Computational and Structural Biotechnology Journal 19 (2021) 2246–2255

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Targeting protein-protein interaction interfaces in COVID-19 drug discovery

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ARTICLE INFO

Article history:
Received 18 January 2021
Received in revised form 30 March 2021
Accepted 1 April 2021
Available online 15 April 2021

Keywords:
COVID-19
PPIIs
Antiviral strategy
Drug discovery
SARS-CoV-2

ABSTRACT

To date, the COVID-19 pandemic has claimed over 1 million human lives, infected another 50 million individuals and wreaked havoc on the global economy. The crisis has spurred the ongoing development of drugs targeting its etiological agent, the SARS-CoV-2. Targeting relevant protein-protein interaction interfaces (PPIIs) is a viable paradigm for the design of antiviral drugs and enriches the targetable chemical space by providing alternative targets for drug discovery. In this review, we will provide a comprehensive overview of the theory, methods and applications of PPII-targeted drug development towards COVID-19 based on recent literature. We will also highlight novel developments, such as the successful use of non-native protein-protein interactions as targets for antiviral drug screening. We hope that this review may serve as an entry point for those interested in applying PPIIs towards COVID-19 drug discovery and speed up drug development against the pandemic.

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https://doi.org/10.1016/j.csbj.2021.04.003

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1. Introduction

COVID-19 has become one of the most formidable public health crises of this century. As of now, over 50 million people have been infected with a death toll of over 1 million individuals worldwide [1]. It has also caused global social and economic disruption, with losses amounting to over US$ 8.5 trillion according to data from the United Nations [2]. COVID-19 is caused by a novel coronavirus named SARS-CoV-2 [3]. Given the urgency imposed by the crisis, several initiatives were quickly set up to gain a better understanding about the virus and to utilize that knowledge to pursue potential therapies and vaccines against the disease [4]. SARS-CoV-2 belongs to the beta-coronavirus subfamily and shares considerable similarity with SARS-CoV and MERS-CoV at the protein level [5]. The viral architecture is essentially identical to that of SARS-CoV and MERS-CoV, comprised of a phospholipid envelope, the structural proteins N (nucleocapsid), M (membrane), S (spike) and E (envelope), and non-structural proteins such as the 3C-like protease and the RNA-dependent RNA polymerase (RdRP). In a fortunate turn of events, the high similarity between SARS-CoV-2 and other coronaviruses allowed researchers to leverage knowledge from past research to speed up development of potential therapies. For example, the interaction between the SARS-CoV-2 S protein and the human angiotensin-converting enzyme 2 (ACE2) receptor, one of the most promising drug targets, was quickly elucidated based on what was known about its SARS-CoV counterpart [6,7].

The battle against COVID-19 has been waged in several forms, but vaccine development and drug design remain two of the most important ones. Vaccines provide the means for large-scale immunization of the population which is essential for a return to normal life. Ye et al. provide an excellent summary of current preclinical efforts and technologies for vaccine development against SARS-CoV-2 [8]. However, given the scale of the pandemic, it may be necessary to vaccinate a large percentage of the global population before life can return to normal. Hence, drugs designed to combat COVID-19 will be required to "hold the fort" during the vaccine development/production/distribution time window. Even after successful implementation of a vaccination program, there is still a need for anti-COVID-19 drugs to deal with sporadic cases.

Several approaches have been applied towards drug development against COVID-19. Gil et al. provide a comprehensive overview of potential drug targets and therapeutic options against SARS-CoV-2 infections [9]. The best-known approach is to inhibit key enzymes in SARS-CoV-2 such as the RNA-dependent RNA polymerase (RdRp) and 3C-like protease (3CLpro). Remdesivir, a drug which has been in the spotlight, is a prime example because it is a nucleotide analogue which inhibits RdRp and has received emergency use authorization against COVID-19 in several countries [10,11]. Although highly effective, this approach relies on targeting the catalytic site, which limits the chemical space that may be explored and may reduce the chances of identifying successful hits. A second popular approach is to disrupt the interaction between proteins which are essential for viral processes [12,13]. In this review, we shall focus on the second approach and discuss various aspects of targeting protein-protein interaction interfaces (PPIIs) for anti-COVID-19 drug discovery.

2. Role of PPIIs in drug development

Nearly all biological processes involve some type of protein-protein interaction (PPI). In human cells, it is estimated that more than 300,000 PPIs participate in processes such as immunity, signal transduction, molecules transportation, and maintenance of cellular organization [14]. Aberrations in these interactions are correlated with many human disorders, including cancer, infectious diseases, autoimmune diseases and neurodegeneration [15–20]. Other organisms, including pathogens, also have their own set of essential PPIs. The ability to target and manipulate these interactions may thus be a viable strategy for drug discovery [21] (Table 1). Paclitaxel, more commonly known as Taxol®, is an anticancer agent extracted from the Pacific yew Taxus brevifolia [22]. Paclitaxel arrests the cell cycle in the mitotic phase by inhibiting microtubule disassembly [23]. Binding of paclitaxel to a hydrophobic patch in β-tubulin stabilizes the interdimer contacts between β-tubulin molecules of adjacent protofilaments, resulting in microtubule stabilization [24–26]. Colchicine, which has been used to treat gout since 1961, utilizes a mechanism opposite to paclitaxel [27,28]. Colchicine inhibits the assembly of microtubules by disrupting the interaction between α- and β-tubulin [29–31]. Although the mechanism of action of the two drugs were elucidated long after they were approved for medical use, recent studies have proven the possibility of targeting PPIs *a priori* for development of new treatments against refractory diseases [32–34].

PPIs usually involve a large and flat interaction interface between the proteins. These protein-protein interaction interfaces (PPIIs) typically have an area of 1500–3000 Å^2 and are often complementary in shape and electrostatic properties [35–37]. Although the interface is usually composed of several amino acid residues, only a small subset of these residues, called "hot spots", contributes significantly to the binding free energy [38,39]. These hot-spots are prime targets for small-molecule orthosteric PPII disruptors, such as colchicine, which act directly on the PPII. Disruption of PPIIs may also be achieved by allosteric means where the small-molecules can bind to sites that are topologically distinct from PPIIs (Fig. 1). BIO8898 is a small-molecule inhibitor of the trimeric cytokine CD40-ligand (CD40L) developed by the company Biogen Idec targeted at autoimmune diseases [40]. The inhibitor intercalates at the trimer interface of CD40L and allosterically disrupts the CD40L/CD40 interaction associated with several types of autoimmune diseases. Caporuscio et al. reported two novel inhibitors against HIV-1 that target the Phe43 pocket in the gp120-CD4 interaction through molecular dynamics simulations, and Zhan et al. summarized the potential compounds against a variety of PPIs in HIV infection [41,42]. Similarly, PPII stabilizers may also act in an orthosteric or allosteric fashion (Fig. 1). For example, FK506 and rapamycin inhibit calcineurin and mTOR kinase activity, respectively, by stabilizing the corresponding interactions between FKBP12 and calcineurin or mTOR [43–45]. Both FK506 and rapamycin bind first to FKBP12 and form part of the interaction interface with calcineurin and mTOR, respectively, and both are considered orthosteric PPII stabilizers [46]. On the other hand, paclitaxel is believed to exert allosteric stabilization in addition to its orthosteric effect in microtubules because the interface

Table 1
Examples of PPII modulators described in the present review.

Name	Protein complex	PPI modulators	PDB code	EC_{50} / IC_{50} (μM)	Ref
Paclitaxel	microtubules	Allosteric stabilizer	1JFF	1.41 ± 0.32 (IC_{50})	[26]
Colchicine	α- / β-tubulin	Orthosteric inhibitor	1SA0	3.2 (IC_{50})	[31]
BIO8898	CD40L/CD40	Allosteric inhibitor	3LKJ	25 (IC_{50})	[40]
Compound 2/4	gp120–CD4	Orthosteric inhibitor	N/A	22/9 (EC_{50})	[40]
FK506	FKBP12/ calcineurin	Orthosteric stabilizer	1TCO	0.047 (IC_{50})	[44]
Rapamycin	FKBP12/mTOR	Orthosteric stabilizer	2RSE	0.002 (EC_{50})	[45]
Nucleozin	Influenza nucleoprotein	Orthosteric stabilizer	3RO5	0.17 (IC_{50})	[54]

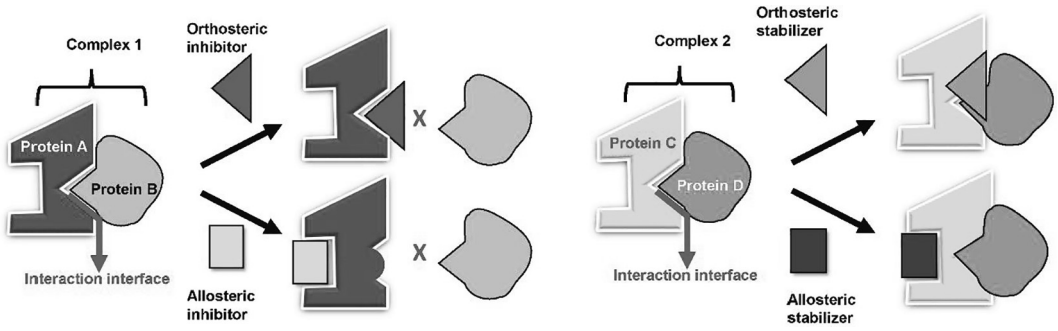

Fig. 1. Different strategies for designing PPI modulators. Modulation of PPIs can be achieved by using inhibitors or stabilizers to target the orthosteric or allosteric sites of the protein-protein complex. (Right) graphic expression of PPI inhibitors. Protein A binds to Protein B to form Complex 1. Orthosteric inhibitors bind directly to the PPII, which hinders Protein B from binding with Protein A. On the other hand, allosteric inhibitors bind to a region distal from the PPII on Protein A, which induces a conformational change to obstruct Protein B from binding with Protein A. (Left) graphic expression of PPI stabilizers. Protein C interacts with Protein D to form Complex 2. Orthosteric stabilizers bind directly to the PPII, which enhances the binding affinity between Protein C and D. Allosteric stabilizers bind to a region distal to the PPII on protein C, which induces a conformational change to enhance the binding affinity between Protein C and Protein D.

between α- and β-tubulin within the same protofilament, which is not part of the paclitaxel binding site, is also affected by paclitaxel binding [47,48].

Orthosteric PPII inhibitors and stabilizers are easier to design as long as there is enough information about the interaction interface as a guide [49]. In contrast, the design of allosteric modulators faces difficulties in identifying a suitable target site [49,50]. However, it may sometimes be difficult to design orthosteric PPII modulators, e.g. when the interface is particularly flat and few hits are available, and allosteric compounds may provide alternative choices for drug design.

Non-native contact modulators comprise a particularly interesting category. Nucleozin induces aggregation of influenza A nucleoproteins in the nucleus of the host cell, which stops viral replication [51–53]. The structure of influenza A nucleoprotein in complex with nucleozin revealed that nucleozin exerted its antiviral activity by stabilizing a non-native interface between two adjacent nucleoprotein trimers, leading to abnormal viral nucleoprotein oligomerization and suppression of the influenza virus [51,54].

3. Strategies for the design of PPII modulators

PPIIs have long been ignored as drug targets because of their flat interacting surfaces, which is not conducive towards conventional methods of drug discovery. Fortunately, technological advancements have made it possible to design and screen potential modulators of PPIIs with therapeutic potential. Several excellent and detailed reviews have been written on the subject [55,56]. For the sake of brevity, we only highlight a few of the strategies:

3.1. Structural studies

Structural biology is arguably one of the most important tools for the design of PPII modulators. Unlike conventional methods where one may not require structural information for screening of potential therapeutic molecules, targeting PPIIs requires intimate knowledge of the interaction interface, which can only be provided by its detailed atomic structure. Energetics analysis of the structural data may provide clues about potential interaction hot spots, which may then be experimentally validated. In recent years, a combination of X-ray crystallography and alanine scanning mutagenesis has become the main strategy for this purpose [34,39]. Even when a lead has been identified, elucidation of the structure of the proteins in complex with the lead molecule is still desirable, since it may yield additional information useful in the optimization process [57–59]. In addition, structural elucidation of the protein-modulator complex is currently the only way to identify allosteric PPII modulators.

3.2. Structure-based drug design

Once the structure of the interface is known, two different strategies may be employed to design PPII modulators. First, novel compounds may be generated through bioisosterism and *de novo* design based on the structure surrounding the hot spots. However, because PPIIs are often flat, a larger interaction surface between the compound and the interface may be desired. This leads to the second approach, peptidomimetic design, which employs small molecules or short peptide derivatives that mimic a binding peptide [60–62]. Human knowledge plays a key role in either approach since interpretation of the structure has to be carried out manually before the design phase.

3.3. High-throughput screening

To accelerate the development of PPII modulators, screening methods which are amenable to a high degree of automation may be employed. High-throughput screening (HTS) experiments and virtual screening are widely used approaches in traditional drug discovery [63,64]. In theory, HTS does not even require the elucidation of the protein structures as long as a suitable valida-

tion assay targeting the PPII is available. Virtual screening, on the other hand, requires that the structures of the interacting proteins are known before hand, but has the advantage of high speed and low cost because the screening is carried out in silico. However, both HTS and virtual screening are limited by the chemical space represented by their compound libraries, and conventional libraries, which were not developed for this use case, may be less effective at screening PPII modulators. Virtual screening further suffers from an interface degeneracy problem, because there may be multiple potential PPIIs, but only one or a few of them are actually physiologically relevant. Although these issues are not trivial, both strategies have been successfully applied towards the identification of compounds regulating PPIs in recent years [65–67].

3.4. Fragment-based drug discovery

The hot spots on the PPII are often scattered at the interface and do not form a continuous surface. This is often a problem because compounds targeting a single hot spot may not bind to the protein tightly enough. Fragment-based drug discovery (FBDD) is an alternative approach that may be employed to solve this problem. FBDD starts from the identification of small fragments (~200 Da) that target a single site at the PPII. Once fragments for several sites have been identified, they may be linked into a single molecule to obtain a 'lead' which has a much higher binding affinity towards the target [68,69]. Compared to HTS, FBDD allows for the initial screening of a smaller library, because N hits could be combined to produce $N \times N$ leads for further screening (assuming two screening sites), thus increasing the combinatorial space. However, optimization of the linker between the fragments by organic methods still requires the structure of the lead-target complex to be solved first, which is not absolutely necessary for HTS.

3.5. Computational tools

In silico strategies offer flexibility and insights which may not be accessible with experimental approaches. Advances in computational and systems biology, combined with the increasing amount of structural knowledge available has ushered several computational tools that may assist in the design of PPII modulators. Databases of small-molecule PPI inhibitors, such as TIMABL (http://www-cryst.bioc.cam.ac.uk/databases/timbal), 2P2I (http://2p2idb.cnrs-mrs-fr) or iPPI-DB (http://www.ippidb.cdithem.fr) contain three-dimensional structures of several protein-protein and protein-inhibitor complexes. These databases may serve as starting points for molecule design, or as resources to explore possible interaction "rules" at the protein-protein interface. Another aspect is the collaborative development of new theoretical and computational modeling approaches, such as OpenMM and other open force-field initiatives [70]. The increasing use of machine learning algorithms, such as those employed on the Alpha Fold system for protein fold prediction [71], may also provide more accurate predictions of binding residues at PPIIs. On the small-molecule side, novel computational methods may help in optimizing desired pharmacophore properties (such as oral bioavailability) and/or expanding the chemical space beyond the "rule of five" at the initial stages of the design process [72].

4. Application to COVID-19 drug discovery

4.1. Potential candidate targets

CoVs share several proteins that are essential in the viral life cycle. Many of these proteins form PPIs with each other or host proteins, making them attractive targets for the design of PPII modulators (Fig. 2). A comprehensive list of potential targets is listed below (Table 2).

4.1.1. PPIIs related to viral entry

Viral entry is initiated through the interaction between the receptor-binding domain of viral spike glycoprotein (S-RBD) and host receptors. Subsequently, host proteases such as TMPRSS2 or furin cleave the S protein and activates membrane fusion. The receptor for MERS-CoV S protein is dipeptidylpeptidase 4 (DPP4), whereas both SARS-CoV and SARS-CoV-2 S proteins bind to ACE2 [73,74]. Being the first stage in viral infection, PPIIs involved in the viral entry process, such as those between S and host receptors/proteases, are considered to be one of the most promising drug development targets. Several PPII inhibitors targeting the interaction between S and host receptors have been identified. For example, Sarafianos et al. identified three compounds that block SARS-CoV entry from a chemical library containing 3000 compounds. One compound (designated SSAA09E2) was found to obstruct the binding of SARS-CoV S protein to ACE2 [75]. In addition, Kao et al. identified 104 compounds that inhibit SARS-CoV-induced cytopathic effects from a library of 50,240 small molecules. 18 compounds were found to block the S-ACE2-mediated entry of SARS-CoV, among which one compound, VE607, inhibited plaque formation of SARS-CoV at low micromolar range [76]. In light of these successful SARS-CoV studies, Bojadzic et al. initiated a screen to find compounds which interfere with the PPI between SARS-CoV-2 S protein and ACE2. They identified methylene blue as an inhibitor of SARS-CoV-2 entry with an IC_{50} of 3.5 μM [77]. Kalhor et al. conducted structure-based virtual screening with FDA approved drug databases to discover the PPI inhibitors against S-ACE2 complex. They identified 6 compounds can bind to the ACE2 binding pocket on SARS-CoV-2 S protein, from which they further proposed Diammonium Glycyrrhizinate as the most potent compound by MD simulation technique [78]. Hanson et al. also developed an AlphaLISA RBD – ACE2 platform to facilitate the screening of PPII inhibitors perturbing this host–pathogen interaction. They identified corilagin as a potential inhibitor against the ACE2 – RBD complex with an IC_{50} of 5.5 μM [79].

In addition to using the screening approach to find small-molecule candidates that block the S-ACE2 interaction, macromolecular PPI inhibitors have also been developed. In this regard, the most common approach is to mask the S-ACE2 interaction through monoclonal antibodies which recognize either the S protein or ACE2 [80–82]; or through the application of recombinant soluble proteins/peptides, which competes with normal proteins for the binding to their respective interacting partners [83,84]. Currently, several antibodies recognizing the S protein have been reported. For example, Shi et al. isolated two specific human monoclonal antibodies from a convalescent COVID-19 patient that exhibited SARS-CoV-2 neutralization activity in vitro. One of these antibodies, termed CB6, further exhibited antiviral activity in a rhesus monkey model. The mechanism behind the antiviral activity of CB6 was further revealed by structural studies, which showed that CB6 interacted with the RBD of SARS-CoV-2 S protein in an orthosteric fashion and interfered with the virus–receptor interaction [80]. Yan et al. also isolated four antibodies from a convalescent patient which neutralized SARS-CoV-2. Two antibodies (B38 and H4) blocked the interaction between the S-RBD and ACE2 via binding to different epitopes on the RBD. Both antibodies were able to relieve the symptoms of infected animals in mouse model experiments. The crystal structure of the RBD-B38 complex revealed that the B38-binding surface on the RBD overlaps with its ACE2-binding interface (Fig. 3A). In addition, Chen et al. identified three SARS-CoV-2 antibodies from 26 recovered COVID-19 patients. Two of them, 311mab-31B5 and 311mab-32D4, exhibited

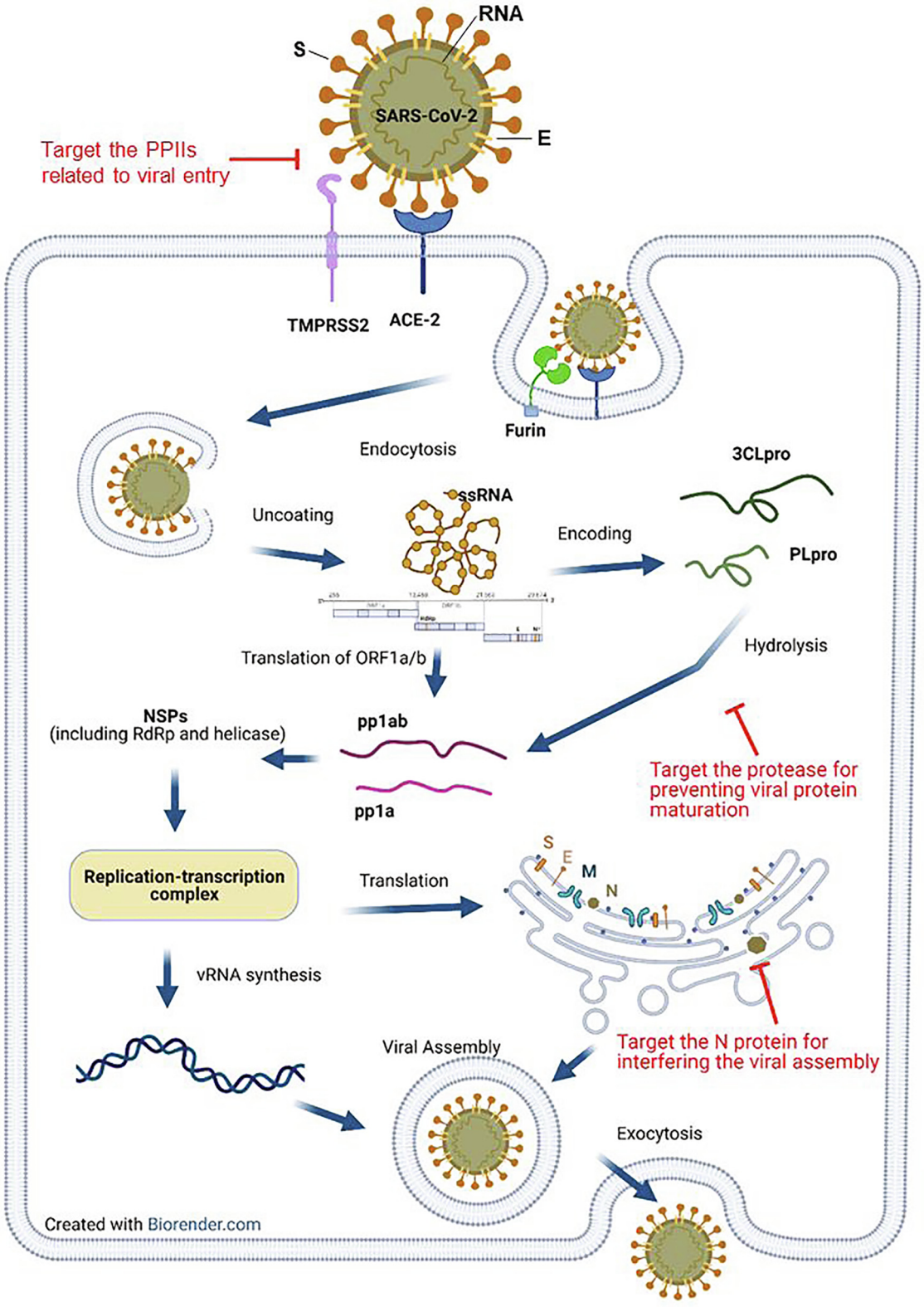

Fig. 2. Critical PPIs and proteins involved in the virus life cycle. The PPIs suitable for inhibitor design are highlighted in red. Host proteins involved in CoV processing might be candidates that can be targeted through the PPII strategy: these include primary cellular receptors for CoV, such as ACE2 or DPP4, and host proteases, such as TMPRSS2 or furin. Host receptors are recognized by CoV spike proteins and the binding of receptor and S1 domain of S protein subsequently activates the conformational changes of S protein. For host proteases, the serine protease TMPRSS2 is responsible for two distinct functions during the CoV infection, including an alternative pathway for viral entry and activation of S protein for virus-cell fusion [128]. In parallel, the protease furin, which is predominantly expressed on the *trans*-Golgi network and intracellular vesicles, activates the S protein by cleaving at the S1/S2 cleavage site, thus facilitating membrane fusion [129,130]. PPII inhibitors targeting host receptors and proteases may provide a potent way to prevent CoV from entering host cells during the early stages of infection. During later stages of infection, N protein dimerization and interaction with viral RNA is required for formation of RNP complexes and viral assembly. Targeting N dimers using a PPII strategy is a potential mechanism of inhibiting late steps of viral production. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Examples of PPII modulators against SARS-CoV/ SARS-CoV-2.

Name	Virus	Type	Mechanism of inhibition	Targets	Ref
SSAA09E2	SARS-CoV	Small molecule	Disturbing S-ACE2 interaction	PPII of S-ACE2 complex	[75]
VE607	SARS-CoV				[76]
Methylene blue	SARS-CoV-2				[77]
Diammonium Glycyrrhizinate	SARS-CoV-2				[78]
corilagin	SARS-CoV-2				[79]
CB6	SARS-CoV-2	Antibody			[80]
B38	SARS-CoV-2				[81]
311mab-31B5 and 311mab-32D4	SARS-CoV-2				[85]
COVA2-15	SARS-CoV-2				[86]
IgG1 ab1	SARS-CoV-2				[87]
hrsACE2	SARS-CoV-2	Soluble peptide analogues of ACE2			[83]
ACE2-Ig	SARS-CoV-2				[88]
EK1C4	SARS-CoV-2	Lipopeptide	Disturbing 6-HB formation of S protein	S protein	[90]
Arbidol	SARS-CoV-2	Small molecule	Modulating S protein trimerization		[96,127]
S471-503	SARS-CoV	Soluble peptide analogues of S	Disturbing S-ACE2 interaction	host ACE2	[97]
438YKYRYL443	SARS-CoV			host ACE2	[98]
Chloroquine	SARS-CoV-2	Small molecule		atypical PPI inhibition	[105]
Octapeptide	SARS-CoV-2	Peptide-based inhibitor	Disturbing intra-dimer of 3CLpro	3C-like protease	[108,109]
P3	SARS-CoV-2	Small molecule	Stabilizing a non-native dimer of N-NTD	Nucleocapsid protein	[113]

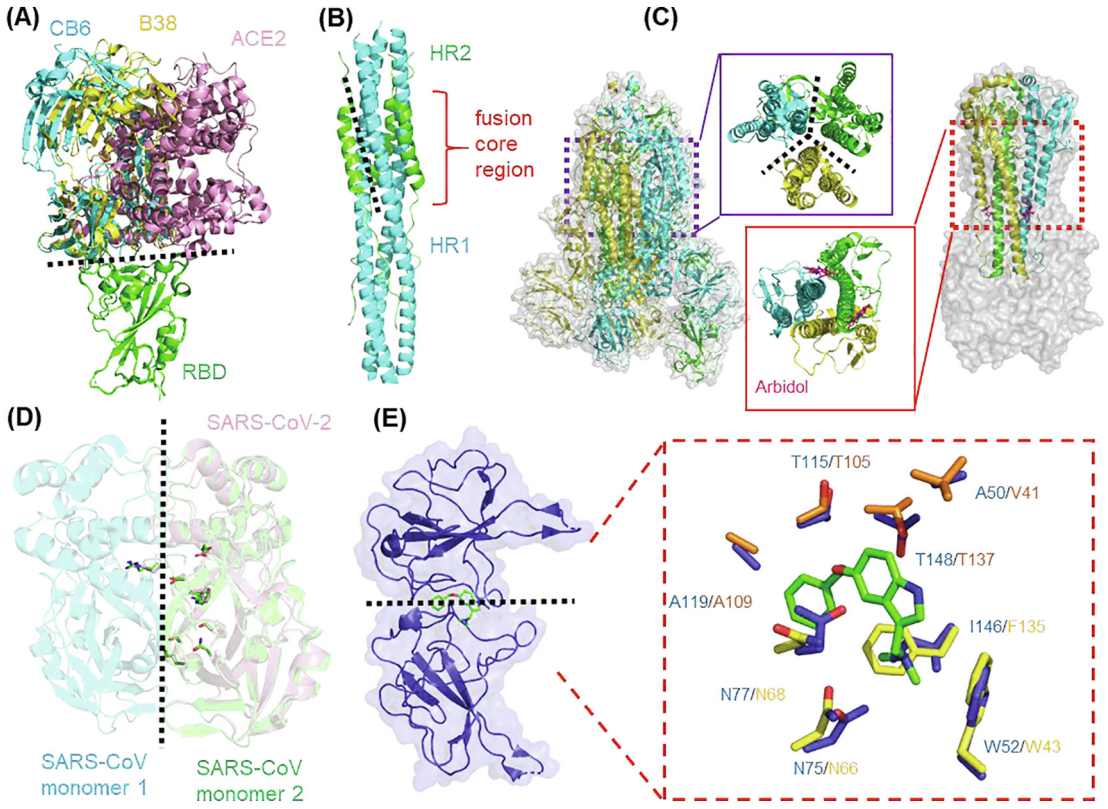

Fig. 3. Main strategies for PPI modulator design against SARS-CoV-2. (A) Hot-spot for PPI inhibitor design against RBD-ACE2 complex. Structures of SARS-CoV-2 S protein RBD in complex with ACE2 (PDB: 6LZG), CB6 (PDB: 7C01) and B38 (PDB: 7BZ5) complex are shown in cartoon. The RBDs of each structure are aligned to show the PPI suitable for modulator design. (B) Hot-spot for PPI inhibitor design against fusion core region of S protein. SARS-CoV-2 6-HB structure is shown in cartoon with HR1 and HR2, colored in green and cyan, respectively (PDB: 6LXT). (C) Hot-spot for PPI modulator design against S protein trimerization. (Left) Structure of SARS-CoV-2 S protein trimer (PDB: 6VSB). The trimeric interface is enlarged in the middle. (Right) Structure of the influenza HA in complex with arbidol (PDB: 5T6N). The arbidol target site of the trimeric interface is enlarged in the middle. (D) Hot-spot for PPI inhibitor design against intra-dimer of 3CLpro. The structure of 3CLpro of SARS-CoV-2 (PDB:6Y2E) is aligned with that of SARS-CoV (PDB: 1UK4). The key residues involved in dimerization are shown in stick representation. (E) Hot-spot for PPI stabilizer design against N-NTD. The structure of SARS-CoV-2 N-NTD (PDB: 6M3M) is aligned to P3: MERS CoV N-NTD complex (PDB: 6KL6), the interacting residues are shown in sticks and highlighted in right box. The residues of SARS-CoV-2 and MERS CoV N-NTD are shown in orange and yellow, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

neutralizing activities in host cells ectopically expressing hACE2. Enzyme-linked immunosorbent assays (ELISA) and flow cytometry-based blockade experiments proved that both antibodies specifically bind to SARS-CoV-2 RBD and may disrupt the PPI between RBD-ACE2 [85]. Brouwer et al. used an ELISA-based approach with SARS-CoV-2 stabilized prefusion S protein to isolate 19 neutralizing antibodies from blood samples of three convalescent COVID-19 patients. One of them, COVA2-15, showed picomolar neutralizing activity against infectious SARS-CoV-2. Single-particle negative-stain electron microscopy (EM) revealed that the COVA2-15 epitope partially overlapped with the binding region of ACE2 in S protein, suggesting that the antibody may block receptor engagement [86]. Li et al. also identified a potent monoclonal antibody (mAb), IgG1 ab1, from large antibody libraries. ELISA experiments revealed that IgG1 ab1 exhibited high-affinity to RBD and competed with ACE2 in vitro. More importantly, IgG1 ab1 showed high therapeutic efficacy in an animal experiment of SARS-CoV-2 infection [87].

Development of soluble peptide analogues of ACE2 is an alternative approach employed to compete with normal ACE2 for the binding to S protein. Monteil et al. have shown that human recombinant ACE2 (hrACE2) reduced the replication of SARS-CoV-2 by a factor 1000–5000 times in a cell model [83]. In addition, Hu et al. connected the extracellular domain of human ACE2 with human IgG-Fc to generate a novel recombinant protein (ACE2-Ig). This chimeric recombinant protein displayed high affinity for binding to RBD of SARS-CoV2 and neutralized SARS-CoV-2 with potent efficacy in vitro [88]. These developments highlight the promise of using PPII inhibitors targeting the S-ACE2 complex as therapeutic agents against SARS-CoV-2.

Another indirect way of disrupting the interaction between the S protein and the host is by targeting regions of the protein involved in membrane fusion (Fig. 3B). The S protein forms a 6-helix bundle (6-HB) fusion core through two heptad repeats, HR1 and HR2, which brings the host and viral membranes close together for fusion and infection [89]. The inhibition of the formation of viral 6-HB could be a strategy to interfere the viral entry. For this purpose, Xia et al designed a series of lipopeptides against HR1 to disturb the formation of viral 6-HB. One of which, termed EK1C4, showed the capacity as a potent fusion inhibitor, which appeared to inhibit infection of several types of coronaviruses in cells, including SARS-CoV-2 [90,91].

Based on the fact that S protein trimerization appeared to be the rate limiting step in other types of coronavirus infections [92], it has been suggested that manipulation of S protein trimerization may be another strategy to block the viral entry of SARS-CoV-2 (Fig. 3C). Kalathyia et al. employed molecular dynamics simulations to identify a highly conserved cavity within the S protein homotrimer of SARS-CoV-2 which may serve as a novel drug target for PPII inhibitor design [93]. Bongini et al. also performed molecular docking to identify eight available compounds targeting the trimer cavity which may interfere with trimerization of SARS-CoV-2 S protein [94]. Arbidol, a broad-spectrum antiviral drug against influenza, targets the trimerization interface of hemagglutinin (HA) and inhibits virus-host cell fusion by stabilizing the prefusion conformation of HA, which prevents further conformational rearrangements required for membrane fusion [95]. Since the role of HA in influenza is similar to S protein in SARS-CoV-2, arbidol is now being used in a clinical trial for treatment of SARS-CoV-2. By employing molecular dynamics and structural analysis, Vandakari suggested that arbidol may target the SARS-CoV-2 spike glycoprotein employing a mechanism similar to that of influenza virus [96].

Host proteins such as ACE2, DPP4, TMPRSS2 or furin are also considered targets for inhibition of viral entry. Liu et al. generated a library comprising of peptides derived from S protein of SARS-CoV for identifying the epitopes of SARS-CoV to target the ACE2 receptors. They found one peptide, S471-503, which specifically interfered with the interaction between the S-RBD and ACE2, and inhibited SARS-CoV entrance in vitro [97]. By a similar approach, Meyer et al. synthesized one hexapeptide (438YKYRYL443), derived from SARS-CoV S-RBD, to bind to ACE2 and inhibit viral entry [98] https://elsevier.proofcentral.com/en-us/landing-page.html?token=c0d27m66064a67522d0470b27d0966 [99]. Several substrate analogues have been proposed to target either furin or TMRRSS2 for the inhibition of influenza. Although the exact mechanism has not been elucidated, these substrate analogues may inhibit viral entry by inhibiting the interaction between furin [100,101] or TMPRSS2 [102] and their respective substrates. Since the host targets exhibit lower mutation rates, these results from previous studies may provide an important basis to develop PPII inhibitors against SARS-CoV-2.

It is worth noting that chloroquine, a repurposed anti-malarial drug that has gained lots of attention in COVID-19 treatment [103–105], has been proposed to reduce the affinity of SARS-CoV S protein to ACE2 [106] by increasing endosomal pH. Hence, chloroquine appears to be one atypical example for PPI inhibition that does not directly involve PPIIs.

4.1.2. 3C-like protease (3CLpro)

The active form of 3CLpro is a dimer which cleaves the peptide bond between a glutamine and a small amino acid (serine, alanine or glycine). It is essential for processing the coronavirus polyprotein into its functional constituents [107]. In addition to the active site, the intra-dimer PPII is also a valid target for drug development (Fig. 3D). As a proof of concept, an octapeptide derived from the N-terminus of SARS-CoV 3CLpro has been shown to disrupt protease dimerization and inhibit viral replication [108,109]. The important residues involved in dimerization of SARS-CoV 3CLpro, Arg4, Ser10, Gly11, Glu14, Asn28, Ser139, Phe140, Ser147, Glu290, Arg298, all are conserved in SARS-CoV-2. It is conceivable that the octapeptide may also be active against SARS-CoV-2, and similar stratagems for drug development against COVID-19 may be gleaned from this example [110].

4.1.3. Nucleocapsid (N) protein

The N protein is a dimer which self-assembles with viral RNA to form the ribonucleoprotein (RNP) particle [111,112]. Our group has recently identified a novel non-native PPII between N protein dimers of MERS-CoV [113]. Formation of the PPII inactivates the N protein by occluding its essential RNA-binding site but requires the presence of a "glue" molecule to stabilize the non-native contacts. The shape of the non-native PPII is highly conserved among other coronaviruses, making it a potential target for broad-spectrum antivirals that may also be effective against SARS-CoV-2 (Fig. 3E). Preliminary in vitro studies showed that at least one compound was effective across MERS-CoV, SARS-CoV and mouse hepatitis virus, and early studies assessing its efficacy against SARS-CoV-2 appear to yield promising results. Elucidation of other non-native PPIs among coronaviral proteins may contribute additional non-canonical targets for drug development.

4.1.4. PPIIs involving other proteins

The proteins listed above represent only a fraction of the possible targets for anti-COVID-19 drug development. For example, the viral membrane (M) protein has long been known to interact with the N protein and is also essential for virion assembly [114,115], implying that the PPII between N-M may be another possible target. On a broader scale, Gordon et al used affinity-purification mass spectrometry to discover 332 protein-protein interactions between SARS-CoV-2 and humans [116]. However, these examples lack the structural characterization of the interaction interface, thus limiting their potential for PPII-based drug development.

4.2. In-silico exploration of potential PPII modulators against COVID-19

Several computational studies have been carried out since the early days of the COVID-19 outbreak. These can be broken down into the following categories:

4.2.1. Identification of PPI networks

PPI networks provide a wealth of information about possible pathogenesis mechanisms and drug interactions that may not be evident using conventional approaches. For example, disease mechanisms were revealed through comparative analyses of various host-coronavirus protein interaction networks [117,118]. These PPI networks may also provide insights into possible drug repurposing [119–121]. In addition, open PPI network databases such as STRING-covid (https://string-db.org/cgi/covid.pl) and IMEx coronavirus interactome [122] may provide novel potential targets for the design of PPII modulators.

4.2.2. Modeling the interaction between potential PPII modulators and binding proteins

Virtual screening through docking is generally cheap but does not provide the free-energy information required for drug binding affinity estimation. However, accurate simulations that do provide the necessary energetic parameters for binding are usually time-consuming and computationally (and monetary) expensive. The Anton supercomputer developed by David Shaw and coworkers promises to vastly reduce the computational time required to conduct such calculations [123]. The Anton has been used to model the structure of several SARS-CoV-2 proteins, including the binding of drug molecules to the trimeric S protein, and all the trajectories are openly accessible and free of charge (for details, see https://www.deshawresearch.com/downloads/download_trajectory_sarscov2.cgi/). Modeling using less esoteric hardware have been carried out for the binding of remdesivir, favilavir, and ribavirin to SARS-CoV-2 RdRp [124]. The interaction between remdesivir, chloroquine, ciclesonide and niclosamide to ACE2 have also been modelled via autodock simulations [125].

5. Future perspectives

The variety of approaches towards COVID-19 drug discovery afforded by targeting PPIIs share a common goal: to find molecular entities that can stop viral activity in its track. In fact, using a combination of drugs targeting different PPIIs and conventional viral targets in a "cocktail" formulation may provide the best chance of inhibiting viral activity. From this perspective, the inherent variability of PPIIs provides an opportunity to diversify the chemical space of COVID-19 drugs and may help avoid drug resistance problems arising from the usage of drugs targeting a single mechanism. Another advantage to targeting PPIIs is the possibility to develop broad-spectrum antivirals which may be useful against other coronaviruses [113]. It is telling that three of the most important emerging diseases of the century (SARS, MERS, and COVID-19) are all caused by coronaviruses and having a coronavirus-specific broad-spectrum drug may help avert the next coronavirus health crisis.

One of the major obstacles to targeting PPIIs for drug discovery is the lack of a starting scaffold for further development [126]. However, the case for COVID-19 is very different. Thanks to a research intensity which has never been seen before in the history of drug development, there are now several candidate molecules available for repurposing tests or as leads for further development. The main issue today is a lack of experimental validation of these molecules. With the number of potential PPII-targeting compounds and biologics on the rise, there is an immediate need for increased validation capacity among laboratories worldwide.

We believe that targeting PPIIs for drug development against COVID-19 is a viable strategy that warrants further consideration from the scientific community. This is especially true in the current crisis, which unfortunately does not appear to be abating any time soon. Development of PPII-targeting drugs may provide an additional piece in the arsenal of anti-coronaviral treatments, and we sincerely hope that further studies in this direction will one day help find a cure for COVID-19.

CRediT authorship contribution statement

Chung-ke Chang: Writing - original draft, Writing - review & editing. **Shan-Meng Lin:** Writing - original draft, Writing - review & editing. **Roshan Satange:** Writing - review & editing. **Shih-Chao Lin:** Writing - review & editing. **Sin-Cih Sun:** Writing - original draft. **Hung-Yi Wu:** Resources. **Kylene Kehn-Hall:** Writing - review & editing. **Ming-Hon Hou:** Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the MOST 109-2327-B-005 -005 and 109ST001C from ENABLE Center, NCHU.

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