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REVIEW

Analysis of the SARS-CoV-2-host protein interaction network reveals new biology and drug candidates: focus on the spike surface glycoprotein and RNA polymerase

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ABSTRACT

Introduction: The COVID-19 pandemic originated from the emergence of anovel coronavirus, SARS-CoV-2, which has been intensively studied since its discovery in order to generate the knowledge necessary to accelerate the development of vaccines and antivirals. Of note, many researchers believe there is great potential in systematically identifying host interactors of viral factors already targeted by existing drugs.

Areas Covered: Herein, the authors discuss in detail the only available large-scale systematic study of the SARS-CoV-2-host protein–protein interaction network. More specifically, the authors review the literature on two key SARS-CoV-2 drug targets, the Spike surface glycoprotein, and the RNA polymerase. The authors also provide the reader with their expert opinion and future perspectives.

Expert opinion: Interactions made by viral proteins with host factors reveal key functions that are likely usurped by the virus and, as aconsequence, points to known drugs that can be repurposed to fight viral infection and collateral damages that can exacerbate various disease conditions in COVID-19.

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV -2) has spread rapidly since its identification in patients with severe pneumonia in Wuhan, China, December 2019. About one year later, 4 December 2020, there has been about 65 million confirmed cases, including over 1.5 million deaths (World Health Organization). As many regions of the world are now facing worsening second waves of infections in late 2020, there is an urgent need to develop therapeutics to combat SARS-CoV-2 and COVID-19.

SARS-CoV-2 is the third coronavirus with epidemic capability to appear in the past 20 years. Compared to MERS-CoV or SARS-CoV, SARS-CoV-2 has a lower fatality rate but a higher transmission rate [1]. To design therapeutic strategies to counteract SARS-CoV-2 infection and COVID-19 (Coronavirus Disease 2019), it is crucial to understand how this coronavirus usurps host functions during infection, characterize mechanisms of key targets for drug discovery, and then apply this knowledge to develop new drugs and repurpose existing ones.

So far, only one drug has been approved by the U.S. Food and Drug Administration (FDA) for treatment of COVID-19. Remdesivir is a nucleoside analog that targets the SARS-CoV-2 RNA-dependent RNA Polymerase (RdRP). However, Remdesivir is only approved for cases requiring hospitalization and its effect has been reported to be modest at most; additional drugs must thus be developed to treat COVID-19. A number of clinical trials are underway for antiviral and vaccine candidates, many holding great promises such as repurposing compounds able to inhibit the human protease TMPRSS2 [2]. Indeed, many researchers believe there is great potential in systematically identifying host interactors of viral factors already targeted by existing drugs. Therapies targeting the host–virus interface, where the occurrence of mutational resistance may be less likely, could potentially present efficient treatments. Unfortunately, limited knowledge of the molecular details of SARS-CoV-2 infection prevents a comprehensive evaluation of small molecule candidates for therapies directed against host proteins.

In this review, we will first discuss a large-scale proteomic study that decipher virus–host protein interactions and evaluate possibilities for repurposing drugs to treat COVID-19. Second, we review data on two key SARS-CoV-2 proteins that are promising targets for the development of therapeutics: The Spike surface glycoprotein that is the target of SARS-CoV-2 neutralizing monoclonal antibodies and the RNA polymerase that is the target of the first FDA-approved drug Remdesivir.

2. The SARS-COV-2-host protein interaction network

In an immense paper, where the interaction interface between SARS-CoV-2 proteins and human proteins was systematically

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Article highlights

- SARS-CoV-2 proteins interact with host cell factors, indicating new biological functions for these proteins.
- Drugs that are known to mod-The Spike surface glycoprotein targets the ACE2 receptor at the surface of host cells, inducing membrane fusion and determining tropism.
- The SARS-CoV-2 RNA-dependent RNA polymerase (RdRP or NSP12) synthesizes viral RNA as part of a viral multisubunit assembly named Replication and Transcription Complex (RTC).
- The Spike surface glycoprotein and the RNA-dependent RNA Polymerase are privileged targets for drug discovery.
- Recombinant vaccines with novel vectors show advantages in multiple gene expression, immunogenicity or immunity enhancement and safety.

This box summarizes key points contained in the article.

mapped, Krogan and colleagues [3] generated important knowledge of SARS-CoV-2 interactions with host proteins, a knowledge that is essential to develop new therapeutics. This group cloned, tagged, and expressed 26 out of the 29 SARS-CoV-2 proteins in cultured human cells and used affinity purification coupled with mass spectrometry (AP-MS) to identify the human proteins physically associated with each. This effort led to the identification of 332 SARS-CoV-2-human protein interactions.

To gain insight into the function of these interactions, the authors then studied the human interactors in regard to their biology, expression patterns, expression modulations during SARS-CoV-2 infection, and in relation to protein interaction maps with other pathogens, and Gene Ontology enrichment. This analysis identified the major cell processes involving the interacting proteins [3]. In addition, binding interfaces were identified by enriching for domain families within the interactors of each tagged viral protein. As an example, DNA polymerase domains are enriched among Nsp1 interactors, while Bromodomains and Extra-Terminal Domain (BET) family domains are enriched among E interactors. This large-scale proteomic analysis clearly revealed new biological functions relating to SARS-CoV-2 infections (see below).

One concern regarding the experiments in Gordon et al. [3] is the absence of reciprocal analysis of the newly discovered interactions (see [4] for a review and [5] for an example). Although such an analysis is not easily possible when studying host interactors of viral proteins, this study is lacking validation of the interactions using reciprocal AP-MS. This is especially relevant when one considers that the protein–protein interactions were studied in HEK-293T cells, a line which is permissive to SARS-CoV-2 infection but is not the primary infection site, which is the lung.

To circumvent this issue, Gordon et al. [3] used different strategies. First, they were able to show that the lung is the tissue where the identified interactors are the most highly expressed relative to all other proteins. Consistent with this, the interacting proteins were enriched in the lung relative to other organs/tissues, and compared to overall RefSeq gene expression in the lung, interactors were expressed at a higher level. This analysis supports the idea that SARS-CoV-2 preferentially usurps proteins expressed in this tissue.

Secondly, the authors also studied the evolution of the host proteins and analyzed protein abundance modulations during SARS-CoV-2 infection [3]. They found that interacting pairs of interactors correlated more strongly than other pairs of viral-human proteins. This analysis suggests that the AP-MS derived interactions are relevant for the target tissue and the context of SARS-CoV-2 infection.

2.1. The protein interaction network unravels new elements of SARS-CoV-2 function

This gigantic piece of work by Krogan and colleagues revealed interactions between SARS-CoV-2 proteins and human proteins that are involved in several protein complexes and biological processes (Figure 1). NSP1 interacts with factors involved in DNA replication; NSP5, NSP8, NSP13 and E bind to epigenetic and gene-expression regulators; NSP2, NSP6, NSP7, NSP10, NSP13, NSP15, ORF3a, E, M and ORF8 are connected to proteins playing a role in vesicle trafficking; S binds to factors involved in lipid modification; NSP8 and N interact with factors controlling RNA processing; ORF10 binds to the ubiquitin ligase machinery; NSP7, NSP8, NSP13, N and ORF9b connect to signaling molecules; NSP9, NSP15 and ORF6 to the nuclear transport machinery; NSP1 and NSP13 are connected to the cytoskeleton; NSP4, NSP8 and ORF9c to mitochondrial factors and NSP9 to the extracellular matrix. Overall, SARS-CoV-2 interactors are mainly connected to central processes, including vesicle trafficking, innate immune signaling, and the ribosome and translation processes.

2.2. Identification of drugs that target host interactors

To perturb the SARS-CoV-2 protein interaction network, Krogan and colleagues [3] searched for chemical compounds that bind to human interactors of viral proteins. Molecules were prioritized using a set of criteria including their availability, selectivity, MiST scores, regulatory approbation status, integration in clinical trials or classification as preclinical candidates. Chemical informatic searches on the human interactors identified 16 approved drugs, 3 drugs in clinical trials, and 18 preclinical candidates. Searches for target- and pathway-specific literature revealed 13 approved drugs, 9 drugs in clinical trials and 10 preclinical candidates. The 332 high-confidence human interactors of tagged viral proteins revealed 69 different drugs for 62 targets (Figure 2).

Using two specific viral assays done at the New York Mount Sinai Hospital, USA and the Institut Pasteur in Paris, France, Gordon et al. [3] also investigated the antiviral activity of these drugs and compounds. Two groups of molecules were found to reduce viral infection: the protein biogenesis inhibitors zotatifin, ternatin-4, and PS3061, and ligands of the sigma-1 and 2 receptors haloperidol, PB28, PD-144,418 and hydroxychloroquine. This analysis unraveled drugs that are candidate for repurposing as a possible way to develop a treatment for COVID-19. We expect that this new knowledge will be invaluable for the development of antivirals targeting SARS-CoV-2.

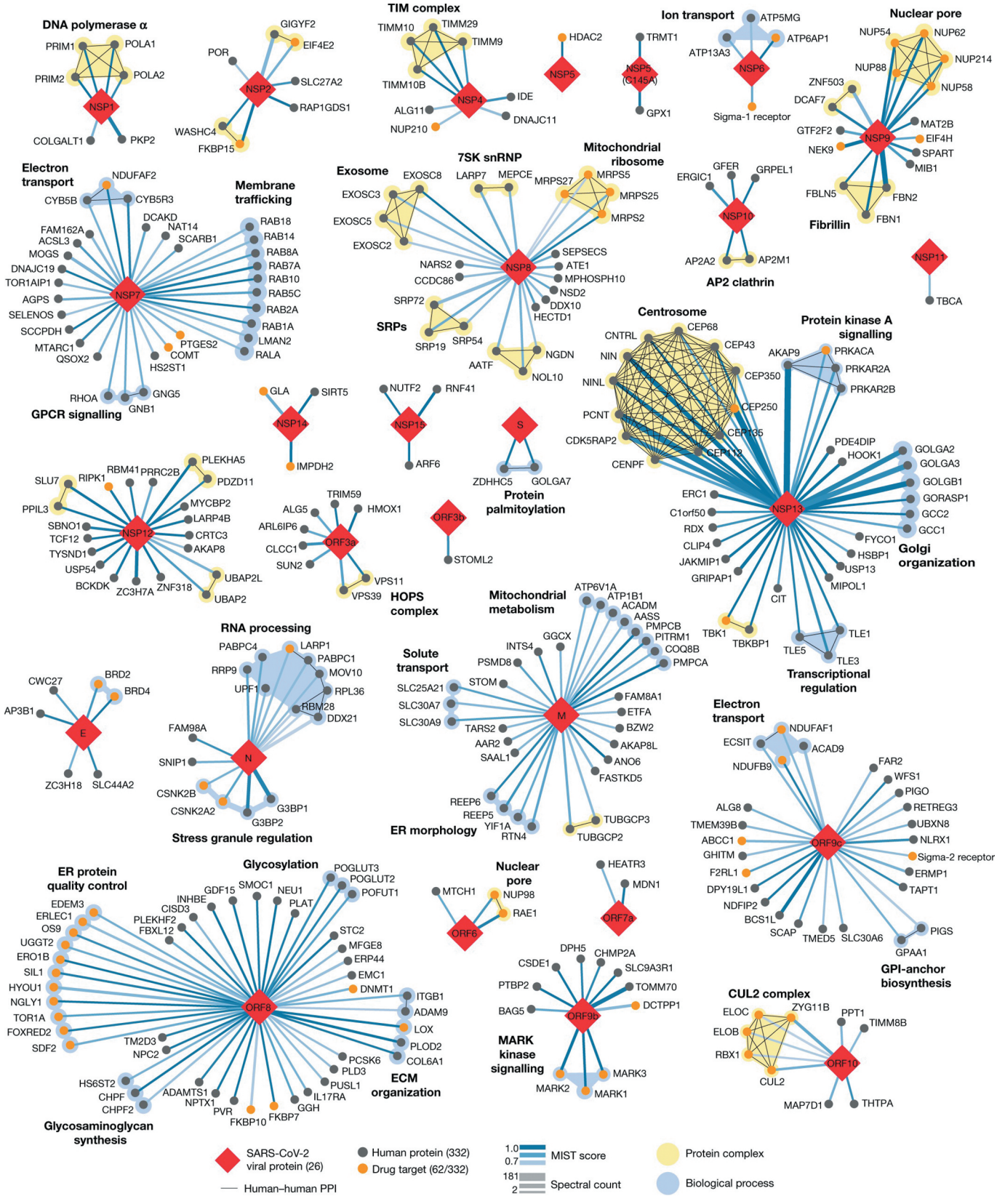


Figure 1. SARS-CoV-2-human protein interaction network.

High-confidence interactions between SARS-CoV-2 proteins (red diamonds) and human proteins (circles; drug targets: orange; protein complexes: yellow; proteins in the same biological process: blue). ECM, extracellular matrix; ER, endoplasmic reticulum; snRNP, small nuclear ribonucleoprotein. for details, see original paper. (reproduced from [3] with permission of springer nature).

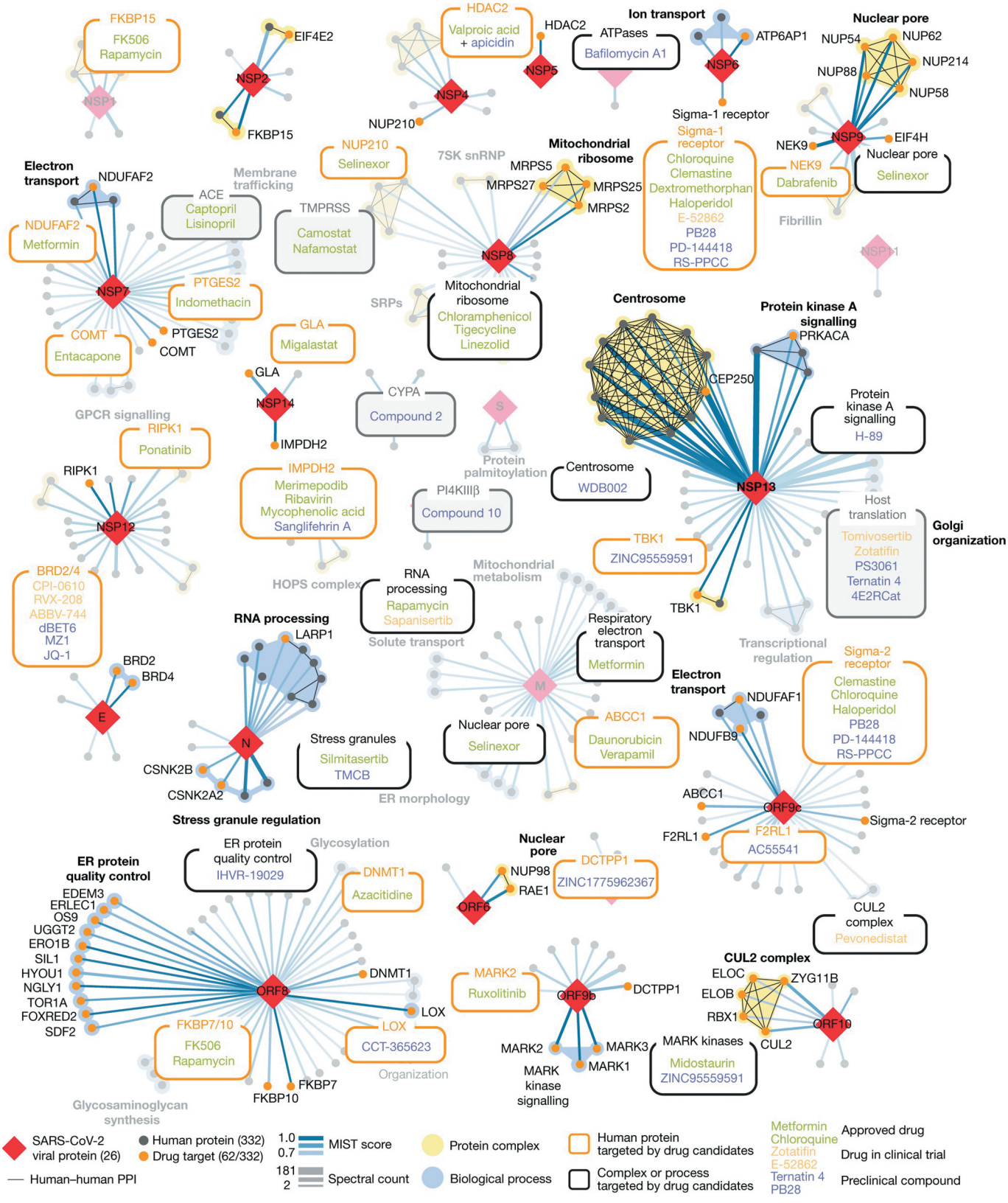


Figure 2. Drug-human target network.

Protein–protein interactions of SARS-CoV-2 proteins with approved drugs (green), clinical candidates (orange) and preclinical candidates (purple) with experimental activities against the host proteins (white background) or previously known host factors (gray background) are shown (reproduced from [3] with permission of springer nature).

Other authors have used computational methods to study SARS-CoV-2-host protein interactions [6–8] and CRISPR screens to identify host genes impacting SARS-CoV-2 infection [9,10]. The results are complementary to what has been described in the preceding paragraphs.

3. Structure and function of the SARS-CoV-2 surface spike (s) glycoprotein: defining viral tropism

The spike (S) protein of SARS-CoV-2 is a type I trimeric transmembrane protein which specifically binds to the host cellular receptor angiotensin-converting enzyme 2 (ACE2) to initiate the infection process. The cryo-electron microscopy (EM) structures of the S protein reveal several functional domains including an N terminal cleavable signal peptide, a densely N-glycosylated ectodomain (60–90 carbohydrates per trimer), a transmembrane region, and a cytoplasmic tail containing S-acylated cysteine residues (Figure 3) [11]. S1 and S2 subunits, which form the ectodomain, are responsible for receptor binding and the membrane fusion catalysis, respectively. More specifically, the C-terminal domain of the S1 subunit consists of the receptor-binding domain (RBD), while the S2 subunit contains a hydrophobic fusion loop and two heptad repeat regions (HR1 and HR2), which facilitate fusion by bridging the viral and host cell membranes [12,13].

As observed with other coronaviruses, the S protein of SARS-CoV-2 has a metastable prefusion conformation that undergoes a substantial structural rearrangement [12,14]. Conformational movements of the RBD transiently hide or expose the determinants of receptor binding in order to engage a host cell receptor [14]. The predominant state of the trimer has one of the RBDs rotated up in a receptor-accessible conformation while the RBD with 'down' conformation has a receptor-inaccessible state [14]. Receptor binding to exposed RBDs leads to an unstable three-RBD 'up' conformation [14]. As the RBD is a key component for binding of SARS-CoV-2 to host cells' ACE2 receptor, RBD-ACE2 interaction has been investigated by several cryo-EM studies and compared with that of SARS-CoV. Both RBD–ACE2 interfaces consist of hydrophilic interactions, such as hydrogen bonds and salt bridges, in addition to multiple tyrosine residues forming hydrogen–bonding interactions with the polar hydroxyl group [15]. Surface plasmon resonance (SPR) assays show that SARS-CoV-2 RBD has higher ACE2 binding affinity as compared to SARS-CoV RBD.

Atomic comparison of the two ligands as well as *in silico* analysis reveals more atomic interactions in SARS-CoV-2-ACE2 than in SARS-CoV-ACE2, correlating with data showing higher affinity of SARS-CoV-2-RBD to ACE2 [16,17]. On the other side, the full-length SARS-CoV-2 S protein has been shown to have similar/lower ACE2 binding affinity than SARS-CoV S protein [18]. This paradox is explained with the dynamic state of the RBD. While SARS-CoV RBD is mostly in the 'up' conformation, the predominant 'down' conformation of SARS-CoV-2 RBD makes it less accessible for receptor binding and results in comparable or lower human ACE2 binding affinity of both S proteins.

After binding to its receptor, the S protein is cleaved into subunits, a process called priming, at the S1/S2 boundary and another site (called S2') within S2 by host proteases trans-

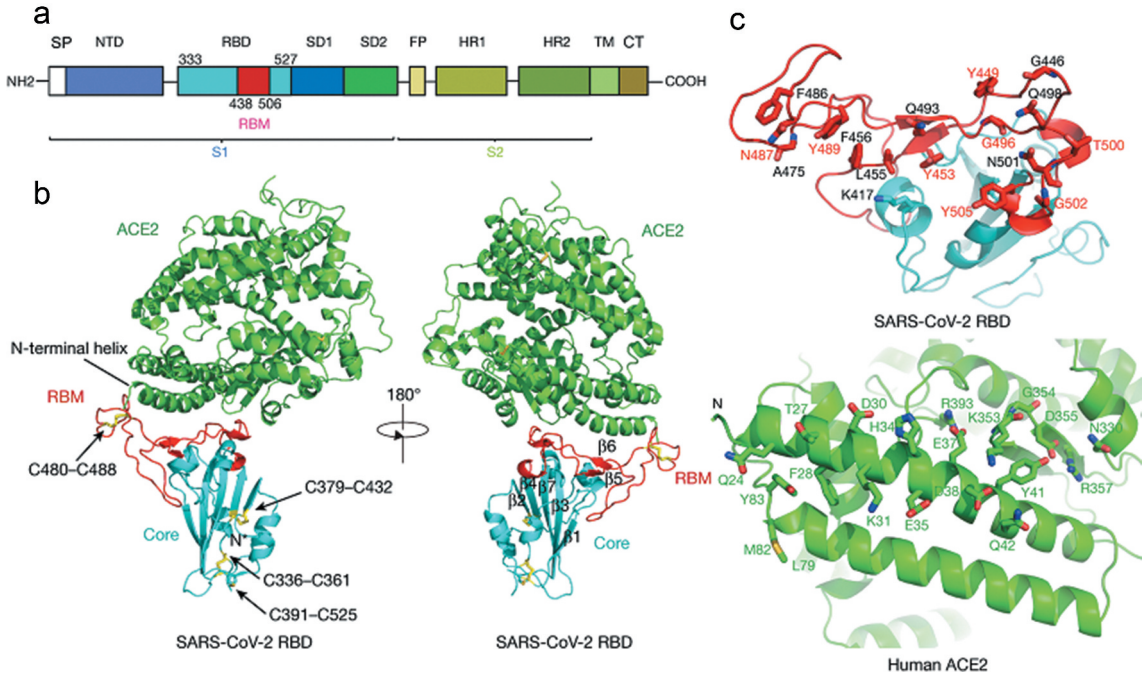


Figure 3. (A) Overall topology of the SARS-CoV-2 spike monomer. SP, signal peptide; NTD, N-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; SD1, subdomain 1; SD2, subdomain 2; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; TM, transmembrane region; CT, cytoplasmic tail. (B) Overall structure of the SARS-CoV-2 RBD bound to ACE2. ACE2 is shown in green. The SARS-CoV-2 RBD core is shown in cyan and RBM in red. disulfide bonds in the SARS-CoV-2 RBD are shown as sticks and indicated by arrows. The N-terminal helix of ACE2 responsible for binding is labeled. (C) Contacting residues are shown as sticks at the SARS-CoV-2 RBD–ACE2 interfaces. positions in RBD that are involved in ACE2 binding are indicated by red labels. Adapted by permission from Springer Nature, Nature, Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. adapted from Lan et al [15]. with permission of springer nature.

membrane protease/serine 2 (TMPRSS 2), cathepsin B and L [19,20]. An additional furin cleavage site at the S1/S2 boundary of the SARS-CoV-2 S protein is a novel feature distinguishing this virus from SARS-CoV [21]. The furin cleavage site is introduced as an insertion of four amino acid residues (PRRA), which is found in hemagglutinin proteins of highly virulent avian and human influenza viruses [22]. Therefore, it is speculated that the furin cleavage site in S protein might play an important role in facilitating the rapid human-to-human transmission of SARS-CoV-2 [23].

3.1. The role of ACE2 in COVID-19

ACE2 is a type I membrane protein regulating heart and kidney function as well as controlling blood pressure by maturation of a peptide hormone, angiotensin [24]. N terminal peptidase domain (PD) of ACE2, which cleaves angiotensin for maturation, is also the region interacting mostly with SARS-CoV-2 S protein [25]. Cryo-EM studies show that two S protein trimers can simultaneously bind to an ACE2 homodimer and each PD accommodates one RBD [26]. ACE2 is a receptor expressed in several organs. Screening of ACE2 mRNA expression in human organs demonstrated that digestive tract intestine displayed the highest expression of ACE2, followed by testis and kidney, which could explain the positive detection of SARS-CoV-2 in patients' feces and urine [27,28]. The lung serves as a main target organ of the virus due to the vast surface area which makes it susceptible to inhaled virus. Moreover, high levels of multiple viral process-related genes in ACE2-expressing alveolar epithelial type II cells (AECII) of lung tissues suggests that virus replication in lung is facilitated by AECII cells [24]. On the other hand, high expression of ACE2 in the heart indicates an intrinsic susceptibility of heart to SARS-CoV-2 infection. Patients with basic heart failure disease exhibited increased expression of ACE2 suggesting high possibility of heart attack and progress to severe condition after infection [27].

Many coding variants of ACE2 in humans have been associated with several diseases such as cardiovascular disorders, hypertension, and diabetes [29,30]. The influence of structural variations in human ACE2 on SARS-CoV-2 S protein/ACE2 interactions was also investigated by employing a comparative modeling and molecular superimposition study [31]. Two ACE2 alleles, rs73635825 (S19P) and rs143936283 (E329G), located at the interaction surface of ACE2 with the S protein showed low binding affinity to SARS-CoV-2 S protein [31].This finding makes the authors suggest that the variations in recovery rate of COVID-19 between different age groups, nationalities and race might be due to the existence of ACE2 variants [31]. It is important to note that the enthalpy and the vibrational entropy calculations for the S protein/ACE2 interaction show no significant changes in neither folding energy of the complex nor the protein–protein interaction energy of the two proteins [32]. Similarly, molecular dynamics simulations for wild-type and a selection of variant ACE2 proteins in Italian population were carried out to predict protein structural changes caused by the ACE2 variants [33]. Two rare variants, so-called Leu351Val and Pro389His, were predicted to have effect on ACE2-spike protein interaction [33]. K26R variant of ACE2 was reported in another structural study with increased RBD binding affinity [34]. Despite the high numbers of structural models built on the interaction of ACE2 variants with RBD, there is a limited number of reports analyzing their interactions in vitro and showing contrary results. For instance, Hashizume et al. performed in vitro studies with ACE2 variants (V184A, S257N, I468V, N638S, L656X, S19P, K26R, and N720D) and demonstrated that the selected variants, except the L656X variant reducing ACE2 expression, did not affect ACE2 binding to RBD [35]. These results suggest that the observed differences in the infectivity and severity of COVID-19 may not be explained with ACE2 polymorphisms [35]. The mutations in S protein and their impact on viral infectivity and antigenicity have been also studied [36,37]. It is important to note that the more infectious variant D614G is located outside the RBD and is characterized with unaltered ACE2 binding affinity and no resistance to neutralizing antibodies. However, the variants with mutations in RBD were shown to be less infectious (e.g. V341I) or more resistant to neutralizing antibodies (e.g. A475V, L452R, V483A, and F490L) [38]. SARS-CoV-2 variants first observed in the United Kingdom (UK) (B.1.1.7) and in South Africa (B.1.351) have gained special attention due to their easy transmission [39]. *In silico* analysis was performed to understand the effects of their observed mutations (the B.1.1.7 variant: 69/70 deletion, P681H, and N501Y; the B.1.351 variant: K417N, E484K, and N501Y) on ACE2 binding affinity of S protein as well as virus transmission and disease severity. K417N and E484K mutations were suggested to be not favorable for the ACE2 interaction [40]. Nevertheless, the adverse effect of E484K mutation on virus recognition by neutralizing antibodies was suggested to increase the pathogenicity [40]. Unlike to these two mutations, N501Y was suggested to enhance the virus transmission and disease severity due to the increased binding affinity of S protein for ACE2 receptor [40].

3.2. Potential therapeutics targeting S protein/ACE2 interaction

Unfortunately, as mentioned above, only one antiviral agent has been approved against the current outbreak. Due to the dramatic number of deaths and confirmed cases of SARS-CoV -2, there is an urgent demand of effective drugs for COVID-19 treatment. Although, polyclonal antibodies from recovered SARS-CoV-2-infected patients have been used to treat SARS-CoV-2 infections, sufficient sera and antibodies can hardly be produced during a large outbreak. Nevertheless, some trials performed at clinical sites suggest reevaluation of convalescent plasma therapy as a standard care as there was no significant difference in clinical status or overall mortality between patients treated with convalescent plasma and those who received placebo [41]. Moreover, human sera from convalescent patients can include non-neutralizing antibodies which may cause an antibody-dependent enhancement (ADE) effect on viral infectivity, as well as other harmful immune responses [42]. Therefore, it is highly

required to develop alternative therapeutic agents including specific antibodies against SARS-CoV-2, which are promising agents in the absence of an effective prophylactic vaccine.

3.3. Inhibitors targeting the S protein

Notably, the interface of SARS-CoV-2 S protein and ACE2 receptor, which is essential in the first stage of viral infection, emerges as a potentially important epitope for development of vaccines and therapeutics [43]. At the beginning of the pandemic, neutralizing monoclonal antibodies against SARS-CoV have been considered as potential inhibitors for SARS-CoV-2. However, studies show that monoclonal antibodies targeting SARS-CoV are not or poorly reactive against SARS-CoV-2. For instance, pseudovirus assays demonstrate that monoclonal antibodies against SARS-CoV-RBD (80 R, m396, S230, CR3022 and N-176-15) fail to neutralize SARS-CoV-2 [44,45]. The difference between RBD sequences of the two viruses is considered the potential reason for limited cross-neutralization. In fact, it has been shown that the interface of SARS-CoV-2 with ACE2 is significantly larger than that of SARS-CoV with a remarkably higher number of interacting residues, whereas SARS-CoV is more flexible in its interaction with ACE2, interacting through fewer contacts [46]. As the conformational flexibility of the RBD in SARS-CoV from high- to low-affinity led to an increased efficacy for inhibiting peptides and antibodies, enhanced rigidity of the SARS-CoV-2 RBD results in failure of SARS-CoV antibodies to neutralize the binding between SARS-CoV-2 and ACE2 [46]. In general, SARS-CoV-2 specific neutralizing antibodies (CV1/CV35, CV30, BD-368-2, B38, H4, P2B-2F6, 4A8, and COVA2-15 etc.) are generated from S protein-specific B cells of SARS-CoV-2 infected patients [2,47–51]. While some antibodies (CV1/CV35 and 4A8) bind to an epitope outside of the RBD, such as the N-terminal domain of S protein, majority of the potent neutralizing antibodies recognize the RBD and neutralizes infection by inhibiting interaction of the S protein with the ACE2 receptor [2,47–51]. Another neutralizing antibody recognizing the RBD was also developed from supernatants of SARS-CoV-2 S protein hybridoma's derived from immunized transgenic mice [52]. Although not many, there are some antibodies with neutralizing activity against SARS-CoV-2 which were directed against the SARS-CoV S protein. These single-domain antibodies (VHHs) directed against the SARS-CoV S protein were isolated from a llama immunized with pre-fusion-stabilized S protein and screened by employing phage display antibody libraries [53]. Phage antibody libraries were also screened against SARS-CoV-2 S protein and resulted in potent S protein/ACE2 inhibitors, which should be tested with SARS-CoV-2 virus neutralization assay [48]. Similar to phage library screening, DNA aptamers against SARS-CoV-2 RBD were identified by employing the SELEX method [54]. Selected aptamers demonstrated a competitive interaction with ACE2, suggesting its applications in diagnosis, prevention and treatment of SARS-CoV-2. As of today, the U.S. FDA issued an emergency use authorization for three neutralizing monoclonal antibodies directed against the spike protein of SARS-CoV-2 for the treatment of COVID-19, highlighting their importance in combating the current pandemic. Casirivimab (REGN10933) and imdevimab (REGN10987) are non-competing monoclonal antibodies produced by Regeneron Pharmaceuticals Inc. The company produces virus-neutralizing antibodies in genetically engineered mice as well as identifies similarly performing antibodies from human COVID-19 survivors. These two antibodies must be administered together for the treatment of mild to moderate COVID-19 in adults and pediatric patients (12 years of age or older) with positive results of direct SARS-CoV-2 viral testing and who are at high risk for progressing to severe COVID-19 [55]. Another investigational neutralizing antibody, bamlanivimab (LY-CoV555), was also authorized for the treatment of mild to moderate COVID-19 in adults and pediatric patients 12 years and older with a positive COVID-19 test, who are at high risk for progressing to severe COVID-19 and/or hospitalization [56]. Bamlanivimab (LY-CoV555) emerged from the collaboration between Eli Lilly and Company (NYSE: LLY) and AbCellera, one of the partners who discovered the antibody with the scientists at the National Institute of Allergy and Infectious Diseases (NIAID) Vaccine Research Center. The antibody was identified from a blood sample taken from one of the first U.S. patients who recovered from COVID-19. Health Canada also granted authorization for the use of bamlanivimab (LY-CoV555) as a treatment of COVID-19.

The ACE2 protein itself was also proposed as an inhibitor of the interaction between S protein and host cell receptor [57,58]. Recombinant ACE2 proteins were produced in various forms, such as soluble human ACE2 and recombinant Fc fusion [57,58]. While all recombinant ACE2 proteins demonstrated neutralizing activity in both pseudovirus and SARS-CoV-2 assays in vitro, the soluble human ACE2 protein had a dose-dependent and incomplete inhibition, suggesting other possible co-receptors/auxiliary proteins or even other mechanisms that viruses employ to enter cells [57]. In a similar manner, ACE2 PD-derived peptide inhibitors were designed by computational tools to block S protein binding to cell receptors [25,59]. Successful employment of computational binding experiments resulted in a novel hybrid peptide from the interface of the human ACE2 protein and based on this peptide scaffold, multiple novel peptide sequences with enhanced affinity toward SARS-CoV-2 RBD were designed [60]. Although the proposed peptide inhibitors are promising therapeutic candidates, wet-lab experimental validation is required.

The 6-HB structure formed by HR1 and HR2 regions of S protein has a crucial role during the viral membrane fusion process [61]. Therefore, it has been another important target for drug development and several designs have been already proposed. A previously designed pan coronavirus fusion inhibitor

peptide, EK1, targeting HR1 region of S protein, was modified with covalently attached cholesterol molecule [62]. The resulting EK1C4 lipopeptide effectively inhibits membrane fusion and entry of SARS-CoV-2 pseudovirus [62]. A similar lipopeptide was also produced by conjugating cholesterol to a HR2 peptide derived from the S fusion protein of SARS-CoV-2 [63]. This lipopeptide demonstrated highly potent activities in inhibiting SARS-CoV-2 pseudoviruses as well as S protein-mediated cell-cell fusion activity [63].

In addition to previously mentioned regions of the S protein, other targetable regions were also suggested for drug development. A well-conserved peptide in the S2 subunit, KRSFIEDLLFNKV, has been one of these targets [64]. This sequence is considered as a likely primary target for vaccines and a basis for drug discovery due to the fact that the mutations are less easily accepted in this region and it is potentially exposable, another important feature required for proteolytic activation cleavage [64]. Allosteric drugs are suggested as an alternative therapeutic to target tightly packed and scarcely accessible interaction interface between S and ACE2. In fact, 10 residues in SARS-CoV-2 allosteric modulation regions (AMR) with wide-ranging allosteric effects on the ACE2 protein were predicted [65]. Hepcidin, a crucial protein for iron regulation, was shown to have a strict structural similarity with the AMR region, suggesting its inhibitory effect on the binding affinity of the spike protein toward the ACE2 protein [65].

By employing bioinformatics, potential interaction between SARS-CoV-2 and upper respiratory track (URT) commensal bacteria was also investigated to propose new antiviral molecules and therapeutic approaches. It has been shown that gram-negative *Proteobacteria* bacteria, whose population decrease due to aging, produce bacterial proteins with potential ability to interact with S, suggesting that the decline in the bacterial population may abolish the ability of the upper respiratory system to trap viral particles [66].

3.4. Other inhibitors

The recombinant RBD protein was shown to block the entry of SARS-CoV-2 into ACE2-expressing cells, suggesting the potential of SARS-CoV-2 RBD protein as a viral attachment or entry inhibitor against SARS-CoV-2 infection [67].

Similar to bacterial peptides, human intestinal defensin 5 (HD5) has been another peptide suggested to interfere with SARS-CoV-2 infection as a part of the innate defense system [68]. HD5 is an antimicrobial peptide produced by intestinal cells where the symptoms of SARS-CoV-2 infection are low. Therefore, Wang et al. [68] suggested inhibitory activity of HD5 against SARS-CoV-2 by cloaking ACE2 receptors. They showed that HD5 interacted with the critical residues of ACE2 involving in S protein binding and the entry of SARS-CoV-2 pseudovirions to human renal proximal tubular epithelial cells could be inhibited. This finding suggests the use of the HD5 as an antiviral against SARS-CoV-2 infection.

Artificial intelligence and other computational tools are currently used to facilitate the lengthy process of drug discovery and development. The FDA-approved drug library of 1234 compounds was screened by a virtual drug design algorithm, and the 13 top scoring compounds were obtained through manual screening [69]. The SPR results confirmed that eltrombopag, one of these compounds, possesses good binding affinity to both the SARS-CoV-2 S protein and human ACE2, indicating its potential to affect both sides of viral entrances [69]. Cell and animal experimental studies still need to be carried out for these drug candidates, though. Molecular docking studies have also suggested Withaferin A (WFA), a steroidal lactone with anti-inflammatory and anti-tumorigenic properties, as a therapeutic agent that could potentially block the interaction between RBD of the S protein and ACE2 [70].

Inhibitors of the proteases that prime S protein have been another group of molecules studied as antivirals. Cathepsin B and L inhibitors, such as ammonium chloride and E-64d, as well as clinically proven serine protease inhibitor camostat mesylate have demonstrated strong inhibitory effects on cell entry of SARS-CoV-2 [2]. However, as several proteases are used for priming, more than one inhibitor is also required for complete inhibition.

4. Structure and function of the SARS-CoV-2 RNA polymerase: the COVID-19 rna synthesizing machine

Upon host cell infection by SARS-CoV-2, replication and transcription of the viral genome made of a single-stranded RNA (ssRNA) molecule is carried out by a multiprotein complex composed of non-structural viral proteins (NSP) (Figure 4). This replication and transcription complex (RTC) is composed of eight NSPs: NSP7-10, NSP12-14 and NSP16; all of which being required for efficient *in vivo* viral propagation as shown for other nidoviruses [71–74]. These SARS-CoV-2 NSPs are highly similar to their SARS-CoV equivalents (93.29% to 100% homology) [75] and are expressed from the cleavage of two polyproteins translated by the host ribosomes from the 5′ portion of the viral genome [76]. Their maturation is carried out by the two viral proteases NSP3 (papain-like proteinase) and NSP5 (3-chymotrypsin-like proteinase) [77,78] that are also promising target for drug development and existing marine natural compounds [79]. Moreover SARS-CoV-2 NSP3 macrodomain was shown to have a stronger interaction with ADP-ribose by docking experiment suggesting also a role in the host antiviral response [8]. Other non-structural proteins are also important in the replication of the viral genome. The nucleocapsid (N) was also shown to be required in viral RNA synthesis of betacoronavirus [80] and SARS-CoV-2 N protein interaction with RNA has been assessed [81].

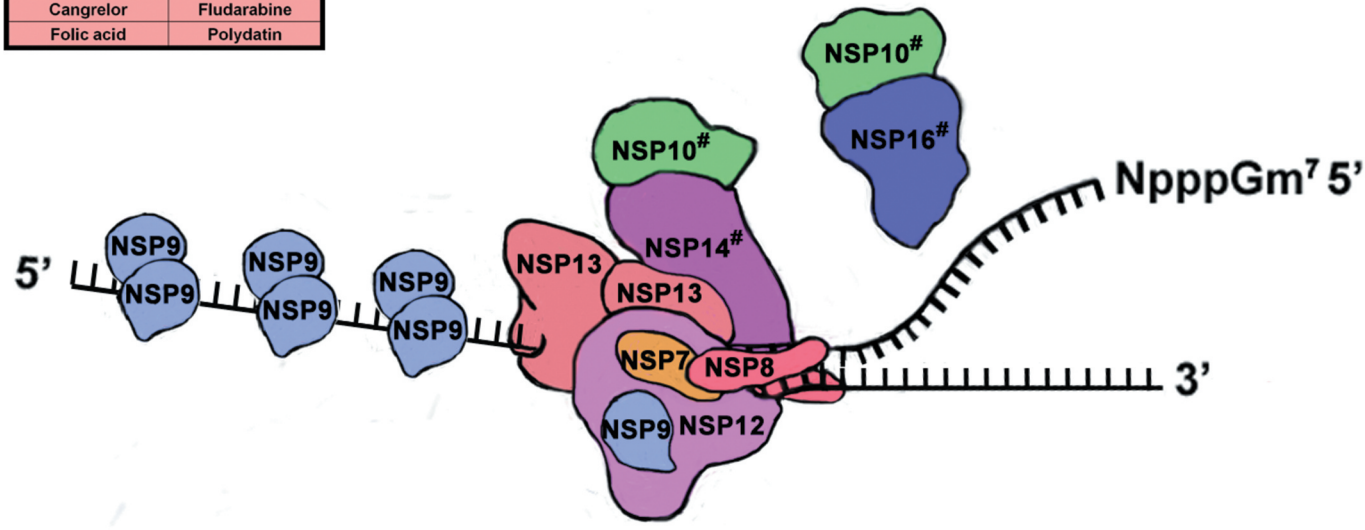
4.1. NSP12 and co-factors

NSP12, the RNA-dependent RNA Polymerase (RdRP) can be divided into two specific domains. One domain found in all nidovirus RdRP with nucleodylation activity, the nidovirus RdRP-associated nucleotidetransferase (NiRAN) is comprised in the SARS-CoV-2 N-terminus [84]. Its exact function needs to be investigated, but *in silico* analysis using DALI server displays similarity with kinase-like fold [76] and is crucial for nidovirus viral propagation [85]. Recently, the NiRAN domain was shown to interact with a NSP9 monomer and act has the second step in viral

NSP13 ATPase inhibitors	
Helicase WT	
Grazoprevir	SSYA10-001
SSYA10-002	Myricetin
Scutellarin	Meclonazepam
Oxiphenisatin	**Simeprevir**
Paritaprevir	**Pemetrexed**
Cidofovir	**Zanamivir**
Sapropterin	**Ertapenem**
Ribavirin	**Lumacaftor** +
Cepharanthine +	**Bismuth salt** +
Helicase mutant (P504L and Y541C)	
Leucovorin	**Methotrexate**
Milrinone	
Helicase WT or mutant	
Cangrelor	**Fludarabine**
Folic acid	**Polydatin**

NSP16 2'-O-Mtase inhibitors		
Hydroxychloroquine	**Chloroquine**	DB02449
Lifitegrast	Entrectinib	DB03067
Antrafenine	Tegobuvir	Aticaprant
TG-100801	Siramesine	**Sonidegib**
PF-04457845	**Ergotamine**	**Dihydroergotamine**
Saquinavir	**Indinavir**	**Digitoxin**
Deslanoside	**Chlorthalidone**	**Dactinomycin**
Irinotecan	**Nilotinib**	**Plicamycin**
Methotrexate	**Eribulin**	**Lanreotide**
Desoximetasone	**Betamethasone**	**Tadalafil**
Zafirlukast	**Paliperidone**	**Ertapenem**
Sinefungin	**Teniposide**	Lifirafenib
Fluocinolone acetonide		**Triamcinolone acetonide**

NSP14 N7-Mtase inhibitors	
Hydroxychloroquine	**Chloroquine**
SAM analogs +	**Paritaprevir**
Simeprevir	Sinefungin
Grazoprevir	



RNA polymerase (RdRP) inhibitors				
Remdesivir @, +	**Favipiravir**	Galidesivir	**Penciclovir**	**Casopitant**
β-D-N4-hydroxycytidine				
ZINC database				
Valganciclovir	**Chlorhexidine**	**Ceftibuten**	Fenoterol	**Fludarabine**
Itraconazole	**Cefuroxime**	**Atovaquone**	**Novobiocin**	Tibolone
Silybin	**Bromocriptine**	**Diphenoxylate**	**Idarubicin**	**Cromolyn**
Cortisone	**Benzylpenicilloyl G**	**Pancuronium Bromide**	**Chenodeoxycholic acid**	**Dabigatran etexilate**
HCV repurposed drugs				
	IDX-184	**Sofosbuvir**	**Ribavirin**	
Natural product database				
Betulonal	Gnidicin	Gniditrin	Andrographiside	Baicalin
Phyllaemblicin B	Sugetriol-3,9-diacetate	14-Hydroxy-cyperotundone	Theaflavin 3,3'-di-*O*-gallate	1,7-Dihydroxy-3-methoxyxanthone
14-Deoxy-11,12,didehydroandrographolide		2*β*-Dihydroxy-3,4-*seco*-friedelolactone-27-oic acid		
2*β*,30*β*-Dihydroxy-3,4-*seco*-friedelolactone-27-lactone		8-(*β*-D-Glucopyranosyloxy)-1,3,5-trihydroxy-9H-xanthen-9-one		
1,2,6-Trimethoxy-8-[(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)oxy] -9*H*-xanthen-9-one		1,8-Dihydroxy-6-methoxy-2-[(6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranosyl)oxy]-9*H*-xanthen-9-one		
2-((1*R*,5*R*,6*R*,8a*S*)-6-Hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylenedecahydronaphthalen-1-yl)ethyl benzoate		(1*S*,2*R*,4a*S*,5*R*,8a*S*)-Formamido-1,4a-dimethyl-6-methylene-5-((E)-2-(2-oxo-2,5-dihydrofuran-3-yl)ethenyl)decahydronaphthalen-2-yl 5-((*R*)-1,2-dithiolan-3-yl) pentanoate		
(*R*)-((1*R*,5a*S*,6*R*,9a*S*)-1,5a-Dimethyl-7-methylene-3-oxo-6-((*E*)-2-(2-ox-2,5-dihydrofuran-3-yl)ethenyl)decahydro-1*H*-benzo[*c*]azepin-1-yl)methyl 2-amino-3-phenylpropanoate		2-(3,4-Dihydroxyphenyl)-2-[[2-(3,4-dihydroxyphenyl)-3,4-dihydro-5,7-dihydroxy-2*H*-1-benzopyran-3-yl]oxy]-3,4-dihydro-2H-1-benzopyran-3,4,5,7-tetrol		

NSP8-NSP12 interaction inhibitors		
NSP8-NSP12 WT		
Tetrahydrouridine	**Dobutamine**	**Saquivanir**
TG100-115	NOP-1A	Thiarabine
NSP8-NSP12 P323L		
Canagliflozin	Epicatechin	Ipragliflozin
Secondary metabolite of morus bark		
NSP8-NSP12 (WT or P323L)		
Nebivolol	RX-3117	
Fenoterol	Glucocorticoid	

Figure 4. Model of SARS-CoV-2 Replication and Transcription Complex (RTC) subunits and lists of potential drugs targeting them.

Model of SARS-CoV-2 Replication and Transcription Complex (RTC) subunits based on previously reported interactions for either SARS-CoV-2 [86,89,98,105] or SARS-CoV (#) [82,122]. lists of potential drugs were obtained according to results of crystallography (@) [87], in vitro experiments (+) [96,108,111,123] or *in silico* docking. catalytic 3D structure (NSP12, NSP13, NSP14 and NSP16) or interaction interface (NSP8-NSP12) were generated *in silico* and screened against a list of potential therapeutic compounds [83,90–93,96,101,108,111–113,118,120,123,125]. Drugs listed in bold are FDA-approved for the treatment of others pathologies.

genomic RNA capping [86]. The RdRP catalytic domain found on the C-terminal part is divided into seven motifs (A to G) that form three specific structures including a thumb domain, a finger domain (F-G motif) required to bind and stabilize the phosphate backbone with the help of the palm domain (A-E motif) [87]. According to Peng et al., the G domain is a feature found in primer-dependent viral polymerase and interacts with the primer to initiate synthesis of the new RNA strand [88]. The palm domain can interact with the NiRAN domain to stabilize RdRP [84]. Hydrophilic residues in the F motif form the NTP entry channel positioned in the backside of the palm domain. The 2′-OH group of nucleotides would interact with amino acids N691, S682, D623, promoting the synthesis of RNA strands [89]. SARS-CoV-2 NSP12 crystallography has shown interaction with the RNA strand via the C motif amino acids D760 and D761 [89] or with Y595 in the pre-translocation complex [87]. The RdRP structure with or without NSP7 and NSP8 was used to study interactions with various known nucleotides analogs such as remdesivir by crystallography [87] or used to screen for new drug candidates using *in silico* modeling [90–95]. Dynamic studies show that remdesivir delays chain termination by blocking RNA synthesis at position +3 after remdesivir incorporation [96]. A steric hindrance with S861 was proposed by Gordon et al. and mutagenesis did support this hypothesis [96]. The initiation complex was suggested to comprise a primase complex including SARS-CoV-2 NSP7 and NSP8. In the absence of SARS-CoV-2 NSP12, the NSP7-NSP8 heterodimer interacts together through a domain similar to a leucine zipper and is stabilized by a phenylalanine residue followed by the formation of a disulfide bridge between two cysteins found on each NSP7 subunit at position 8 [97]. However, the RTC stoichiometry (NSP(7 + 8) + NSP8 + NSP12) was shown to possess polymerase activity in cells [98]. This raises the question as to whether the heterodimer could exist in the presence of NSP12 and whether it could affect some cellular pathways [99]. Nevertheless, Wang et al. proposed a model in which the primase activity is driven by a SARS-CoV-2 NSP7-NSP8 hexadecameric complex [87]. The SARS-CoV-2 NSP7-NSP8-NSP12 crystal has revealed the interaction between the RdRP and the NSP7-NSP8 heterodimer [88]. Binding of the heterodimer stabilizes the thumb domain [88] and increases the polymerase activity as shown by Hillen et al [89]. A second NSP8 subunit interacts with the finger domain and both interacting NSP8 create positively charged extensions that interact with the RNA backbone [89]. Importantly, SARS-CoV-2 NSP8 K58 could affect viral replication [100] and this amino acid is required to interact with the nascent RNA strand [89]. Moreover, Multu et al. targeted the interaction between NSP8 and NSP12 by using computational structure-based models [101]. SARS-CoV-2 NSP12 mutations (A97V, A185V, I201L, P323L, L329I A466V and V880I) were detected in different patients [102]. A97V and A185V had a small effect on the secondary structure whereas P323L affects RdRP stability [102,103]. These mutations should be taken into account for drug discovery because they could affect the potency of candidate RdRP targeting drugs. In fact, the man-made RdRP S861G mutation could affect the chain termination effect of remdesivir [104].

SARS-CoV NSP9 can dimerize through a α-helix GXXXG interaction domain which is conserved in SARS-CoV-2 NSP9 as demonstrated by crystal structures [105]. This domain was shown to be essential for viral replication for SARS-CoV [106] but remains to be fully characterized for SARS-CoV-2 NSP9. Moreover, SARS-CoV-2 NSP9 interactions with components of the nuclear translocation machinery were demonstrated by mass spectrometry [3]. SARS-CoV-2 NSP9 contains several conserved residues which are positively charged that would allow interaction with the simple strand RNA backbone [75] but that interaction was not confirmed *in vitro* [105]. However, interaction with RNA backbone was demonstrated for SARS-CoV NSP9 dimer [107].

SARS-CoV-2 NSP13 is part of the superfamily 1B helicase and is composed of five domains giving it a triangle appearance composed of two RecA-like domains, a 1B domain, a Stalk domain and a zinc binding domain (ZBD) [76]. SARS-CoV-2 NSP13 was shown to possess an NTPase activity (amino acid Lys288, Ser289, Asp374, Glu375, Gln404 and Arg567) in addition to a helicase activity [108] which is essential for the spread of the SARS-CoV virus [109]. Moreover, interaction with epigenetic, gene regulatory pathways and cytoskeleton was shown [3]. The crystal structure was resolved recently for the MERS-CoV virus [110] and study of the crystal composed of the SARS-CoV-2 holo-RdRP and NSP13 confirmed that a stable complex could be formed [98]. The interaction of the two proteins is thought to be mediated by the ZBD domain of SARS-CoV-2 NSP13 and the extension of the NSP8 subunit. Targeting the ATP binding pocket with Bismuth salt was shown to inhibit the NTPase and the helicase in a dose-dependent matter [108]. Otherwise, multiple drugs targeting the ATP binding pocket analyzed *in silico* were identified in which some are already approved by the FDA [94,111–113]. However, the capacity of these candidates to block SARS-CoV-2 NSP13 ATPase activity remains to be tested.

4.2. NSP14 and co-factors

NSP14 and NSP16 are required for the sequential incorporation of a cap structure at the 5′ end of the viral genome necessary for recognition by the ribosomal machinery [76] and the activity of NSP14 would be essential for viral spread, a situation not observed in the SARS-CoV virus [72]. Both enzymes interact with the NSP10 cofactor and his interaction with SARS-CoV-2 NSP14 and NSP16 is crucial for their enzymatic activity [114]. Rogstam et al. have shown that SARS-CoV-2 NSP10 is folded in a structure unique to coronavirus, stabilized by two zinc fingers [114]. A S-adenosyl methionine (SAM) binding pocket and an RNA binding groove were identified by studying the SARS-CoV-2 NSP16-NSP10 crystal and NSP16 configuration was the one of a canonical SAM-methyltransferase (SAM-MTase) enzyme [115,116]. High conservation of the MTase site between different nidoviruses

supports the idea that the catalytic site is targeted with compounds such as sinefongin [115]. Other drugs were also proposed as MTase inhibitor candidates after *in silico* analysis of SARS-CoV-2 NSP16 [117,118]. Moreover, interaction between SARS-CoV-2 NSP10 and NSP16 occurs between Val42 and Leu45 of NSP10 and two hydrophobic pockets found in NSP16. Hydrogen bridges produced from the amino acids Lys93, Thr106, Ala107 and Ser105 are also present [115]. In addition to being necessary for its MTase activity, SARS-CoV-2 NSP14 possesses an exonuclease (ExoN) activity and the NSP14-NSP10 complex interacts with the RTC [76]. In SARS-CoV, the ExoN activity provides a proofreading mechanism that can reduce the formation of undesired mutations in the viral genome [119]. SARS-CoV-2 NSP14 requires three specific domains which are found in the superfamily of DEDD proteins requiring Mg^{2+} ion coordination [120] and the ExoN activity could be increased following NSP10 interaction in SARS-CoV [121]. This function could protect the viral genome and reduce the potency of nucleotide analogs [122]. Drugs were developed to target the ExoN or the N7-methyltransferase activity to increase mutation rate or inhibit RNA cap formation, respectively. Various compounds were obtained through chemistry such as adenine dinucleoside SAM analogs [123] or defined by *in silico* modeling to find molecules with the strongest affinity [112]. Nonetheless, modeling of the SARS-CoV-2 NSP14 active site revealed that remdesivir would create a steric clash, suggesting a less efficient excision of this drug [124]. Gentile et al. proposed that chloroquine and hydoxychloroqiune could interact in the ExoN and SAM-MTase activity site [125]. SARS-CoV-2 NSP10 and NSP16 mutations were detected in some patients and *in silico* models suggest that it could have an impact on the stability of the complex [116,126].

In conclusion, several drugs, some already FDA-approved, have been proposed based on SARS-CoV-2 RdRP structure and function. Morevover, the FDA-approved remdesivir, that shows inhibiting effects on SARS-CoV-2 RdRP polymerase activity and was tested in various cohorts, is not recommended anymore by WHO guidelines because uncertainties remain regarding its clinical efficacy [127]. Thus, there still remains much work to accomplish to ascertain their *in vivo* antiviral efficacy.

5. Expert opinion

The development of vaccines and antivirals to fight COVID-19 requires a detailed molecular understanding of the viral replication cycle and of the interface between the virus and host factors. Defining interactions between viral factors and host proteins identifies pathways that are usurped by the viral machinery (see Figure 1). Close to half of SARS-CoV-2-interacting proteins are connected to endomembrane compartments or vesicle trafficking pathways. SARS-CoV-2 proteins interact with innate immune signaling pathways, the host translation machinery, Cullin ubiquitin ligase, and bromodomain proteins. This information helps to understand mechanisms of viral infection and propagation. They also inform strategies for drug development. In fact, drugs known to target host interactors constitute key candidates for repurposing and generating antivirals (see Figure 2). A total of 62 interactors of viral factors are the target of 69 different drugs, investigational drugs or preclinical molecules that modulate them and can be overlaid on their protein-interaction network. After follow-up studies, two classes of molecules were found to reduce viral infection: the protein biogenesis inhibitors zotatifin, ternatin-4 and PS3061; and the ligands of the sigma-1 and sigma-2 receptors haloperidol, PB28, PD-144,418 and hydroxychloroquine. Importantly, these drugs can possibly be repurposed to treat COVID-19 and collateral damages exacerbating various disease conditions.

We also think that detailed mechanisms of S and RdRP function, including protein–protein interactions, serve to understand the function of candidate drugs. The S protein is the main target of neutralizing monoclonal antibodies developed against SARS-CoV-2 (see Figure 3). Remdesivir, a drug that has been approved by the U.S. FDA to treat severe cases of COVID-19, interferes with RNA synthesis by the viral RdRP (see Figure 4). Drug discovery requires such knowledge to combat COVID-19.

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Declaration of interest

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