due to an essential mutation of the hydrophobic residue Val404, present in SARS-CoV, to the positively charged residue Lys417 in SARS-CoV-2[31,34].

Amin et al. also identified a complementary negative electrostatic potential on the surface of the binding site of ACE2[33].

Taking advantage of our previous experience dealing with nanoparticles (NPs) specifically tailored to cross the blood brain barrier (BBB) and target the brain tissue we can speculate the potential strategies of COVID-19 to enter into the brain. Indeed, the dimension and the surface properties of the COVID-19 is similar, in terms of adhesion and cell membrane crossing abilities, to those shown by the nanoparticles specifically designed for BBB crossing[29,36]. So the parallelism between COVID-19 and the strategies adopted to let nanoparticles cross the BBB can be useful to hypothesized the ways used by the virus to enter into the brain. Therefore, an increase of the number of the positive amino acids of the COVID-19 envelope might increase in a significant manner the adhesion properties of the COVID-19 crossing the BBB and entering the brain.

In order to quantify the difference in the binding affinity of the two complexes (SARS-CoV-2:ACE2 and SARS-CoV:ACE2), their binding free energy was calculated. The results showed that SARS-CoV-2 S protein binds to the host ACE2 receptor with a 30% higher

binding energy than the SARS-CoV S protein. It has also been observed that the electrostatic contribution to the total binding free energy is the dominant term in the SARS-CoV-2:ACE2 interaction. Hence, this data supports the qualitative analysis of the electrostatic potential of the structures presented above and the quantitative data shown in previous studies.

According to the bioinformatics data regarding the possible interaction between virus Spike protein and ACE2 protein, it is suggested that SARS-CoV-2 is probable to adhere with higher efficiency to the cells through a non-specific interactions which have a major impact on cell adhesion[28] due to 1) SARS-CoV-2 electrostatic properties and, 2) binds with higher affinity to the host ACE2 receptor through specific interactions. In fact, our findings revealed that the Spike protein of SARS-CoV-2 binds to the host ACE2 receptor with a significant higher binding energy than the SARS-CoV S protein, indicating the electrostatic contribution to the total binding free energy is the dominant term in the SARS-CoV-2:ACE2 interaction.

As previously described, Spike protein and ACE2 represents the key, but not the exclusive, site of entry of the virus into the cell, thus non-ACE2 pathways for virus infection of neural cells also cannot be excluded[37]. Whether Covid-19 infects neurons, astroglial cells and enters astrocytes by endocytosis remains to be studied. Overall, considering the computational assay that have been performed in this study we suggest that Spike protein dependent pathway is thought to be more important than clathrin-dependent endocytosis for cell entry and BBB crossing. Therefore, Spike dependent pathway should be taken into account in therapeutic strategies for specific antibodies or vaccine production research.

Regardless of how the virus enters the brain, there are some CNS complications in patients with COVID-19 that should be taken into consideration.

The presence of the virus in the brain stem may affect chemo-sensing neural cells related to the respiration as well as respiratory center neurons thus damaging the lung ventilatory function[37].

It has been shown that SARS-CoV downregulate ACE2 protein expression in a replication dependent manner[38]. Supporting these finding, it has been revealed that SARS-CoV infections and the Spike protein of the SARS-CoV reduced the ACE2 expression and the injection of SARS-CoV Spike into mice worsened acute lung failure in vivo, which was attenuated by blocking the renin-angiotensin pathway[39].

Considering the high similarity of SARS-CoV and COVID-19, and higher binding energy of COVID-19 than the SARS-CoV S protein to bind the ACE2, it has been hypothesized that SARS-CoV-2 also can downregulate ACE2 in different organs including brain[40,41]. This downregulation might be a part of this complicated story; inhibition of ACE2 activity, reduces the sensitivity of the baroreceptor reflex control of heart rate as well as increase in sympathetic tone, eventually resulting in the blood pressure elevation and cardiac dysfunction. On the other hand, increasing of inflammatory cytokines during lung injury, hypoxemia and elevation of sympathetic tone through ACE2 downregulation leads to CNS hyper-activation which might play a crucial role in etiopathogenesis of neurogenic pulmonary edema (NPE)[42], a life-threatening complication following a neurologic insult[43], and finally deteriorating the respiratory and cardiovascular complications in these patients (see figure 5).

Supporting the idea of brain infection, more recently, in a case report one patient was described with no past medical history showed frequent seizures probably due to COVID-19 infection[44]. Several mechanisms for the etiology of seizure have been taken into consideration, including the direct infiltration of brain tissue, production of toxins by the virus or increasing of inflammatory cytokines by the brain[45]. Recently, It has been reported that COVID-19 initiates the inflammatory cascade and as a result, releases inflammatory cytokines[46] which is called cytokine storm syndrome[47]. Consecutively, these cytokines can drive neuronal hyper-excitability via activation of glutamate receptors and play a role in the development of acute seizures[48–50].

In addition, in a case report study, it was presented a case of self-limited encephalitic associate with SARS-CoV-2. The authors suggested that with the clearance of virus and the use of mannitol, the CSF pressure might gradually decrease and the patient's consciousness will improve[51].

In a recent study, neurologic features in severe Covid-19 patients who admitted to the hospital has been reported. Magnetic resonance imaging (MRI) of the brain was performed in 13 patients in this evaluation. Although these patients did not have focal signs that suggested stroke, they underwent MRI because of unexplained encephalopathic features. Two of 13 patients who underwent brain MRI showed single acute ischemic strokes. Authors concluded that their data were not enough to recognize which of these features were due to critical illness–related encephalopathy, cytokines, or the effect or withdrawal of medication, and which features were directly due to SARS-CoV-2 infection.

Post-viral anosmia which is also named olfactory dysfunction[52,53] and ageusia[54] are other neurologic symptoms have been reported in patients with COVID-19. More recently in a

cross-sectional study in Iran on 10.069 cases, coincidence of COVID-19 epidemic and olfactory dysfunction has been reported[55]. In this context, recently, Lechien et al. reported that olfactory and gustatory dysfunctions are prevalent in patients with mild-to-moderate COVID-19 infection[56]. Some mechanisms have been raised to explain this association including 1) injury at the level of the neuro-epithelium of olfactory receptor cells in the nasal roof or in the central olfactory processing system[55] 2) damage of the central olfactory routs and other regions of the brain[57–59] 3) inflammation or the possible damages to the nasal epithelium cells that required for normal olfactory function[60]. Therefore, both epithelial damage and CNS involvement, have been reported as the possible causes; however, its exact pathophysiology remains yet to be elucidated[53,61].

In accordance with the neurotrophic mechanism proposed by Baig et al.,[10] which hypothesizes the COVID-19 brain access via the transcribrial route, as documented for other CNS targeting pathogens, we suppose a possible entry of the virus from the olfactory bulb and, exploiting the blood microcirculation, the COVID-19 may have access to the cerebral circulation and interact with ACE2 receptors expressed on neuronal cells.

Conclusion

Considering the neurological manifestations of patients with COVID-19 and in light of the bioinformatics findings of this study indicating more positive charged spike protein structure and higher binding free energy of the SARS-CoV-2:ACE2 interaction, it is expected that COVID-19 possess higher efficiency than SARS-CoV to enter the cells and reaching the brain. This neuro-invasive characteristic, should be taken into account in the basic and clinical research as well as prioritization and individualization of therapeutic approach.

Methods:

Spike protein sequence alignment and analysis

The sequence alignment of the S protein of SARS-CoV-2 (UniProt ID P0DTC2) and SARS-CoV (UniProt ID P59594) was conducted in the webserver BLASTp[62] using the Needleman-Wüncsh algorithm with the default substitution matrix (BLOSUM62)[63] (see Figure 1). For illustrative purposes, the resulting sequence alignment was downloaded as a text file from BLASTp and converted into an ALI format file in order to visualize and produce the sequence alignment images on the Molsoft Browser 3.9 [64,65]. In order to analyze the divergence in the amino acidic content of the S protein from SARS-CoV-2 and SARS-CoV, the number of each residue present in each protein sequence was counted using the "str_count" function in RStudio 3.6.3 (Table1)[66].

Homology modeling and structure comparison

Homology models of the complete 3D structures of SARS-CoV-2 and SARS-CoV S protein, both in the open and close conformation, were built in the MODELLER 9.23 [67] program by using a sequence alignment extracted from BLASTp[62] and template structures obtained from the PDB[68] (Supplementary data). Homology models of SARS-CoV-2 and SARS-CoV S protein in the close conformation were superimposed using the structure comparison tool Match Maker in the software UCSF Chimera 1.14 [69]

Calculation of the electrostatic potential

Electrostatic potentials of the homology models were calculated using the program Adaptive Poisson-Boltzmann Solver (APBS)[70] and were displayed in PyMol 2.3.4 [71] as a

color-coded electrostatic potential molecular surface (Solvent-Excluded Surfaces (SESs)) by using the APBS 1.5 plugin [72] (see Figure 2).

Binding free energy calculation

In order to quantify the difference in the binding affinity of complexes SARS-CoV-2:ACE2 and SARS-CoV:ACE2, their binding free energy was calculated using the Molecular Mechanics-Poisson Boltzmann Surface Area (MM-PBSA) approach[73] implemented in the GROMACS-5.0.7 tool g_mmpbsa[74]. MM-PBSA is a fully atomistic method for the calculation of binding free energies that combines a molecular mechanics description of the protein complex with a continuous solvent model. It is widely used to evaluate interaction energies between proteins and biomolecules in general[75].

The structures of the SARS-CoV-2 S protein in complex with ACE2 receptor (PDB ID 6LZG) and the SARS-CoV S protein in complex with ACE2 receptor (PDB ID 6ACG) were subjected to geometry optimization in GROMACS-5.0.7 [76] prior to the calculation. A relative dielectric constant ε=80 was used to model the water solvent while ε=2 was used for the protein in the solution of the Poisson-Boltzmann equation.

Supporting Information

Table of detailed sequence alignments used for homology modelling is available (PDF) as supporting information.

Author Contributions

Kambiz Hassanzadeh: Designing, investigation and Writing the original draft; Helena Perez Pena: Methodology, investigation, figures and tables preparation; Jessica Dragotto: Prepared figures and writing the original draft; Lucia Buccarello: Revising the manuscript critically for important intellectual content; Federico Iorio: Performed the literature search; Stefano Pieraccini: Designing and methodology; Giulio Sancini: Co-supervision of project; Marco Feligioni: Conceiving the idea, Designing the study and supervision of project, funds . The manuscript was reviewed by all authors.

Conflict of interest

The authors declare that there is no conflict of interest

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Table 1. Number of each residue present in the S protein of SARS-CoV-2 and SARS-CoV. The different residues are represented with the one letter code. Positively charged residues are highlighted in light blue and negatively charged residues in light red.

Residuo	SARS-CoV-2	SARS-CoV
A	79	84
R	42	39
N	88	81
D	62	73
C	40	39
Q	62	55
E	48	42
G	82	79
H	17	15
I	76	78
L	108	99
K	61	60
M	14	20
F	77	83
P	58	57
S	99	96
T	97	99
W	12	11
Y	54	54
V	97	91

Figure Legends:

1. **Sequence alignment of SARS-CoV-2 and SARS-CoV S protein.** Conserved residues are labelled in dark green, same residues in lighter green and residues with similar properties in yellow.

2. **Electrostatic potential of Spike protein in SARS-CoV-2 vs SARS-CoV.** Electrostatic potential of (A) SARS-CoV-2 and (B) SARS-CoV S protein in the (1) close and (2) open conformations mapped onto their molecular surface. This region represents the top side of the protein where the RBD is located, thus, the opposite side to the one that is attached to the surface of the virus. The negative electrostatic potential is shown in red, the neutral in white, and the positive in blue. Values ranging from -kT/e (red) to +kT/e (blue).

3. **Electrostatic potential of (A) SARS-CoV-2 (PDB ID GLZG) and (B) SARS-CoV (PDB ID 6ACJ, A) S protein** RBD section mapped onto its molecular surface when in complex with human ACE2 receptor (transparent green). The negative electrostatic potential is shown in red, the neutral in white, and the positive in blue. Values ranging from -kT/e (red) to +kT/e (blue).

4. **Electrostatic potential of human ACE2 receptor.** Electrostatic potential of human ACE2 receptor mapped onto its molecular surface when in complex with SARS-CoV-2 (cyan) (PDB ID GLZG) shown from different perspectives. The negative electrostatic potential is shown in red, the neutral in white, and the positive in blue. Values ranging from -kT/e (red) to +kT/e (blue).

5. **Brain and lung crosstalk in the COVID-19 infection.** COVID-19 employs ACE2 as receptor for viral cell entry and induction of lung injury through increasing the immune system cytokines. It can downregulate the central ACE2 protein expression; inhibition of ACE2 activity, reduces the sensitivity of the baroreceptor reflex control of heart rate as well as increase in sympathetic tone which eventually resulting in the blood pressure elevation and cardiac dysfunction. In addition, concerning the neuroprotective property of ACE2, its downregulation may disturb the balance of neurotoxicity/neuroprotection inside the brain. Increasing of inflammatory cytokines during lung injury, hypoxemia and elevation of sympathetic tone trough ACE2 downregulation leads to CNS hyper-activation which might play a crucial role in etiopathogenesis of neurogenic pulmonary edema which may play a role in the COVID-19 pulmonary complications in patients. ACE2: Angiotensin-Converting Enzyme 2, NPE: Neurogenic Pulmonary Edema, NP: Neuroprotection, NT: Neurotoxicity.

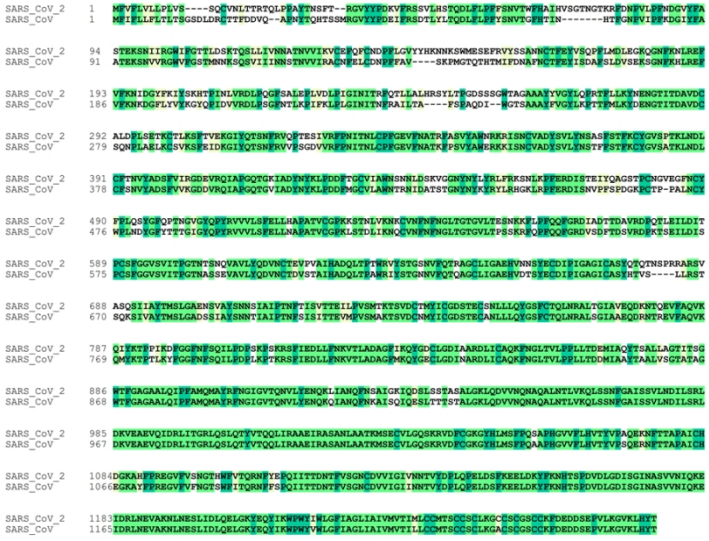

Figure 1

338x190mm (96 x 96 DPI)

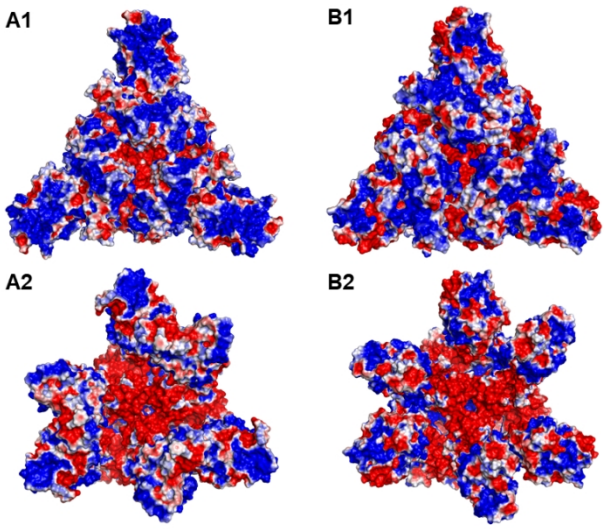

Figure 2

338x190mm (96 x 96 DPI)

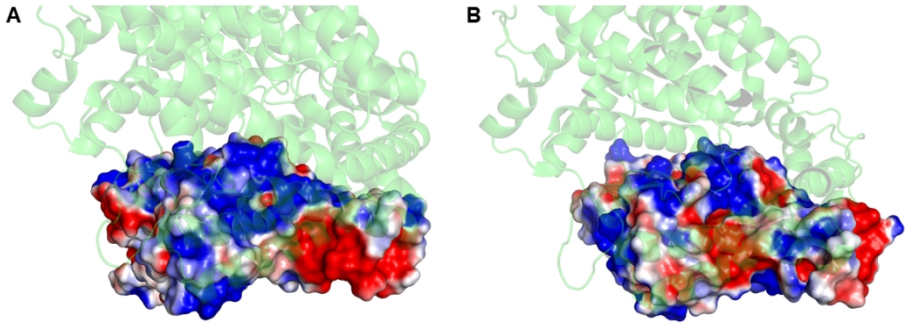

Figure 3

338x190mm (96 x 96 DPI)

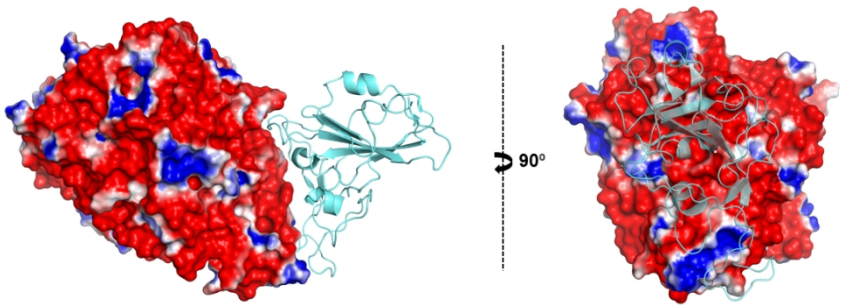

Figure 4

338x190mm (96 x 96 DPI)

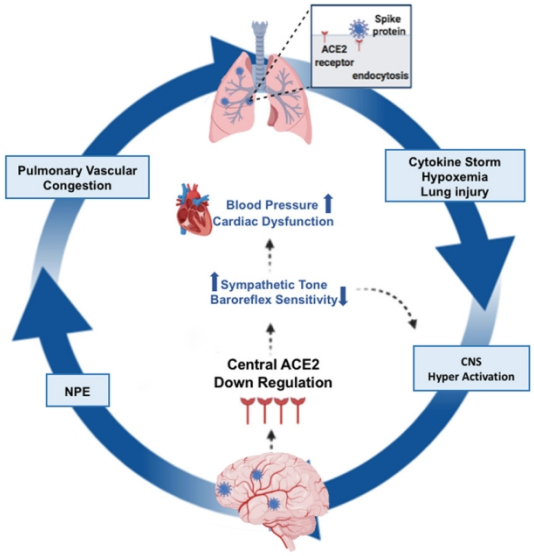
Spike protein
ACE2 receptor
endocytosis
Cytokine Storm
Hypoxemia
Lung injury
CNS
Hyper Activation
Central ACE2
Down Regulation
Sympathetic Tone
Baroreflex Sensitivity
Blood Pressure
Cardiac Dysfunction
Pulmonary Vascular Congestion
NPE

Figure 5