Structural Perspective on Molecular Interaction of IgG and IgA with Spike and Envelope Proteins of SARS-CoV-2 and Its Implications to Non-Specific Immunity

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a ruthless killer of the human population and highly transmissible, has become a big threat to public health by spreading one of the most infectious coronavirus diseases (COVID-19). Vaccine production is of paramount importance at present, albeit it is a gradual and time taking process. Since the predicament demand is immediate prevention, we hypothesized the utility of IgG2a LA5 and IgA antibodies developed inside the body after vaccination to assess its protective effects as non-specific immunity against SARS-CoV-2. Identifying the vaccine for repurposing, we considered the C-terminal domain of spike protein (S1-CTD) and envelope (E) protein for molecular interactions with aforesaid antibodies using computational and Bioinformatics tools in order to elucidate its practicality and applicability. Our in silico findings exhibited the involvement of S1-CTD and E-protein hotspot residues as key players in molecular interaction with IgG2a LA5 and IgA and exhibited better binding efficiency (higher negative ΔG and lower Kd values) in comparison to their cognate host receptors (ACE2 and MPP5). Detailed hotspot residue analysis of S1-CTD and E-protein with IgG2a LA5 and IgA indicates that the existing vaccine could be used as a preventive measure against SARS-CoV-2.

Keywords: SARS-CoV-2; COVID-19; IgG2a LA5; IgA; ACE2; MPP5.

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1. Introduction

The recent outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously known by the provisional name 2019 novel coronavirus (2019-nCoV), in the city of Wuhan in China’s Hubei province in 2019–2020 has been causing significant numbers of mortality and morbidity in humans with the coronavirus infection disease (COVID-19) showing fever, severe respiratory illness, and pneumonia [1]. The exponential growth of COVID-19 cases throughout the world has put tremendous pressure on the scientific fraternity to discover and produce preventive and effective treatments in a short span of time. With over 22,000 publications and >2000 ongoing clinical trials for vaccine and/or oral antiviral drugs,
they show tireless efforts to develop effective therapy against SARS-CoV-2 infection [2]. Moreover, identification and complete annotation of about 56 proteins cognate to humans and SARS-CoV-2 facilitate extra edge on its underlying infection mechanism [3].

Deciphering and distributing a promising vaccine to enhance the immunity of billions of people worldwide due to the relatively high rate of infection caused by intensive SARS-CoV-2 and subsequent mortality is highly desirable at the earliest. This has led to unprecedented scientific collaborations and the exchange of scientific information worldwide among the leading pharmaceutical industries and research laboratories to achieve the common goal of procuring an effective vaccine at the earliest. In this context, various developing vaccines seem to produce non-specific immune effects apart from showing pathogenic specificity.

Further, a lot of curiosity has also been seen in looking retrospectively at the already developed antiviral drugs and vaccines for their effectiveness against the SARS-CoV-2 [4-10]. The presently available live attenuated vaccines viz., smallpox vaccine, Bacillus Calmette-Guérin (BCG), Measles, and oral Poliovirus vaccine (OPV) also induce non-specific effects on the immune system [11-13]. It may be noted that various immunological mechanisms mediating non-specific effects include the induction of protective immune response and clonal expansion of lymphocytes against non-self pathogens, thereby boosting the innate immunity via inheritable changes and metabolic adaptability [14].

SARS-CoV-2, one of the seventh coronaviruses known to spread disease in human, is basically a cytoplasmic replicating pathogen with positive-polarity, single-stranded RNA (ssRNA) genome, structurally consists of four major membrane-bound proteins viz., spike (S), envelope (E), and membrane (M) and nucleocapsid (N) glycoproteins along with few subsidiary proteins [15,16]. Virus entry into the host cells is mediated through interactions between spike (S) glycoprotein, a homotrimer, and angiotensin-converting enzyme 2 (ACE2). S-protein consists of two domains S1 and S2, in which S1 contains the N-terminal domain (NTD) and C-terminal domain (CTD), collectively known as receptor binding site. Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) previously used S1-CTD as a receptor-binding domain (RBD). At the same time, S1-NTD interacts with its cognate receptor in mouse hepatitis coronavirus. However, identifying the exact receptor binding site of SARS-CoV-2 with its host receptor ACE2 is underway [17-19].

Notwithstanding targeted vaccine pursuit, clinical assessment of non-specific vaccines, e.g., BCG against COVID-19 in some countries like Australia and The Netherlands, is underway. The logic behind this pursuance was a comparison of deaths occurring in countries getting a predominant neonatal vaccination exhibited lower incidence as compared to the countries lacking deep-rooted vaccination [7]. It is well established through pre-clinical and clinical studies that the BCG vaccine exhibited non-specific immune response; however, due to lack of experimental proof and clinical relevance of its protective effect against COVID-19 WHO does not recommend it [8-10].

Since all viral pathogens use some cellular receptors to enter the host cell, it is imperative to explore the receptor interaction site for virulence susceptibility. Being a vital part of pathogen-host entry gateway and cellular adapter building blocks, the glycoproteins have been gaining high momentum towards designing immunotherapeutics and prophylactic vaccines against COVID-19. However, the traditional route of vaccine development taking between 5-10 years correspondingly put forth a formidable challenge. Nonetheless, great
strides have been made by the Coalition for Epidemic Preparedness Innovations (CEPI) and GAVI, the Vaccine Alliance, to take the challenge in a coordinated fashion in financing, development, production, and distribution of vaccines for SARS-CoV-2 [5,6,20].

In the light of scientific data supporting non-specific protective immunity, it is highly desirable to look into the common interface residues of viral glycoproteins and host receptor molecules. Subsequent identification of the pathogen's interacting residues at the interface of their cognate neutralizing antibodies of host developed upon exposure of past infection seems to establish the most probable structural insight into the underlying mechanism of non-specific immunity. This study considers two antibodies (Abs) IgG2a LA5, and IgA for molecular interaction studies with SARS-CoV-2 S-and E-glycoproteins through molecular docking simulation. Identified residues at the interface of S1-CTD protein and the most plausible binding site of E-protein with aforesaid Abs shed light on the elucidation of viral pathogenesis at the structural level and predict important clues for the establishment of underlying non-specific defense mechanisms.

2. Materials and Methods

2.1. Structure retrieval of viral and cellular proteins.

3D structure of S1-CTD protein co-crystallized with host receptor ACE2 (PDB ID: 6LZG) [15], Fragment antigen-binding (Fab) domain of IgG2a LA5 (PDB ID: 4EBQ) [21], Fab fragment of IgA (5KW9) [22], and MPP5 (https://covid-19.uniprot.org/uniprotkb/Q8N3R9) a cellular interactant of E-protein (PDB ID: 4UU5) [23] were retrieved from research collaboratory for structural bioinformatics (RCSB) protein data bank (PDB) Due to the unavailability of E-protein 3D structure in RCSB PDB, we checked it through Zhang Lab at University of Michigan (https://zhanglab.ccmb.med.umich.edu/COVID-19/), and Swiss model repository (SMR) where they predicted its structure using I-Tasser tool and Swiss model server (https://covid-19.uniprot.org/uniprotkb/P0DTC4), respectively.

2.2. E-protein model evaluation and validation.

We evaluated and validated the predicted model of E-protein through PROCHECK [24], Qualitative Energy ANalysis (QMEAN) [25], and Protein quality prediction (ProQ) [26] tools. The PROCHECK tool checks the stereochemical quality of the model by analyzing residue-by-residue geometry and overall structure geometry, QMEAN evaluates to structure in terms of Global Score. In contrast, ProQ tool uses Levitt–Gerstein (LG) and Maximum Substructure (MaxSub) score [27, 28].

2.3. Protein preparation and optimization.

PDB and predicted structure of viral and cellular proteins were prepared as inputs for molecular docking through removing undesired heteroatoms, ions, and molecules. The CHARMM force field was assigned to them and carried out two steps of energy minimization to remove the bad steric clashes using steepest descent and conjugate gradient algorithm for 1000 steps at an RMS gradient of 0.01 and 0.05, respectively [29].
2.4. **Computational simulation.**

We used Hex 8.0.0 [30], ZDOCK [31], ClusPro [32, 33], and Patchdock [34] tools for rigid protein docking, and HADDOCK for flexible protein docking [35]. The Hex 8.0.0 tool uses a spherical polar Fourier (SPF) algorithm along with rotational correlations [36], an improved version of the traditional fast Fourier transform (FFT) scoring function. The ZDOCK produces the top 10 docking models on the basis of shape complementarity, desolvation, and electrostatic energy terms [37]. The ClusPro implies FFT correlation integrating a multistage protocol like-rigid body docking, energy-based filtering, ranking the retained structures based on clustering properties, and finally yields best models with minimum energy and large clusters size [38]. The PatchDock relies on a shape complementarity-based geometry algorithm along with an atomic desolvation energy term followed by the removal of redundant decoys through clustering of root mean square deviation (RMSD) [39]. The HADDOCK deals with flexible protein docking based on various energy terms (van der Waals energy, electrostatic energy, desolvation energy, and restraint violation energy), HADDOCK score, cluster size, Z-score, and buried surface area [40].

2.5. **Binding affinity prediction.**

In the case of flexible protein docking, the binding propensity of all docking interactants was evaluated through various HADDOCK parameters [40]. PROtein binDIng enerGY (PRODIGY) server was used to analyze the molecular interaction tendency and stability for both flexible and rigid docking in terms of binding affinity (ΔG) and dissociation constant (K_d) [41, 42]. Docked complexes have the least RMSD, and greater binding affinity was taken forward for further docking analysis.

2.6. **Visualization of docked complexes.**

The surface structure and interacting residues of docked complexes were visualized using CHIMERA, developed at the University of California, San Francisco (UCSF), and DIMPLOT of LigPlot+ respectively [43, 44]. The DIMPLOT uses 3D coordinates of interacting molecules and generates 2D schematic diagrams wherein dashed lines and arcs exhibiting H-bonds and hydrophobic interactions, respectively. A postscript file is generated that can be converted to a desirable image format or portable document format.

2.7. **Validation of docking methodology.**

To validate our docking procedure, extracted predicted docking pose of S1-CTD from the S1-CTD-ACE2 complex was and subsequently superimposed to its native structure (PDB ID: 6LZG). The lowest deviation between docked and experimentally determined pose was identified [45-48].

3. **Results and Discussion**

3.1. **E-protein model evaluation and validation.**

Ramachandran plot of PROCHECK tool exhibited that 54.43% residues of the I-Tasser model and 84.4% residues of SMR model were located in its core region. However, a predicted model with >90% residues in the core region is considered good [24]. QMEAN tool predicted...
a consensus-based distance constraint (DisCo) score called global score (GS) [25]. Global scores of both models were found 0.34 ± 0.11 and 0.36 ± 0.05, respectively. The possible error pertinent to the GS is based on the predicted and reference models' local Distance Difference Test (IDDT). The value of IDDT >0.6 is considered promising [49]. LG- and MaxSub-scores of I-Tasser model were found to be 4.098 and 0.257, respectively. However, the SMR model depicted LG- and MaxSub-scores of 0.879 and 0.029, respectively. 3D structure having LG- and MaxSub-scores, respectively >4 and 0.8 is categorized as one of the best models [27, 28]. In the aforesaid data purview, we analyzed that SMR model is supposedly better in comparison to the I-Tasser and therefore was selected for further docking studies.

3.2. Rigid protein-protein docking.

The rigid molecular docking of S1-CTD and E-protein with Fab fragment of IgG2a LA5 and IgA, as well as its host proteins ACE2 and MPP5, respectively, was carried out using Hex 8.0.0, ZDOCK, ClusPro and Patchdock tools [30-34]. It is known that the Hex predicts molecular interactions with the lowest free energy (Etotal) using an SPF algorithm allowing rigorous search space sampling to cluster solutions depicting similar binding orientation [30, 50]. Findings of Hex docking revealed that S1-CTD exhibited strong interaction with IgG2a LA5 as depicted by its Etotal (-758.0 kJ/mol), followed by IgA (-721.8 kJ/mol) and ACE2 (-354.3 kJ/mol). Docking stability of these interactants was calculated in terms of ∆G (kcal mol⁻¹) and Kd (M) [41, 42] that followed the order as- IgG2a LA5 (∆G: -17.5, Kd: 1.4x10¹³) > IgA (-17.1, 3.1x10¹³) > ACE2 (-12.4, 8.3x10¹⁰). Likewise, E-protein showed a strong interaction with IgA (Etotal: -696.6 kJ/mol) followed by IgG2a LA5 (-694.2 kJ/mol) and MPP5 (-545.9 kJ/mol). Further, a similar pattern was also observed in docking stability (∆G and Kd) which was as follows- IgA (∆G: -24.4, Kd:1.4x10¹⁸) > IgG2a LA5 (-16.2; 1.4x10⁻¹²) > MPP5 (-9.4, 1.9x10⁻⁷) (Table 1).

Findings of ZDOCK Server highlighted that S1-CTD exhibited strong interaction with IgG2a LA5 as depicted by its Z-dock score (2268) followed by IgA (1525), and ACE2 (1457) [37, 51]. Further, docking stability of these proteins was checked in terms of ∆G (kcal mol⁻¹) and Kd (M) [41, 42] that followed the order as- IgG2a LA5 (∆G: -21.1, Kd: 1.0x10¹⁶) > IgA (-20.2, 1.5x10¹⁵) > ACE2 (-18.7, 2.0x10¹⁴). Moreover, E-protein showed a strong interaction with IgG2a LA5 (Zdock score: 1897) followed by IgA (1497), and MPP5 (1375). Further, a similar pattern was also detected in docking stability (∆G and Kd): IgG2a LA5 (∆G: -20.7, Kd: 6.7x10¹⁶) > IgA (-18.1; 5.5x10¹⁴) > MPP5 (-15.7, 3.2x10¹²) (Table 1).

ClusPro performed rigid docking in two stages; the first stage generates 1,000 low energy docked conformations using pairwise interaction potentials. The second stage includes retaining top 30 clusters showing minimum energy [32,33,38]. Findings of ClusPro docking revealed that S1-CTD exhibited strong interaction with IgA as depicted by its lowest energy function (-2268), followed by IgG2a LA5 (-844.5) and ACE2 (-790.7). Further, docking stability of these proteins was checked in terms of ∆G (kcal mol⁻¹) and Kd (M) [41, 42] that followed the order as- IgA (∆G: -19.2, Kd: 7.8x10¹⁵) > ACE2 (-17.7, 1.5x10¹⁰) > IgG2a LA5 (-17.2, 2.5x 10¹³). Moreover, E-protein showed a strong interaction with IgA (Lowest energy: -1021.9) followed by IgG2a LA5 (-968.6) and MPP5 (-914.5). Similarly, a consistent pattern was also exhibited in docking stability (∆G and Kd) which was as follows- IgG2a LA5 (∆G: -19.3, Kd: 6.9x10¹⁵) > IgA (-18.5; 2.9x10¹⁵) > MPP5 (-11.2, 6.6x10⁹) (Table 1).
Findings of PatchDock exhibited that S1-CTD exhibited strong interaction with IgG2a LA5 as depicted by its PatchDock score (16318) followed by IgA (15520), and ACE2 (14786) [39]. Likewise, E-protein showed a strong interaction with IgG2a LA5 (PatchDock score: 14612) followed by IgA (13982), and MPP5 (11662). Similarly, stability pattern was also consistent that followed the order as - IgG2a LA5 (∆G: -26.3, Kd: 6.2x10^{-20}) > IgA (-22.7; 4.2x10^{-16}) > MPP5 (-21.8; 5.6x10^{-15}) (Table 1).

It is evident from the above findings that both viral proteins (S1-CTD and E-protein) depicted strong and stable binding interactions with antibodies compared to their cognate host receptor proteins except ClusPro docking, wherein stability of S1-CTD with IgG2a LA5 was predicted lower to ACE2. Detailed rigid protein-protein docking analysis of all protein molecules and their stabilities are shown in Table 1.

### 3.3. Flexible protein-protein docking.

The flexible molecular docking of S1-CTD and E-protein with both antibodies and their host proteins ACE2 and MPP5 was carried out using HADDOCK tool that allows backbone and side-chains flexibility during docking execution of interacting molecules [35, 52,53].

### Table 1. Docking analysis of S1-CTD and E-protein.

| Proteins   | A^c | B^o | D^- | \(\Delta G\) (kcal mol^{-1}) | Kd (M)^5 |
|------------|-----|-----|-----|-----------------------------|----------|
|            | Etotal (kJ/mol) | Dock score | Energy Score | Dock score | A^c | B^o | C^o | D^- | A^c | B^o | C^o | D^- |
| S-ACE2     | -354.3 ± 17.3 | 1457 | -790.7 | 14786 | -12.4 | -18.7 | -17.7 | -23.0 | 8.3x10^{-10} | 2.0x10^{-14} | 1.5x10^{-10} | 1.4x10^{-17} |
| S-IgG     | -758.0 ± 19.2 | 2268 | -844.5 | 16318 | -17.5 | -21.1 | -17.2 | -28.2 | 1.4x10^{-11} | 1.0x10^{-16} | 2.5x10^{-13} | 2.3x10^{-22} |
| S-IgA     | -721.8 ± 15.2 | 1525 | -2268 | 15520 | -17.1 | -19.2 | -19.2 | -25.2 | 3.1x10^{-11} | 1.5x10^{-15} | 7.8x10^{-15} | 3.4x10^{-19} |
| E-MPP5    | -545.9 ± 13.7 | 1375 | -914.5 | 11662 | -9.2 | -15.7 | -11.2 | -21.8 | 1.9x10^{-6} | 3.2x10^{-12} | 6.6x10^{-9} | 5.6x10^{-15} |
| E-IgG     | -694.2 ± 19.7 | 1897 | -968.6 | 14612 | -16.2 | -20.7 | -19.3 | -26.3 | 1.4x10^{-12} | 6.7x10^{-16} | 6.9x10^{-15} | 6.2x10^{-20} |
| E-IgA     | -696.6 ± 10.1 | 1497 | -1021.9 | 13982 | -24.4 | -18.1 | -18.5 | -22.7 | 1.4x10^{-18} | 5.5x10^{-14} | 2.9x10^{-15} | 4.2x10^{-18} |

*S-Spike C-terminal domain (S1-CTD),

*IgG- Fab fragment of IgG2a LA5,

*stability predicted by prodigy server.

*findings related to column A, A’ and A” predicted by Hex tool.

*findings related to column B, B’ and B” predicted by ZDOCK tool.

*findings related to column C, C’ and C” predicted by ClusPro tool.

*findings related to column D, D’ and D” predicted by PatchDock tool.

### Table 2. Flexible docking analysis of S1-CTD and E-protein.

| Proteins   | HADDOCK score | Cluster size | Docking RMSD | Z-score | VdW energy | Electrostatic energy | Desolvation energy | Restraints violation energy | of Buried surface area | \(\Delta G^3\) (kcal mol^{-1}) | Kd (M)^6 |
|------------|---------------|--------------|--------------|---------|-------------|----------------------|---------------------|---------------------------|------------------------|-----------------------------|----------|
| S-ACE2     | -89.6 ± 17.3  | 9            | 0.5 ± 0.3    | -1.8    | -79.1 ± 10.6 | -315.9 ± 12.5        | -18.5 ± 3.5         | 213.7 ± 145.5            | 2708.8 ± 57.7 | -15.5 | 4.3x10^{-14} |
| S-IgG     | -79.8 ± 15.9  | 11           | 18.6 ± 0.2   | -1.4    | -52.3 ± 4.4  | -59.2 ± 54.9         | -27.2 ± 2.8         | 2208.1 ± 116.4           | 2262.3 ± 42.8 | -16.4 | 9.7x10^{-14} |
| S-IgA     | -125.5 ± 19.2 | 4            | 21.0 ± 0.2   | -1.2    | -111.9 ± 4.9 | -206.6 ± 69.8        | -40.0 ± 4.5         | 3187.1 ± 139.5           | 3146.6 ± 104.8 | -19.4 | 6.4x10^{-11} |
| E-MPP5    | -56.1 ± 10.8  | 7            | 1.5 ± 0.9    | -1.7    | -73.6 ± 2.6  | -170.0 ± 44.4        | -40.3 ± 5.2         | 932.8 ± 86.9              | 2886.7 ± 154.9 | -12.9 | 3.4x10^{-10} |
| E-IgG     | -19.9 ± 13.9  | 8            | 10.2 ± 0.1   | -1.4    | -87.1 ± 8.5  | -94.8 ± 9.7          | -43.2 ± 4.2         | 1294.0 ± 150.4           | 3727.2 ± 57.5 | -15.8 | 2.5x10^{-10} |
| E-IgA     | -6.6 ± 20.0   | 7            | 0.5 ± 0.3    | -1.6    | -107.3 ± 4.8 | -84.4 ± 33.7         | -70.0 ± 4.5         | 2014.2 ± 189.9           | 3989.1 ± 85.9 | -16.0 | 1.9x10^{-11} |

*S-Spike C-terminal domain (S1-CTD),

*IgG- Fab fragment of IgG2a LA5.

*stability predicted by the prodigy server.

HADDOCK score of S1-CTD with IgA (-125.5 ± 19.2) was more than ACE2 (-89.6 ± 17.3), and IgG2a LA5 (-79.8 ± 15.9), while E-protein exhibited stronger interaction with MPP5 (-56.1 ± 10.8) rather than IgG2a LA5 (-19.9 ± 13.9), and IgA (-6.6 ± 20.0). Z-scores of S1-CTD-ACE2 and E-MPP5 complexes were predicted more than antibodies. In terms of

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different energy functions (van der Waals-, electrostatic-, desolvation-, and restraint violation energy) docking RMSD, and buried surface area, no consistent docking pattern was observed between viral and cellular proteins (Table 2).

In addition to, docking affinity and stability of complexes having maximum HADDOCK scores were further calculated in terms of $\Delta G$ (kcal mol$^{-1}$) and $K_a$ (M) wherein, S1-CTD showed the order as: IgA ($\Delta G$: -19.4, $K_a$: 6.4x10$^{-15}$) > IgG2a LA5 (-16.4, 9.7x10$^{-12}$) > ACE2 (-15.5, 4.3x10$^{-12}$). Likewise, E-protein also showed strong binding affinity with IgA ($\Delta G$: -16.0, $K_a$: 1.9x10$^{-12}$) followed by IgG2a LA5 (-15.8; 2.5x10$^{-12}$) > MPP5 (-12.9, 3.4x10$^{-10}$) [Table 2]. Detailed flexible protein-protein docking analysis of all protein molecules and their binding stabilities are shown in Table 2.

3.4. Interface residues analysis.

Findings obtained through Hex docking tool [30] exhibited that S1-CTDS1-CTD residues namely-ALA475, GLY476, ASN487, PHE456, TYR473, ASN489, LYS417, LEU455, GLU484, PHE486, PHE490, GLN493, TYR505, TYR449, GLY496, GLN498, THR500, ASN501, TYR453, LEU455, and TYR502 interact with GLN24, SER19, THR27, PHE28, TYR83, ASP30, LYS31, HIS34, LEU79, MET82, ARG393, GLY354, LYS353, GLU37, ASP38, GLN42, TYR41, LEU45, ASP355, ARG357, and ASN330 residues of ACE2, which is similar (except ALA36) to the interface binding pattern reported earlier by Wang et al., 2020 (Table S1) [15]. Two (GLY476 and PHE490) (Table S2) and six residues (ALA475, GLY476, ASN487, TYR473, PHE486, and ASN501) (Table S3) of S1-CTD were missing at the interface of IgG2a LA5, and IgA, respectively. Residues of S1-CTD making hydrophobic contacts and H-bondings with ACE2, IgG2a LA5, and IgA are shown in Figures 1-3.

![Figure 1](image1.png)  
**Figure 1.** Docked complex of S1-CTD and ACE2 (a) Surface view-S1-CTD in green and ACE2 in yellow (b) DIMPLOT in which hydrophobic contacts and H-bonds are shown by dashed lines and arcs, respectively.

![Figure 2](image2.png)  
**Figure 2.** Docked complex of S1-CTD and IgG2a LA5 (a) Surface view-S1-CTD in green, H, and L chain of IgG2a LA5 respectively in blue and magenta (b) DIMPLOT in which hydrophobic contacts and H-bonds are shown by dashed lines and arcs, respectively.
Likewise, Hex docking results highlighted that E-protein residues namely-GLU8, GLY10, THR11, LEU12, ILE13, VAL14, ASN15, SER16, VAL17, LEU18, LEU19, LEU21, ALA22, PHE23, VAL25, PHE26, and VAL29 interact with ILE265, GLY268, LYS285, VAL284, LEU267, ALA288, PHE266, GLY287, LYS291, GLY286, ALA89, ASP264, PHE318, SER322, THR270, ALA269, VAL217, ARG272, VAL314, VAL317, GLU316, ASN315, ASP319, VAL271, ARG272, ARG28, THR270, SER281, ASN273, ASP313, MET275, and GLU274 residues of MPP5 (Table S4). All residues of E-protein are involved in molecular interaction with IgG2a LA5, and IgA (Table S5 and Table S6).

Figure 3. Docked complex of S1-CTD and IgA (a) Surface view-S1-CTD in green, H, and L chain of IgA respectively in cyan and purple (b) DIMPLOT in which hydrophobic contacts and H-bonds are shown by dashed lines and arcs, respectively.

Figure 4. Docked complex of E-protein and MPP5 (a) Surface view-E-protein in red and MPP5 in orange (b) DIMPLOT in which hydrophobic contacts and H-bonds are shown by dashed lines and arcs, respectively.

Figure 5. Docked complex of E-protein and IgG2a LA5 (a) Surface view-E-protein in red, H, and L chain of IgG2a LA5 respectively in blue and magenta (b) DIMPLOT in which hydrophobic contacts and H-bonds are shown by dashed lines and arcs, respectively.
Only one residue VAL29 of E-protein is not involved in an interaction with IgG2a LA5. Residues of E-protein making hydrophobic contacts and H-bondings with MPP5, IgG2a LA5, and IgA are respectively shown in Figures 4-6.

![Docked complex of E-protein and IgA](image)

**Figure 6.** Docked complex of E-protein and IgA (a) Surface view E-protein in red and H and L chain of IgA respectively in cyan and purple (b) DIMPLOT in which hydrophobic contacts and H-bonds are shown by dashed lines and arcs, respectively.

ZDOCK tool showed the involvement of almost all residues except three (ALA475, GLY476, and TYR473) in ACE2, and only one residue (GLY476) missing in IgG2a LA5. ClusPro docking exhibited the involvement of all residues in ACE2, missing all residues in IgG2a LA5, and six residues (ALA475, GLY476, ASN487, TYR473, LYS417, and PHE486) missing in IgA. PatchDock tool showed three residues (ASN487, PHE486, and PHE490) missing in ACE2, five residues (ALA475, GLY476, ASN487, TYR473, and PHE486) missing in IgG2a LA5, and missing of all residues in IgA except one (TYR505). HADDOCK tool exhibited missing of all residues except one (THR500) in ACE2, nine residues (ASN489, GLU484, PHE486, PHE490, GLN493, TYR449, GLY496, GLN498, and THR500) missing in IgG2a LA5, and four residues (ALA475, GLY476, TYR473, and LYS417) missing in IgA. It is evident from residue analysis that most of the tools predicted true positive results, which favors the engagement of S1-CTD and E-proteins key residues with other proteins. Overall interacting and missed residues of S1-CTD and E-protein with their cognate receptors and Abs are summarized in Table 3 and Table 4.

### Table 3. S1-CTD interface residues analysis.

| S. No. | Residues | Hex | ZDOCK | ClusPro | PatchDock | HADDOCK |
|--------|----------|-----|-------|--------|-----------|---------|
| 1.     | ALA475   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 2.     | GLY476   | ✓   | x     | x      | x         | ✓       |
| 3.     | ASN487   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 4.     | PHE456   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 5.     | TYR473   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 6.     | ASN489   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 7.     | LYS417   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 8.     | LEU455   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 9.     | GLU484   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 10.    | PHE486   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 11.    | PHE490   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 12.    | GLN493   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 13.    | TYR505   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 14.    | TYR449   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 15.    | GLY496   | ✓   | ✓     | ✓      | ✓         | ✓       |
| 16.    | GLN498   | ✓   | ✓     | ✓      | ✓         | ✓       |
The year 2020 and the last quarter of the year 2019 have witnessed >22,000 articles and >2000 ongoing trials on COVID-19. Moreover, high-throughput sequencing and interdisciplinary approaches have provided advanced and quick information about 53 humans and viral proteins, accelerating the scientific fraternity to uncover the curtain from the tangled tale of the COVID-19 outbreak [2,3]. Scientists are using every preventive and therapeutic strategy, whether it is testing of clot-busting drugs, monoclonal antibodies, or convalescent plasma, to develop substantial drugs and vaccine candidates. Moreover, a comparison among different countries having mandated and not mandated BCG policies and its effectiveness against the COVID-19 outbreak was made by Berg et al., 2020. The findings of their study reveal that cases of COVID-19 and its related deaths were observed less in the BCG mandated countries in comparison to the non-mandated BCG countries [58].

Our study explored the hotspot residues of SARS-CoV-2 S1-CTD interacted with ACE2 reported previously [15], whether they are involved at the interface with other receptor proteins or not. These findings consistently highlighted the S1-CTD, and E-protein hotspot residues as key players in molecular interaction with IgG2a LA5, and IgA. The majority of the protein-protein interaction tools are based on the simple scoring functions like shape complementarity of interacting partners, excluding key structural properties like pH, temperature, and $K_d$ [54, 55]. Therefore, we also checked the binding strength of viral proteins with the inclusion of structural features in terms of $\Delta G$ and $K_d$ [42]. Abs's binding affinity with
aforesaid viral proteins, is reflected greater than their cognate host protein responsible for cellular entry and subsequent modification and survival (Tables 1-2). This interface attributes playing key roles in the host entry might be promising therapeutic sites and immunogen for novel vaccine candidate design as in SARS-CoV and MERS-CoV, where their receptor binding proteins induced to generate an ample amount of neutralizing Abs [56, 57]. The study’s findings obtained through different docking tools are almost consistent with respect to the binding affinity and engagement of hotspots residues that might confer the establishment of non-specific immunity. Moreover, the study also provides structural insight into the existing vaccines at the molecular level. It supports Berg et al., 2020 findings [58].

3.5. Validation of docking simulation.

To validate our docking procedure, extracted S1-CTD from its crystal structure and re-docked using Hex, ZDOCK, ClusPro, and PatchDock tools. Superimposition of the best pose obtained from each docking tools into its PDB pose exhibited minimum deviation. The molecular binding orientation of all the poses into the same binding cavity of ACE2 validates our docking procedures (Figure 7).

![Figure 7. Validation of docking methodology by showing the same binding orientation of the native S1-CTD (green) co-complexed with ACE2 (orange) and that after re-docking the same (Hex-yellow, ZDOCK-cyan, Cluspro-blue, and PatchDock-magenta).](image)

4. Conclusions

According to the statistics, till October 10, 2020 (12:46 GMT), 37,175,477 cases of COVID-19 were reported, in which 1,073,673 people lost their lives globally (https://www.worldometers.info/coronavirus/). So, the earliest possible prevention and therapeutic measures are the prime demand of the current predicament. Towards this direction, an attempt was made to show the structural insight into the binding pattern of S1-CTD, and E-protein with their host receptors ACE2 and MPP5, respectively, along with their neutralizing antibodies IgG2a LA5 and IgA. Findings depicted that Abs interacted more efficiently compared to ACE2 and MPP5 and formed a relatively stable structure with greater $\Delta G$, indicating the use of existing vaccines as a preventive measure against COVID-19. Further,
detailed hotspot residue analysis and its key role in molecular interaction will help design and develop non-specific vaccine candidates and immunotherapeutics against SARS-CoV-2.

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Conflicts of Interest
The authors declare no conflict of interest.

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## Supplementary data

### Table S1. Interface residues between S1-CTD and ACE2 using the Hex tool.

| S1-CTD | ACE2          |
|--------|---------------|
| ALA475 (3)$ | GLN24, SER19, THR27 |
| GLY476 (2) | GLN24, SER19 |
| ASN487 (3) | PHE28, GLN24, TYR83 |
| PHE456 (3) | ASP30, LYS31, THR27 |
| TYR473 | THR27 |
| ASN489 (5) | GLN24, TYR83, LYS31, THR27, PHE28 |
| LYS417 (2) | ASP30, HIS34 |
| LEU455 (3) | HIS34, ASP30, LYS31 |
| GLU484 | LYS31 |
| PHE486 (2) | LEU79, MET82 |
| PHE490 | LYS31 |
| GLN493 (3) | HIS34, GLU35, LYS31 |
| TYR505 (5) | ARG393, GLY354, ALA386, LYS353, GLU37 |
| TYR449 (2) | GLN42, ASP38 |
| GLY496 (2) | LYS353, ASP38 |
| GLN498 (5) | ASP38, LEU45, GLN42, LYS353, TYR41 |
| THR500 (7) | LEU45, LYS353, ASP355, ARG357, TYR41, ASN330, GLY354 |
| ASN501 (4) | TYR41, LYS353, ASP355, GLY354 |
| TYR453 | HIS34 |
| LEU455 (3) | HIS34, ASP30, LYS31 |
| TYR502 (3) | ASP355, LYS353, GLY354 |

$\text{Number in parenthesis denotes number of times it is interacting with host residues.}$

E36 is missing in ACE2 as per the data reported [15].

### Table S2. Interface residues between S1-CTD and IgG using the Hex tool.

| S1-CTD | IgG$^a$          |
|--------|------------------|
| ALA475 (2)$ | HIS81, LYS20 |
| ASN487 | LYS20 |
| PHE456 (6) | SER22, HIS81, THR79, SER8, CYS23, LYS24 |
| TYR473 (2) | SER77, ASP74 |
| ASN489 (5) | HIS81, SER22, GLU11, ILE21, LYS20 |
| LYS417 (9) | SER26, GLN4, SER78, THR79, SER77, CYS23, ALA25, LYS24, GLN6 |
| LEU455 (8) | THR79, SER22, GLN6, GLN7, GLY9, LYS24, SER8, CYS23 |
| GLU484 | GLU11 |
| PHE486 (3) | SER18, GLN83, LYS20 |
| GLN493 (7) | GLY113, GLU11, THR114, PRO10, SER8, GLY9, GLN7, |
| TYR505 (8) | SER42, VAL109, LEU5, ARG45, GLY111, ALA112, SER43 (L), TRP110 |
| TYR449 (6) | PRO156, THR158, PRO207, PRO10, VAL157, THR115 |
| GLY496 | GLY41 (L) |
| GLN498 (2) | GLY41 (L), PRO40 (L) |
| THR500 (6) | SER42 (L), ASP165 (L), LYS39 (L), SER168 (L), PRO40 (L), GLY41 (L) |
| ASN501 (4) | PRO40 (L), SER43 (L), SER42 (L), GLY41 (L), |
| TYR453 (3) | GLN7, SER8, GLN6 |
| LEU455 (8) | THR79, SER22, GLN6, GLN7, GLY9, LYS24, SER8, CYS23 |
| TYR502 (2) | SER42, ARG45 |

$^a\text{IgG- Fab fragment of IgG2a LA5.}$

$\text{Number in parenthesis denotes the number of times it is interacting with host residues.}$

‘L’ in parenthesis denotes the light chain of Ab.

GLY476 and PHE490 residues are missing in S1-CTD as per the data reported by [15].

### Table S3. Interface residues between S-CTD and IgA using the Hex tool.

| S-CTD | IgA          |
|-------|--------------|
| PHE456 | LYS217 |
| ASN489 (2)$ | ASP219, LYS217 |
| LYS417 | LYS212 |
| LEU455 (3) | LYS312, ASN210, LYS217 |
| GLU484 (5) | ASP219, SER167, ASN208, ASN166, ILE206 |
| PHE490 (6) | ASP219, ASN208, LYS217, GLY168, ASN166, SER167 |
| GLN493 (10) | VAL162, LYS217, VAL163, GLY168, ASN210, VAL209, ASN208, ASP164, SER164, SER167 |
| TYR505 (6) | VAL161, PRO213, GLU159, LEU119, THR162, PRO160 |
| TYR449 (11) | HIS175, LEU170, THR176, VAL163, SER191, THR171, VAL174, GLY168, PHE177, TRP165, SER164 |

$^a\text{IgA- Fab fragment of IgA2a LA5.}$

$\text{Number in parenthesis denotes the number of times it is interacting with host residues.}$

‘L’ in parenthesis denotes the light chain of Ab.
Table S4. Interface residues between E-protein and MPP5 using the Hex tool.

| E-protein | MPP5 |
|-----------|------|
| GLU8 (11) | ILE265, GLY268, LYS285, VAL284, LEU267, ALA288, PHE266, GLY287, LYS291, GLY286, ALA89 |
| GLY10     | ILE265 |
| THR11 (3) | PRO266, ILE265, ASP264 |
| LEU12 (3) | ASP264, PRO266, ILE265 |
| ILE13     | PRO266 |
| VAL14 (3) | GLY268, PRO266, VAL284 |
| ASN15 (3) | PRO266, PHE318, SER322 |
| SER16 (2) | PHE318, PRO266 |
| VAL17 (2) | THR270, PHE318 |
| LEU18 (7) | ALA269, PRO266, GLY268, THR270, PHE318, VAL217, ARG272 |
| LEU19 (8) | VAL314, VAL317, GLU316, ASN315, THR270, ASP319, PHE318, VAL271 |
| LEU21 (5) | ARG272, ARG282, THR270, SER281, VAL271 |
| ALA22 (4) | ARG272, ASN273, VAL271, VAL314 |
| PHE23 (3) | ASP313, VAL314, ASN315 |
| VAL25 (4) | ASN273, ARG272, SER281, VAL271 |
| PHE26 (6) | ARG272, ASN273, ASP313, MET275, VAL314, GLU274 |
| VAL29 (2) | MET275, ASN273 |

Number in parenthesis denotes number of times it is interacting with host residues.

Table S5. Interface residues between E-protein and IgG using Hex tool.

| E-protein | IgG |
|-----------|-----|
| GLU8 (3)  | GLN44, GLY43, PRO42 |
| GLY10     | LEU177 |
| THR11 (3) | VAL176, LEU177, PRO42 |
| LEU12 (9) | PRO42, SER89, ARG41, TYR182, THR117, LEU177, VAL116, SER92, VAL118 |
| ILE13 (6) | TYR182, LEU177, ALA175, GLU155, VAL176, PRO42, |
| VAL14 (2) | VAL94, PRO42 |
| ASN15 (10)| THR115, THR114, VAL94, PRO42, THR117, VAL116, ALA93, GLN40, ARG41, SER92, |
| SER16 (4) | TYR182, VAL94, THR115, GLU155, |
| VAL17 (5) | GLY41 (L), PRO174, ASP165 (L), GLU155, ALA175 |
| LEU18 (10)| SER42 (L), PRO42, GLN38 (L), GLN40, TYR96, VAL94, GLY41 (L), THR115, PRO40 (L), ALA112 |
| LEU19 (8) | VAL94, VAL112, GLY113, GLY41 (L), THR115, TYR95, THR114, TYR96 |
| LEU21 (5) | SER42 (L), GLY41 (L), LYS39 (L), ASP165 (L), PRO40 (L) |
| ALA22 (5) | GLY41 (L), GLY113, SER42 (L), ALA112 |
| PHE23 (5) | GLY113, GLY41 (L), PRO10, ALA112, PRO156, |
| VAL25 (2) | GLY41 (L), SER42 (L) |
| PHE26 (6) | LEU5, ALA112, GLY111, TRP110, GLN4, GLN6 |

IgG- Fab fragment of IgG2a LA5.

Table S6. Interface residues between E-protein and IgA using Hex tool.

| E-protein | IgA |
|-----------|----|
| GLU8 (7)  | THR162, PRO160, GLY47 (L), VAL163, VAL161, THR176, GLY42, |
| GLY10 (7) | PRO160, LEU119, GLU159, ALA9, THR118, VAL93, VAL120, |
| THR11 (7) | LEU119, GLN116, GLU159, PRO160, GLY47 (L), TYR95, VAL93 |
| LEU12 (12)| VAL120, GLY42, VAL93, TYR187, GLN39, PRO41, ALA40, THR121, GLU159, ALA92, THR91, LEU119 |
| ILE13 (12)| ALA92, GLY42, PRO41, VAL93, GLY47 (L), TYR95, LEU119, GLN39, GLN43, GLN44 (L), ALA40, GLN48 (L) |
| VAL14 (5) | GLU171 (L), GLY47 (L), SER46 (L), PRO41, GLY42 |
| E-protein | IgA |
|----------|-----|
| ASN15 (5) | GLN43, GLY42, PRO41, ALA40, ALA179 |
| SER16 (6) | GLY44, GLN39, PRO41, ALA40, GLY42, GLN43 |
| VAL17 (8) | GLY47 (L), GLU171 (L), LYS109 (L), SER46 (L), GLY42, LYS45 (L), VAL91 (L), GLN43, |
| LEU18 (5) | ARG148 (L), VAL169 (L), THR170 (L), GLU171 (L), PRO178 |
| LEU19 (2) | GLY42, ARG48 (L) |
| LEU21 (10) | GLU171 (L), ARG148 (L), THR170 (L), LYS109 (L), GLN172 (L), ASP11 (L), VAL110 (L), VAL89 (L), TYR179 (L), SER10 (L) |
| ALA22 (4) | LYS109 (L), SER10 (L), ARG148 (L), GLU149 (L) |
| PHE23 (5) | ARG148 (L), SER10 (L), LEU11 (L), ASP9 (L), LYS109 (L) |
| VAL25 (9) | ALA12 (L), SER10 (L), PRO147 (L), TYR146 (L), ARG148 (L), GLU149 (L), TYR179 (L), LEU11 (L), ASP111 (L) |
| PHE26 (2) | GLU149 (L), SER10 (L) |
| VAL29 | GLU149 (L) |

5Number in parenthesis denotes the number of times it is interacting with host residues.

‘L’ in parenthesis denotes light chain.