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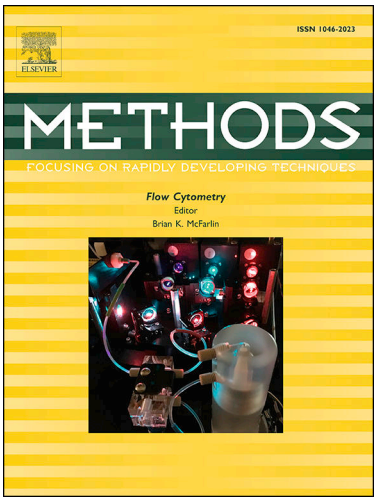

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Swatantra Kumar, Marc H.V. van Regenmortel, Ariel L. Rivas, Shailendra K Saxena

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Structural and Molecular perspectives of SARS-CoV-2

Swatantra Kumar[1#], Marc H. V. van Regenmortel[2], Ariel L. Rivas[3], Shailendra K Saxena[1#*]

[1]Centre for Advanced Research (CFAR), Faculty of Medicine, King George's Medical University (KGMU), Lucknow 226003, India.
[2]Medical University of Vienna, 1090 Vienna, Austria.
[3]Center for Global Health, School of Medicine, University of New Mexico, Albuquerque, NM 87131, USA.

[#]Contributed equally as first author

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*** Correspondence:**
Prof. Shailendra K Saxena
Centre for Advanced Research (CFAR), Faculty of Medicine,
King George's Medical University (KGMU),
Lucknow 226003, India.
Tel: 91 522 2257450;
Fax: 91522 2257450;
E-mail: shailen@kgmcindia.edu
http://orcid.org/0000-0003-2856-4185

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Abstract

Recent emergence of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transpired into pandemic coronavirus disease 2019 (COVID-19). SARS-CoV-2 has been rapidly transmitted across the globe within a short period of time, with more than 106 million cases and 2.3 million deaths. The continuous rise in worldwide cases of COVID-19, transmission dynamics of SARS-CoV-2 including re-infections and enormous case-fatality rates emphasizes the urgent need of potential preventive and therapeutic measures. The development of effective therapeutic and preventive measures relies on understanding the molecular and cellular mechanism of replication exhibited by SARS-CoV-2. The structure of SARS-CoV-2 is ranging from 90/120 nm that comprises surface viral proteins including spike, envelope, membrane which are attached in host lipid bilayer containing the helical nucleocapsid comprising viral RNA. Spike (S) glycoprotein initiates the attachment of SARS-CoV-2 with a widely expressed cellular receptor angiotensin-converting enzyme 2 (ACE2), and subsequent S glycoprotein priming via serine protease TMPRSS2. Prominently, comprehensive analysis of structural insights into the crucial SARS-CoV-2 proteins may lead us to design effective therapeutics molecules. In the present article, we will emphasize the mechanistic insights of SARS-CoV-2 replication including insights into the structural perspective of crucial SARS-CoV-2 proteins.

Keywords: COVID-19, SARS-CoV-2, Spike-glycoprotein, ACE2, Mpro, nucleocapsid, nsp10/nsp16 2′-O-methylase

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Highlights:

- Spike glycoprotein undergoes two conformational states i.e. receptor-inaccessible state and receptor-accessible state upon binding to ACE2.
- S1 subunit involved in the attachment to the peptidase domain (PD) of the ACE2 receptor via RBD and S2 subunit involved in cell and viral membrane fusion.
- SARS-CoV-2 and SARS-CoV RBDs use eight identical interacting residues for attachment to the ACE2 receptor.
- Main protease (Mpro) doesn't share cleavage sites with any human encoded proteases; therefore, it can be one of the best drug targets for combating CoVs.

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1. Introduction

Unprecedented global emergence of novel coronavirus (nCoV) coined as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late 2019 in Wuhan, China has led the whole world susceptible towards its infection causing a pandemic Coronavirus disease 2019 (COVID-19) [1]. According to the World Health Organization (WHO), SARS-CoV-2 has infected at least 106 million people worldwide with 2.3 million deaths affecting at least 220 countries, areas and territories [2]. The uninterrupted rise in worldwide cases of COVID-19, transmission dynamics of SARS-CoV-2 including re-infections and enormous case-fatality rates emphasizes the urgent need of effective therapeutic and preventive measures [3]. Coronaviruses (CoVs) are a family of positive sense enveloped RNA viruses known to infect a wide range of species including avian, humans, livestock and companion animals. Until 2019, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) were the only two known CoVs which causes a severe respiratory illness in humans [4]. SARS-CoV-2 is the 7th CoV which is known to infect human where SARS-CoV and MERS-CoV, SARS-CoV-2 infection results in severe disease and HKU1, OC43, NL63 and 229E were found to cause mild symptoms [5]. Human transmission of SARS-CoV-2 is primarily exhibited via direct, indirect or close contact with infected secretions including respiratory droplets, saliva generated when an infected individual coughs, sneezes, talks or sings [6]. However, other routes of transmission occur via fomite, blood borne, fecal-oral, mother-to-child, and animal-to-human transmission [7].

SARS-CoV-2 primarily targets the respiratory systems, however, complication in other systems including cardiovascular, neurological and renal also contributes to the mortality [8]. So

far, the clinical characteristics are considerably heterogeneous which ranges from asymptomatic to mild, moderate and severe disease forms [9]. SARS-CoV-2 infection is characterized by various immunopathological features including lymphopenia, lymphocyte activation and dysfunction, increased level of neutrophils, cytokine storm, depletion and exhaustion of lymphocytes [10]. Still lack of specific treatment for COVID-19 is the major cause of mortality since the treatment mainly depends on supportive care of the patients. Interestingly, a number of therapeutic molecules including repurposed drugs are found efficient for the treatment of COVID-19 [11, 12 and 13]. Although a large number of studies and clinical trials are being performed [14, 15], a comprehensive understanding of SARS-CoV-2 structure and replication is decisive for designing effective preventive and therapeutic measures.

2. SARS-CoV-2 morphology

Vero cells infected with SARS-CoV-2 subjected to transmission electron microscopy using ultrathin sections have allowed a morphological understanding of the structure of SARS-CoV-2. The tomograms generated by electron microscopy revealed that SARS-CoV-2 is of oval shape. The SARS-CoV-2 and SARS-CoV are not only phylogenetically related but are also similar morphologically. The negative staining of electron microscopy of SARS-CoV-2 revealed a diameter of 90/120 nm which is identical to that of SARS-CoV (86.5/118.5 nm) [16]. The host membrane-derived lipid bilayer of SARS-CoV-2 incorporates structural proteins encapsulating viral RNA. The genome size of SARS-CoV-2 is ranging from 27 to 32 kb which comprises of 6-11 open reading frames (ORFs) where six functional ORFs are arranged from 5' to 3' as replicase (ORF1a/ORF1b), spike (S), envelope (E), membrane (M) and nucleocapsid (N) encodes for structural proteins as well as seven putative ORFs arranged between the structural genes and

encodes for accessory proteins [17]. The replicase gene covers 67% of the 5′ genome which encodes for a large polyprotein (pp1ab) that gets proteolytically processed into 16 non-structural proteins (nsps). The nsps comprises various viral cysteine proteases namely as chymotrypsin-like, papain-like protease (nsp3), 3C-like, or main protease (nsp5), RNA binding protein (nsp9), RNA-dependent RNA polymerase (nsp12), helicase (nsp13) and others involved in the replication and transcription of SARS-CoV-2. Moreover, structural proteins and nsps derived from the processed polyprotein, SARS-CoV-2 encode for nine accessory factors from sub-genomic ORFs (3, 3a, 6, 7a, 7b, 8, 9b, 9c and 10) [18] (Figure 1).

3. Mechanisms of SARS-CoV replication

SARS-CoV-2 infection initiates upon attachment of spike glycoprotein with the ACE2 receptor and priming of S glycoprotein via host cell serine protease TMPRSS2 [19]. Following the entry of SARS-CoV-2, viral RNA is released and immediately undergoes a translation process to generate ORF1a and ORF1b [20]. These polyproteins are processed into nsps which form viral transcription and replication processes. During the synthesis of nsps, organelles undergoing viral replication form distinctive perinuclear double-membrane vesicles (DMVs), convoluted membranes (CMs) and small open double-membrane spherules (DMSs) that all together creates a defensive microenvironment for transcription of subgenomic (sg mRNAs) and viral RNA replication [21]. The structural proteins get transported into the endoplasmic reticulum (ER) membranes following transportation to Golgi apparatus through ER-to-Golgi intermediate compartment (ERGIC) [22]. Finally, SARS-CoV-2 progeny virions get released from the infected cell by the process of exocytosis [23] (Figure 2).

4. SARS-CoV-2 Spike Glycoprotein Structure

Spike glycoprotein (S-glycoprotein) is a trimeric fusion protein of class I type that exists in a metastable prefusion conformation of club-like shape of ~20 nm in length (Figure 3A) [24]. S-glycoprotein mediates the attachment and entry into the host cells through widely expressed cellular receptor angiotensin-converting enzyme 2 (ACE2) [25]. S-glycoprotein consists of two functional subunits S1 and S2 where S1 subunit comprises the N-terminal domain (NTD), signal sequence and receptor binding domain (RBD) whereas, S2 subunit is involved consists of heptad-repeat (HR) regions as HR-N and HR-C crucial for the generation of coiled coil structure inside the protein ectodomain [25, 26]. Several of the cleavage sites including furin, protease and S2′ protease are present at the border of S1 and S2 [27]. The S1 subunit involve in the in the attachment to the peptidase domain (PD) of the ACE2 receptor via RBD and S2 subunit involved in the cell and viral membrane fusion. The processing of S-glycoprotein occurs at the boundary of S1 and S2 subunits that is non-covalently associated in prefusion conformation [24]. The ectodomain consists of a head which is connected to the membrane a slender stalk [28]. The three RBDs are conformationally variable which are shielded by the N-terminal domains (NTDs). Upon binding to the ACE2 receptor, the RBD undergoes hinge-like conformational arrangements that temporarily exposes or hide the binding receptors [29]. These two states are denoted as down i.e. receptor-inaccessible state or up conformation i.e. receptor-accessible state [30].

5. Interaction of RBD and ACE2

The interaction between RBD and ACE2 receptor was found to be similar to the polar interactions observed in SARS-CoV [31]. ACE2 is a type I membrane protein expressed in various tissues including lungs, kidney, heart and intestine [32]. ACE2 protein is a transmembrane protein

comprised of N-terminal PD and C-terminal collectrin-like domains (CLD). The X-ray crystallographic structure of RBD-ACE2 complex of SARS-CoV-2 revealed that each PD domain interacts with one RBD where an extended loop region of the RBD interacts by forming a bridge spanning the arch shape α1 helix of the ACE2-PD [33]. Whereas, only limited contributions were observed from the α2 helix and β3 and β4 antiparallel strands. The interaction of RBD with PD can be categorized in 3 clusters where the two edges of the bridge interacts with N and C terminal of α1 helix including few areas of α2 helix and β3 and β4. The crucial interacting residues involved are Gln 498, Thr 500, and Asn 501 from the N-termini of the RBD with Tyr 41, Gln 42, Lys 353, and Arg 357 from ACE2, respectively. The middle of the bridge involves interactions of Lys 417 and Tyr 453 from the RBD with Asp 30 and His 34 of ACE2, respectively. Whereas, the C termini of α1 helix involves interactions of Gln 474 and Phe 486 of the RBD interacting with Gln 24 and Met 82 of ACE2 via H-bonding and van der Waals forces respectively (Figure 3B) [34]. Interestingly, structure-guided sequence alignment has revealed that SARS-CoV-2 and SARS-CoV RBDs use eight identical interacting residues for attachment to the ACE2 receptor.

6. Structure of main protease (Mpro, also called 3CLpro)

The Mpro acts on at least 11 Leu-Gln ↓ (Ser, Ala, Gly) cleavage sites present on the pp1ab (↓ marks the cleavage site) [35]. Interestingly, Mpro doesn't share these cleave sites with any human encodes proteases, with the result that Mpro has been found as one of the best-characterized drug targets for combating CoVs [36]. The structure of SARS-CoV-2 Mpro was found to be similar as SARS-CoV Mpro due to 96% sequence identity and was found to structurally diverge by only 0.53 Å. Mpro is characterized as six-stranded antiparallel β barrels which exhibit the substrate-binding site. The structure of Mpro resembles that of chymotrypsin (residues 10 to 99) as well as

picorna 3 C protease-like domains = I and II (residues 100 to 182). Whereas, the domain III (residues 198 to 303) forms five helices of globular shape which involve in the dimerization of Mpro exhibited via salt bridge interaction among Arg 4 of one protomer with Glu 290 residue of the other (Figure 3C) [37]. Dimerization of Mpro has been shown to be crucial for its catalytic activity due to NH2-terminal residues (N-finger) of all protomers interacting with Glu 166 required for substrate specific binding site, S1 pocket of the enzyme.

7. Structure of nucleocapsid protein RNA binding domain

The nucleocapsid (N) protein of coronavirus is a multi-faceted RNA-binding protein crucial for replication and transcription of SARS-CoV-2 [38]. The primary role of N protein is binds to SARS-CoV-2 RNA and form the ribonucleoprotein (RNP) complex. Crystal structure of 47-173 residues of N protein of SARS-CoV-2 revealed that SARS-CoV-2 N-NTD packed into an orthorhombic crystal form while the interaction between the interfaces occurs via residues of β-hairpin fingers and palm regions. The core of the protein is made up of five antiparallel β-strands where a single short helix exists just ahead of β2 strand and protruding β-hairpin between strands β2 and β5 (Figure 3D) [39].

8. Structure of nsp10/nsp16 2′-O-methylase

Among the nsps, nsp10 and nsp 16 have been shown to form a protein complex that requires for methylation at ribose 2′-O position of intermediate nucleotide of SARS-CoV-2 RNA cap [40]. This methylation is crucial for the conversion of cap-0 to cap-1 viral RNA to mimic the cellular mRNAs for translation and a host defense mechanism. The crystal structure of the protein complex was solved at 2.5 Å resolutions. The two complex proteins were found similar to a RMSD of ~ 0.2Å. Altogether, the nsp10/nsp16 2′-O-methylase was found as nsp16 monomer sitting on

top of nsp10 monomer where the nsp16 is consists of seven α-helices, twelve β-strands and five 3_{10} helices. These secondary structures assemble together to form a core comprised of helices αZ, αA, αD, and αE and strands β1–β7. Whereas, bottom of the nsp10 consists of three α-helices, three β-strands and two 3_{10} helices where two zinc-fingers have been found to stabilizes the structure (Figure 3E). Within the heterodimer complex, nsp10 is crucial due to its role as a co-factor for nsp16 methylase [41].

9. Conclusions

Although, some studies and clinical trials have been performed with SARS-CoV-2, further studies are still required for designing effective preventive and therapeutic measures. The SARS-CoV-2 and SARS-CoV are not only phylogenetically related but are morphologically similar. Among the structural proteins, the spike which is a trimeric glycoprotein has only been studied extensively for a short period of time although it is essential for the attachment to the cellular receptor ACE2. In addition to spike, several of the proteins including Mpro, nucleocapsid protein RNA binding domain and nsp10/nsp16 2′-O-methylase are crucial targets for developing antiviral drugs.

10. Future perspectives

The continuous rise in the COVID-19 cases and its severity emphasizes the urgent need of effective preventive and therapeutic measures which crucially depends upon the comprehensive understanding of SARS-CoV-2 structures and replication. Structural perspectives of SARS-CoV-2 biology therefore play a vital role in both identification of effective antiviral therapy and structure

based antigenic determinants for potential vaccine candidates. So far, the crucial structures of SARS-CoV-2 have been resolved in a very short span of time which made it possible to design potential effective therapies and a few vaccines. The current vaccines being developed are mostly based on the spike glycoprotein which should be further investigated with the other potential SARS-CoV-2 proteins.

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12. Author Contributions

SKS conceived the idea. S.K. and S.K.S. collected the data, devised the initial draft and reviewed the final draft. S.K.S., S.K., M.H.V.V.R. and A.L.R. finalized the draft for submission. All authors read and approved the final version of the manuscript.

13. Conflict of Interest

The authors declare no competing financial interest. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. We have not received any specific funding for this work. SK Saxena is supported by CCRH, Government of India. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Legends to the figures:

Figure 1: Genome and Structure of SARS-CoV-2. The genome size of SARS-CoV-2 encodes for 6-11 open reading frames (ORFs) where six functional ORFs are arranged from 5' to 3' as replicase (ORF1a/ORF1b), spike (S), envelope (E), membrane (M) and nucleocapsid (N) encodes for structural proteins as well as seven putative ORFs interspersed between the structural genes and encodes for accessory proteins. The replicase gene covers 67% of the 5′ genome which encodes for a large polyprotein (pp1ab) that gets proteolytically processed into 16 non-structural proteins (nsps). SARS-CoV-2 has surface structural proteins, namely, spike glycoprotein (S), viral membrane glycoprotein (M) and envelope (E) of which are embedded in host membrane-derived lipid bilayer encapsulating the helical nucleocapsid viral RNA.

Figure 2: Replication of SARS-CoV-2. SARS-CoV-2 infection initiated upon attachment of Spike glycoprotein with ACE2 receptor followed by receptor mediated internalization. Following entry of SARS-CoV-2, viral RNA releases in and immediately undergoes translation process to generate ORF1a and ORF1b which gets processed to generate into nsps which forms viral replication and transcription complex. During the synthesis of nsps, viral replication organelles comprises of characteristic double-membrane spherules (DMSs) that all together creates a protective microenvironment for genomic viral RNA replication and transcription of subgenomic RNAs. The structural proteins get translocate into the endoplasmic reticulum (ER) membranes and assembled into nucleocapsid and viral envelope at ER-to-Golgi intermediate compartment (ERGIC). SARS-CoV-2 progeny virions released from the infected cell by the process of exocytosis.

Figure 3: Structures of SARS-CoV-2 proteins. A. Trimeric spike glycoprotein structure showing the closed state of the protein. **B.** Interaction of RBD and ACE2 is showing the involvement of SARS-CoV-2 receptor binding motif (RBM). **C.** Structure of main protease (Mpro, also called 3CLpro) has been shown. **D.** Nucleocapsid protein RNA binding domain has been shown in tetramer form. **E.** Structure of the nsp10/nsp16 2′-O-methylase showing the nsp16 monomer sitting on top of nsp10 monomer.

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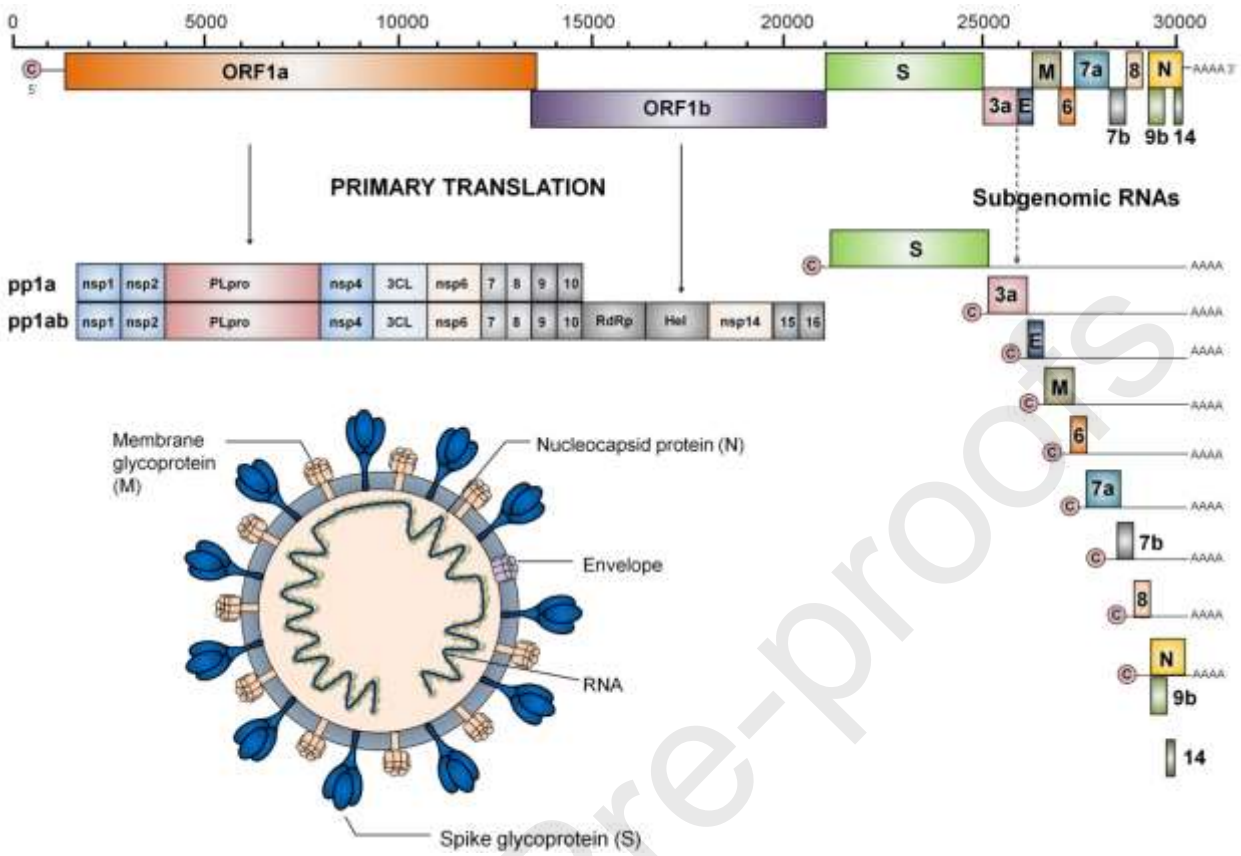

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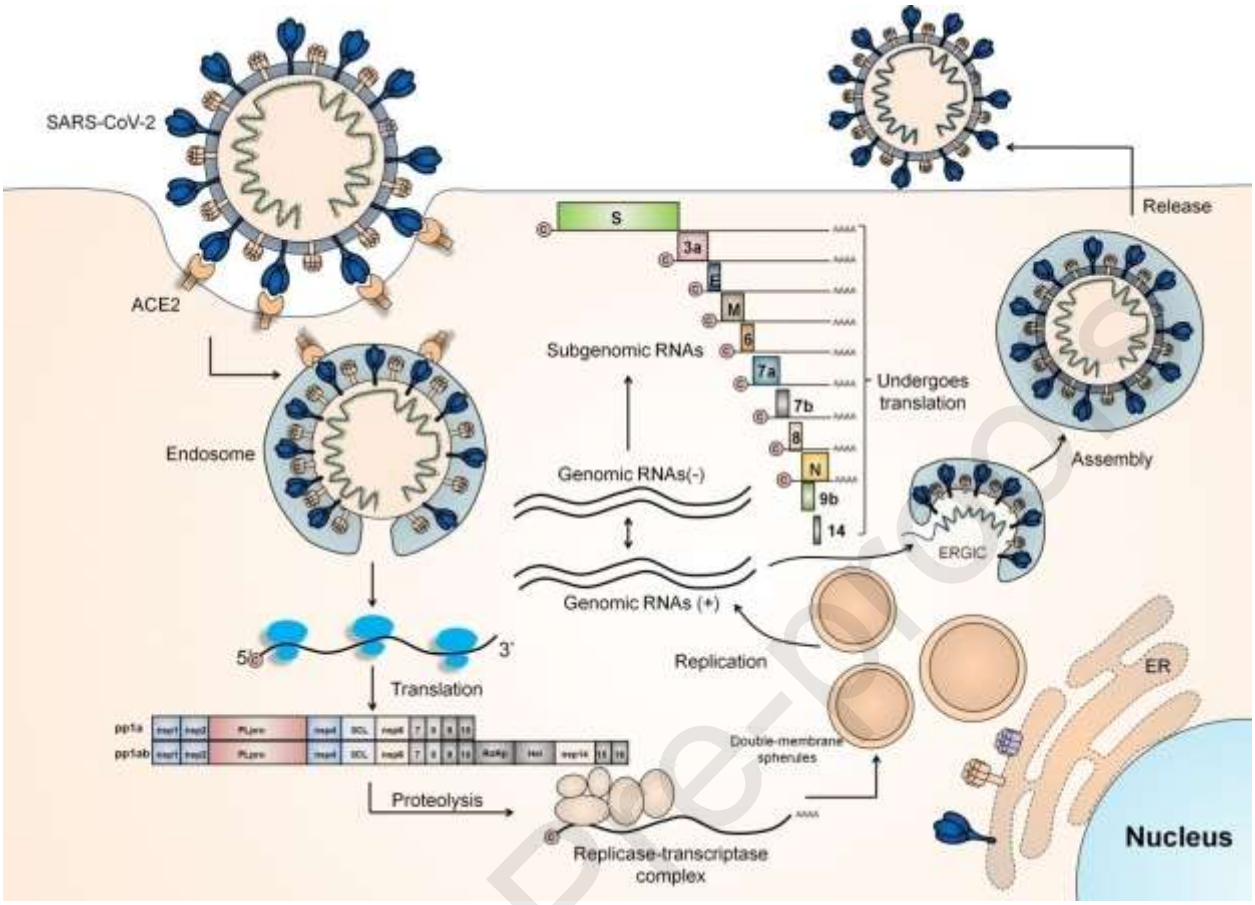

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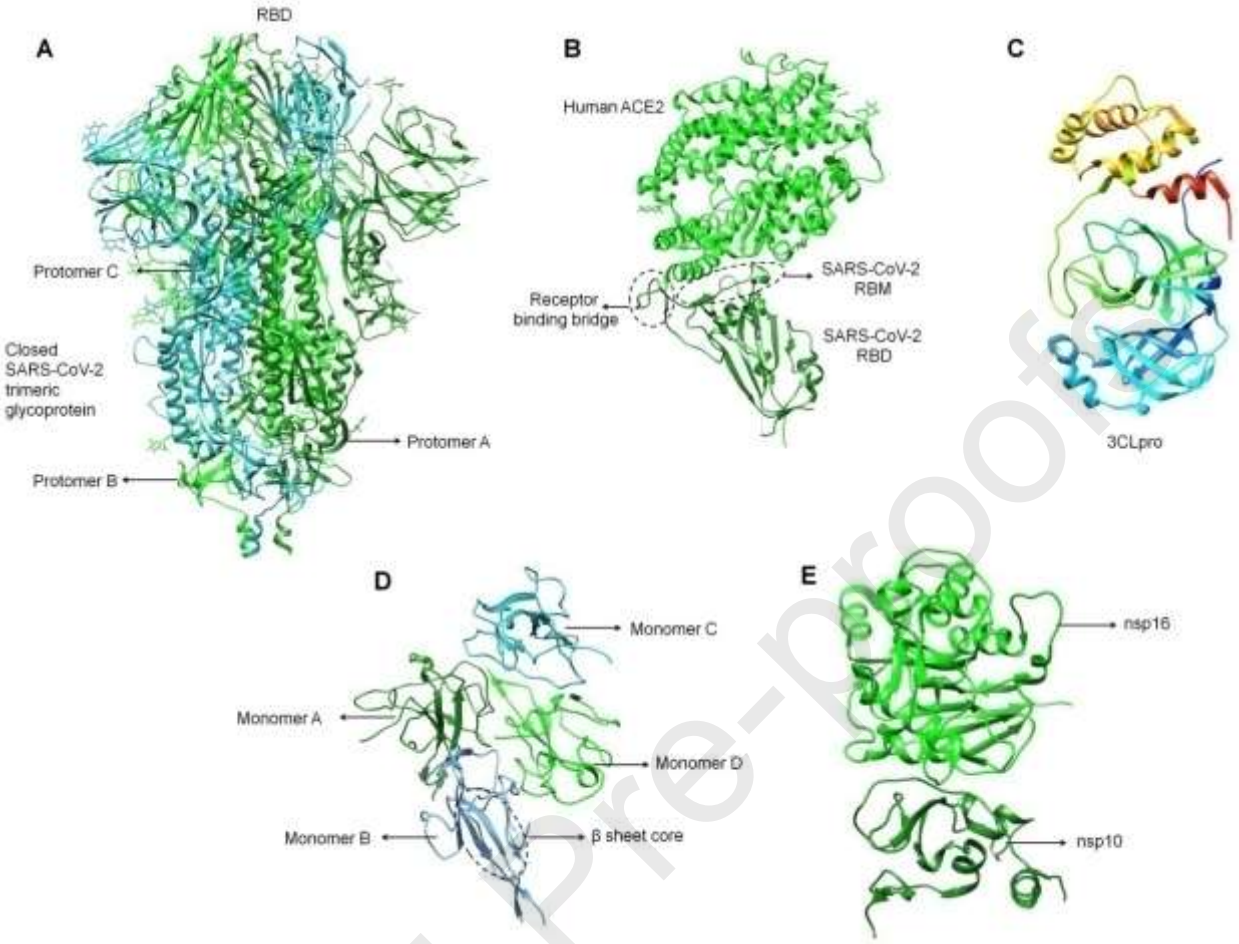

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Highlights:

- Spike glycoprotein undergoes two conformational states i.e. receptor-inaccessible state and receptor-accessible state upon binding to ACE2.
- S1 subunit involved in the attachment to the peptidase domain (PD) of the ACE2 receptor via RBD and S2 subunit involved in cell and viral membrane fusion.
- SARS-CoV-2 and SARS-CoV RBDs use eight identical interacting residues for attachment to the ACE2 receptor.
- Main protease (Mpro) doesn't share cleavage sites with any human encoded proteases; therefore, it can be one of the best drug targets for combating CoVs.

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