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Research Article

Molecular Docking - Study of Potential Cyclophilin-Dependent Mechanism of SARS-CoV-2 Viral Entry

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Abstract

Objectives: The aim of this article is to analyse interaction of the SARS-CoV2 fragments with cyclophilins and to validate the mechanism of the viral entry involving spike glycoprotein or membrane proteins.

Coronavirus Disease: COVID-19 is a disease caused by RNA virus that got potential to cause respiratory and intestinal illness in humans as well as animals. There are 7 different type of Coronavirus diseases (229E, NL63, OC43, HKU1, MERS-CoV, SARS-CoV, SARS-CoV-2) reported in humans that are known to cause illness ranging from common cold to more severe forms [1]. The epicentre of the COVID-19 disease was Wuhan city, Huban province of China where a cluster of unexplained pneumonia cases of unknown aetiology were officially reported on Dec 31 2019 [2]. Though Covid-19 has a lower mortality rate (2.3%) than SARS (9.5%) or MERS (34.4%) it is highly transmissible and has rapidly progressed from epidemic to pandemic scale [3].

Keywords: SARS-CoV-2; Covid-19; Docking

SARS-CoV-2 infection causes a wide spectrum of symptoms from mild disease to severe respiratory distress, hospitalization, and mortality. Since the appearance of SARS-Cov-2 a number of structures have been proposed as potential sites for drug and vaccine development. A key to preventing the spread of SARS CoV-2 and the resultant pandemic is to gain a clear understanding of its mechanism of cell entry. Mapping virus–host protein interactions can provide important clues on the initial stages of infection. Coronavirus entry into host cells is an important determinant of viral infectivity and pathogenesis [4-6]. It is also a major target for host immune surveillance and human intervention strategies [5,7].

SARS-CoV-2 is an enveloped spherical or pleomorphic particle containing single-stranded (positive-sense) RNA. The viral genome encodes four structural proteins, the envelope (E), the nucleocapsid (N), the membrane (M), and the spike (S) proteins [8,9]. The envelope (E) and membrane (M) proteins that, along with the spike, form the virus protein interface to the external environment [8,10]. To enter host cells, coronaviruses needs firstly bind to a cell surface receptor for viral attachment before internalization into cells.

The spike glycoprotein attaches the virion to the host cell membrane and has two functional units, S1 and S2. S1 domain of SARS-CoV-2 spike glycoprotein potentially interacts with the human host cell targets such as ACE2, CD147, cyclophilins, CD26 and other cell adhesion factors [11]. The surface spike glycoprotein has three S1-S2 heterodimers. S2 segment of spike protein is acting as viral fusion protein to mediate the membrane fusion of virus and cells. The receptor binding domain (RBD) locates on the head of S1. It has been shown that the spike protein is present in two differential conformations: open state 6VYB and closed state 6VXX [12,13].

Cyclophilins

Cyclophilins are ubiquitous proteins found in both prokaryotes and eukaryotes and have been structurally conserved throughout evolution and most of them have PPIase activity. They are found in mammals, plants, insects, fungi and bacteria. The human genome consists a total of 16 cyclophilin isoforms. 7 major cyclophilin isoforms in humans are hCypA (hCyp-18a), hCypB (hCyp-22), hCypC, hCypD, hCypE, hCyp40 (40 kDa) and hCypNK (first identified from human natural killer cells) [14].

Cyclophilins are present in all cellular compartments, and are also involved in processes such as protein trafficking and maturation, receptor complex stabilization, apoptosis, receptor signaling, RNA processing, muscle differentiation, detoxification of reactive oxygen species (ROS), immune response, spliceosome assembly, miRNA activity and RISC assembly. Cyclophilins also have proposed functions in facilitating protein folding and trafficking [14]. Some of the cyclophilin members may serve as scaffolding proteins for assembly of large supramolecular complexes.

Secreted cyclophilins (CypA, CypB, CypC)

Cyclophilin A (CypA) is involved in protein folding and trafficking, cell signalling [15]. It can induce signalling via two distinct pathways: PPIase activity and extracellular binding to CD147. The major signalling pathways are associated with CypA/CD147 include Akt, ERK1/2, MAPK, and NF-κB [15]. Interaction of CD147 with the virion-associated CypA plays a functional role in facilitating invasion of host cells by SARS-CoV and as such may be implicated in host cell invasion by the closely related SARS-CoV-2 virus [16]. PPIA, encoding CypA, has been predicted from 32 datasets, the top ranked gene in Sars-Cov-2 infection [17].

Cyclophilin B (CypB) is the other characterized member of the cyclophilin family. CypB was found to interact with platelets; it is bound to the peripheral blood lymphocytes and enhances adhesion of platelets to collagen [18].

High levels of both proteins were recovered in biological fluids in pro inflammatory states (severe sepsis, HIV infection, oxidative stress) [19]. CypA stimulates production of pro-inflammatory cytokines IL-6, IL-8, IL-1β, MCP-1 and TNFα by monocytes but no such activity was found for extracellular cyclophilin B (eCyPB) [20]. For extracellular cyclophilins and signalling through CD147, isomerization of Pro211 bond by extracellular cyclophilin A (eCypA) and Pro180 bond by extracellular cyclophilin B (eCypB) takes place, although it remains possible that eCypB also targets Pro211 [20].

The third member of the cyclophilin family that can be secreted from cells is CypC. Similar to CypB, CypC localizes to the ER and is released to the extracellular space by cyclosporine (CsA) treatment [20].

Cyclophilins in viral infections

CypA facilitates or inhibits the replication of viruses [21,22]. Cyclophilins play a central role in the lifecycle of viruses from different families, including Human Immunodeficiency virus (HIV), Hepatitis C virus (HCV), Hepatitis B virus (HBV) Dengue virus (DENV), Japanese encephalitis virus (JEV), Yellow fever virus (YFV), Cytomegalovirus (CMV), Human Papillomavirus, Influenza A virus (IAV) or vesicular stomatitis virus (VSV) [21]. CypA was found to interact functionally with HCV NS5A, West Nile virus (WNV) NS5 and YFV NS4B [23]. CypA is a pro-viral factor for HCV, HIV-1, and SARS-CoV, and an anti-viral factor for IAV [17]. It has been shown that the lifecycles of human coronaviruses 229E (HCoV-229E), NL-63 (HCoV-NL63), feline infectious peritonitis coronavirus (FPIV) and SARS-CoV were highly dependent on CypA (and possibly also CypB for FPIV) [21,24,25].

CypA interacts with HIV-1 proteins. CypA-CD147 complex regulates HIV-1 attachment to host cells. CyPA can be specifically incorporated into the virions of HIV-1 and it can enhance an early step of cellular HIV-1 infection. The Gag polyprotein of human HIV-1 was shown to bind CypA [21,26,27].

CypA was suggested to be also an interaction partner of the SARS-CoV nucleocapsid (N) protein using the binding affinity of purified SARS-CoV N protein and CypA and co-immunoprecipitation of purified CypA with the SARS-CoV N protein. CypA was also shown to bind to cellular CD147. The presence of CypA using a mass-spectrometry-based approach was confirmed in SARS-CoV virions [23]. By use of electronic microscopy, CyPA was present on or around the surface of SARS-CoV and as such CypA may act in the process of invasion of host cells by SARS-CoV. It is accepted that

CyPA bound to viral proteins in the core can be relocated to the viral surface during maturation of the virus [16]. The SARS-CoV and MERS-CoV virions bring with them quantities of CypA sufficient for the achievement of their lifecycle, allowing them to compensate for a defect in cell production in their target cells [24].

CypB bound to HCV NS5B polymerase promoted RNA binding activity, and CyPB-NS5B interaction was reduced by the presence of cyclosporin [25,28]. There is similarity in the structure of NS5B polymerases. For instance, MERS CoV polymerase shares more than 20% sequence identity with HCV and the active site is conserved between the two proteins, with two consecutive aspartates that are crucial in the nucleotide transfer reaction [29]. An interaction between CypB and JEV NS4A was also documented [23].

CsA inhibits the replication of HCoV-229E, HCoV-NL63, FPIV, mouse hepatitis virus (MHV), avian infectious bronchitis virus (IBV), SARS-CoV (the virus genetically closest to SARS-CoV-2) and HCV [24,25,30]. Cyclophilin inhibitors (CsA and alisporivir) activate host innate immunity by induction of interferon-λ via activation of interferon regulatory factor 1 (IRF1) and as such efficiently decrease Middle East respiratory syndrome Coronavirus (MERS-CoV) replication *in vitro* and *in vivo* [31].

Mechanism of action

Many viral pathogens have evolved to utilize glycans as attachment factors including SARS-CoV and MERS-CoV. For Sars-Cov-2 a model of heparan sulphate-dependent (HS-dependent) enhancement of binding to ACE2 has been proposed. Cellular heparan sulphate (HS) has a role in SARS-CoV-2 infection [32] because of insufficient abundance of CD147 and very low affinity of cyclophilin-CD147 interaction. Docking studies suggested a presence of heparin/HS binding site in the receptor-binding domain (RBD) where both ACE2 and heparin can bind independently to spike protein. HS acts as a co-receptor priming the S for ACE2 interaction and the putative HS-binding site is fully exposed in the open state of spike protein [32]. When HS may stabilize the open conformation of the S protein, RBD/S1 S1-HS binding is likely to be in a sulfation-dependent manner (related to HS sulfation position and degree) [33]. In an interaction between the SARS-CoV-2 Spike S1 protein receptor binding domain (SARS-CoV-2 S1 RBD) and the polysaccharide heparin SARS-CoV-2 S1 RBD undergo conformational change. There are several potential heparins binding as basic amino acids patches are exposed on the SARS-CoV-2 S1 surface [34].

The docking simulation presented in this article suggests that additionally to 'opened' RBD domain direct interactions with heparin, there could be CypA/CypB dependent interactions with HS in the 'closed' inactive RBD conformation. As such, I postulate that initial step of Sars-Cov-2 attachment to target cells may rely also on CypA/CypB proteins. HS acts not only as a coreceptor priming the S for ACE2 interaction [32] but may also acts as attachment for CypA/CypB coated mature virus particles. Taking into consideration docking results, the Sars-Cov-2 attachment to target cell surfaces not only depend on ACE2 entry receptor, but may use an initial interaction of spike bound CypA/CypB proteins with HS proteoglycans (HSPGs) or with CD147 proteins or initial interaction with heparans which might facilitate subsequent binding to CD147. Upregulation of CD147 expression on activated T cells removes the heparinase sensitivity of eCyPA-induced signalling [20]. As such, CD147 role in viral internalisation needs further investigation.

CD147 is a transmembrane glycoprotein which has been documented to facilitate the entrance of viruses namely measles, malaria and HIV into human host cells [11]. The infection of T cells by SARS-CoV-2 with low values of ACE2 in T lymphocytes highlight the feasibility of CD147 as an alternative site for the viral entrance [35].

In human CypA a loop from Lys118 to His126 and four β strands (β3-β6) make up the binding site for CsA [36]. Based on presented here docking simulation residues which form a compact hydrophobic core has involvement in RBD domain interactions. It is consistent with findings of other group, which has shown that the CypA can bind to RBD at the ACE2 binding sites, this binding involves direct hydrophobic interactions and interaction between CypA and RBD was blocked by CsA [37]. For HIV virions, it has been shown that CypA is exposed at the viral membrane, mediates HIV-1 attachment and CypA-deficient HIV virus does not replicate [38]. CypA and cell surface heparans represents the initial step of HIV-1 attachment before gp120-binding to CD4 [38].

Docking studies presented here suggest strong interactions not only between the top of the spike (RBD domain) and CypA but also between the top of the spike (RBD domain) and CypB protein.

The structure of human CypB (hCypB) resembles that of human CypA (hCypA), with difference in the two loop regions (residues 19-24 and 152-164) and at the amino and carboxyl termini [36]. The presence of binding sites for CyPB on T lymphocytes, platelets and endothelial cells have been described. T cells adherence to the extracellular matrix is eCyPB dependent and integrin-mediated [19]. CyPB is a high-affinity ligand for cell surface HS present at the surface of T cells [39]. In the presence of CyPB, cell adhesion was increased in CD4+ and CD8+ subsets. CyPB is promoting adhesion of CD4+ lymphocytes to fibronectin and CD147 is required for the enhancing effect of CyPB on T cell adhesion [19]. Proteoglycan syndecan was shown to interact with the extracellular domain of CD147 [39] as well as eCyPB protein [19]. CypB and CD147 are co-expressed at the same time on human erythrocytes [40]. Syndecan-1 (HS proteoglycan) is physically associated with CD147 in CD4+/CD45RO+ T cells [39].

The N-terminal extensions of CyPA and CyPB differ [20]. eCyPB binds to oligosaccharides via the N-terminal stretch of lysines (3LysLysLys5), whereas eCyPA relies for this purpose on basic amino acids (Arg148, Lys151, Lys154, and Lys155) in the C-terminal part of the protein [20]. Binding of both eCyPA and eCyPB to most cells depends on heparan sulfate proteoglycans (HSPGs), and heparinase treatment abrogates signaling by both cyclophilins [20].

There is also some possibility of external M protein interaction with CypA or CypB, however further experiments are required to confirm/exclude these findings.

Materials and Methods

The molecular docking was carried out using on-line docking program ClusPro 2.0 https://cluspro.bu.edu/home.php and COVID-19 Docking Server https://ncov.schanglab.org.cn/index.php. PDB files for analysis have been obtained from the Protein Data Bank (PDB) https://www.rcsb.org/ or http://ncov.schanglab.org.cn server.

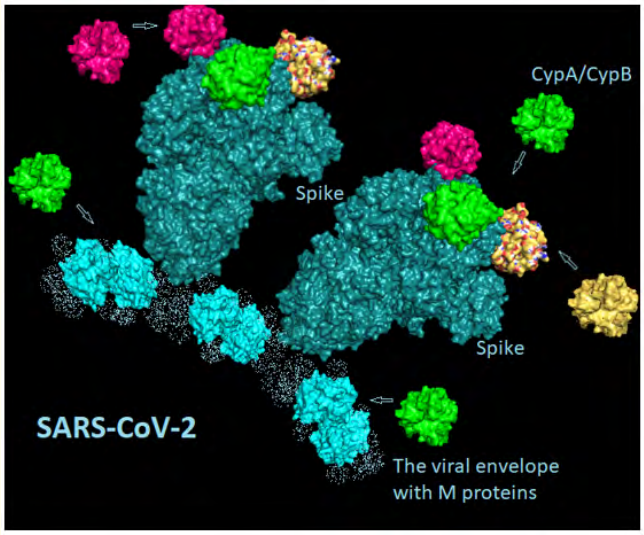


Figure 1: External Sars-CoV-2 proteins interactions with extracellular human proteins (CypA/CypB).

This study using molecular docking has shown that CypA/CypB interact with the top of the spike protein and likely there are also some interactions with M proteins. The viral envelope and spikes in cyan, cyclophilins in pink/green/yellow colour.

Sars-Cov-2 proteins chosen for molecular docking

The externally located proteins of Sars-Cov-2 virus which comes in direct contact with the host cells are considered to be the entry spot and ideal for the study. Structures of SARS-CoV-2 viral proteins (or fragments) which were used as targets for molecular docking with PDB ID when available (4-character unique identifier of every entry in the Protein Data Bank) are presented in figure 2, 3, 4.

Proteins/macromolecules modifications/preparation for docking

The top part of trimer 6VYB and trimer 6VXX as well as RBD domain (6M0J) pdb files were downloaded from the http://ncov.schanglab.org.cn server (Figure 3). Where, the RBD domain (6M0J) was provided from the structure of spike RBD of SARS-CoV-2 in complex with human ACE2 released by Wang and Zhang's group in Tsinghua University [13].

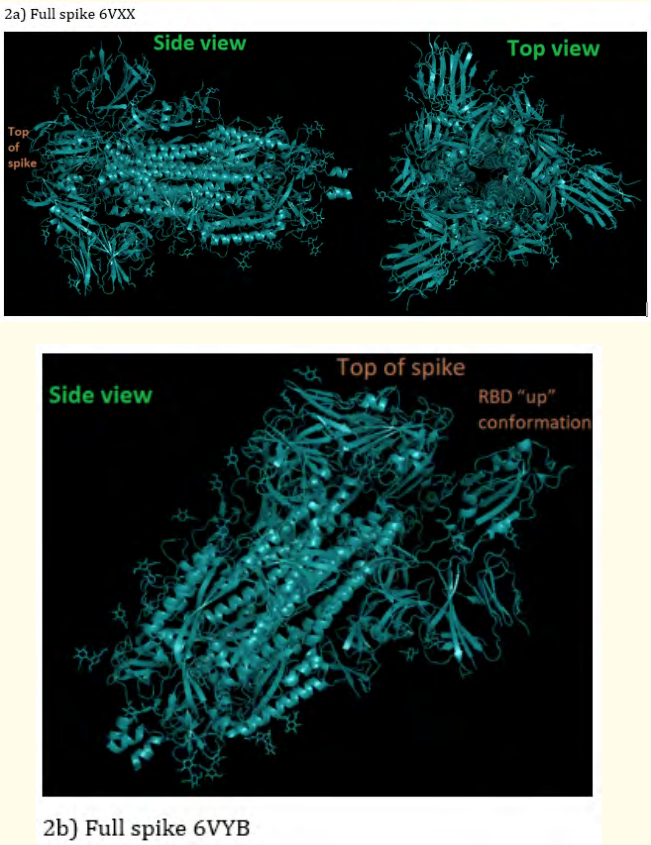


Figure 2: SARS-CoV-2 spike protein.

The theoretical models of full Sars-Cov-2 spike protein (the SARS-CoV-2 2P S GenBank: YP_009724390.1, UniProt: P0DTC2 SPIKE_SARS2) [12] were downloaded from PDB (Protein Data Bank) (https://www.rcsb.org/) as described in Wallis., *et al*. [41]). The cryoEM maps and atomic models are deposited at the Electron Microscopy Data Bank and the Protein DataBank with accession codes EMD: 21452 and PDB: 6VXX (closed SARS-CoV-2 S), as well as EMD: 21457 and PDB: 6VYB (SARS-CoV-2 S with one domain B (SB) open) [41].

2a) Full SARS-CoV-2 spike protein 6VXX where all RBD domains are buried inside the trimer (closed state).

2b) Full SARS-CoV-2 spike protein 6VYB where the spike RBD domain is in opened state.

The structure of membrane protein (M protein) prepared for peptide or antibody docking was also downloaded from the http://ncov.schanglab.org.cn server. The M protein is required for assembly of coronaviruses [42].

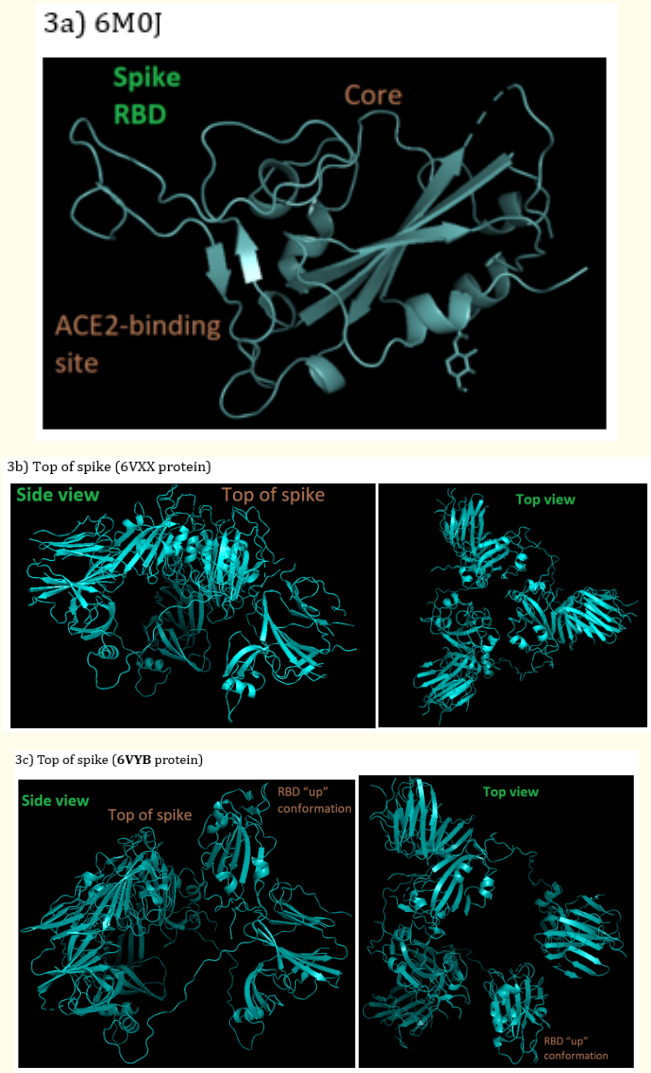


Figure 3: Fragments of SARS-CoV-2 spike protein downloaded from the http://ncov.schanglab.org.cn server.

3a) The RBD domain 6M0J.

3b) The top part of the CoV-2 spike protein 6VXX (three-fold symmetry) where all RBD domains are buried inside the trimer (closed state).

3c) The Top part of the Cov-2 spike protein in open conformation 6VYB with an upright RBD.

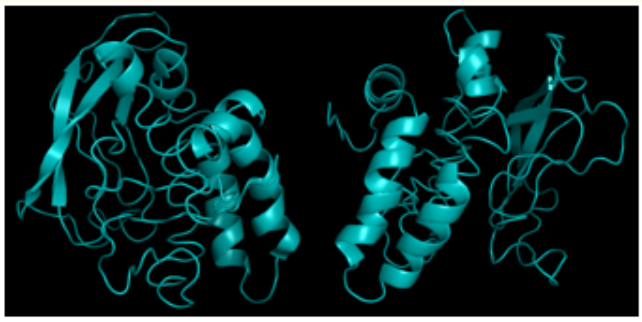


Figure 4: Membrane protein (M protein) (GenBank: YP_009724393, UniProt: P0DTC5, VME1_SARS2)) [8,9] was downloaded from the http://ncov.schanglab.org.cn server. Visualisations from different angles. PDB ID is not available.

Human proteins in docking studies

All pdb files of proteins used in docking studies have been obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/) and structures are shown in Figure 5. It includes angiotensin-converting enzyme 2 (ACE2 PDB: 1R42, GenBank: BAB40370, UniProt database: Q9BYF1, ACE2_HUMAN), CypA (PPIA PDB: 3K0N, GenBank: CAG32988, UniProt: P62937) and CypB (PPIB PDB: 3ICH, GenBank: CAG33110 UniProt: P23284).

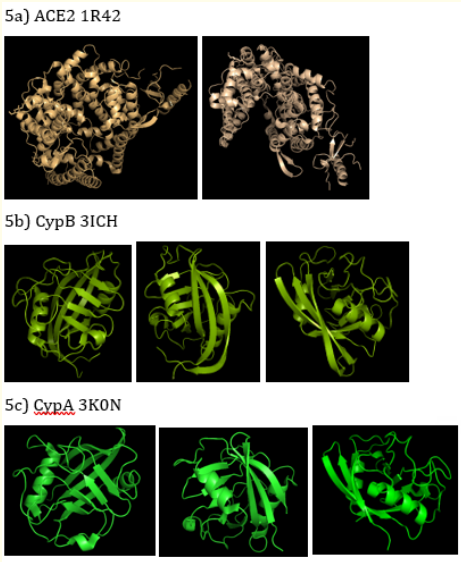


Figure 5: Structure of proteins (view from different angle) which were used for molecular docking with PDB ID. Source: Human.

5a) ACE2 1R42 5b) CypB 3ICH 5c) CypA 3K0N.

Experiment design

Molecular docking studies between the S protein (or its fragments) and CypA/CypB/ACE2 or molecular docking between the M protein and CypA/CypB were performed in year 2020 using on-line docking program ClusPro 2.0 https://cluspro.bu.edu/home.php and COVID-19 Docking Server version 2020 https://ncov.schanglab.org.cn/index.php.

The ClusPro server is a web-based server for the direct docking of two interacting proteins with availability on https://ClusPro.org [References ClusPro 2.0 Docking Server] [43-45]. CPU server with basic settings has been chosen. Following equations have been used to compute cluster scores as well as to predict the lowest binding energy

$E=0.40E_{rep}+-0.40E_{att}+600E_{elec}+1.00E_{DARS}$ (ClusPro balanced-favored model) and

$E=0.40E_{rep}+-0.40E_{att}+600E_{elec}+2.00E_{DARS}$ (ClusPro hydrophobic-favored model)

The repulsive {rep}, attractive {att}, electrostatic {elec} forces and interactions extracted from the decoys as the reference state {DARS}, are measured using molecular docking study.

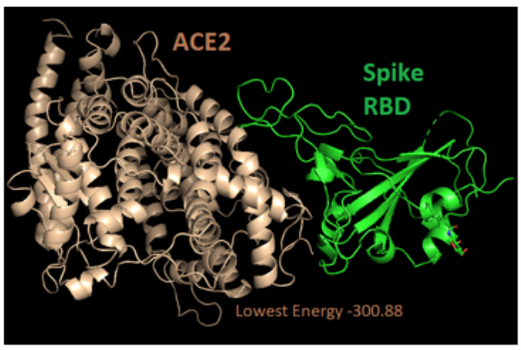
The COVID-19 Docking Server is a web server that predicts the binding modes between COVID-19 targets and the ligands including small molecules, peptides and antibodies where structures of proteins involved in the virus life cycle were collected or constructed based on the homologs of coronavirus, and prepared ready for docking with availability on http://ncov.schanglab.org.cn [References COVID-19 Docking Server] [46-50] Peptide or antibody mode with no constraint option has been chosen.

Final visualization

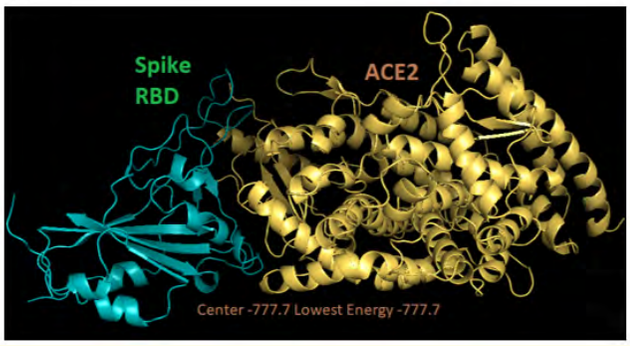
PyMOL Molecular Graphics System v 2.4.0 has been used which is a visualization system, maintained and distributed by Schrödinger.

Results

6a)



6b)



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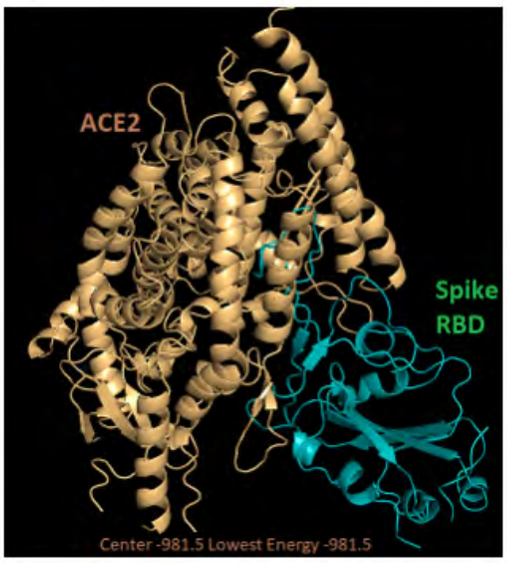

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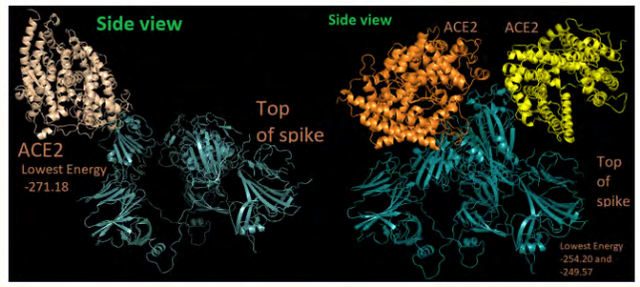
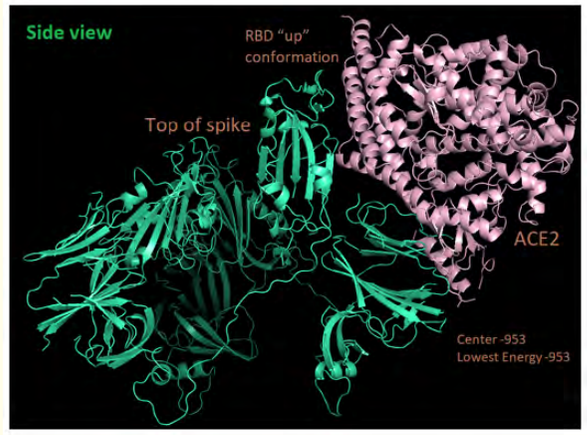


6e)



6f)

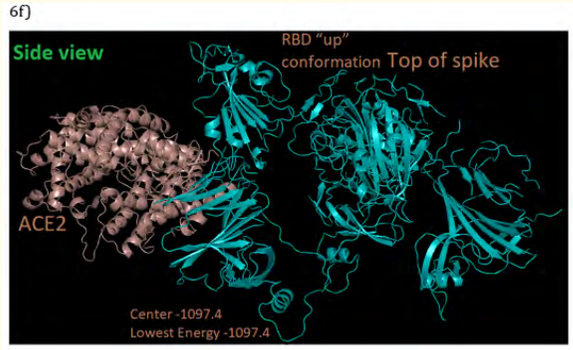


Figure 6: Complex models of spike fragments bound to ACE2 (known Sars-Cov-2 receptor) presented as ribbon structure where spike fragments are in green or cyan colour and cyclophilins CypA or CypB are in multicolour presentation. Values obtained here where used to interpret results of CypA/B docking with the spike/spike fragments or the M protein.

6a) RBD/ACE2 (COVID-19 docking server).

6b) RBD/ACE2 (ClusPro docking server - balanced model).

6c) RBD/ACE2 (ClusPro docking server - hydrophobic model).

6d) The top of the spike (open conformation)/ACE2 (COVID-19 docking server).

6e) The top of the spike (open conformation)/ACE2 (ClusPro docking server - balanced model).

6f) The top of the spike (open conformation)/ACE2 (ClusPro docking server - hydrophobic model).

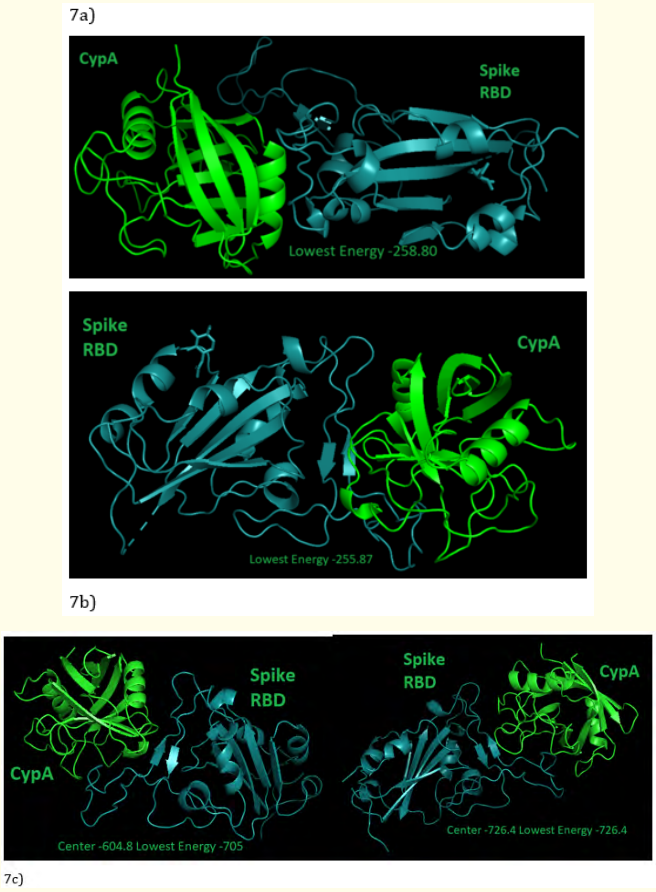


Figure 7: Complex models of RBD domain bound to CypA 3K0N presented as ribbon structure where the spike RBD domain is in dark cyan colour, and CypA protein is in light green colour

7a) RBD domain/CypA (COVID-19 docking server)

7b) RBD domain/CypA (ClusPro docking server - balanced model)

7c) RBD domain/CypA (ClusPro docking server - hydrophobic model).

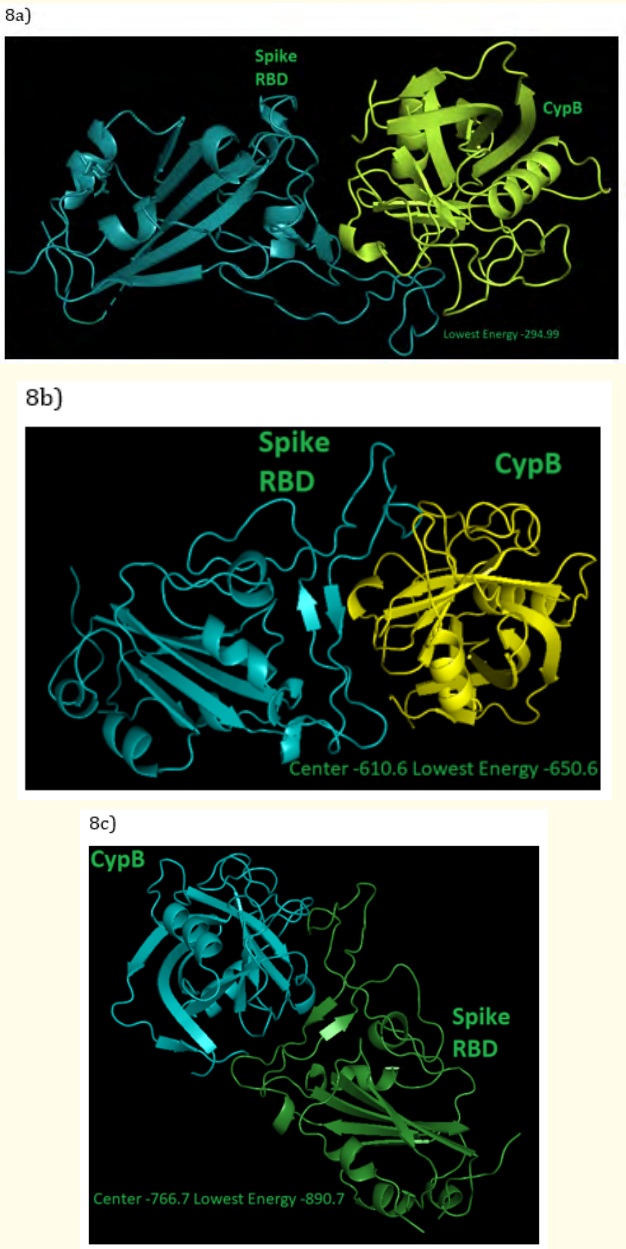


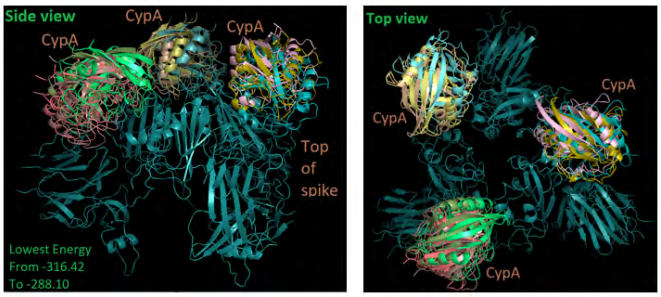
Figure 8: Complex models of RBD domain bound to CypB 3ICH presented as ribbon structure where the spike RBD domain is in dark cyan or green colour, and CypB protein is in yellow/yellow-green/cyan colour.

8a) RBD domain/CypB (COVID-19 docking server)

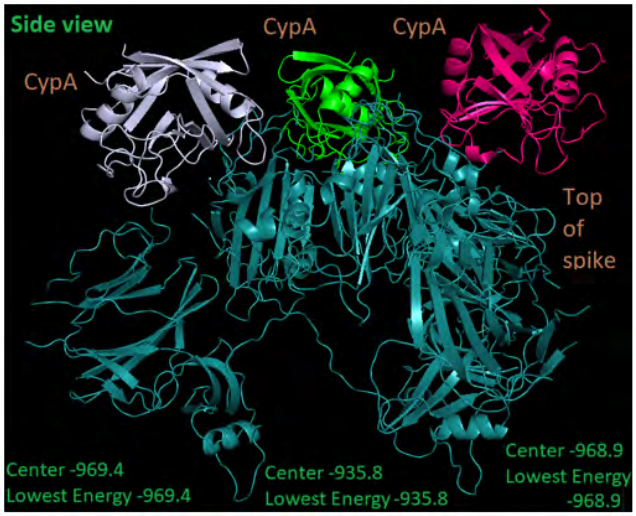
8b) RBD domain/CypB (ClusPro docking server - balanced model)

8c) RBD domain/CypB (ClusPro docking server - hydrophobic model).

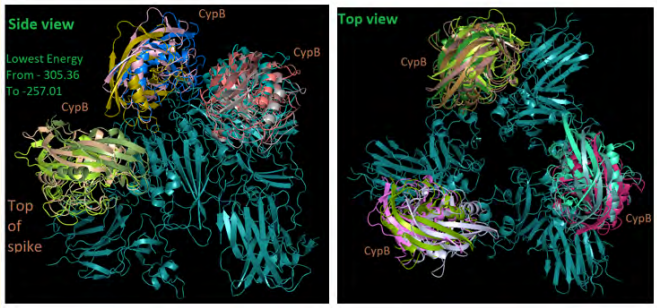
9a)



9b)



9c)



9d)

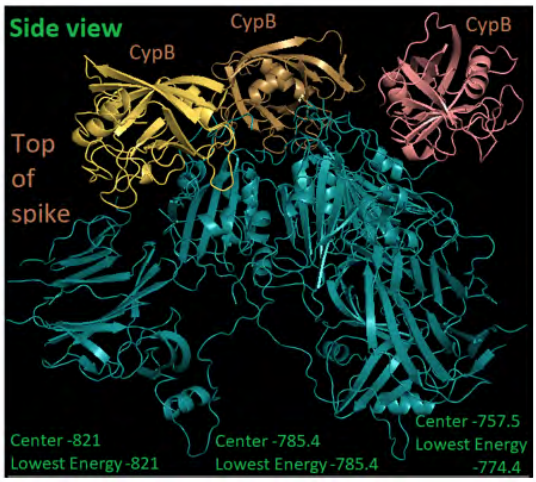


Figure 9: Complex models of interaction between the top of the spike protein (in closed conformation) and CypA 3K0N or CypB 3ICH proteins where part of Sar-Cov-2 protein used in docking studies is in cyan colour and cyclophilins CypA or CypB are in multicolour presentation

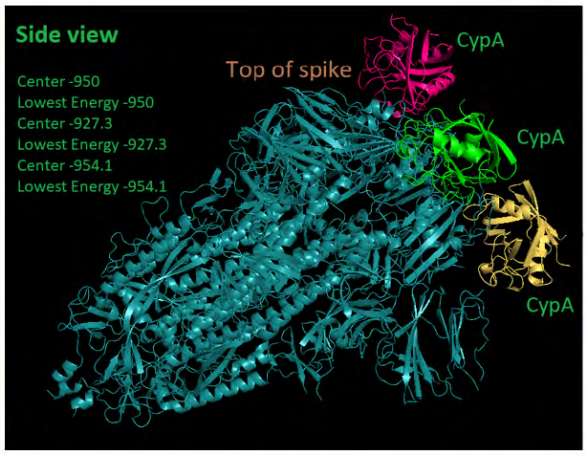
9a) The top of the spike (closed conformation)/CypA (COVID-19 docking server)

9b) The top of the spike (closed conformation)/CypA (ClusPro docking server - hydrophobic model)

9c) The top of the spike (closed conformation)/CypB (COVID-19 docking server)

9d) The top of the spike (closed conformation)/CypB (ClusPro docking server - hydrophobic model).

10a)



10b)

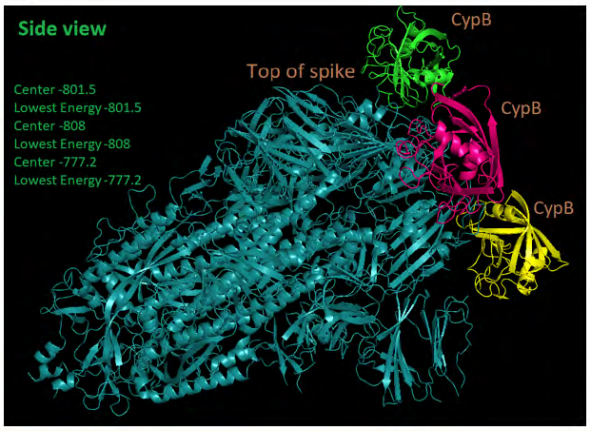
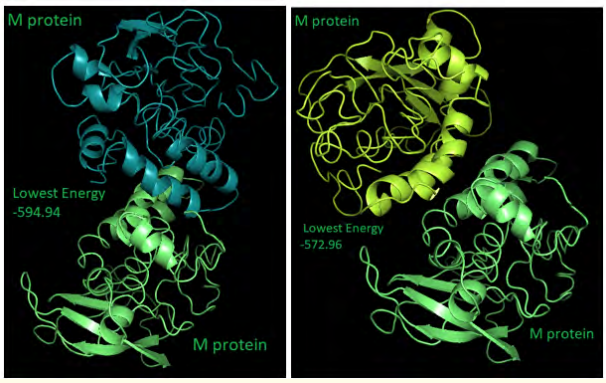


Figure 10: Complex models of full spike (closed conformation 6VXX) bound to CypA 3K0N or CypB 3ICH proteins where full spike protein is in cyan colour and cyclophilins CypA or CypB are in multicolour presentation

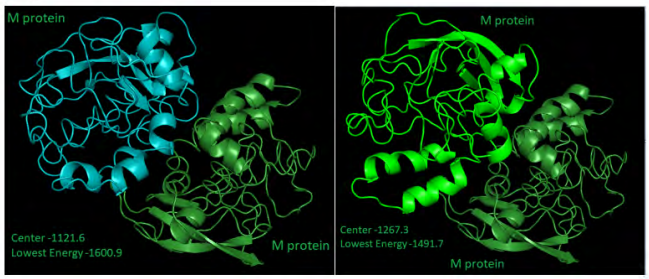
10a) Full spike (closed conformation)/CypA (ClusPro docking server - hydrophobic model)

10b) Full spike (closed conformation)/CypB (ClusPro docking server - hydrophobic model).

11a)



11b)



11c)

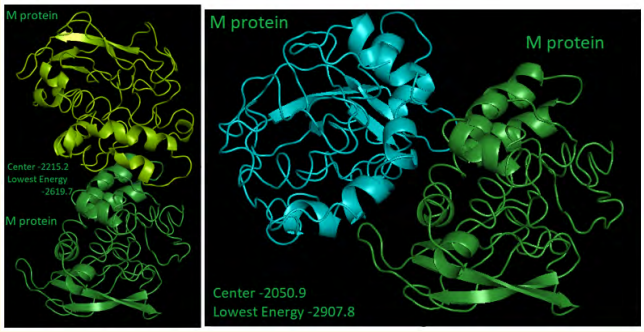


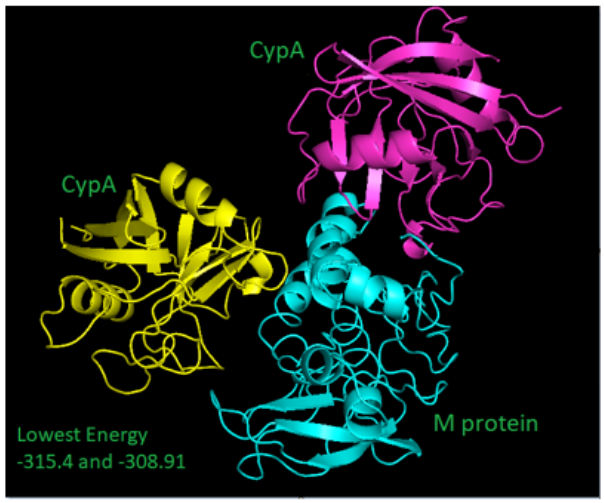
Figure 11: Complex models of interactions between the M protein where M proteins are in cyan/green colours. Values obtained here where used to interpret results of CypA/B docking with the M protein.

11a) M protein dimers (COVI-19 docking server)

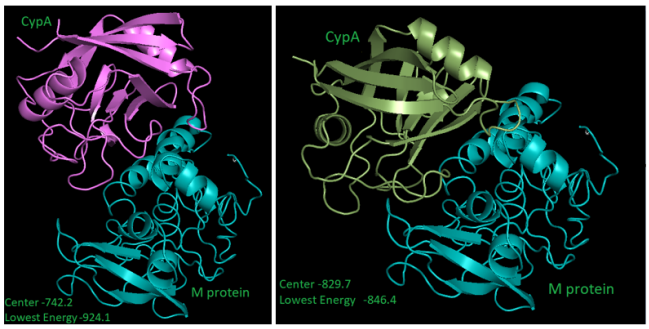
11b) M protein dimers (ClusPro docking server - balanced model)

11c) M protein dimers (ClusPro docking server - hydrophobic model).

12a)



12b)



12c)

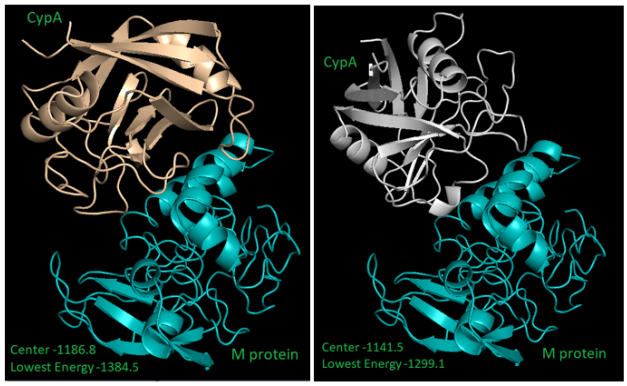


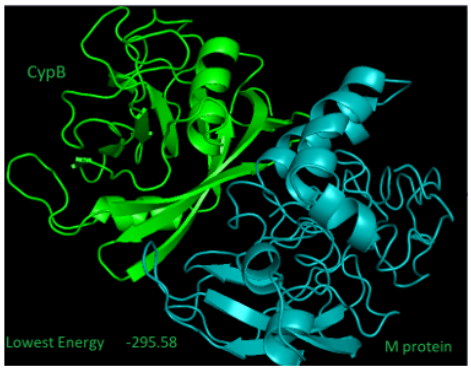
Figure 12: Complex models of bound M protein to cyclophilin A 3K0N where the M protein used in docking studies is in cyan colour and cyclophilins CypA are in multicolour presentation.

12a) M protein/CypA (COVID-19 docking server).

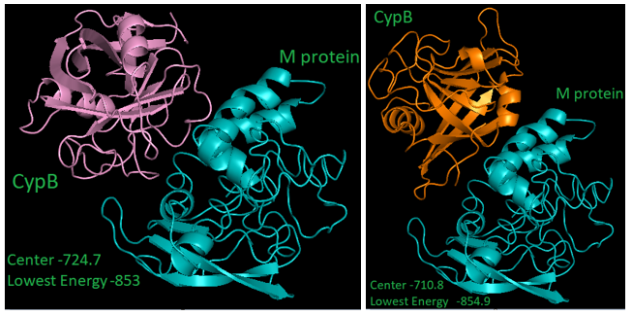
12b) M protein/CypA (ClusPro docking server - balanced model).

12c) M protein/CypA (ClusPro docking server - hydrophobic model).

13a)



13b)



13c)

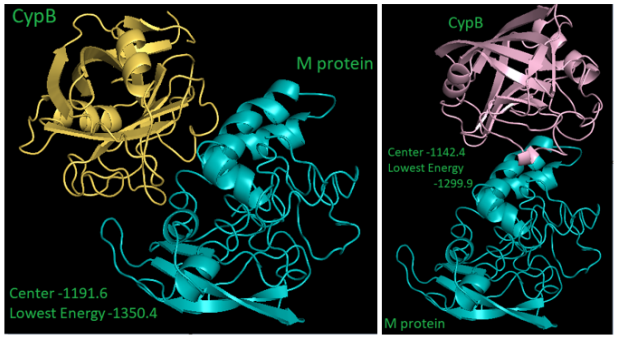


Figure 13: Complex models of M protein bound to cyclophilin B 3ICH where the M protein used in docking studies is in cyan colour and cyclophilins CypA are in multicolour presentation.

12a) M protein/CypB (COVID-19 docking server).

12b) M protein/CypB (ClusPro docking server - balanced model).

12c) M protein/CypB (ClusPro docking server - hydrophobic model).

Results are summarized in figure 6, 7, 8, 9, 10, 11, 12, 13 and in summary tables below.

Summary tables

Interaction of ACE2 with the spike trimer in open state mediates entry of SARS-CoV-2 into host cells. Values are negative numbers and the lowest binding energies are for the most likely models.

1a)

	Rank	1	2	3	4	5	6	7	8	9	10
RBD / ACE2 1R42	Score	-	-	-	-	-	-	-	-	-	-
	Value	300.88	264.91	259.42	259.42	258.21	255.83	254.74	253.53	253.47	250.48
RBD / CypA 3K0N	Score	-	-	-	-	-	-	-	-	-	-
	Value	258.80	255.87	251.67	247.21	238.77	236.60	235.90	235.47	234.86	234.01
RBD / CypB 3ICH	Score	-	-	-	-	-	-	-	-	-	-
	Value	294.99	288.85	278.97	272.01	266.53	266.43	264.16	258.29	254.05	248.26

1b)

	Rank	1	2	3	4	5	6	7	8	9	10
Top part of spike (open) /ACE2 1R42	Score	-	-	-	-	-	-	-	-	-	-
	Value	271.18	269.57	261.68	258.06	254.20	254.17	253.34	253.23	252.19	249.57
Top part of spike (closed) / CypA 3K0N	Score	-	-	-	-	-	-	-	-	-	-
	Value	316.42	308.21	301.13	298.69	295.86	294.76	294.00	291.25	288.31	288.10
Top part of spike (closed) / CypB 3ICH	Score	-	-	-	-	-	-	-	-	-	-
	Value	305.36	301.02	298.71	276.17	272.16	267.91	262.20	259.44	258.32	257.01

Table 1: Top 10 docking results (COVID-19 Docking Server).

1a) RBD bound to cyclophilin A/B. Binding energy is comparable (for CypB) or a little bit higher (for CypA) when comparing with the ACE2/RBD interaction.

1b) Spike trimer in closed state (the top of spike) bound to cyclophilin A/B where binding energy is lower for CypA/CypB than in case of ACE2 interaction with the spike trimer in open state. All interactions encompass the RBD domain.

2a)

	ClusPro docking balanced model	ClusPro docking hydrophobic model
RBD / ACE2 1R42	Center -777.7 Lowest Energy -777.7	Center -981.5 -886.9 Lowest Energy -981.5 -886.9
RBD / CypA 3K0N	Center -604.8 -726.4 Lowest Energy -705 -726.4	Center -938.1 Lowest Energy -938.1
RBD / CypB 3ICH	Center -610.6 Lowest Energy -650.6	Center -766.7 Lowest Energy -890.7

2b)

	ClusPro docking balanced model	ClusPro docking hydrophobic model
Top of spike (open) / ACE2 1R42	Center -950.7 -953 Lowest Energy -950.7 -953	Center -1097.4 Lowest Energy -1097.4
Top of spike (closed) / CypA 3K0N	-	Center -969.4 -935.8 -968.9 Lowest Energy -969.4 -935.8 -968.9
Top of spike (closed) / CypB 3ICH	-	Center -821 -785.4 -757.5 Lowest Energy -821 -785.4 -774.4
Full spike glycoprotein (closed) / CypA 3K0N	-	Center -950 -927.3 -954.1 Lowest Energy -950 -927.3 -954.1
Full spike glycoprotein (closed) / CypB 3ICH	-	Center -801.5 -808 -777.2 Lowest Energy -801.5 -808 -777.2

Table 2: Docking (ClusPro Docking Server - balanced/hydrophobic models).

2a) RBD bound to cyclophilin A/B, where binding energies are a little bit higher or comparable with the ACE2/RBD interaction

2b) Spike trimer in closed state (the top of the spike) or full spike bound to cyclophilin A/B where binding energies are a little bit higher or comparable with the ACE2/spike trimer in opened state (the top of the spike) interaction. All interactions encompass the RBD domain.

	Rank	1	2	3	4	5	6	7	8	9	10
Top part of spike (open) / ACE2 1R42	Score	-	-	-	-	-	-	-	-	-	-
	Value	271.18	269.57	261.68	258.06	254.20	254.17	253.34	253.23	252.19	249.57
M protein dimerization	Score	-	-	-	-	-	-	-	-	-	-
	Value	594.94	581.46	572.96	568.64	555.07	552.08	546.24	543.07	535.56	534.86
M protein / CypA 3K0N	Score	-	-	-	-	-	-	-	-	-	-
	Value	315.46	308.91	294.78	290.21	282.66	266.44	263.84	263.61	260.40	258.45
M protein / CypB 3ICH	Score	-	-	-	-	-	-	-	-	-	-
	Value	295.58	281.84	281.40	271.07	271.07	270.55	267.14	263.78	262.97	261.86

Table 3: Top 10 docking results (COVID-19 Docking Server) - M protein dimers or M protein interaction with Cyp A/B.

M protein dimers (The M-M interaction in viral particle) have twice lower score values then interactions predicted for Cyp A/B with M protein. However, Cyp A/B and M protein binding energies are lower in comparison with the ACE2/top part of spike protein (open state) binding energy calculated by the same server.

	ClusPro docking balanced model			ClusPro docking hydrophobic model		
Top of spike (open) / ACE2 1R42	Center	-950.7	-953	Center	-1097.4	
	Lowest Energy	-950.7	-953	Lowest Energy	-1097.4	
M protein / M protein	Center	-1121.6	-1267.3	Center	-2215.2	-2050.9
	Lowest Energy	-1600.9	-1491.7	Lowest Energy	-2619.7	-2907.8
M protein / CypA 3K0N	Center	-742.2	-829.7	Center	-1186.8	-1141.5
	Lowest Energy	-924.1	-846.4	Lowest Energy	-1384.5	-1299.1
M protein / CypB 3ICH	Center	-724.7	-710.8	Center	-1191.6	-1142.4
	Lowest Energy	-853	-854.9	Lowest Energy	-1350.4	-1299.9

Table 4: Docking (ClusPro Docking Server - balanced/hydrophobic models) - the M protein dimers or the M protein interaction with Cyp A/B. M protein dimers (The M-M interaction in viral particle) have lower binding energies then interactions predicted for Cyp A/B with the M protein. However, for ClusPro docking hydrophobic model Cyp A/B and M protein binding energies are lower in comparison with the ACE2/top part of spike protein (open state) binding energy calculated by the same server.

Summary of findings

Sars-Cov-2 spike protein

To understand Sars-Cov-2 mechanism of cell entry CypA and CypB binding to external proteins was studied. For predictions two independent prediction tools have been used. Comparison has been done with the binding energy of ACE2/RBD or ACE2/spike trimer in open state (6VYB) using the same docking servers. The spike glycoprotein in open state is known to interact with the ACE2 protein. Within S1 fragment in closed state, docking of CypA or CypB appears in positions encompassing RBD domains. The energy of these interactions is comparable to energy of ACE2 interaction with the spike trimer in open state. S1 domain interacts directly with the cell membrane during early stages of cell invasion and externally located cyclophilins will have role in adhesion of viral particles to body cells. Not only CypA but also CypB is predicted here to be involved in Sars-Cov-2 attachment to target cell surfaces. Docking analysis for CypA and CypB and Sars-Cov-2 external proteins showed that there is strong interaction of CypA or CypB with the RBD domain and these interactions may increase when the top of the spike is in a closed state (6VXX). As effect of these interactions each spike trimer can have 3 cyclophilins attached. Recent docking studies and experiments (the structure analysis/ the MILF strip assay/far-western blotting and surface plasmon resonance (SPR)) with the CypA protein only [37] reported by Korean scientists are consistent with results obtained in this study.

It has been shown that CypA interacts strongly with the viral SARS-CoV-2 receptor-binding domain (RBD) and the CypA/spike protein complex suppressed the binding of RBD to the ACE2 receptor [37]. These findings bring Sars-Cov-2 mechanism of attachment closer to that one seen in HIV. CypA is incorporated into the virions of HIV-1 and attachment to target cells is mediated via heparans [38]. HIV-associated CypA interacts also with CD147 [26].

Additionally, there is some potential role of CypB/CypA bound to Sars-Cov-2 virions in dynamic hypercoagulation/hyperfibrinolysis observed in COVID patients infected with the original Wuhan Sars-Cov-2 strain. Extracellular cyclophilin A (eCyPA) enhances platelet adhesion to the vascular wall and thrombus formation [51]. CyPB was found to interact with platelets. CypB is bound to the peripheral blood lymphocytes [18] and enhances adhesion of platelets to collagen [19]. Extracellular cyclophilins initiate a signal transduction cascade leading to production of pro-inflammatory factors. Sars-Cov-2 virons coated in CypA or CypB without cell invasion may activate the same processes as free cyclophilins.

Sars-Cov-2 M protein

In docking studies presented here, it is shown that M protein dimers which have very low binding energy are preferred state [52]. However comparing binding energy of CypA or CypB to the M protein with results for the ACE2/spike model (open state), it is clear that they are comparable. Overlap of CypA/CypB/M protein interaction area with M protein/M protein dimer interface, makes it more likely that cyclophilins interacting with M proteins may attach to N-terminal side located closer to nucleocapsid proteins then external viral surface. Taking into consideration that the M protein in coronaviruses is functionally dynamic and can acquire a homodimeric form [53] there is possibility of interaction with CypA or CypB within the same surface as the M protein dimer utilise for dimer stability. Further experiments are required to confirm/exclude these findings.

Understanding affinity to cyclophilins in new Sars-Cov-2 viral strains seems to be crucial for prediction of clinical presentation. There is also some likelihood that cyclophilin coated Sar-Cov-2 virus particles on early stages of infection may be poorly recognised by immune system. That feature may be a reason for great infectivity and delayed immune response.

Further research study should be carried out to look into the role of cyclophilins and proteins interacting directly with CypA or CypB in Sars-Cov-2 life cycle.

Conflict of Interest Statement

The author declares no competing financial interest.

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