An insight into SARS-CoV-2 Membrane protein interaction with Spike, Envelope, and Nucleocapsid proteins

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Abstract

Intraviral protein-protein interactions are crucial for replication, pathogenicity, and viral assembly. Among these, virus assembly is a critical step as it regulates the arrangements of viral structural proteins and helps in the encapsulation of genomic material. SARS-CoV-2 structural proteins play an essential role in the self-rearrangement, RNA encapsulation, and mature virus particle formation. In SARS-CoV, the membrane protein interacts with the envelope and spike protein in Endoplasmic Reticulum Golgi Intermediate Complex (ERGIC) to form an assembly in the lipid bilayer, followed by membrane-ribonucleoprotein (nucleocapsid) interaction. In this study, using protein-protein docking, we tried to understand the interaction of membrane protein's interaction with envelope, spike and nucleocapsid proteins. Further, simulation studies performed up to 100ns agreed that protein complexes M-E, M-S, and M-N were stable. Moreover, the calculated free binding energy and dissociation constant values support the protein complex formation. The interaction identified in the study will be of great importance, as it provides valuable insight into the protein complex, which could be the potential drug targets for future studies.

Keywords: SARS-Co-2 structural protein, Protein-protein interactions, binding free energy, simulation, Membrane Protein, Envelope, Nucleocapsid, Spike Glycoprotein

Introduction

Seven types of coronaviruses infect humans, among which severe acute respiratory syndrome (SARS-CoV), middle east respiratory syndrome (MERS-CoV), and SARS-CoV-2 viruses are primarily focused [1–3]. The coronaviruses structural proteins make up the viral symmetry and enclose the positive-sense single-stranded RNA of ∼30-kb size [1]. The S protein consists of S1 and S2 subunit, which recognizes the human receptor ACE-2 and mediates the viral membrane fusion with the host plasma membrane [4,5]. Whereas the N protein is phosphorylated and highly basic, which primarily function is associated with the packaging of viral genomic RNA [6,7]. The CoV N protein contains two RNA-binding domains: the N-terminal domain and the C-terminal domain, linked by a serine/arginine-rich domain (SRD) [8–11]. The role of SRD is vital for effective virus replication [12]. The M protein is a transmembrane protein consisting of N-terminal ectodomain and a C-terminal endodomain [13–15].

Viruses use Protein-Protein interactions (PPI) to reach out and hijack its host cellular network [16,17]. The virus-host PPI map is invaluable, as it provides insight into the virus behavior and its mode of action [18–20]. Recently, targeting of virus (SARS-CoV-2)-host PPI shows 66 druggable human proteins/host factors targeted by 69 compounds [16]. Experimental techniques such as biomolecular fluorescence complementation, co-immunoprecipitation, and yeast two-hybrid have extensive use to detect virus-host PPI, which also shed light on the intraviral PPI [21–24]. The M protein expressed in higher propensity during infection interacts with N protein and plays a vital role in assembling virus particles [25–27]. The M-M interaction occurs by the transmembrane domain [28]. Further, the C-terminal endodomain is the hotspot for protein-protein interaction with N and S proteins [27,29–32]. Besides the role of M protein's C-terminal in M-N interactions, multiple regions of M protein are responsible for M-E and M-S interactions [26]. In SARS-CoV, the amino acids 168–208 in the N protein are essential for oligomerization and N-M interactions [25]. PPI plays a critical role in stabilizing N protein-RNA interactions [33]. However, the N protein interaction with the C terminal of M protein involves multiple M endodomain regions [28]. But it is not known in the case of SARS-CoV-2 whether these regions interact or not?

On the other side, computational techniques such as protein-protein interaction networks based on phylogeny methods and structure-based protein-protein docking are now very impactful and

faster to identify the interaction sites in protein [34,35]. In this context, we propose to study the protein-protein interaction of M-E, M-S, and M-N of SARS-CoV-2 with protein-protein docking and molecular dynamics simulation (MDS) methods. The primary goal to perform docking is to reveal interaction sites and the generation of protein-protein complexes. Further, atomic-level MD simulations help to characterize the structure and dynamics of protein-protein complexes [36]. In this study, MD allows us to understand the association-dissociation propensity of protein complex during a single trajectory. Moreover, the study's outcome will highlight the mechanistic details, i.e., intermediates and transition state, along with the protein complex's association-dissociation, which could be used as a potential drug target to counter the pathogenicity associated with SARS-CoV-2.

Material and Methods

Protein structure modeling and preparation: Many SARS-CoV-2 proteins structure, i.e., spike, protease, and RdRp, reported by X-ray crystallography or Cryo-EM techniques [37–39]. However, several other proteins, such as full-length nucleocapsid, envelope, and membrane, do not have structure available yet. Therefore, we have utilized the structure models of the envelope, and membrane proteins, generated by the Zhang lab using the I-Tasser web server [40]. Here we also built the model for the full-length 3D structure of S protein and used for protein-protein docking. Firstly, the protein structures prepared using the protein preparation wizard and docked using Schrodinger LLC using our previously defined protocols [41,42].

Protein-protein docking

The PIPER program embedded in the BioLuminate module of Schrodinger for protein-protein docking was implemented to docking M protein with E, S, and N proteins [43,44]. A detailed methodology has been given in our previous report [41]. PIPER performs a global search with Fast-Fourier Transform (FFT) approach and reduces the false-positive results. Among 1000 conformations of input structures, the top 50 clusters were selected with a cluster radius of 9 Å. The docking outcomes based on cluster size were evaluated. With the most massive cluster size, the docked complex out of 5 complexes was selected for molecular dynamics simulation. A total of 70,000 rotations were allowed to generate five docked complexes for all setups.

MD Simulations of protein-protein complexes

For MD simulation of docked protein-protein complex, three setups were generated for M-E, M-N, and M-S proteins. The binding and their interacting stability were observed for a 100 ns timescale. Simulation of these complexes carried in the Desmond simulation package, which utilizes OPLS 2005 forcefield to calculate bonded and non-bonded parameters and energy parameters [45,46]. Previously, the C-terminal region of SARS-CoV M protein was found to interact with N protein [26]. Therefore, in our study, simulation of the M-N protein complex was provided with a lipid bilayer (POPE; 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine) environment around M's transmembrane regions. All systems fed up with the TIP4P water model, 0.15 M NaCl salt, neutralizing counterions, and minimized for 5000 iterations using the steepest descent method. Final production run carried out at an average temperature of 310K, and 1 bar pressure maintained using Noose-Hover chain thermostat and Martyna-Tobias-Klein barostat methods.

Binding energy calculation

PRODIGY (PROtein binDIng enerGY) webserver used to calculate the binding free energy (ΔG) and to predict the dissociation constant (K_d) of the protein-protein complexes [47].

Results

Membrane-Envelope interaction

As shown in **figure 1A**, the protein-protein complex of M and E proteins have been formed by multiple aromatic hydrogen bonds and a pi-pi stacking through N-terminal residues Cys33, Phe37, Tyr39, and His125 of Membrane protein **(figure 1A, Table 1)**. The binding energy calculated for M-E docked complex from the PRODIGY server was -10.1 kcal/mol. Further, the complex was subjected to MD simulations for 100 ns and analyzed for its stability **(Supplementary movie 1)**. We have also calculated the simulated frames' binding energy at every 25ns of the trajectory (**Table 2**). From **figure 1B**, the M-E complex was relatively stable with RMSD at ~6Å up to half simulation time and showed upward fluctuation up to 9Å. The mean changes of M and E protein residues within the interaction site were less compared to the non-interacting region. Similarly, the number of hydrogen bonds found increased between both proteins throughout the simulation period, with an average of ~5.

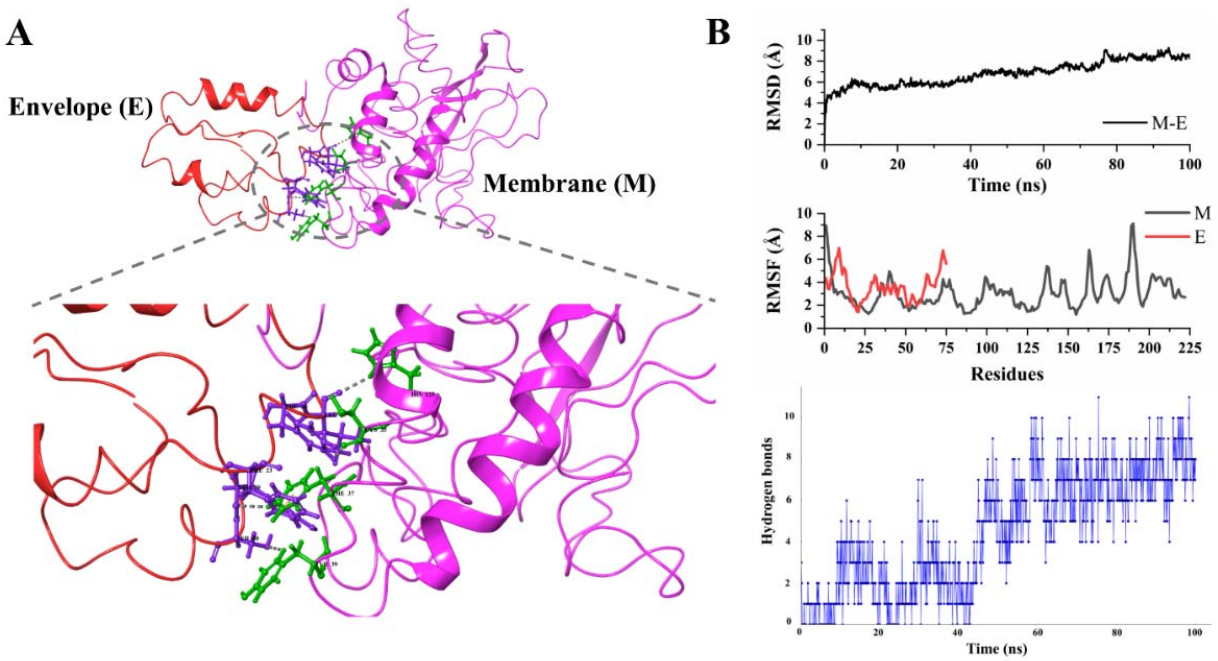

Figure 1: A. Protein-protein docking of M and E proteins structure models. The dashed lines represent the interactions and interacting residues highlighted with ball and stick form in different colors (green of membrane and blue of envelope proteins, respectively). **B.** Molecular dynamic simulation analysis of M-E proteins complexes up to 100 ns depicting RMSD in the upper panel, RMSF in the middle, and Hydrogen bonds formed between these two proteins in the lower forum.

Table 1: Interaction analysis of protein-protein complexes from computational docking.

Proteins	**Membrane Residue**	**Envelope Residue**	**Interaction Type**
Membrane-Envelope	CYS33	PHE26	Aromatic H-bond
	PHE37	PHE23	Pi-pi stacking
	PHE37	TYR59	Aromatic H-bond
	TYR39	SER60	Aromatic H-bond
	HIS125	LUE27	Aromatic H-bond
Membrane-Spike	**Membrane Residue**	**Spike Residue**	

	TRP31	CYS1241	Aromatic H-bond
	TRP31	LYS921	Pi-cation
	TYR39	TYR1209	Pi-pi stacking
	PHE53	CYS1247	Aromatic H-bond
	TRP55	ASP796	Aromatic H-bond
	TRP58	ASN925	Aromatic H-bond
Membrane-Nucleocapsid	**Membrane Residue**	**Nucleocapsid Residue**	
	TRP58	ARG107	Pi-cation
	ASP163	LYS256	H-bond
			Salt bridge
	SER184	TYR268	H-bond and
			Aromatic H-bond

Membrane-Spike interaction

The S protein interacts with M in ERGIC; therefore, these two proteins' docked complex show promising interactions viz. multiple aromatic hydrogen bonds, pi-cation, and pi-pi stacking **(figure 2A)**. The interacting residues of S proteins are at C-terminal, which are represented and tabulated in **table 1**. The binding energy of the M-S docked complex was -18.4 kcal/mol. Further, we have investigated the M-S complex's binding stability through MD simulations upto 100 ns **(Supplementary movie 2)**. The RMSD values from MD simulation trajectory were trending upward from 5 to 20Å up to 40ns but stabilized till the rest of the simulation period. The RMSF plot of the loosely packed S protein model with 1273 residues showed massive fluctuations near 400th-500th residues up to 40Å (**figure 2B**). However, the changes in interacting site residues of S protein's C-terminal is relatively less around 8Å. The binding free energy from the simulation trajectory of M-S complexes represented in **table 2.** The last frame at 100ns has

shown ΔG value -20.3 kcal/mol, and K_d value of 5 * 10^{-15} M. In final, the average number of hydrogen bonds were ~16 in M-S complex simulation setup throughout the MD period.

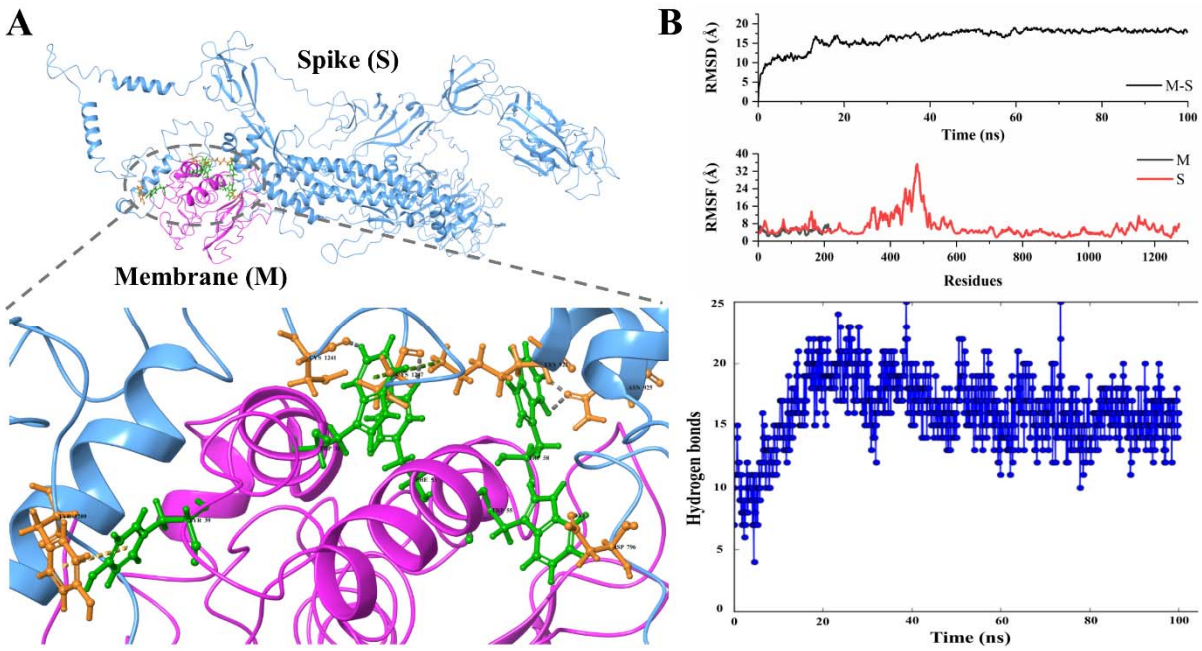

Figure 2: A. Protein-protein docking of M and S proteins structure models. The ball and stick represent the interacting residues in different colors (green of membrane and orange of spike). **B.** Molecular dynamics simulation analysis of M-S proteins complex up to 100ns depicting RMSD in the upper panel, RMSF in the middle, and Hydrogen bonds formed between these two proteins in the lower forum.

Table 2: Binding energy calculation of protein-protein complexes from PRODIGY webserver. The complexes are selected at every 25ns of simulation trajectory and compared with the docked complex (obtained from protein-protein docking).

Protein-protein complex	**MD Frame**	**Binding energy (kcal/mol)**	**Predicted dissociation constant (K_d) (M)**
Membrane-Envelope	Docked Complex	-10.1	8.1 * 10^{-8}
	25ns	-8.9	5.4 * 10^{-7}
	50ns	-9.2	3.5 * 10^{-7}
	75ns	-9.5	2.1 * 10^{-7}

	100ns	-7.6	$4.6 * 10^{-6}$
Membrane-Spike	Docked Complex	-18.4	$1.1 * 10^{-13}$
	25ns	-19.2	$2.7 * 10^{-14}$
	50ns	-19.2	$2.9 * 10^{-14}$
	75ns	-19.6	$1.5 * 10^{-14}$
	100ns	-20.3	$5 * 10^{-15}$
Membrane-Nucleocapsid	Docked Complex	-8.3	$1.4 * 10^{-6}$
	25ns	-8.9	$5 * 10^{-7}$
	50ns	-11.5	$7.2 * 10^{-9}$
	75ns	-11.9	$4.2 * 10^{-9}$
	100ns	-11.3	$1.2 * 10^{-8}$

Membrane-Nucleocapsid interaction

The protein-protein docking of M-N complex showed a total of three residues of N protein viz. Arg107, Lys256, and Tyr268 are interacting with residues Trp58, Asp163, and Ser184 of M protein (**Figure 3A, Table 1**). The docked complex M-N has attained the binding energy of -8.3 kcal/mol. Based on simulation analysis, the M-N protein-protein complex was found stable **(Supplementary movie 3)** with an average RMSD of approx. 6.8 Å **(Figure 3B)**. The number of intermediate hydrogen bonds formed within the simulation setup was ~ 7 up to 100ns timescale. However, there was a fluctuating trend in RMSF values throughout the simulation from 2Å to 6Å in N protein residues. These fluctuations may be due to high disorder propensity in N protein. The RMSF values of interacting residues of M protein were 1.7 Å (Trp58), 1.2 Å (Arg107), 2.1 Å (Asp163) and for N protein 4.9 Å (Lys256), 2.2 Å (Ser184), and 2.9 Å (Tyr268) for 100ns simulation period **(Figure 3B)**. The binding free energy of complexes from the simulation trajectory was higher than the complex obtained from protein-protein docking (-8.3 to 11.3 kcal/mol, **Table 2**).

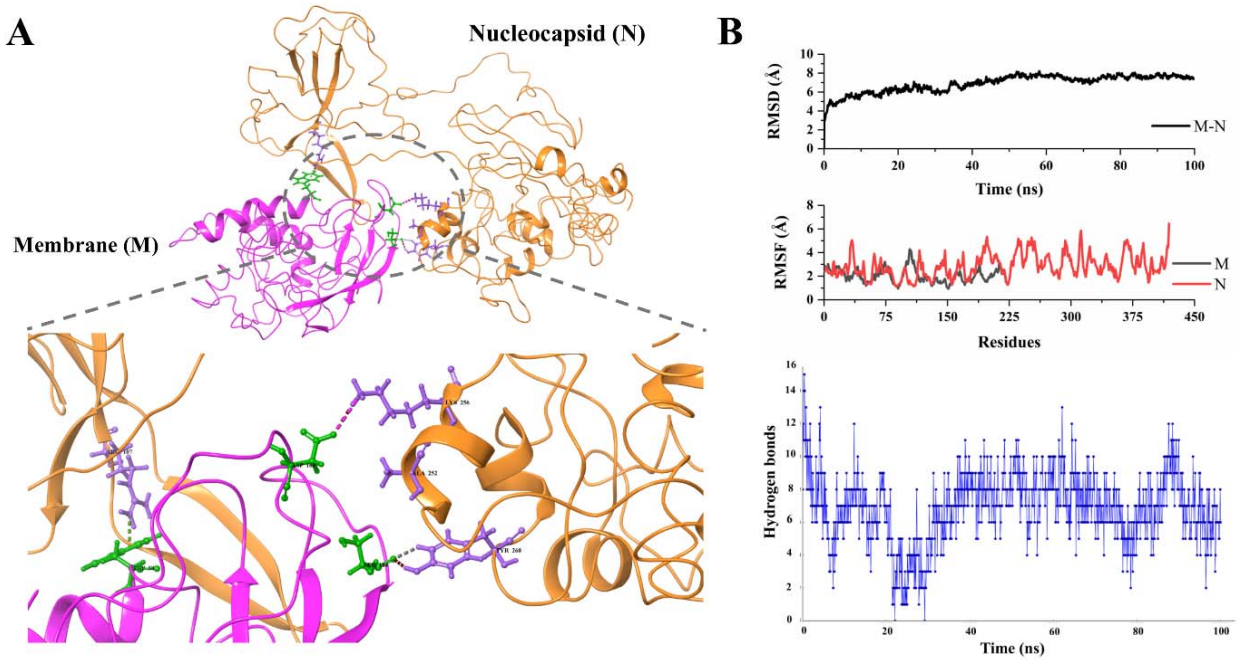

Figure 3: A. Protein-protein docking of M and N proteins structure models. The ball and stick represent interacting residues in different colors (green of Membrane and purple of Nucleocapsid). **B.** Molecular dynamics simulation analysis of M-E proteins complex up to 100ns depicting RMSD in the upper panel, RMSF in the middle, and Hydrogen bonds formed between these two proteins in the lower forum.

Discussion

Intraviral Protein-Protein interactions play an essential role in the coronavirus life cycle, specifically during the replicating complex formation as elucidated from several structural studies [48–50]. The RNA dependent RNA polymerase (nsp12) of SARS-CoV interacts with nsp7 and nsp8 and increases the RNA-synthesizing activity [48]. The nsp12-nsp7-nsp8 also associate with the nsp14 (proofreading enzyme) [48]. The cryo-EM studies showed that the nsp7 and nsp8 heterodimers stabilize RNA binding regions of nsp12, while the second subunit of nsp8 plays a vital role in polymerase activity [49]. Further, structural studies showed that nsp10 interacts with the N-terminal domain of nsp14 to stabilize it and stimulate its activity [50].

Similarly, the SARS-CoV structural proteins have been reported to interact with each other and play an essential role in virus assembly [6,15,28]. Therefore, in this study, we report the

intraviral PPI among structural proteins of SARS-CoV-2, where we have computationally shown that the M proteins interact with other structural proteins to form complexes of M-E, M-S, and M-N, which responsible for the proper virus assembly. We have performed protein-protein docking to identify the regions and residues which interact during these bindings. We have investigated these in membrane protein with several interacting structural proteins such as envelope, spike, and nucleocapsid proteins, respectively. Previously, in SARS-CoV, mutation-based studies showed that M protein is vital for virus assembly and interact with other structural proteins [26]. The entire C-terminus domain of M proteins was found to interact with N protein [26,29,31]. Similarly, two transmembrane domains and the cytoplasmic domain of M protein were reported to interact with E protein [26]. There are multiple regions of M protein that interact with spike glycoprotein [26]. Therefore, we have considered the M protein as a receptor and S, E, and N proteins as protein ligands, in this study. The M protein is a triple spanning membrane protein, and cytosolic side region is solely responsible for the interaction of M-N; therefore, in the case of M-N docking, cytosolic part of M protein was targeted for interaction with N protein.

To understand the stability of docked complexes and formed interactions, we have performed long MD simulations. The simulation studies showed resilience in docked protein complexes of M-E, M-S, and M-N. The binding energy was found in good agreement with the results and allowed good binding of intraviral structural proteins. Our computational studies agree with previous reports, where particle assembly occurs in the Endoplasmic Reticulum-Golgi intermediate compartment (ERGIC) and finally trafficked for release via exocytosis [51] (**Figure 4)**

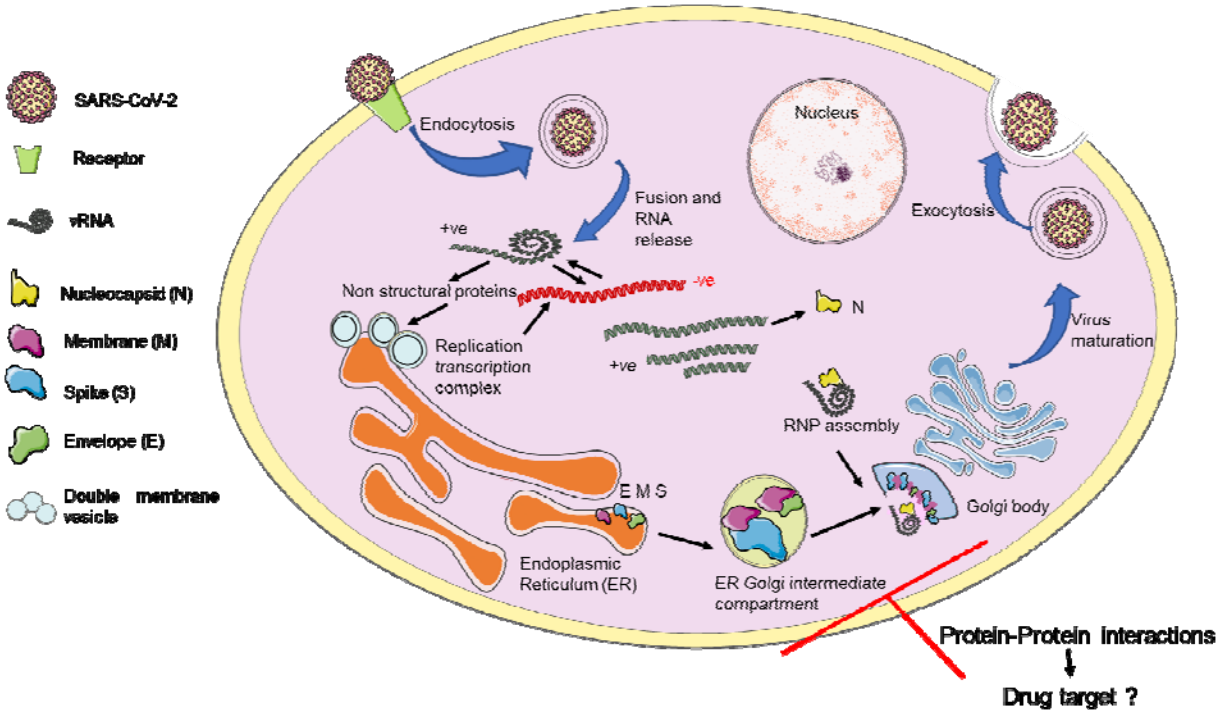

Figure 4. Schematic representation of protein-protein interactions among SARS-CoV-2 structural proteins (Membrane, Spike, Nucleocapsid, and Envelope).

Conclusion

Despite the small genome of viruses, they are highly pathogenic/infectious, and their genome integrity allows them to hijack the cellular machinery. Viruses for rapid infection and replication follow multiple pathways. In between regulating host cellular system, it is essential to co-ordinates among own proteins for proper assembly and genome encapsulation. Here, PPI plays an important role in coronaviruses where structural protein interacts with each other, encapsulate the genome, and forms mature viruses. It could be a great interest to study these PPIs in drug targeting, as disruption of virus assembly will lead to immature virion formation. In this context, the present study may help to design the mutation-based study to understand PPI in SARS-CoV-2. Further, it is still an open question of how these structural proteins interact specifically in the presence of several host proteins? What are the driving forces which lead to the formation of proteins assembly and virus particle formations? Additional studies on binding mechanism and energy favorable interaction of structural protein could help us develop new strategies against protein-protein inhibition.

Author Contribution

RG, NG: study supervision and designed the experiment. AK and PK acquisition and interpretation of computational data. AK, PK, and RG contributed to paper writing. # Authors contributed equally.

Declaration of competing interest

All authors affirm that there are no conflicts of interest.

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Conflict of Interest

All authors affirm that there are no conflicts of interest.

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