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Review Article

Computer-aided discovery, design, and investigation of COVID-19 therapeutics

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Abstract

Coronavirus disease 2019 (COVID-19) pandemic is currently the most serious public health threat faced by mankind. Thus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes COVID-19, is being intensively investigated. Several vaccines are now available for clinical use. However, owing to the highly mutated nature of RNA viruses, the SARS-CoV-2 is changing at a rapid speed. Breakthrough infections by SARS-CoV-2 variants have been seen in vaccinated individuals. As a result, effective therapeutics for treating COVID-19 patients is urgently required. With the advance of computer technology, computational methods have become increasingly powerful in the biomedical research and pharmaceutical drug discovery. The applications of these techniques have largely reduced the costs and simplified processes of pharmaceutical drug developments. Intensive and extensive studies on SARS-CoV-2 proteins have been carried out and three-dimensional structures of the major SARS-CoV-2 proteins have been resolved and deposited in the Protein Data Bank. These structures provide the foundations for drug discovery and design using the structure-based computations, such as molecular docking and molecular dynamics simulations. In this review, introduction to the applications of computational methods in the discovery and design of novel drugs and repurposing of existing drugs for the treatments of COVID-19 is given. The examples of computer-aided investigations and screening of COVID-19 effective therapeutic compounds, functional peptides, as well as effective molecules from the herb medicines are discussed.

Keywords: *Bioinformatics, Coronavirus disease 2019, Molecular docking, Molecular dynamics simulations, Severe acute respiratory syndrome coronavirus 2*

Introduction

Coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is currently the most serious public health threat to mankind leading to a worldwide humanity disaster. SARS-CoV-2 is one of the seven coronaviruses (CoVs) known to infect human. Among the seven, four of them, human CoVs OC43, HKU1, 229E, and NL63, cause common cold [1]. Infection with SARS-CoV-1 or Middle East Respiratory Syndrome (MERS)-CoV, on the other hand, results in serious symptoms leading to high fatality rates of approximately 10% and 47% in infected individuals, respectively [2]. However, the seriousness and high mortality rates of the infections are considered disadvantages for virus transmission, as the infected individuals are less mobile, thus reducing the virus transmission efficiency and are easily identified, as a result quarantined. The SARS-CoV-2 seems to be a "smarter" virus. Infected individuals with severe symptoms account for only <20%, and the fatality rate is indicated to be approximately 1%–6% in confirmed cases, depending on the regions where the cases are reported [3-7]. The large proportion of the mild symptomatic and asymptomatic infections increases the difficulty in the identification of infected individuals, and a large number of infected individuals are still able to travel actively, thus speed up the disease spreading. There have been several vaccines approved by authorities worldwide for clinical use [8], and dozens of vaccine candidates are under investigations and clinical trials [9]. Although some of the vaccines were tested to be effective in preventing symptomatic infections, none of these vaccines is able to 100% terminate the virus infection. In

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addition, owing to the nature of single-stranded RNA viruses, the mutation rate of SARS-CoV-2 is high. The evolution of the virus might lead to decreased or lost protective efficacy of the vaccines in a very near future [9,10]. Therefore, it is always necessary to have feasible therapeutic strategies in hands for treating COVID-19 patients. With the advancement of computer technology, bioinformatic tools are nowadays very powerful in the development of pharmaceutics and have been applied in the design of therapeutics for COVID-19. This article aims to review recent studies of computer-assisted design, analysis, and developments of therapeutics against SARS-CoV-2 infections and to give examples to applications of the computational approaches related to these issues.

Current Understanding of Severe Acute Respiratory Syndrome Coronavirus 2 Infection and Pathogenesis

It is believed that human-to-human transmission of SARS-CoV-2 is mainly through respiratory droplets and indirect contact through contaminated surfaces. SARS-CoV-2 is currently suggested to have a zoonotic origin. Sequence analysis has indicated a >75% sequence identity of the virus spike glycoprotein between SARS-CoV-2 and several bat CoVs [11], among which, bat CoV RaTG13 has the highest spike protein sequence identity of 97.56% [11]. SARS-CoV-2 and pangolin CoV share 92.3% amino acid identity in their spike proteins [12-14]. Despite these, there are still arguments about the roles played by bat and pangolin CoVs in SARS-CoV-2 evolution [15,16]. SARS-CoV-2, approximately 125 nm in diameter [17], contains four structural proteins, the spike, envelope, membrane (M), and nucleocapsid proteins. The virus infection to host cells is initiated when the receptor-binding domain (RBD) of spike protein engages to the host cell receptor angiotensin-converting enzyme 2 (ACE2) [18]. The spike protein is then primed by the cellular transmembrane serine protease 2 (TMPRSS2) [19] allowing the release of a fusion peptide to facilitate the SARS-CoV-2 entry into the host cells. In the host cells, the released positive-stranded virus genomic RNA is directly translated into a polyprotein by exploiting cellular machinery. The polyprotein is then processed to produce nonstructural proteins (NSPs) which form replicase-transcriptase complex. Following these processes, negative-sense RNA templates are generated for genomic RNA replication. Sub-genomic RNA then encodes structural proteins, spike, M, and envelope, which are inserted in the endoplasmic reticulum (ER), and are transported to ER-Golgi intermediate compartment (ERGIC). In cytosol, the newly synthesized viral genomic RNA is encapsulated by nucleocapsids formed by nucleocapsid protein, and the RNA containing nucleocapsids are condensed with the envelope components in ERGIC. The assembled viruses are released from the host cell through exocytosis and spread to other cells and organs [17]. The sensing of the SARS-CoV-2 infection by the host should follow the similar pathway as those for other CoVs, in which Toll-Like receptors, cytosolic retinoic acid-inducible gene I, and melanoma differentiation-associated protein are involved [14]. The sensing of the virus triggers signaling cascades resulting in the activation of immune cells, including dendritic cells, macrophages, and polymorphonuclear neutrophils, and elevated productions of complex combinations of pro-inflammatory and anti-inflammatory cytokines [14,17], including interleukin (IL)-1β, IL-1RA, IL-2RA, IL-6, IL-7, IL-8, IL-9, IL-10, basic FGF, G-CSF, GM-CSF, HGF, interferon gamma, MCP-1, MIP-1a, MIP-1b, PDGF, tumor necrosis factor-alpha (TNF-α), IP-10, and MCP-1 are measured in mild and moderate cases [17,20]. In severe cases, patients encounter a deadly acute respiratory distress syndrome (ARDS), which is thought to be caused by a cytokine storm, most likely induced by the stimulation and activation of the IL-6-STAT3 pathway or NF-κB signaling [21]. A variety of therapeutic strategies targeting the virus lifecycle or reducing effects caused by the virus are currently under investigations. As computer technology is nowadays a powerful tool for solving problems human encountered, it has currently been applied for drug design. Intensive and extensive researches have yielded structures of the host receptor ACE2, as well as most of the SARS-CoV-2 proteins with X-ray crystallography, cryo-electron microscopy (cryo-EM), or NMR experiments. These structures have been deposited in the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https://www.rcsb.org/), allowing computational investigation and design of possible/potential therapeutics, based on the analysis of interactions between SARS-CoV-2 and host proteins. Among many *in silico* approaches in used for drug discovery and modifications, molecular docking and molecular dynamics (MD) simulations are the most applied techniques and will be discussed in the next few paragraphs.

Molecular Docking and Molecular Dynamics Simulations

Molecular docking is currently one of the most important computational techniques in drug discovery which allows effective investigation on interactions between two or more molecules and prediction of how these molecules fit together. The molecules studied can be proteins and their ligands, which can be small molecules, nucleic acids, or other proteins. Molecular docking is structure based. It describes the binding between ligand and protein, including orientations and poses. As a result, to perform this technique, structures of the molecules of interest are required. The protein structures can be determined by X-ray crystallography, NMR spectroscopy, cryo-EM, or computational homology modeling. In general, molecular docking includes rigid-body docking and induced-fit docking. In rigid-body docking, the ligand and protein are set rigid, thus the docking is fast and requires lower computing cost. However, it is relatively less accurate. On the other hand, in induced-fit docking, the ligand and protein are set flexible, thus the docking requires higher computing cost but is able to provide a higher accuracy. Molecular docking programs generate all possible poses, which are potential orientations and conformations of the protein interacted with its ligand(s). To find out the best fits, scoring functions are introduced. Most scoring functions are physical chemistry-based molecular mechanics force fields which calculate the free energy of the poses. The lowest calculated free energy indicates the most

possible binding orientation. In general terms, the binding free energy of the protein with its ligand in solvent can be expressed as [22,23]:

$$\Delta G_{bind} = G_{complex} - \left(G_{protein} + G_{ligand}\right) \cong \Delta E_{MM} - T\Delta S + G_{polar} + G_{non-polar} \quad (1)$$

Where, $G_{complex}$ is the total free energy of the protein-ligand complex, and $G_{protein}$ and G_{ligand} are total free energies of the isolated protein and ligand in solvent, respectively; T is temperature and S denotes the entropy; E_{MM} is the vacuum potential energy calculated based on the molecular mechanics (MM) force-field parameters. The free energy for each individual entity (G_x) can be given by [22]:

$$G_x = \left\langle E_{MM} \right\rangle - TS + \left\langle G_{solvation} \right\rangle \quad (2)$$

where $\left\langle G_{solvation} \right\rangle$ is free energy of solvation.

EMM is expressed as:

$$E_{MM} = E_{bonded} + E_{non-bonded} = E_{bonded} + \left(E_{elec} + E_{vdw}\right) \quad (3)$$

Where, E_{bonded} is the energy of bonded interactions consisting of bond, angle, dihedral, and improper interactions. The energy of nonbonded interactions ($E_{non-bonded}$) includes both electrostatic (E_{elec}) and van der Waals (E_{vdw}) interactions [22]. The energy of nonbonded interactions also includes hydrogen bond energy. In a broad sense, hydrogen bond and van der Waals interactions are both dipole-dipole interactions, and in energy point of view, there is no clear "energy border" which distinguishes hydrogen bond and van der Waals interactions [24]. As a result, in many of the estimations, the hydrogen bond energy is included in the term E_{vdw}. In E_{MM} calculations, E_{elec} is modeled with the Coulomb potential function, and E_{vdw} is modeled using the Lennard-Jones potential function, which also includes hydrogen bond energy in general force fields. In a single trajectory, the conformation of protein and ligand in the bound and unbound forms is assumed to be the same. As a result, ΔE_{bonded} is set as zero. The solvation free energy in equation 2 can then be expressed as G_{polar} and $G_{non-polar}$ (in equation 1), which are the electrostatic and nonelectrostatic contributions to the solvation free energy. ΔG_{polar} is electrostatic solvation energy and $\Delta G_{non-polar}$ is the nonelectrostatic solvation energy and is considered proportional to the solvent accessible surface area (SASA) [22,23]:

$$\Delta G_{non-polar} = \gamma \times SASA + \beta \quad (4)$$

Molecular docking relies on approximations, and in most of the time, the receptor flexibility is not included in the docking process. As a result, in some cases, MD simulations, which calculate more detailed interaction energies, are required for providing complementary to molecular docking and more reliable results [25]. MD simulations can be used independently for investigations of conformational changes of specific molecules over a period of time [26,27]. These simulations can also be applied to optimize the structures of final complexes from molecular docking and provide insights of the ligand binding mechanism [23,25,28].

Molecular docking and MD simulations can be used to study interactions between drugs and their receptors, for providing clues for drug designs and for virtual screening of new compounds for specific protein biomolecular targets. In the following sections, the examples of applications of the computational tools in drug discovery for the treatments of COVID-19 are discussed.

Repurposed Therapeutic Compounds

Since the first cases reported, the SARS-CoV-2 infection has spread around the world at an extremely high speed, causing serious medical and humanity crisis in almost all the regions on this planet. Finding effective therapeutic strategies for treating COVID-19 patients has thus become a crucial and urgent task. Some existing drugs, such as hydroxychloroquine (HCQ), chloroquine, remdesivir, and lopinavir/ritonavir, were repurposed and evaluated for their antiviral activities against SARS-CoV-2 [29-32]. Remdesivir [Figure 1a], a nucleoside analog clinically trialed for the treatment of Ebola virus infection previously, was designed to inhibit RNA-dependent RNA polymerases (RdRp) of viruses and also showed possible inhibitory effects on MERS and SARS. Therefore, it was considered for COVID-19 treatments. The treatment using remdesivir was found to improve the clinical condition of some COVID-19 patients [33] and was one of the only few authorized means for COVID-19 treatments. However, the actual therapeutic improvements by this drug to COVID-19 patients were found to be only slightly better than that of the patients receiving placebo [34]. A report from NIH clinical trials indicated that the treatment of remdesivir can only speed up the disease progression and shorten the recovery time in infected patients [35]. The mortality rate for the group receiving remdesivir versus that for the placebo group was not statistically different [35]. Antimalarial drugs chloroquine and [HCQ, Figure 1b] have also been proposed to be potential drugs for treating COVID-19 [32]. However, the actual antiviral mechanism of chloroquine and HCQ in the human body is still not clear, although an *in vitro* study suggested that they might inhibit the acidification of endosome important for virus infection and replication [36]. The effects of HCQ in clinical applications remain controversial, despite the positive results obtained in the *in vitro* tests. While an open-label nonrandomized clinical trial suggested that HCQ treatment was significantly associated with viral load reduction or disappearance in COVID-19 patients [37], randomized controlled open-label clinical trials indicated that the patients received HCQ did not have a lower incidence of death or an improved clinical status as compared to those who only received usual care [38,39]. In addition to RdRp, interactions between nucleoside analog drugs and the equilibrative nucleoside transporters (ENTs), which function in nucleoside and nucleobase uptake, were analyzed with computational methods. Molnupiravir [Figure 1c, EIDD-2801], a synthetic nucleoside analog originally developed to treat influenza, is currently in clinical trials for COVID-19 treatments in many countries [40-42] and has been approved for medical use in the United Kingdom in October 2021 [43]. Molnupiravir is able to inhibit the replication of certain RNA viruses and is suggested to have a potent ability to inhibit RdRp of SARS-CoV-2. On the other hand, Miller *et al*. investigated ENT-drug interactions on nucleoside analogs

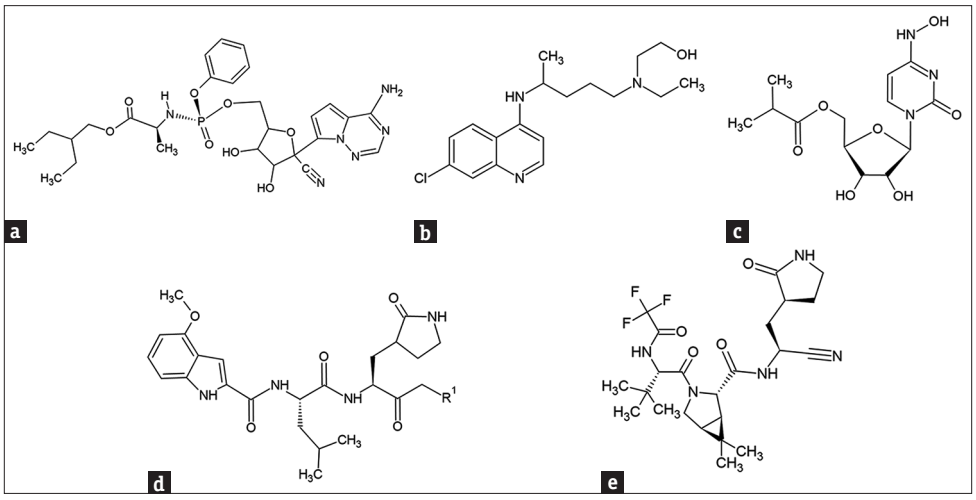

Figure 1: Structures of representative potential antiviral compounds against severe acute respiratory syndrome coronavirus 2. (a) remdesivir. (b) hydroxychloroquine. (c) molnupiravir. (d) PF-00835231 (R^1: hydroxyl group)/PF-07304814 (R^1: phosphate). (e) PF-07321332

remdesivir, molnupiravir, and molnupiravir's metabolite β-D-N^4-Hydroxycytidine (EIDD-1931) by using Bayesian machine learning models, which constructed statistical models based on Bayes' Theorem, to identify potential interactions with the transporters [44]. Together with *in vitro* experiments, the authors found that remdesivir and EIDD-1931 are substrates of ENTs 1 and 2 and are potent inhibitors of ENT-mediated uridine cellular uptake [44]. SARS-CoV-2 main protease (M^{pro}, or 3C-like protease), which cleaves the virus polyprotein at 11 conserved sites, is a crucial enzyme for the productions of mature virus proteins. A molecular docking and MD simulation study by Mishra *et al*. found that HCQ/remdesivir/tetrahydrocannabinol might interact and inhibit the SARS-CoV-2 M^{pro} [45]. Based on the computational study, they also modified the structures of the original compounds, leading to the designs of 18 derivatives. Among these derivatives, two of them showed great affinity to the M^{pro}, and as a result, were suggested to have the potential to be developed into SARS-CoV-2 inhibitory drugs [45]. The outbreak of SARS in 2003 had triggered intensive research into the treatments of SARS-CoV-1 infection. A homology model of SARS-CoV-1 M^{pro} constructed based on the crystal structures for human coronavirus 229E M^{pro} was published [46], and a M^{pro} inhibitor rupintrivir, originally developed for the treatment of human rhinovirus, was investigated for its potential for inhibiting SARS-CoV-1 M^{pro} [47]. A rupintrivir derivative, PF-00835231 [Figure 1d], was designed and selected as a development candidate for SARS-CoV-1 treatments. However, the project was ended with the ending of 2003 pandemic. Following the COVID-19 outbreak, PF-00835231 was again considered as a promising drug and has been tested for its inhibitory activity against SARS CoV-2 M^{pro}. A cocrystal structure (PDB: 6XHL) with PF-00835231 bound in the M^{pro} active site has been solved [47], and the preclinical characterization of PF-00835231 and its prodrug PF-07304814 [lufotrelvir, Figure 1d] has been published [48]. This drug is currently under clinical trials [49]. PF-07321332 [Figure 1e] is developed as an orally administered SARS-CoV-2 inhibitor by Pfizer, Inc. and is currently under clinical trials [50]. The binding mechanism of PF-07321332 onto M^{pro} has been investigated with MD and binding-free energy simulations [51] and its affinity toward the M^{pro} were found to be greater than those of α-ketoamide, lopinavir, and ritonavir [51]. These analyses might be helpful for future development and optimization of specific compounds targeting COVID.

The development of novel therapeutic drugs is a long and complex process and is sometimes too slow to deal with emerging health threats. In addition, as the drug development costs are high and most of the drug development failed in between the drug discovery and being put on market, the pharmaceutical industries normally invest into new drug developments with great cautiousness and are sometimes reluctant to do so. Repurposing existing drugs for new applications is therefore considered as a practical and fast-track approach for combating newly emerging medical situations [52], because of the fact that these drugs have already been tested for their safety and fulfilled many of the requirements set by the authorities. In 2021, a study carried out by Jang *et al*. [53] virtually screened 6218 approved and clinical trial drugs against M^{pro} and RdRp of SARS-CoV-2 using molecular docking and MD simulations. They also introduced a filtering strategy to reduce false-positive results. This mentioned study identified 15 and 23 potential drug candidates targeting the M^{pro} structure [Figure 2a, PDB: 6Y2F [54]] and the RdRp structure [Figure 2b, PDB: 6M71 [55]], respectively. Cellular experiments showed that 7 of these drugs were able to inhibit SARS-CoV-2 replication in Vero cells, and 3 of them, emodin, omipalisib, and tipifarnib, showed inhibitory effects on SARS-CoV-2 in human lung cell line Cali-3 [53]. It was also found that the anti-SARS-CoV-2 activity of omipalisib [an anti-cancer PI3K/mTOR inhibitor also known as GSK2126458, Figure 3a] is much greater than that of remdesivir in Calu-3 cells [53]. As mentioned previously, the deadly ARDS in severe COVID-19 patients is caused by cytokine storm. As a result, COVID-19 associated cytokines and their receptors are also considered as targets for drug developments. An *in silico* study indicated

that the FDA approved drugs rifampicin [Figure 3b] and letermovir [Figure 3c] have the potential to be repurposed for COVID-19 treatments [56]. In addition to targeting M^{pro}, these two drugs were found to show excellent affinity to TNF-α, IL-6, and IL-1β [56]. Computational methods were also applied for large-scale screening of potential lead drugs. A method of deep representation learning on heterogeneous drug networks has been established for the discovery of anti-inflammatory agents for COVID-19 patients [57]. With this method, 22 anti-inflammatory drugs for COVID-19 were identified, in which 9 of them were suggested to be involved in TNF-α related mechanism, 12 of them interact with mechanisms related to IL-6, and a drug acarbose binds to both TNF-α and IL-6 [57]. The TMPRSS2 enzyme of SARS-CoV-2 is also considered a target for the suppression of virus infection. Elbadwi *et al*. applied structure-based virtual screening to search for drugs with the potential to target SARS-CoV-2 TMPRSS2, and identified 5 commercially available drugs amikacin, isepamicin, butikacin, lividomycin, and paromomycin, possibly having inhibitory abilities against the TMPRSS2 enzyme [58]. Hamdy *et al*. applied an iterated virtual screening method to re-screened M^{pro} effective compounds against a TMPRSS2 structure (PDB: 2OQ5) using molecular docking [59]. After MD simulations, five compounds were identified to possess dual-binding affinity to M^{pro} and TMPRSS2, and one of them were tested to exhibit an improved *in vitro* antiviral activity and safety [59].

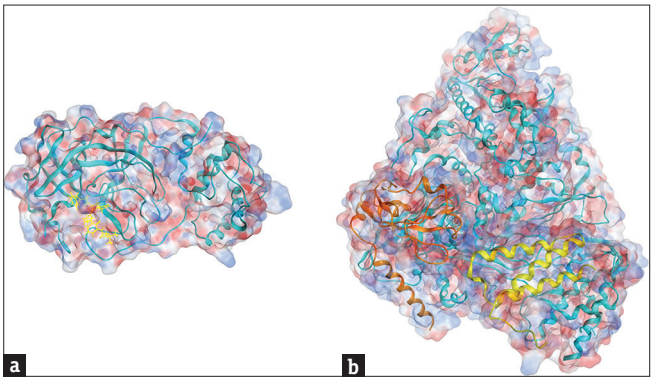

Figure 2: Resolved structures of severe acute respiratory syndrome coronavirus 2 M^{pro} and RdRp. (a) A crystal structure of M^{pro} (PDB: 6Y2F [54]). The structure in cyan color is the M^{pro}; the molecule in yellow color is a α-ketoamide inhibitor binding to the protein. (b) A cryo-EM structure (PDB: 6M71 [55]) of RdRp (cyan) in complex with co-factors non-structural protein 7 (yellow) and non-structural protein 8 (brown). Blue and red colors on the sphere presentation of the protein structures indicate the positive and negative charged force fields, respectively

Because of the nature of RNA viruses, the SARS-CoV-2 is a virus with a high mutation rate. The changes in its genomic RNA sequence cause the changes in the structures of target viral proteins, leading to the possible losses of efficacies of vaccines and therapeutics in use or under clinical trials. Efficient approaches for creating the structures of mutant proteins will provide great help in the future development of vaccines and therapeutics for emerging and mutated viruses. Alphafold by DeepMind, now part of Google's parent firm, is a sequence-based artificial intelligence (AI) algorithmic prediction tool for constructing tertiary structures of proteins with outstanding accuracy [60,61]. Robertson *et al*. created the structure models of SARS-CoV-2 M^{pro} with the Alphafold2 (Alphafold version 2) program and evaluated the concordance of the X-ray and AlphaFold models of M^{pro} with the results from residual dipolar couplings measured in solution [62]. The results showed that although the structures from X-ray crystallography and Alphafold predictions were similar, as compared to the best crystal structures, AlphaFold M^{pro} models agreed more closely with the experimental results of solution residual dipolar couplings [62], suggesting that the AI tools can provide new opportunities for structure-based analysis and simulations for drug discovery and design. SARS-CoV-2 uses its NSP6 to interact with the host cell sigma receptors involved in lipid remodeling and ER stress response. Pandey *et al*. utilized an Alphafold created NSP6 structure to study the binding mechanism of dextromethorphan, a cough suppressant, and haloperidol, an antipsychotic drug, unto the NSP6 with molecular docking and MD simulations [63]. It was found that the binding of dextromethorphan, identified previously to have pro-viral activity [64], destabilized the structure of drug-NSP6 complex and led to an increase in conformational dynamics and energetic frustrations [63]. On the other hand, the strong binding of the haloperidol, found to be antiviral, caused minimal structural and dynamical perturbations to NSP6 [63]. As a result, haloperidol was concluded in this mentioned study to be a potential candidate drug for COVID-19. The M protein of SARS-CoV-2 is crucial for virus assembly, and is also considered as a drug target. Peele *et al*. [65] applied an Alphafold created SARS-CoV-2 M protein structure to screen approved drugs in SuperDRUG2 database for drug repurposing. A total of 3639 SuperDRUG2 database drugs and 14 potential SARS-CoV-2 drugs were selected for examinations. After molecular docking screening, nine drugs were found to bind to the M protein active site. MD simulation analyses and binding free energy calculations suggested that 4 of the 9 bound to M protein with desired

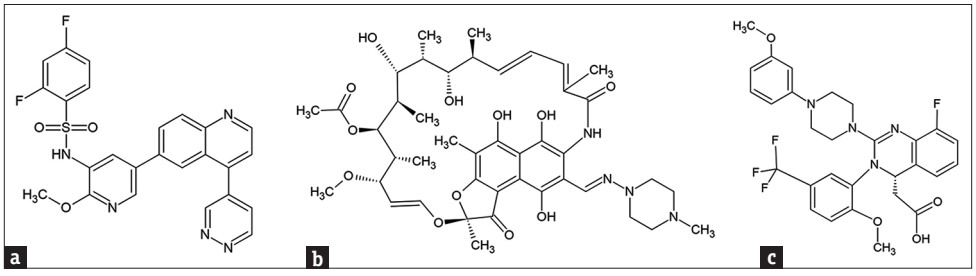

Figure 3: Examples of approved drugs with potential for repurposing to treat severe acute respiratory syndrome coronavirus 2 infection. (a) omipalisib. (b) rifampicin. (c) letermovir

binding stability. Among these four, colchicine, normally used to treat gout flares and Familial Mediterranean fever, was found to be the top most binder to the M protein [65]. Because of this, the authors searched colchicine-like substructures in PubChem database (https://pubchem.ncbi.nlm.nih.gov/) for the identification of effective compounds with less toxicities. Among 683 compounds retrieved, 10 were found to have better binding affinity to the M protein than colchicine, as revealed by docking analyses. The pharmacokinetic properties of these compounds were further calculated with an online software SwissADME [66], and the calculations indicated that 4 of the compounds display comparable pharmacokinetic properties with that of colchicine. The compound with PubChem ID 6711380 (IUPAC: N-[(7S)-1,2,10-trimethoxy-9-oxo-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6,7-dihydro-5H-benzo[a]heptalen-7-yl]acetamide) was calculated to be the best among all selected derivatives [65].

For the screening and study of repurposed drugs against COVID-19, computational tools have proved themselves to be powerful aids in understanding drug-target interactions, revealing unknown mechanisms of known effective drugs, finding new functions of existing drugs, as well as screening of possible drug candidates from drug libraries.

Therapeutic Peptides

Although there are currently effective monoclonal antibody therapies developed for direct targeting the virus spike protein [67], these therapies are extremely expensive. As a result, they may not be the solutions for the worldwide crisis. Relatively inexpensive therapeutics is required. Peptides are biomaterials assembled with amino acids. As they can be easily chemically synthesized with different amino acid sequences, functional peptides are emerging as a popular group of agents for therapeutic purposes. There have been attempts in designing peptides for interrupting the interactions between host ACE2 and SARS-CoV-2 spike protein by using computational approaches. Han and Král [68] analyzed the interactions between host ACE2 and virus spike protein RBD interface using a resolved complex structure [Figure 4a, PDB: 6M17 [69]]. They identified that in total 15 residues of ACE2 interact with the virus spike protein RBD. These residues include Q24, T27, D30, K31, H34, E35, E37, D38, Y41, and Q42 of α1 helix, M82 of α2 helix, K353, G354, D355, and R357 from the linker between β3 and β4. They designed four inhibitory peptides [inhibitor 1–4 in Table 1]. By using classical MD simulations, they found that the inhibitors formed by two sequential self-supporting α-helices (α1 and α2) derived from the protease domain of ACE2 bind to the SARS-CoV-2 spike protein RBD, and the α-helical peptides maintain their secondary structure and provide a highly specific and stable binding [68]. On the other hand, Cao *et al*. applied two strategies for the design of mini-proteins to neutralize the SARS-CoV-2 spike protein RBD [70]. They firstly designed mini-protein incorporated with a derived helix of ACE2 (residues from 23 to 46) responsible for the interactions with the virus RBD by using the Rosetta blueprint builder [73]. They also *de novo* designed RBD-binding proteins by using rotamer interaction field docking [74] with large *in silico* mini-protein libraries [75], followed by the design to generate binders to the distinct regions of the RBD surface [70], the sequences of peptides designed are shown in Table 1 (AHB1-2, LCB1-8). The neutralization activity of the designed mini-proteins was experimentally measured with a focus reduction neutralization test on cell monolayers. Effective concentration (EC_{50}) values of less than 50 nM were achieved [70]. Karoyan *et al*. designed human ACE2 peptide-mimics composed of 27 residues based on the computational analysis of a crystal structure [Figure 4b, PDB: 6M0J] of SARS-CoV-2 spike protein RBD bound with ACE2. They identified that amino acid sequence from S19 to L45 of the hACE2 H1 helix interacts with SARS-CoV-2 spike protein, and 12 residuals in the sequence are important for the interaction. From these finding, they designed/optimized 12 peptide mimics for *in vitro* and cellular experimental tests. 3 peptide-mimics [P8-10 in Table 1] were found to be able to block SARS-CoV-2 pulmonary cell infection with an inhibitory concentration (IC_{50}) of within nanomolar range [71]. For screening of peptides, Chitsike *et al*. designed several candidate peptides [72] from motifs in ACE2 and spike protein RBD by analyzing a crystal complex structure (PDB: 6LZG). Peptides with and without modifications (indicated with # in Table 1) to the native sequences were screened for their inhibitory potential to ACE2-RBD binding with a proximity-based AlphaScreen™ assay [72]. The sequence between the 21th amino acid to the 45th amino acid of ACE2 is commonly found in the results from different research groups to interact with SARS-CoV-2 spike protein RBD and should be an important consideration for future peptide drug design. As mentioned, M^{pro} is also an important target for the development of anti-SARS-CoV-2 drugs. Peptide inhibitors have been investigated using bioinformatic approaches for their applications against CoV M^{pro} [76]. For SARS-CoV-2 specific inhibitors, the previously identified CoV inhibitor M3 peptide [76] was analyzed for its interactions with SARS-CoV-2, and the interactions were further proved by X-ray crystallography [Figure 4c, PDB: 6LU7] [77]. In addition, analysis of large biological data sets with computational approaches to extract meaningful information has been applied for the identification of virus inhibitory peptides. Several machine learning approaches have been developed for predicting antiviral peptides [78-88]. Among these, the recently published few methods have been specifically developed for the prediction of peptides with anti-coronavirus activities [84,85,87]. A neural network-based method developed by Timmons and Hewage, with an external test accuracy of 93.9%, was found to outperform other methods [87].

Phytochemicals

Herbal medicines and their active phytochemicals have long been important sources for the developments of therapeutic drugs. Large-scale screenings of SARS-CoV-2 inhibitory compounds have been performed in several research groups. Yang *et al*. applied computational molecular docking to screen 1800 natural compounds for the identification of SARS-CoV-2 spike protein inhibitors [89]. A compound corilagin derived from an annual perennial herbal species *Phyllanthus*

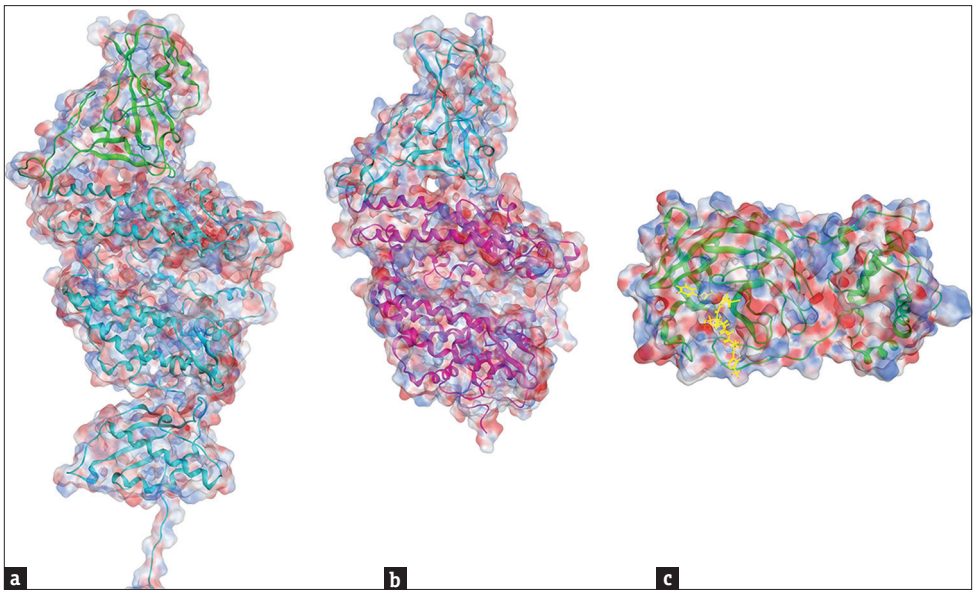

Figure 4: Molecular docking of severe acute respiratory syndrome coronavirus 2 proteins with receptors/ligands. (a) A cryo-EM structure of severe acute respiratory syndrome coronavirus 2 spike protein RBD (green) in complex with human ACE2 (cyan) (PDB: 6M17 [69]). (b) A crystal structure of severe acute respiratory syndrome coronavirus 2 spike protein RBD (cyan) bound with ACE2 (pink) (PDB: 6M0J [18]). (c) A crystal structure of M^{pro} (green) in complex with a peptide-like inhibitor N3 (yellow) (PDB: 6 LU7 [77]). Blue and red colors on the sphere presentation of the protein structures indicate the positive and negative charged force fields, respectively

Table 1: Examples of inhibitory peptides targeting spike protein - angiotensin-converting enzyme 2 interactions

Sequences (names)	Sequence source
^{21}IEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNIT^{55} (inhibitor 1)	ACE2 [68]
^{21}IEEQAKTFLDNFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQEI^{88}	ACE2 [68]
^{349}WDLGKGDFR^{357}(inhibitor 2)	
^{21}IEEQAKTFLDNFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQALTVKLQLQALQQNGS^{105}	ACE2 [68]
^{323}MTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCT^{362} (inhibitor 3)	
^{21}IEEQAKTFLDNFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQALTVKL^{95}	ACE2 [68]
^{335}DPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNGANEGF^{400} (inhibitor 4)	
DEDLEELERLYRKAEEVAKEAKDASRRGDDERAKEQMERAMRLFDQVFELAQELQEKQTDGNRQKATHLDKAVKEAADELYQRVR (AHB1)	de Novo [70]
ELEEQVMHVLDQVSELAHELLHKLTGEELERAAYFNWWATEMMLELIKSDDEREIREIEEEARRILEHLEELARK (AHB2)	de Novo [70]
DKEWILQKIYEIMRLLDELGHAEASMRVSDLIYEFMKKGDERLLEEAERLLEEVER (LCB1)	de Novo [70]
SDDEDSVRYLLYMAELRYEQGNPEKAKKILEMAEFIAKRNNNEELERLVREVKKRL (LCB2)	de Novo [70]
NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFELADKAYKNNDRQKLEKVVEELKELLERLLS (LCB3)	de Novo [70]
QREKRLKQLEMLLEYAIERNDPYLMFDVAVEMLRLAEENNDERIIERAKRILEEYE (LCB4)	de Novo [70]
SLEELKEQVKELKKELSPEMRRLIEEALRFLEEGNPAMAMMVLSDLVYQLGDPRVIDLYMLVTKT (LCB5)	de Novo [70]
DREQRLVRFLVRLASKFNLSPEQILQLFEVLEELLERGVSEEEIRKQLEEVAKELG (LCB6)	de Novo [70]
DDDIRYLIYMAKLRLEQGNPEEAEKVLEMARFLAERLGMEELLKEVRELLRKIEELR (LCB7)	de Novo [70]
PIIELLREAKEKNDEFAISDALYLVNELLQRTGDPRLEEVLYLIWRALKEKDPRLLDRAIELFER (LCB8)	de Novo [70]
SALEEQLKTFLDKFMHELEDLLYQLAL (P8)	Derived* [71]
SALEEQYKTFLDKFM HELEDLLYQLSL (P9)	Derived* [71]
SALEEQYKTFLDKFMHELEDLLYQLAL (P10)	Derived* [71]
^{19}STIEEQAKTFLDKFNHEAEDLFYQSSL^{45}	ACE2 WT [72]
^{24}QAKTFLDKFNHEAEDLFYQSS^{44}GLGKGDFR	ACE2 WT [72]
QVKYFLDKFNHEAEDRDYQSSL	ACE2 MT [72]
PFLEKLLHEAEDLLYQLELA	ACE2 MT [72]
PFLEKLLHEc^{d}EDCLYQLELA	ACE2 MT [72]
^{483}VEGFNCYFPLQSYGFQPTNGVGY^{505}	RBD WT [72]

*Peptides derived from ACE2 sequence"^{19}STIEEQAKTFLDKFNHEAEDLFYQSSL^{45}". WT: Wild type, MT: Mutant, c^{d}: D-cysteine, ACE2: Angiotensin-converting enzyme 2, RBD: Receptor-binding domain

urinaria was identified to have a strong binding affinity to both the SARS-CoV-2 spike protein RBD and human ACE2. The binding was further confirmed by experimental methods such as biolayer interferometry (BLI), ELISA and

immunocytochemistry assay [89]. Zhang *et al*. screened a library of 1871 natural compounds by using molecular docking combined with BLI measurements, and identified 4 compounds, epigallocatechin gallate, isobavachalcone, salvianolic acid A, and isoliensinine, to have effective inhibitory effects on the SARS-CoV-2 entry. The effects were further proven by plaque formation assay in Vero E6 cells [90]. Perrella *et al*. identified two natural polyphenols, polydatin and resveratrol, to possess activities to interact with the spike protein of SARS-CoV-2 [91]. Molecular docking simulations revealed that both polyphenols can bind to the spike protein, ACE2 and the ACE2: spike protein complex [91]. SARS-CoV-2 M^{pro} has been mentioned previously to be a popular target for inhibitory compound screening. Kumar *et al*. applied molecular docking and MD simulations to screen effective compounds from purple nutsedge (*Cyperus rotundus*) against M^{pro} of SARS-CoV-2 [92]. Two compounds β-amyrin and stigmasta-5,22-dien-3-ol were identified to exhibit excellent binding abilities to the virus M^{pro} and were suggested by the authors to be possible inhibitors for SARS-CoV-2 [92]. Giofrè *et al*. screened 14 natural compounds from limonoids and terpenoids for their ability to inhibit the key target proteins of SARS-CoV-2 by using molecular docking and MD simulations, and identified two limonoids, deacetylnomilin and ichangin, able to directly interact with the catalytic dyad of M^{pro} [93]. Silva *et al*. investigated the pharmacokinetic and toxicological properties of molecules in a natural products database of Brazilian semiarid region and performed site prediction and druggability analysis on the SARS-CoV-2 M^{pro} [94]. After molecular docking and MD simulation, among 10 molecules selected, two of them were suggested to have better potential to interact with the M^{pro} and to be worth further studying [94]. Gupta *et al*. screened more than 53,500 bioactive natural molecules from six different natural product databases for the identification of effective molecules against M^{pro} [95]. The top three screened molecules were further validated by MD simulations, and one of the three was found to possess highest binding affinity as indicated by relative binding energy analysis [95]. The effects of chromenes, flavonoids, and hydroxamic acid compounds on SARS-CoV-2 M^{pro} have been investigated [96], and compounds in two herbal methanolic extracts, from Averrhoa carambola (star fruit) leaves and Ageratum conyzoides aerial part, were found to demonstrate significant inhibition on SARS-CoV-2 M^{pro}. In this study, the *in vitro* experiment results were supported by in silico molecular docking analysis [96]. Li *et al*. applied ensemble and cooperative docking, as well as molecular simulations, to investigate potential interactions of more than 600 compounds from an herbal medicine with eight SARS-CoV-2 proteins including spike protein, nucleocapsid protein, M^{pro}, Papain-like protease, RdRp, NSP3, and cat/human ACE2 [97]. This study identified more than nine compounds which may effectively bind to SARS-CoV-2 proteins [97]. In addition, it was found that some of these compounds simultaneously bind to the same target sites. Thus, these compounds might serve as cooperative inhibitors for SARS-CoV-2 proteins [97]. Altogether it has been demonstrated that computational methodologies not only provide useful tools for systematically assess potential antiviral activities of molecules but also indicate new avenues for the search of cooperative compounds to target SARS-CoV-2-related proteins.

Conclusion

With the advanced capability of computer technology, computational methods have become powerful tools for biomedical investigations. The computational approaches also provide meaningful, rapid, and cost-effective ways in drug design and screening. They speed up the process of understanding how structurally complicated molecules interact with one another. Owing to the great efforts of research scientists around the world, structures of the major proteins of SARS-CoV-2 have been resolved with biophysical techniques such as X-ray crystallography and NMR, providing the foundation for applying structure-based computational methods, such as molecular docking and MD simulations, to study virus-host protein interactions for the design of therapeutic drugs against COVID-19. These structure-based methods can also be applied to identify effective molecules from compound/drug banks as well as from traditional medicines. Among all the SARS-CoV-2 proteins, the most frequently used targets are the spike protein RBD and M^{pro}. Owing to the nature of RNA viruses, the SARS-CoV-2 proteins are mutating at a fast speed, thus changing their structures. These might result in their escape from the targeting of specific therapeutics. To combat these situations, powerful AI tools, such as Alphafold, will play increasing important roles in the future for the generations of viral protein structures for investigations. In addition to structure-based analytical tools, machine learn algorithms have been applied in drug discovery for the effective treatments of COVID-19. Efforts by research scientists have been focused on the discovery and design of novel drug candidates, or repurposing and modifying existing approved drugs. Projects on theses purposes have been either exclusively computational or computational-experimental combined studies. It is expected that the computer-aided methods will continue to play central and crucial roles in the battle against COVID-19.

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Conflicts of interest

There are no conflicts of interest.

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