The 3a protein of SARS-CoV-2 is a potential calcium binding protein

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Short Report

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The 3a protein of SARS-CoV-2 is a potential calcium binding protein

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Abstract

The 3a protein is an accessory protein of SARS-CoV-2. We present here the result of bioinformatics analysis based on cryo-EM structure of 3a protein that has predicted calcium binding, majorly in cytoplasmic domain of 3a protein, thereby providing crucial information for further understanding of viral pathogenesis in context to its effect on cellular calcium signaling. The prediction analysis has also shown binding of antiviral drug amantadine with 3a protein of SARS-CoV-2.

The SARS-CoV-2 being the etiological agent of COVID-19 has overtly presented symptoms beyond pulmonary injury, where patients have conspicuously shown gastrointestinal problems. The RNA genome of SARS-CoV-2 has several accessory proteins encoded by open reading frames (ORFs) that are unique to human coronaviruses. Among them is the 3a protein, encoded by ORF3a of SARS-CoV-2, has been known to share 72% sequence similarity with the 3a protein of SARS-CoV-1. Being a membrane protein with three transmembrane domains and a large cytoplasmic domain, the 3a protein of SARS-CoV-1 has been shown to form an ion channel (viroporins) functioning in modulation of virus release [1]. It has been previously shown that the cytoplasmic domain of SARS-CoV-1 3a protein binds calcium and undergoes significant changes in its secondary structure [2]. Although structural information on SARS-CoV-1 3a protein is lacking an account of the absence of crystal structure data but Kern *et al* have presented structural analysis of the dimeric as well as tetrameric SARS-CoV-2 3a protein in lipid nanodiscs through cryo-electron microscopy (cryo-EM) [3].

Calcium has been a predominant 'second messenger' in our signaling cascade that has been agreeably studied to be exploited by many viruses for their various requirements of multiplication cycle [4]. An example of rotavirus NSP4illustrates how effectively the virus calcium binding protein engages the cellular calcium signaling for virus replication resulting in severe gastrointestinal complications [4]. The NS1-2 protein of Tulane virus act as viroporin that interrupts cellular calcium signaling for its own replication benefit [4]. The viroporin p7 of HCV also functions as Ca^{2+} ion channel [4].

The cryo-EM study on SARS-CoV-2 3a protein has reported that the ion channel formed by the protein has modest selectivity for Ca^{2+} and K^{+3}. Since, the cytoplasmic domain of SARS-CoV-1 3a protein has been shown to bind Ca^{2+}, we carried out the prediction of potential Ca^{2+} binding sites on the 3a protein of SARS-CoV-2 [2].The three-dimensional cryo-EM structure of SARS-CoV-2 3a protein (PDB ID: 6XDC) was retrieved from RCSB Protein Data Bank (https://www.rcsb.org) and used as a model for molecular docking. The 3a protein has three transmembrane domains with a long cytoplasmic domain (Figure 1A). The prediction for binding of $Ca^{2+}$was done on Metal Ion-Binding Site Prediction (MIB) and Docking Server [5]. Using the fragment transformation method that searches for structure similarity between SARS-CoV-2 3a protein and available Ca^{2+} binding residue templates, the web server predicted five potential Ca^{2+} binding molecular sites (S1, S2, S3, S4 and S5) in the 3a protein of SARS-COV-2. This was further elaborated to generate the 3D metal ion

bound state of protein structure (Figure 1B). The threshold score for Ca^{2+} binding at S1, S2, S3, S4 and S5 were 1.288, 1.529, 1.265, 1.644 and 1.32 respectively (Figure 1C).

The M2 viroporin of influenza A virus has been shown to be disrupted by antiviral amantadine [6]. A case study on COVID-19 patients with neurological diseases though declaring limitation of small sample size has advocated the use of amantadine in support of the finding that the antiviral drug might interfere with lysosomal gene expression [7, 8]. We therefore, conducted molecular docking on Auto Dock Vina wherein amantadine was used as ligand against the 3D structure of SARS-CoV-2 3a protein[9]. The resultant receptor-ligand complex was visualized through PyMOL and UCSF chimera 1.9[10, 11]. Amantadine binding displayed interactions with the participant amino acids 75K, 76G, 78H, 79F, 123I, 122R 126R, 139L of SARS-CoV-2 3a protein(Figure 2). The Gibbs free energy value for the binding of amantadine with 3a protein of SARS-CoV-2 is -4.8 kcal/mol.

Many enteric viruses have been studied for their requirement of higher cytosolic Ca^{2+} that facilitates in their replication [12]. Viroporins like calcivirus NS1-2 are actively involved in anomalous Ca^{2+} signaling [12]. Hepatitis C virus (HCV) has been reported to upsurge mitochondrial Ca^{2+}uptake leading to apoptosis [13]. Moreover, the 3a protein of SARS-CoV-2 has been shown to induce apoptosis in cells [14]. The 2002-2003 SARS-CoV-1 infection presented diarrhoea as one of the major clinical manifestation due to intestinal tropism of the virus, this led to amplification of virus transmission route through oral-faecal mode [15]. Furthermore, COVID-19 patients have also shown diarrhoea where SARS-CoV-2 has been reported to be detected in the stool samples of the patients [16].

Today, world is facing tremendously high rate of SARS-CoV-2 infection. In the absence of a certified antiviral drug, the outbreak of COVID-19 has put pressure on our healthcare system. Our finding predicts 3a protein of SARS-CoV-2 to be a potential calcium binding viroporin showing good binding probability with amantadine. We strongly recommend further structural and functional studies based on the calcium binding potential of SARS-CoV-2 3a protein aiming at disentangling the silent aspects of the viral pathogenesis.

Acknowledgments

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be constructed as a potential conflict of interest.

Methods

The 3Dstructure of SARS-CoV-2 3a protein was retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB)(https://www.rcsb.org)(PDB ID: 6XDC) and used as model for molecular docking.

Molecular docking of Ca^{2+} on 3a protein

The online server Metal Ion-Binding Site Prediction and Docking Server was used to predict the potential Ca^{2+} binding sites on 3a protein [5]. The server predicted residues with metal ion-binding sites through the method of fragment transformation wherein the protein structure in query is subjected to comparison with various metal ion-binding templates in the database and generated 3D structure of 3a protein in Ca^{2+} bound state.

Molecular Docking of 3a protein with Amantadine

The structure of antiviral drug amantadine was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) and was converted to PDBQTs with PyRx [17]. The execution of amantadine docking with 3a protein was carried out on Auto Dock Vina [9]. The resultant 3a protein- Ca^{2+} complex was visualized and studied on PyMOL and UCSF chimera [10, 11].

References:

1. Lu W, *et al.* Severe acute respiratory syndrome-associated coronavirus 3a protein forms an ion channel and modulates virus release. *Proc Natl Acad Sci U S A* **103**, 12540-12545 (2006).

2. Minakshi R, Padhan K, Rehman S, Hassan MI, Ahmad F. The SARS Coronavirus 3a protein binds calcium in its cytoplasmic domain. *Virus Res* **191**, 180-183 (2014).

3. Kern DM, *et al.* Cryo-EM structure of the SARS-CoV-2 3a ion channel in lipid nanodiscs. *bioRxiv*, (2020).

4. Zhou Y, Frey TK, Yang JJ. Viral calciomics: interplays between Ca2+ and virus. *Cell Calcium* **46**, 1-17 (2009).

5. Lin YF, Cheng CW, Shih CS, Hwang JK, Yu CS, Lu CH. MIB: Metal Ion-Binding Site Prediction and Docking Server. *J Chem Inf Model* **56**, 2287-2291 (2016).

6. Thomaston JL, Polizzi NF, Konstantinidi A, Wang J, Kolocouris A, DeGrado WF. Inhibitors of the M2 Proton Channel Engage and Disrupt Transmembrane Networks of Hydrogen-Bonded Waters. *J Am Chem Soc* **140**, 15219-15226 (2018).

7. Smieszek SP, Przychodzen BP, Polymeropoulos MH. “Amantadine disrupts lysosomal gene expression; potential therapy for COVID19”. *bioRxiv*, 2020.2004.2005.026187 (2020).

8. Rejdak K, Grieb P. Adamantanes might be protective from COVID-19 in patients with neurological diseases: multiple sclerosis, parkinsonism and cognitive impairment. *Mult Scler Relat Disord* **42**, 102163 (2020).

9. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* **31**, 455-461 (2010).

10. Seeliger D, de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *J Comput Aided Mol Des* **24**, 417-422 (2010).

11. Pettersen EF*, et al.* UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605-1612 (2004).

12. Strtak AC, Perry JL, Sharp MN, Chang-Graham AL, Farkas T, Hyser JM. Recovirus NS1-2 Has Viroporin Activity That Induces Aberrant Cellular Calcium Signaling To Facilitate Virus Replication. *mSphere* **4**, (2019).

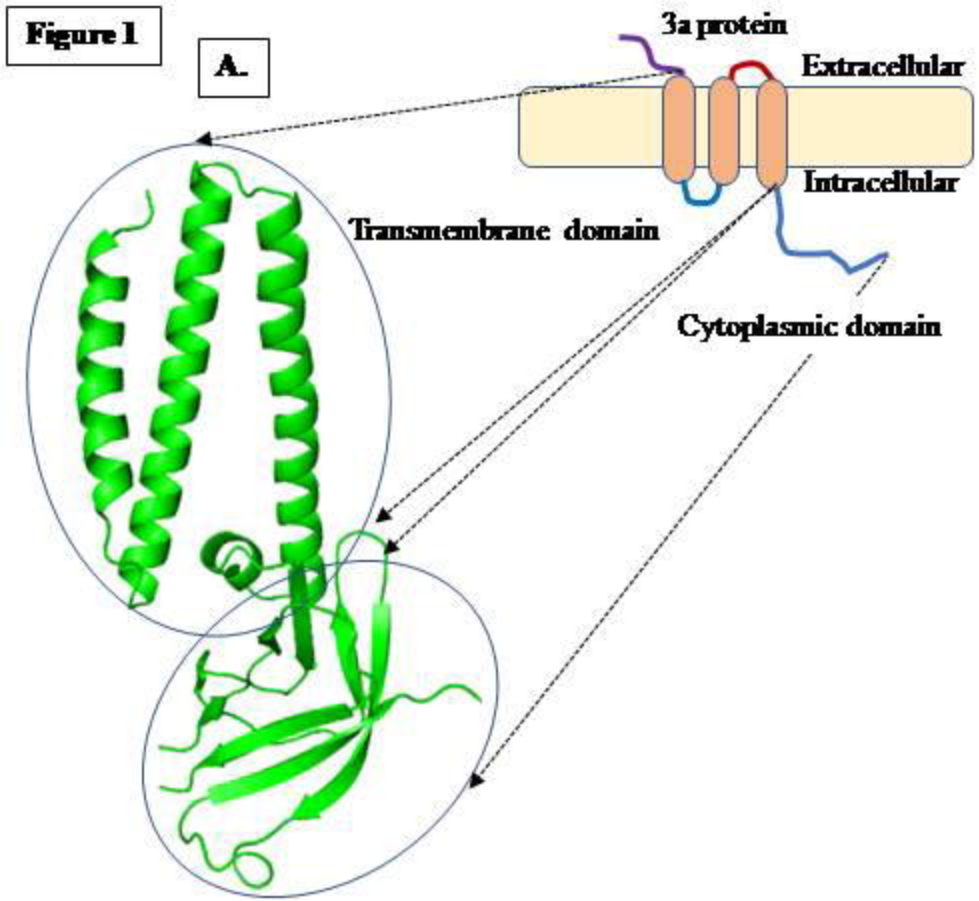
13. Brault C, Levy PL, Bartosch B. Hepatitis C virus-induced mitochondrial dysfunctions. *Viruses* **5**, 954-980 (2013).

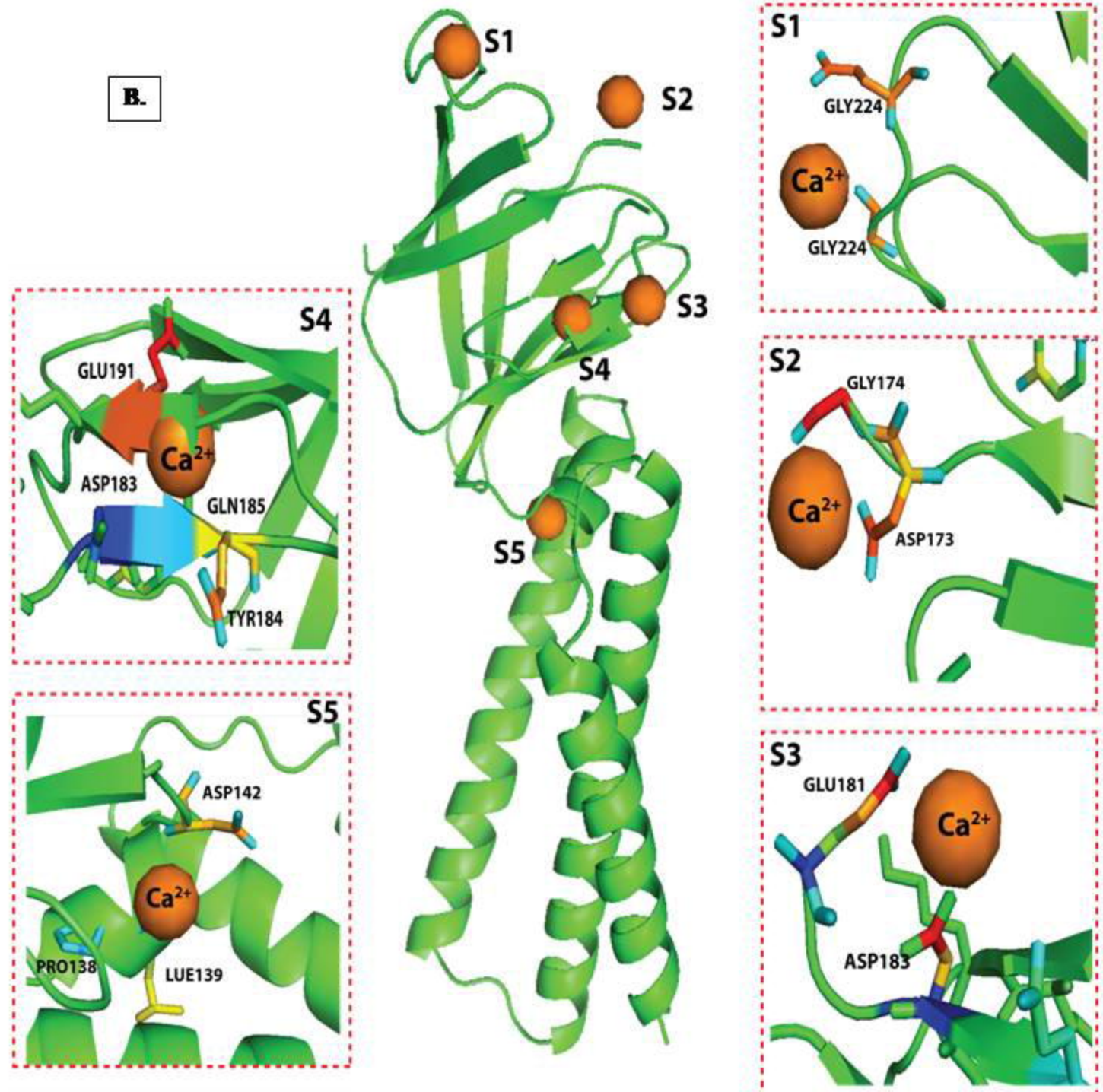
14. Ren Y*, et al.* The ORF3a protein of SARS-CoV-2 induces apoptosis in cells. *Cell Mol Immunol* (2020).

15. Leung WK*, et al.* Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. *Gastroenterology* **125**, 1011-1017 (2003).

16. Gu J, Han B, Wang J. COVID-19: Gastrointestinal Manifestations and Potential Fecal-Oral Transmission. *Gastroenterology* **158**, 1518-1519 (2020).

Figure 1





C.

Sites	Binding Residues	Template PDB ID	Binding Score
S4	183D, 184Y, 185Q	1djwB2	1.644
S2	173D, 174G	1cvmA1	1.529
S5	138P, 139L, 142D	1byh_0	1.321
S1	224G, 226E	1guaB0	1.288
S3	181E, 183D	1lf0A1	1.265

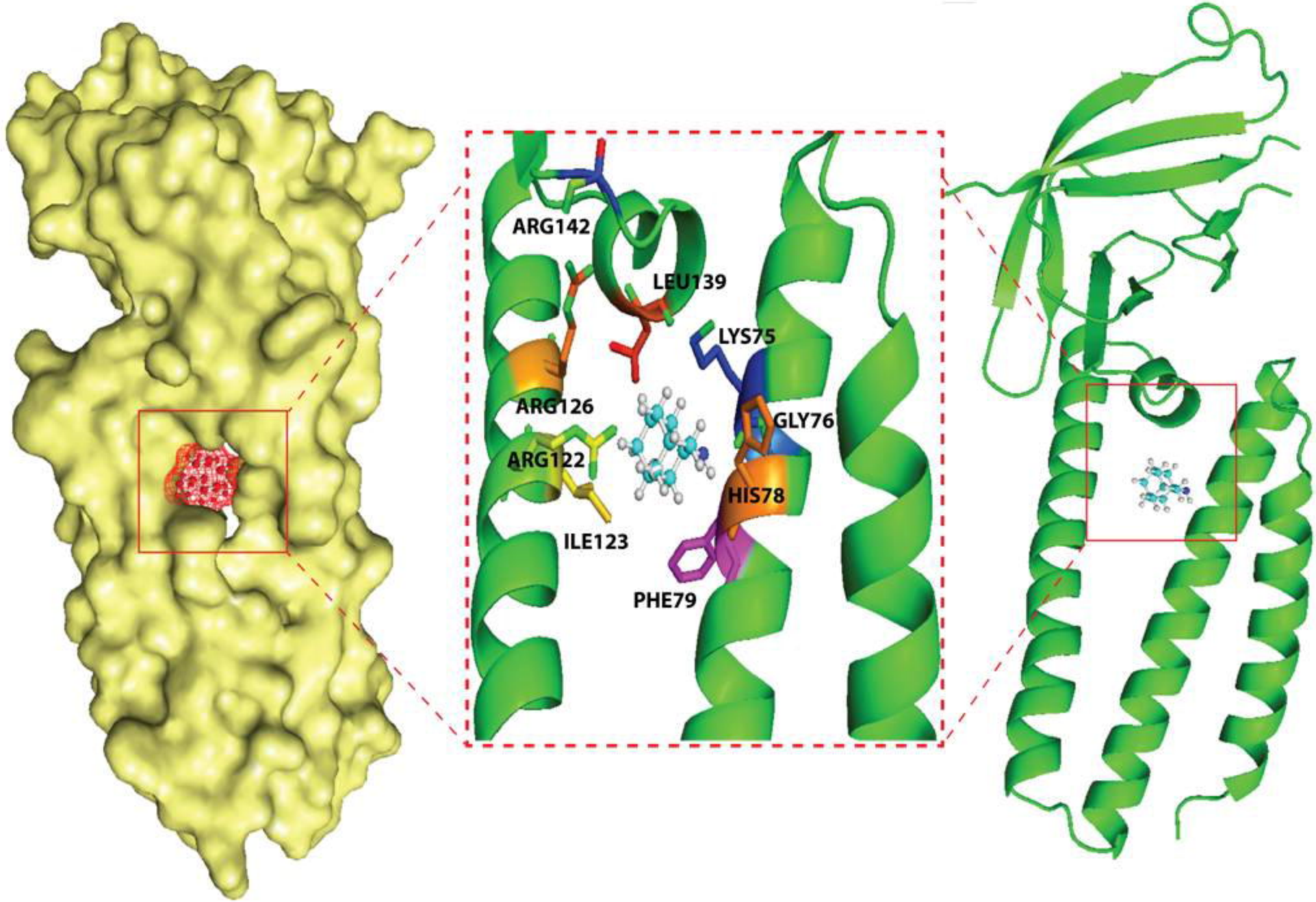

ARG142
LEU139
LYS75
ARG126
GLY76
ARG122
HIS78
ILE123
PHE79

Figure 2

Figures

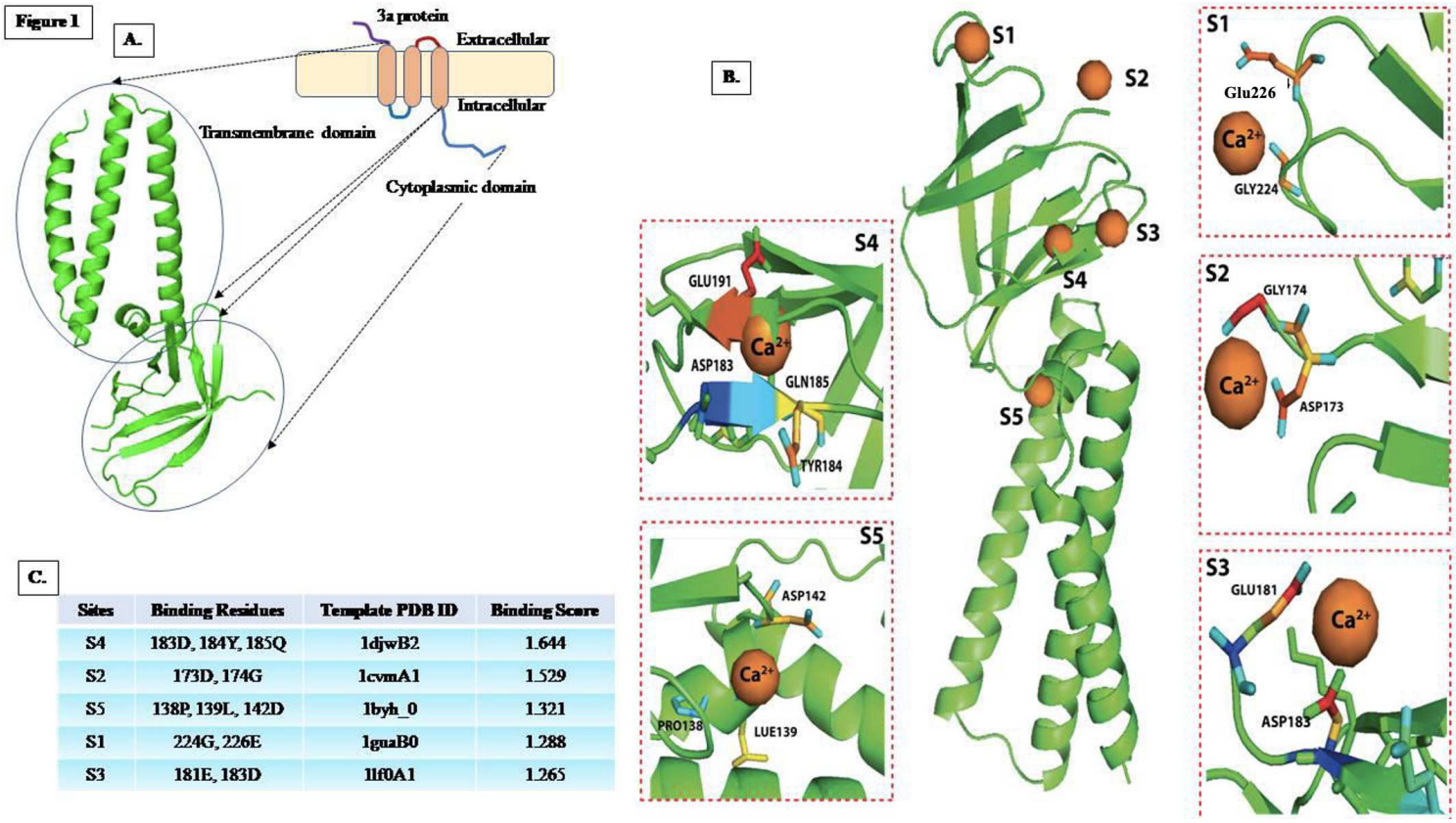


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Figure 1

(A) Cartoon of membrane spanning 3a protein of SARS-CoV-2. The transmembrane domain has three potential helices whereas the cytoplasmic domain is rich in beta sheets. (B) The docking analysis shows five potential calcium binding motifs in various regions of 3a protein. Ca^{2+} binding between residues are S1: Glycine 224 and Glutamic acid 226, S2: Aspartic acid 173 and Glycine 174, S3: Glutamic acid 181 and Aspartic acid 183, S4: Aspartic acid 183, Tyrosine 184 and Glutamine 185, S5: Proline 138, Leucine 139 and Aspartic acid 142. (C) The relative binding scores of all the potential Ca^{2+} binding sites in 3a protein.

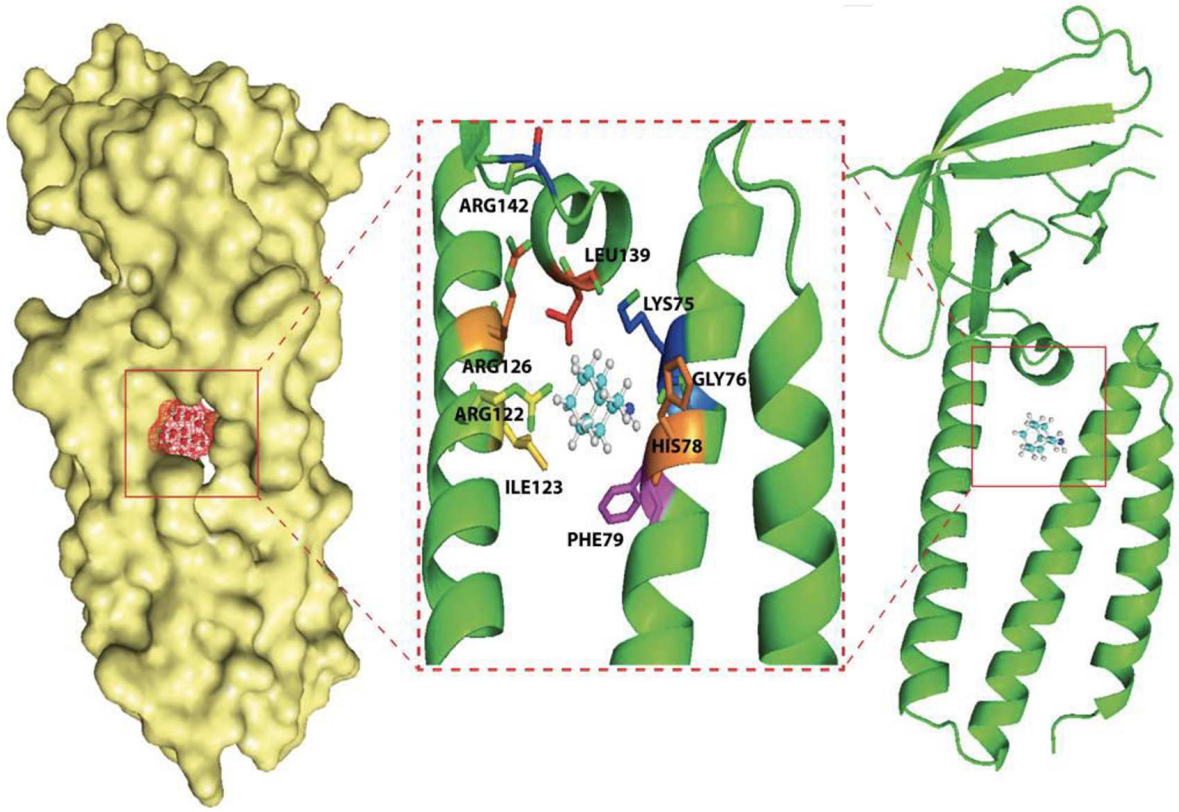

Figure 2

Figure 2

Molecular docking of 3a with amantadine showing the participating amino acid residues in the hydrophilic face of potential pore channel formed by 3a protein.