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Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment

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ABSTRACT

In the past two decades, the world has faced several infectious disease outbreaks. Ebola, Influenza A (H1N1), SARS, MERS, and Zika virus have had a massive global impact in terms of economic disruption, the strain on local and global public health. Most recently, the global outbreak of novel coronavirus 2019 (SARS-CoV-2) that causes COVID-19 is a newly discovered virus from the coronavirus family in Wuhan city, China, known to be a great threat to the public health systems. As of 15 April 2020, The Johns Hopkins University estimated that the COVID-19 affected more than two million people, resulting in a death toll above 130,000 around the world. Infected people in Europe and America correspond about 40% and 30% of the total reported cases respectively. At this moment only few Asian countries have controlled the disease, but a second wave of new infections is expected. Predicting inhibitor and target to the COVID-19 is an urgent need to protect human from the disease. Therefore, a protocol to identify anti-COVID-19 candidate based on computer-aided drug design is urgently needed. Thousands of compounds including approved drugs and drugs in the clinical trial are available in the literature. In practice, experimental techniques can measure the time and space average properties but they cannot be captured the structural variation of the COVID-19 during the interaction of inhibitor. Computer simulation is particularly suitable to complement experiments to elucidate conformational changes at the molecular level which are related to inhibition process of the COVID-19. Therefore, *computational simulation* is essential tool to elucidate the phenomenon. The structure-based virtual screening computational approach will be used to filter the best drugs from the literature, the investigate the structural variation of COVID-19 with the interaction of the best inhibitor is a fundamental step to design new drugs and vaccines which can combat the coronavirus. This mini-review will address novel coronavirus structure, mechanism of action, and trial test of antiviral drugs in the lab and patients with COVID-19.

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
Coronavirus; computational simulation; coronavirus Spike; ACE2 receptor; antiviral drugs; COVID-19


1. Introduction

Virus circulates in human, bird and animal, causing seasonal epidemic and occasional pandemic outbreaks. In the past century, the world has faced five pandemic respiratory diseases caused by different subtypes of influenza virus and pigs serve as major reservoirs of these influenza viruses. The 1918 H1N1 (Spanish flu) which originated in Spanish killed around 50 million people worldwide, 1957 H2N2 (Asian flu) that originated in china killed around 4 million people worldwide, the 1968 H3N2 (Hong Kong flu) killed 1 million people worldwide, the 2005 H5N1 (Bird flu) which affected more birds and humans and 2009 H1N1 (Swine flu) which caused 18,000 human deaths and encircled over 100 countries infect humans, pigs and birds. Another pandemic has born from family of coronavirus, two regional epidemics are severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS) in 2001 to 2003 and 2012 to 2015,

respectively. In 2003, SARS had killed 774 people, while MERS killed 858 people between 2012 and 2019 (Centers for Disease Control & Prevention, 2005; World Health Organization. Middle East respiratory syndrome coronavirus (MERS-CoV), 2019).

Coronavirus is a family of the virus and can cause illness such as the common cold, severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) (Amanat & Krammer, 2020). In 2019, a new virus identified in china namely novel coronavirus disease 2019 (COVID-19). On 11 March 2020, the World Health Organization characterized the COVID-19 as a pandemic disease (WHO Director-General's remarks at the media briefing on 2019-nCoV on 11 February 2020., n.d.). The COVID-19 is rapidly expanding in 194 countries, Europe, North America, Asia, Middle East, Africa, and Latin America (*Coronavirus COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE) at*

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Johns Hopkins University, Coronavirus COVID-19 Global Cases by the Center for Systems Science & Engineering, 2020). In particular, more than 620,000 peoples in the united states are infected, resulting in 27000 deaths till 15 April 2020. So far, existing drugs could not be alleviated the disease. The disease symptoms are fever, cough, sore throat, runny nose and difficulty breathing. The china international exchange and promotive association for medical and health care (CPAM) recommend using anti-SARS properties of lopinavir and ritonavir capsule for older age COVID-19 patients with the underlying condition and serious symptoms, these capsules should be used for emergency cases (Chu et al., 2004; Huang et al., 2020; Y. H. Jin et al., 2020). We used COVID-19 instead of SARS-CoV-2 in the following text.

2. Mechanism of action for COVID-19

Human have long been infected by coronavirus as it is one of those responsible for the common cold. It is a contagious viral infection that can be spread through inhalation or ingestion of viral droplets as a result coughing and sneezing and touching infected surface are primary sources of infection. The coronavirus genome is comprised of ~30000 nucleotides. It encodes four structural proteins, Nucleocapsid (N) protein, Membrane (M) protein, Spike (S) protein and Envelop (E) protein and several non-structural proteins (nsp) (Figure 1). The capsid is the protein shell, inside the capsid, there is nuclear capsid or N-protein which is bound to the virus single positive strand RNA that allows the virus to hijack human cells and turn them into virus factories. The N protein coats the viral RNA genome which plays a vital role in its replication and transcription. The N-terminal of the N protein which is binding to genomic and sub-genomic RNAs in MHV and IBV virions and process the viral replication and transcription. This is one of the important open research problems the developing of an effective drug targeting to prevent the contacts between N-terminal of N-protein and single positive RNA strand which can stop viral replication and transcription. Sarma et al. (2020) reported that two important class of compounds, theophylline and pyrimidone drugs as possible inhibitors of RNA binding to the N terminal domain of N protein of coronavirus, thus opening new avenues for in vitro validations.

The M-protein is most abundant in the viral surface and it is believed to be the central organizer for the coronavirus assembly. The S-protein is integrated over the surface of the virus, it mediates attachment of the virus to the host cell surface receptors and fusion between the viral and host cell membranes to facilitate viral entry into the host cell (Kirchdoerfer et al., 2016). The E-protein is a small membrane protein composed ~76 to 109 amino-acid and minor component of the virus particle, it plays an important role in virus assembly, membrane permeability of the host cell and virus-host cell interaction (Gupta et al., 2020). A lipid envelop encapsulates the genetic material. Hemagglutinin-esterase dimer (HE) have been located on the surface of the viral. The HE protein may be involved in virus entry, is not required for replication, but appears to be important for infection of the

natural host-cell (Lissenberg et al., 2005). State-of-the-art cryo-EM experiments have revealed the full structure of the Spike (S) protein in the close [pdb id: 6VXX] (Walls et al., 2020) and open (prefusion) states [pdb id: 6VYB] (Wrapp et al., 2020). Such glycoprotein is made of three identical chains with 1273 amino acid each and it is composed by two well-defined protein domain regions: S1 and S2 subunits which are associated to cell recognition and the fusion of viral and cellular membranes respectively. The latter process occurs through different protein conformational changes that remain still uncharacterized.

The mechanism of viral entry and replication and RNA packing in the human cell is mapped in Figure 2. The coronavirus spike (S) protein attaches to angiotensin converting enzyme 2 (ACE2) receptors that is found on the surface of many human cells, including those in the lungs allowing virus entry. The coronavirus S protein is subjected to proteolytic cleavages by host proteases (i.e. trypsin and furin), in two sites located at the boundary between the S1 and S2 subunits (S1/S2 site). In a later stage happens the cleavage of the S2 domain (S2' site) in order to release the fusion peptide. This event will trigger the activation of the membrane fusion mechanism. Searching for antibodies can find support on molecular targeting which can utilize the structural information (aa sequence) of the binding region which is found in angiotensin-converting enzyme 2 receptor. In this way this protocol could device a treatment to block the viral entry. Typically, human cell ingests the virus in a process called endocytosis. Once entered the cytoplasm, it has been suggested most likely that COVID-19 employs a unique three-step method for membrane fusion, involving receptor-binding and induced conformational changes in Spike (S) glycoprotein followed by cathepsin L proteolysis through intracellular proteases and further activation of membrane fusion mechanism within endosomes (Simmons et al., 2005). Then, the endosome opens to release virus to the cytoplasm, and uncoating of viral nucleocapsid (N) is started via proteasomes which typically can hydrolyse endogenous proteins, but they are also capable of degrading exogenous proteins such as the SARS nucleocapsid protein (Q. Wang et al., 2010). A different two-step mechanism has been suggested (Li, 2016) and in this case the virion binds to a receptor on the target host cell surface through its S1 subunit and the Spike is cleaved by host proteases (Hasan et al., 2020) and then it is expected the fusion at low pH between viral and host target membranes via S2 subunit. Finally, the viral genetic material a single stranded RNA is fully released into the cytoplasm. There takes place the replication and transcription processes which are mediated by the so-called replication/transcription complex (RTC). Such complex is encoded in the viral genome and it is made of non-structural proteins (nsp). The RTC is believed to induced double-membrane structures in the cytoplasm of the infected cell (Van Hemert et al., 2008). Following the positive RNA genome is translated to generate replicase proteins from open reading frame 1a/b (ORF 1a/b) (see Figure 1). These proteins use the genome as a template to generated full-length negative sense RNAs, which subsequently serve as templates in generating

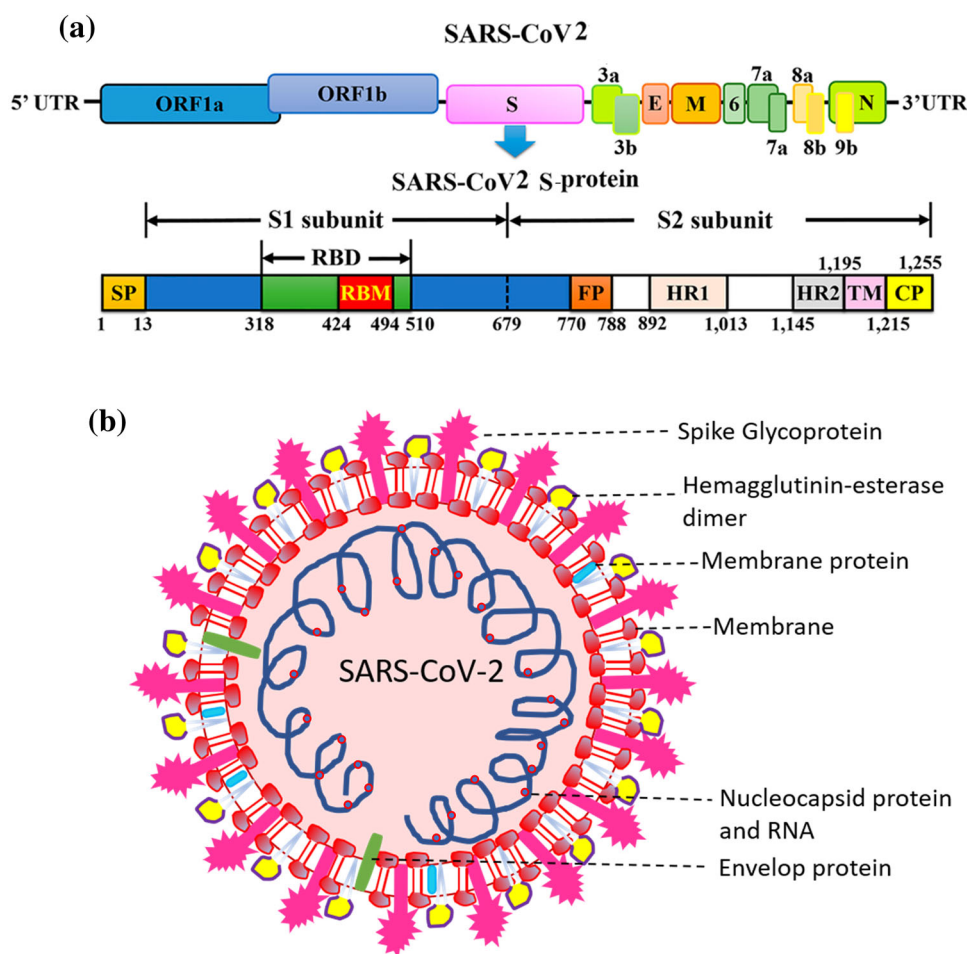


Figure 1. A) Schematic representation of the genome organization and functional domains of S protein for COVID-19. The single-stranded RNA genomes of COVID-19 encode two large genes, the ORF1a and ORF1b genes, which encode 16 non-structural proteins (nsp1–nsp16). The structural genes encode the structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N). The accessory genes denoted in shades of green. The structure of S protein is shown beneath the genome organization. The S protein is consisting of the S1 and S2 subunits. The S1/S2 cleavage sites are highlighted by dotted lines. In the S-protein, cytoplasm domain (CP); fusion peptide (FP); heptad repeat (HR); receptor-binding domain (RBD); signal peptide (SP); transmembrane domain (TM) are shown B) The viral surface proteins, spike, envelope and membrane, are embedded in a lipid bilayer. The single-stranded positive-sense viral RNA is associated with the nucleocapsid protein.

addition full-length genomes. Structural viral proteins, M, S and E are synthesized in the cytoplasm and then inserted into the endoplasmic reticulum (ER) (Figure 2), and transfer to endoplasmic reticulum-Golgi intermediate compartment (ERGIC) (Masters, 2006; Song et al., 2004). Also, in the cytoplasm nucleocapsids are formed from the encapsidation of replicated genomes by N protein, and as a result they coalesce within the ERGIC membrane in order to self-assemble into new virions. Finally, novel virions are exported from infected cells by transport to the cell membrane in smooth-walled vesicles and then secreted via a process called exocytosis, so that can infect other cells. In the meantime, the stress of viral production on the endoplasmic reticulum eventually leads to cell death. However, the mechanism of action for novel COVID-19 is still unknown (Masters, 2006).

3. Crystal structure of COVID-19 M^{pro}

The COVID-19 replicase gene encoded two polyproteins, pp1a and pp1ab with molecular weight 450 and 750 KD respectively, these polyproteins are required for viral replication and transcription (Wu et al., 2020; Zhou et al., 2020). In

the proteolytic process, the functional polypeptides of spike, membrane, envelop, nucleoprotein, replicase and polymerase are released from polyproteins. This process was carried out by a chymotrypsin-fold proteinase namely main protease (M^{pro}) (Anand et al., 2003). The M^{pro} is a vital role in polyprotein processing and virus maturation, hence, it is considered to be an attractive target for antiviral drug design as an approach toward COVID-19 treatment (Anand et al., 2003; Pillaiyar et al., 2016; Yang et al., 2003). The Chinese scientists have first published the genome and with overseas collaboration have obtained the crystal structure of three-dimensional novel COVID-19 main protease (M^{pro}) and deposited in the protein data bank (Z. Jin et al., 2020). The crystal structure of COVID-19 M^{pro} has 306 amino acids with the three regions, in the first region represented in blue contains 8-101 residues, second region in green are 102-184 residues and third region in orange are 201-303 residues. The first and third regions have an antiparallel β -sheet structure, third region has five alpha helices arranged into a cluster. The third region is connected to second region through a long loop region contains 185-200 residues. Mass spectroscopy determined the weight of COVID-19 M^{pro} is 33797.0 Da.

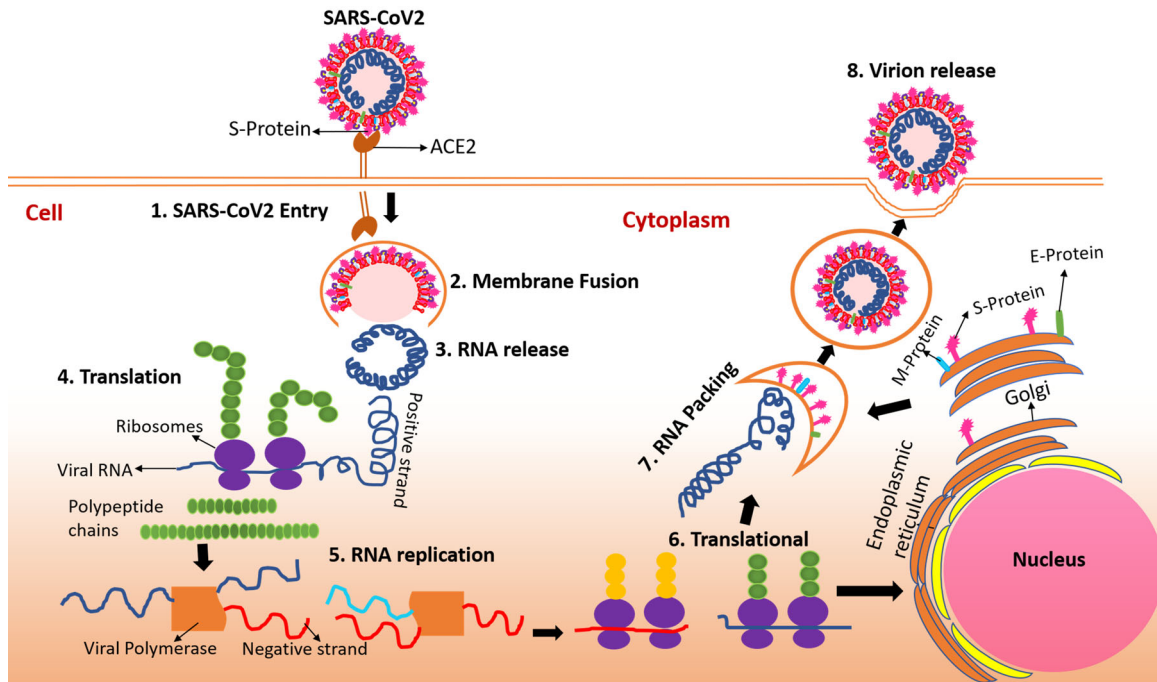


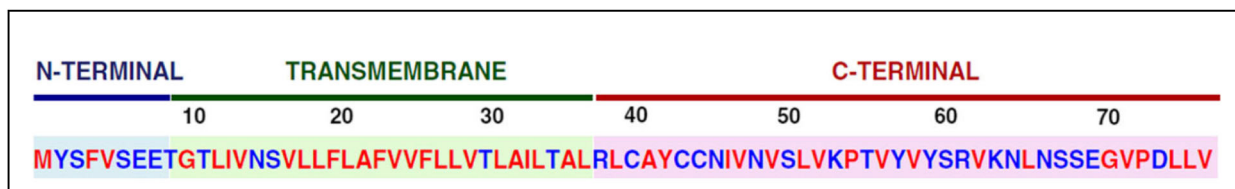
Figure 2. The schematic diagram of the mechanism of COVID-19 entry and viral replication and viral RNA packing in the human cell.

Theoretical studies confirmed the weight is 33796.8 Da (Z. Jin et al., 2020). Furthermore, in the literature, several crystal structures of COVID-19 M^{pro} (see Figure 3) in complex with different inhibitors are deposited in the protein data bank (<https://pdj.org/featured/covid-19>) by using X-ray diffraction technique at resolution between 1.31 Å and 3.084 Å (COVID-19 featured content, 2020). On the other hand, the HIV-1 protease inhibitors, tipranavir, saquinavir, ritonavir, nelfinavir, lopinavir, indinavir, darunavir, atazanavir, and amprenavir are widely reported to be able to deactivate M^{pro} (Yang et al., 2003).

4. E-Protein ion channel activity

The COVID-19 E-protein is a short and integral membrane protein that contains 76-109 amino acids, size ranging between 8.4 and 12 kDa, consist of 35 α -helices and 40 loops. The protein has short hydrophilic amino terminus consisting of 7-12 amino acids, followed by a large hydrophobic transmembrane domain of 25 amino acids long

hydrophilic C-terminal domain (shown in box). The hydrophobic region can generate oligomerization and form an ion-conductive pore in membranes, it plays a significant role in the assembly of the viral genome. This protein is involved in several aspects of the virus life cycle, such as assembly, budding, envelope formation, and pathogenesis (Schoeman & Fielding, 2019). E protein's ion channel activity is found in the transmembrane region of the protein (Verdiá-Báguena et al., 2012; Wilson et al., 2004). The membrane potential has been regulated by E-protein controlling the ion flow between the intracellular and extracellular environment. The ion conductivity triggered by E-protein via the manipulation of COVID-19 genome seems to be a novel route involved in virus pathogenesis. Although, the E-protein has been interacted with other coronavirus proteins as well as host cell proteins. E-protein's ion channel activity and the alteration of coronavirus cell ion balance by E-protein is a necessary process for virus production but the effect of E-protein ion channel activity in virus pathogenesis remains elusive.



Few efforts have been found that mutation of the E-protein in the extracellular membrane could disrupt the ion-conductivity and the normal viral assembly (Torres et al., 2007; Verdiá-Báguena et al., 2012), hence control the E protein dynamics is a promising target for preventing pathogenesis associated with the COVID-19 (Pervushin et al., 2009). For

example, Nieto-torres et al. (2014) have demonstrated that, Mice infected with COVID-19 viruses exhibited E-protein ion channel activity, with wild-type E-protein sequence, that restored ion transport, resulting in the mice death with the loss of weight. In contrast, mice infected with mutant E-protein showed lack of ion channel activity and has result they

recovered from the disease and most survived. This evidence confirmed that ion channel activity correlated well with the virus growth. The strong E-protein ion channel activity is generated a higher chance for the mortality of mice is observed. Hence, inhibitor target to E-protein process can help to prevent virus production. In this respect, Wilson et al., showed that hexamethylene amiloride inhibitor has shown to block the E-protein ion channel conductance in cell membrane and inhibits replication of the parent coronavirus in cultured cells (Wilson et al., 2006). Recently, Gupta et al. (2020) have identified the medicinal properties of Belachinal, Macaflavanone E and Vibsanol B inhibitors which are successfully passed the ADMET test and Lipinski's rule 5s. These compounds are reduced the random motion of the human COVID-19 E protein in terms of inhibiting the function of the human "COVID-19 E" protein. So far, our understanding about the mechanism of the E-protein ion channel activity in the viral assembly is an ongoing debate. This is one of the most important research topics to

investigated which deals with E-protein ion channel activity in viral production to reduce the mortality rate of diseased human by deletion of E-protein by inhibitor.

5. Small molecules interact with COVID-19 M^{Pro}

Yang et al. (Z. Jin et al., 2020; F. Wang et al., 2017; Yang et al., 2005) have designed Michael acceptor N3 inhibitors are found to have an optimal pharmaceutical activity against viral protease through mechanism-based irreversible inhibition. They also evaluated a series of wide-spectrum inhibitors targeting the M^{Pro} proteases of multiple coronaviruses including SARS AND MERS (Ren et al., 2013; F. Wang et al., 2017; Xue et al., 2008; Yang et al., 2005), and also exhibited an antiviral activity for infectious bronchitis virus in an animal model(Xue et al., 2008), it fights to porcine epidemic diarrhea virus which causes high mortality in pigs as well as COVID-19 (F. Wang et al., 2017). Recently, Jin et al. (2020) have constructed a homology model for COVID-19 M^{Pro} and they used molecular docking to monitor whether the N3 binds with it. The docking showed that N3 binds with the COVID-19 M^{Pro} as shown in right side Figure 4. Subsequently, the crystal structure of COVID-19 M^{Pro} in complex with N3 inhibitor have been determined at 2.3 Å resolution and deposited in the protein data bank [pdb id: 6LU7], where the N3 inhibitor binds with the residues of 164-168 in the long strand 155-168 residues, and with residues 189-191 of the loop connecting between second and third region. Further, it can form several hydrogen bonds with the main chain of the residues in the substrate-binding pocket. The contact between N3 and COVID-19 M^{Pro} is locked the inhibitor inside the substrate binding pocket. Several X-ray studies (COVID-19 featured content, 2020) demonstrated that small antiviral compound including lopinavir (Zhao et al., 2008) is locked in the same position of N3 in the substrate-binding pocket as shown in left side of Figure 4 (Figure S1 and Table S1). The second region of the COVID-19 M^{Pro} is the favorable position for the inhibitors binding (Yang et al., 2003; Zhao et al., 2008). In the literature, the number of small bioactive

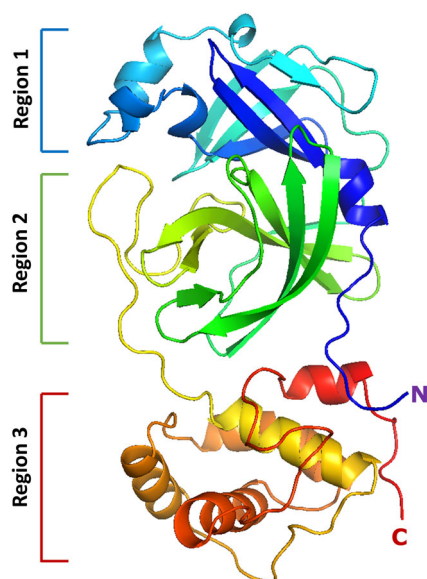


Figure 3. Three-dimensional structure of COVID-19 M^{Pro} (Jin et al., 2020).

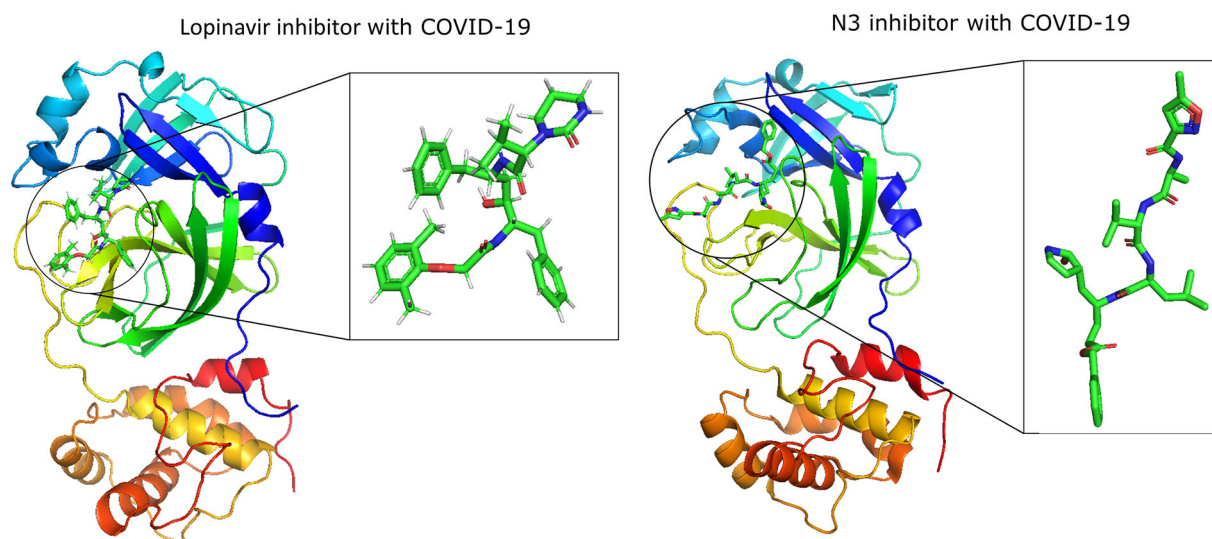


Figure 4. Cartoon representation of COVID-19 M^{Pro} with Antiviral inhibitors, Lopinavir and N3 highlighted in box.

compounds reported has reached 135 million (Boström et al., 2018). In this regard, researchers should devise strategies to identify an anti-COVID-19 M^{PRO} properties candidate based on structure-based virtual screening computational approach.

6. Trial test of the FDA-approved drugs for COVID-19 patients

Nowadays, the drug repurposing has been used to identify potential drugs against coronavirus. Enormous efforts have been paid for the ability to reuse FDA approved/preclinical trial drugs for the disease. In the literature, we find several promised drugs candidate targeted to multiple virus protein (Graham et al., 2013; Khan et al., 2020; Yang et al., 2003). The WHO has provided the permission to doctors and scientist carry out the trial test with the combination of different FDA-approved drugs for COVID-19 treatment. In the view of urgency and current need, to reduce the cost, time and risks of the drug development process, scientists are involved in reusing already approved drug candidates to test in COVID-19 patients. In a short time, the response of the scientific community is such that involve enormous efforts to develop a novel therapy and treatment. For example, Chloroquine and Hydroxychloroquine, old drugs, have been used to treat malarial, rheumatoid arthritis, lupus and sun allergies for more than sixty years. The activity of hydroxychloroquine on viruses is probably same as that of chloroquine since the mechanism of the action of these two molecules is identical. Chloroquine as an antimalarial and autoimmune disease drugs has shown a synergistically enhancing effect as antiviral drugs *in vivo* studies (Savarino et al., 2006; Yan et al., 2013). It interferes with terminal glycosylation of cellular receptor, angiotensin-converting enzyme 2. This may negatively influence-receptor binding and abrogate the infection, with further ramifications by the elevation of vesicular pH, resulting the spread of SARS-Cov in cell culture has been prevented (Vincent et al., 2005).

The hydroxychloroquine has shown a potent efficacy in treating patients with COVID-19 pneumonia. More than hundred patients showed the superiority of chloroquine compared with treatment of standard care in terms of reduction of exacerbation of pneumonia, viral load and symptoms (Chinese Clinical Trial Registry, n.d.; Gao et al., 2020). Colson group have suggested that chloroquine and hydroxychloroquine as available weapons to fight COVID-19 (Colson et al., 2020). In 2014-2017, ivermectin antiviral drug used for phase 3 treatment of dengue infection, daily oral dose significantly reduced in serum level of viral NS1 protein. Caly et al. (2020) have demonstrated ivermectin-treatment can reduce ~5000-fold viral RNA load compared to the control sample in cell cultural at 48 h, but no further reduction observed at 72 h.

Mulangau et al. (2019) have conducted a trial of four investigational therapies for Ebola virus diseased patients. A total of 681 patients were registered between November 18, 2018 and August 9, 2019. The patients were assigned 1:1:1:1 ratio to receive ZMapp (a triple monoclonal antibody agent), remdesivir (a nucleotide analogue RNA polymerase inhibitor), Mab114 (a single human monoclonal antibody derived from

an Ebola survivor), and REGN-EB3 (a coformulated mixture of three human IgG1 monoclonal antibodies). Both Mab114 and REGN-EB3 groups were superior than ZMAP and remdesivir group in reducing mortality for Ebola patients, primary groups have faster rate of viral clearance than later groups. Remdesivir has been promised antiviral drug against RNA viruses, including SARS/MERS, in cultured cell, mice and non-human primate model (Sheahan et al., 2017). It is under the clinical development for Ebola virus treatment and typically it is distributed in the entire human body including lung after oral administration. Sheahan et al. (2017) tested a Remdesivir inhibitor that has shown activity against Ebola virus as a potential agent to be used to combat coronaviruses. Unfortunately, it could not be help to Ebola patients during the 2019 outbreak in the Democratic Republic of the Congo. A coronavirus research at the university of Iowa suggested that Remdesivir antiviral acting much more potential if patient takes it early, patients with mild symptoms (Kai Kupferschmidt & Cohen, 2020). Importantly, M. Wang et al. (2020) study reported that remdesivir and chloroquine are highly effective in the control of COVID-19 infection *in vitro* experiments.

The first COVID-19 patient, 35-year-old in Washington, admitted to a hospital on 15 January 2020, in the united states because of dry cough, two-day history of nausea and vomiting (Holshue et al., 2020). He reported that he had no shortness of breath or chest pain. The important signs, fever, cough, rhinorrhea, fatigue, nausea, vomit, diarrhea and abdominal discomfort, were in normal condition. He had high fever 39.4 °C with cough at the eleventh day of hospitalized, on the evening of same day, clinical began treatment with intravenous remdesivir, next day, his clinical condition had improved. As January 30, he was still hospitalized but his symptoms had resolved exception of cough, which has decreased in severity.

Cao et al. (2020) have conducted trial test using lopinavir-ritonavir antiviral drugs for COVID-19 patients. A total of 199 patients were enrolled for the test, 99 patients were assigned to the lopinavir-ritonavir group, remaining 100 patients to be treated in the standard care group, resulting the mortality rate was similar in both lopinavir-ritonavir group and standard care group. They found that lopinavir-ritonavir treatment failed to significantly accelerate clinical improvement, reduce mortality, diminish throat viral RNA detectability in patients with serious COVID-19. In case of the severe COVID-19 adult patients, no benefit was observed with lopinavir-ritonavir treatment in comparison to the standard care group. Such study has found that lopinavir-ritonavir does not seem to be effective for COVID-19 patients and these combinations has produced more side effects (Cao et al., 2020). In summary, the antiviral drugs, alone and in combination, have been tested in human body, all showed some evidence of effectiveness against SARS and MERS, they seem not to be effective for COVID-19 (Baden & Rubin, 2020).

Azithromycin has been displayed an antiviral property against Zika and Ebola virus *in vitro* (Bosseboeuf et al., 2018; Madrid et al., 2015; Retallack et al., 2016). It can prevent severe respiratory tract infection in the case of viral infected

patients (Bacharier et al., 2015). Gautret group have treated successfully chronic disease patients with long-term hydroxychloroquine medicines with 600 mg per day for 12 to 18 months. As 17 march 2020, A paper reported (Gautret et al., 2020), they are monitored the viral load in the COVID-19 patients with and without receiving drugs for the six days. They have examined a total of 36 patients, 20 hydroxychloroquine-treated patients and 16 control patients. Among hydroxychloroquine-treated patients, six patients received azithromycin with 500 mg on day 1 followed by 250 mg per day for the next four days. At day 6, 100% patients treated with hydroxychloroquine and azithromycin combination have cured comparing with 57.1% in patients treated with hydroxychloroquine only, and 12.5% in the control group. Finally, they found that hydroxychloroquine and azithromycin combination have cured the COVID-19 patients but the drawback of this study is used a very small size of survey.

Z. Jin et al. (2020) have screened a library of ~10,000 compounds which are consisting of approval drugs, clinical trial drugs and natural products. They were used combined structural-based abinitio, drug design, and high-throughput screening to identified two drugs of these compound are Ebselen and thiadiazolidinone-8 (TDZD-8). Quantitative real-time RT-PCR demonstrated that in comparison to treatment in the absence of inhibitor, treatment of Ebselen reduced the amount of COVID-19 by 20.3-fold, and TDZD-8 and N3 (N3 inhibitor designed by Z. Jin et al. (2020) that can inhibit multiple Coronavirus M^{Pro} S including those from COVID-19 and MERS-CoV) showed 10.9-fold and 8.4-fold reduction in COVID-19 growth, respectively. These data suggested that Ebselen as a potential antiviral inhibitor for COVID-19 treatment.

Doctor's team reported, a large doses of the flu drug oseltamivir combined with HIV drugs lopinavir and ritonavir could improve the conditions of several COVID-19 patients at the Rajavithi Hospital in Bangkok (<https://www.the-scientist.com/news-opinion/flu-and-anti-hiv-drugs-show-efficacy-against-coronavirus-67052>). Based on the report, Gromica et al., (Muralidharan et al., 2020) have utilized computational docking approach to analysed the effect of synergism of these drugs against the COVID-19 main protease. They found that the combination of three drugs, lopinavir, oseltamivir and ritonavir, showed a better binding energy than that of individual drug, and they reported the protein in complexed with three drugs are stable during the simulations.

The CPAM (china international exchange and promotive association for medical and health care) recommends to take lopinavir 400 mg, ritonavir 100 mg (two tablets by mouth twice in daily) or chloroquine (500 mg tablet by mouth twice in daily) for older patients or patients with underlying conditions and serious symptoms. In case chloroquine is unavailable, consider to use of hydroxychloroquine (400 mg by mouth once daily). It cannot be recommended to take ribavirin and interferon drug because of the high risk for side effects but these drugs may be considered if treatment with lopinavir, ritonavir, chloroquine or hydroxychloroquine are ineffective (Chu et al., 2004; Gao et al., 2020; Huang et al., 2020; Y. H. Jin et al., 2020). To this end, some drugs

have become promises, other are being ruled out including lopinavir and ritonavir, mix of two drugs used to treat HIV disease. So far, trial test results are confirmed as FDA-approved therapeutics or drugs have not helped to treat, cure or prevent COVID-19.

7. Plasma therapy

Other most important research on plasma therapy is ongoing. Several convalescent patients are donating plasma against COVID-19 based on the positive results of another coronavirus (Koenig, 2015; Mair-Jenkins et al., 2015). Surprisingly, it has preliminarily obtained favourable results in severe COVID-19 patients. On the other hand, the recombinant human monoclonal antibody is a straightforward path to neutralize viral load of COVID-19. CR3022 is a coronavirus-specific human monoclonal antibody that can bind to the receptor-binding domain of COVID-19, this has the potential to be developed as candidate therapeutics of COVID-19 disease (Tian et al., 2020). Other monoclonal, m396, CR3014 antibodies, neutralizing COVID-19 that may be an alternative for the treatment of more severe cases (Zhang & Liu, 2020).

8. Conclusion

Effective antiviral-COVID-19 candidates are essential for reducing disease severity, viral load and transmission, thus helping to prevent the coronavirus outbreaks. There are several drugs against SARS-CoV, MERS-CoV tested in animals, and infected viral cell in lab and proteins vaccines (Graham et al., 2013). These investigations are in progress but it requires months to years to develop the vaccines for COVID-19. So far, the infected virus patient's population is growing rapidly. The organized effort of the computational community is becoming very essential to infer about the structural characteristics of the virion with and without the interaction of drug molecules that may help to experimental/clinical scientist identify effective inhibitor using in vitro studies. Both the computational and experimental outcomes in terms of novel drugs should be recommended a new drug to test in vivo experiments that may be suitable for the disease patient. Following fundamental knowledge of research is required by using computer simulations

1. To elucidate the role of E-protein ion channel activity in virus pathogenesis by study the E-protein ion conductivity with the manipulation of COVID-19 genome.
2. To identify inhibitor block the E-protein ion channel activity as well as inhibits COVID-19 RNA polymerase.
3. To study the energetic binding affinity of COVID-19 M^{Pro} with each inhibitor of ritonavir, lopinavir, Azithromycin, hydroxychloroquine, N3, ribavirin and new inhibitors based on free energy calculations.
4. To investigate the structural properties, flexibility, conformational changes of the COVID-19 M^{Pro}, and study interaction pattern between virus and membrane, virus and inhibitor.
5. To monitor the thermodynamics (virus-water interaction) properties of COVID-19 M^{Pro} in the presence/absence of the antiviral inhibitor.
6. To develop an effective drug targeting to inhibit the contacts between N-protein and single positive RNA

strand in order to stop viral replication and transcription. 7. To characterize mechanical profile and energetic affinities between Spike (S) protein and angiotensin converting enzyme 2 (ACE2) in order to devise novel targeted molecular treatment which can aid vaccine developments.

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