Hydrophobic Pocket of SARS-Cov-2 Spike Glycoprotein are Potential as Binding Pocket

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Abstract. Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 was recently spread all over the world. Spike glycoprotein of SARS-CoV-2 (SARS-CoV-2 S-glycoprotein) is the main agent for host cell recognition. Finding the potential of binding pocket of S-glycoprotein may help to find the specific anti-coronavirus drug. Here we analysed potential binding pocket of SARS-CoV-2 Spike-glycoprotein which is suitable for anti-SARS-CoV-2. In pursuit this aim, dogsitescorer, site finder, and DEPTH were used for binding pocket prediction. Molecular interaction protein-ligands were performed using MOE 2009.10. Based on pocket prediction by Dogsitescorer, there are seven out of eleven pockets which have druggability score above 0.8. Molecular interaction studies revealed that interaction between six potential pockets and ligands resulted in negative scores at all. Our result shows that pocket_4 and pocket_6 are located on upper of SARS-CoV-2 S-glycoprotein and have big volume, 878.94 and 683.05 (Å³) respectively, yet lower number of hydrogen bond. Hydrophobic pocket zero, three, and five which is located in the middle of S-Glycoprotein have high number of interaction. These suggest that hydrophobicity of pocket and both upper and middle positions of S-Glycoprotein pocket are considered for developing anti-coronavirus drugs. We propose that hydrophobic pocket of SARS-CoV-2 S-glycoprotein is important for drug design.

1. Introduction
A novel respiratory SARS-CoV-2 was recognized at December 2019 and become an outbreak in Wuhan, China [1]. It has been reported by World Health Organization (WHO) that 17.889.134 cases have been confirmed and at least 686.145 have died [2]. Thus, an effective drug is urgently required.

SARS-CoV-2 is a novel beta-coronavirus and enters the host cell as SARS-CoV through the Spike glycoprotein (S-glycoprotein) [3]. S-glycoprotein forms homotrimers (chain A, B, and C) [4] and consist of two functional subunits; S1 subunit for binding with host receptor and S2 subunit for the membrane fusion machinery [5]. SARS-CoV-2 employed human Angiotensin-Converting enzyme 2 (ACE2) receptors to enter human host cell. The type II transmembrane serine protease (TMPRSS2) located on the surface of the host cell cleave SARS-CoV-2 S-glycoprotein to allow the viral entry [6] (Figure 1).

S-Glycoprotein’s activity is dominated in binding pocket which consist key residues for various interactions with ligand [7]. Thus, finding a potential drug/ligand that could prevent interaction SARS-CoV-2 to ACE2 is one of the strategies that we could employed.

Many in silico strategies could be used in SARS-CoV-2 drug discovery process, such as analysis of binding pockets and docking. Binding pocket analysis is an important step in the drug discovery process. To this date, analysis binding pockets of S-glycoprotein had not been exposed yet. Therefore, this research aims to determine the potential binding pocket from the S-glycoprotein for antiviral inhibition.
We analysed the binding pocket using drugability score and location of the pockets. Furthermore, we clarified this potential pocket using molecular docking of nafamostat, nelfinavir, and chloroquin diphosphate. We demonstrated that hydrophobic pockets facilitated good binding with both hydrophilic and hydrophobic ligand.

2. **Material and Methods**

2.1. **Binding pocket analysis**

SARS-Cov-2 S-glycoprotein(PDB ID: 6VYB) retrieved from the Protein Data Bank (PDB) database (http://www.rcsb.org/pdb/) that was used to assess the binding pocket. Binding pocket of the SARS-Cov-2 S-glycoproteinsurface were predicted using dogsite scorer server (https://proteins.plus/)[8], Site Finder MOE2009.10 software, and Depth server (http://cospi.iiserpune.ac.in/depth)[9]. DoGSiteScorer server provides not only potential pockets prediction of a protein but also volume, surface area, druggability, and depth of the pockets[8].

2.2. **Preparation of SARS-Cov-2 S-glycoprotein structure and standard ligands**

The native ligands and water molecules were removed from the SARS-Cov-2 S-glycoprotein structure (PDB ID: 6VYB). Preparation of SARS-Cov-2 S-glycoprotein were performed by MOE 2009.10 software using current forcefield as partial charges method, adjust hydrogen and lone pairs as required, adjust H and LP, gradient 0,05, and forcefield partial charges calculation. We choosed nafamostat, nelfinavir, and chloroquin diphosphate as positive control ligands based on their good binding result in previous research[10][11][12]. These three ligand structure were obtained from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) and then optimized by MOE2009.10 software[13].

2.3. **Molecular docking**

Molecular docking was used to predict and evaluate interaction between protein and ligands. This process was performed using the MOE 2009.10 software with several parameters; triangle matcher with 2500000 iterations as placement, one time rescoring, London dG, 100 repetitions for the first retain, and force field refinement[13][14][15].

![Figure 1. Mechanism of SARS-Cov-2 entry into human host cell. a) SARS-Cov-2 virus structure. b) SARS-Cov-2 virus bind to human ACE2 and fuse into cell trigerring by TMPRSS2](image-url)
3. Result

3.1 Binding pocket prediction
In order to analyse the potential pocket for binding with ligand, we carried out analysis of SARS-Cov-2 S-glycoprotein (PDB ID: 6VYB) using Dogsitescorder server. Table 1 shows the ranking of pockets based on its volume and druggable. There are seven out of eleven pockets high volume score which have druggable score > 0.8 (Table 1). Recently, the cutoff of 0.7 used for druggability score as the based on the average score[16]. In the present study, we utilized the score > 0.8 for the top scorers instead providing better discerning between the druggable and non-druggable pockets.

The pocket 0 (P_0) was the biggest pocket which has 4450.65 volume (A³). P_0 had 0.81 of the druggability score and high polar and nonpolar residues percentages. The pocket 5 (P_5) was predicted as the highest potential drug binding pocket followed by P_6, P_10, P_4, P_0, P_3, and P_14.

Table 1. The Potential binding site of SARS-Cov-2 S-glycoprotein (PDB ID: 6VYB) based on drugability analysed by Dogsitescorder

| Pocket | Volume (A³) | Druggability | Nonpolar (%) | Polar (%) | Charge (%) |
|--------|-------------|--------------|--------------|-----------|------------|
| P_5    | 715.03      | 0.85         | 50           | 42        | 8          |
| P_6    | 683.05      | 0.83         | 57           | 32        | 11         |
| P_10   | 562.54      | 0.83         | 44           | 36        | 20         |
| P_4    | 878.94      | 0.82         | 50           | 32        | 18         |
| P_0    | 4450.65     | 0.81         | 41           | 41        | 18         |
| P_3    | 973.74      | 0.81         | 47           | 40        | 13         |
| P_14   | 446.04      | 0.81         | 50           | 41        | 9          |
| P_2    | 1102.81     | 0.80         | 39           | 44        | 17         |
| P_9    | 583.67      | 0.80         | 50           | 25        | 25         |
| P_11   | 535.13      | 0.80         | 55           | 33        | 12         |
| P_15   | 442.61      | 0.80         | 48           | 41        | 11         |

*Pocket 1, Pocket 2 etc are shown as P_1, P_2, etc*

Identification of potential binding site was analysed based not only on the drugability of the pockets, but also on their position and interaction with the ligand. Then, we next evaluated the pocket position on the SARS-Cov-2 S-glycoprotein. Figure 2 shows the position of each pocket. P_3, P_4, P_6, and P_9 are located on upper, P_0, P_2, P_5, P_10, and P_11 are located on the middle, while P_14, P_15 are located on lower site of the SARS-Cov-2 S-glycoprotein. As shown in figure 1, the lower pocket of the SARS-Cov-2 S-glycoprotein is attached on the surface of virus, therefore the P_14 and P_15 are not suitable for drug binding site. Together with analyzing of the drugability P_0, P_3, P_4, P_5, P_6, and P_10 were selected as the potential pockets for anti-S-Glycoprotein in our study.
All ligands are expected to bind in the binding pocket of SARS-CoV-2 S-glycoprotein. By analyzing the amino acids in the pockets, the ligand/inhibitor of SARS-CoV-2 S-glycoprotein would be easy to developed. In order to explore amino acid residues in pocket and its properties, we analyzed the amino acids residues on P_0, P_3, P_4, P_5, P_6, and P_10 using dogsitescorer. All binding sites of SARS-CoV-2 S-glycoprotein have hydrophobic area rather than hydrophilic (Table 1 and Table 2). To confirm the dogsitescorer result, a pocket analysis was also performed using sitefinder MOE, and Depth. Unexpectedly, hydrophobicity pockets are strongly predicted in that of P_0, followed by P_5, P_3 and P_10. This result indicated that hydrophobicities of the pocket are varied, yet tends to have polar pocket.

Perola et al (2012) showed that among the pockets of drug targets analysis, the median hydrophobicity was found to be around −0.13 for the drug target set [17], suggesting highly hydrophobic pockets are preferable for drug discovery.

Table 2. Amino Acid Residues in binding pockets of SARS-CoV-2 S-glycoprotein along with its properties

| Pocket | Hydrophobic | Hydrophilic | Charges | Special charges |
|--------|-------------|-------------|---------|-----------------|
| P_0    | TYR_A756    | LEU_B962   | GLN_A762 | GLN_B1011       |
|        | PHE_A759    | PHE_B970   | GLN_A954 | GLN_C762        |
|        | LEU_A763    | ILE_B997   | GLN_A957 | GLN_C954        |
|        | ALA_A766    | LEU_B1001  | THR_A961 | GLN_C957        |
|        | ILE_A770    | LEU_B1004  | GLN_A965 | THR_C961        |
|        | VAL_A951    | TYR_B1007  | THR_A998 | GLN_C965        |
|        | ALA_A955    | VAL_B100   | GLN_A1002 | THR_C998 | ARG_B765 |
|        | LEU_A962    | LEU_B1012  | SER_A1003 | GLN_C1002 | LYS_B776 |
|        | PHE_A970    | ILE_B1013  | GLN_A1005 | SER_C1003 | LYS_B947 |
|        | LEU_A1004   | ALA_B1015  | THR_A1006 | GLN_C1005 | ARG_B995 |
|        |             |             |         | GLU_C1017 | PRO_A728 |
|        | TYR_A1007   | ALA_B1016  | THR_A1009GL | THR_C1006 | ARG_B1014 |
|        | VAL_A1008   | ILE_B1018  | N_A1010  | THR_C1009 | ARG_B1019 |
|        | LEU_A1012   | ALA_B1020  | SER_A1021 | GLN_C1010 | ARG_C765 |
|        | LE_A1013    | TYR_C756   | THR_C732 | GLN_C1011 | ARG_C995 |
|        | ALA_A1015   | PHE_C759   | THR_C734 | SER_C1021 | ARG_C1014 |
|        | ALA_A1016   | LEU_C763   | SER_C735 | ASN_C1023 | ARG_C1019 |
|        | ILE_A1018A  | ALA_C766   | SER_C738 | GLN_B762 | GLU_B776 |
|        | LA_A1020M   | ILE_C770   | GLN_B762 | GLN_B774 | GLU_B774 |
|        | ET_B731VA   | VAL_C951   | GLN_B762 | GLN_B954 | GLU_B955 |
|        | L_B736     | ALA_C958   | GLN_B954 |             | PRO_B728 |
|        | TYR_B756   | LEU_C962   | ASN_B955 |             |             |
|     |     |     |     |     |
|-----|-----|-----|-----|-----|
|     |     |     |     |     |
| PHE_B759 | PHE_C970 | GLN_B957 |
| LEU_B763 | ILE_C997 | THR_B961 |
| ALA_B766 | LEU_C1001 | GLN_B965 |
| LEU_B767 | TYR_C1007 | THR_B998 |
| ILE_B770 | VAL_C1008 | GLN_B100 |
| ALA_B771 | LEU_C1012 | SER_B1003 |
| VAL_B951 | ILE_C1013 | GLN_B1005 |
| ALA_B956 | ALA_C1016 | THR_B1006 |
| ALA_B958 | ILE_C1018 | THR_B1009 |
| LEU_B959 | ALA_C1020 | GLN_B1010 |

| MET_A740 | LEU_A996 | ASN_A856 | SER_C591 | ARG_A1000 | ASP_A745 | CY5_A743 |
| TYR_A741 | ILE_A997 | SER_A967 |
| ILE_A742 | VAL_C320 | SER_A975 |
| PHE_A855 | PHE_C541 | ASN_A978 |
| LEU_A966 | LEU_C546 | GLN_C321 |
| VAL_A976 | PHE_C565 | THR_C547 |
| ALA_A977 | LEU_C570 | THR_C549 |
| ILE_A980 | ILE_C587 | THR_C572 |
| ILE_A993 | PHE_C592 | THR_C573 |

| VAL_B350 | TYR_B423 | SER_B399 | LYS_B378 | ASP_B398 | CY5_B379 |
| TYR_B351 | LEU_B425 | GLN_B409 | ARG_B403 | GLU_B406 | GLY_B381 |
| ALA_B352 | PHE_B429 | ASN_B422 | LYS_B424 | GLU_B425 | GLY_B404 |
| TRP_B353 | VAL_B433 | THR_B430 | ARG_B466 | PRO_B412 |
| TYR_B380 | ALA_B435 | SER_B514 | ARG_B509 | PRO_B426 |
| PHE_B400 | TRP_B436 | GLY_B431 |
| VAL_B401 | PHE_B464 | CY5_B432 |
| ILE_B402 | TYR_B508 |
| VAL_B407 | VAL_B510 |
| ILE_B410 | VAL_B511 |
| ALA_B411 | VAL_B512 |
| ILE_B418 | LEU_B513 |

| VAL_A736a | LEU_A1004 | SER_A735 | LYS_A733 | ASP_A737 | CY5_A738 |
| LEU_A763a | ALA_A766a | ASN_A764 | THR_A768 | GLY_A857 |
| LEU_A767a | ILE_C312 | THR_A859 | PRO_A862 |
| ALA_A771 | TYR_C313 | GLN_C314ASN | PRO_A863 |
| VAL_A772 | PHE_C592 | C517 | GLY_C593 |
| LEU_A858 | LEU_C611 | SER_C596 | GLY_C594 |
| LEU_A861 | ALA_C647 | GLN_C613 | PRO_C665 |
| LEU_A864 | ILE_C666 | GLY_C667 |

| PHE_B338, | PHE_B392 | THR_B376 | LYS_B356 | ASP_B398 | CY5_B336 |
| VAL_B341, | PHE_B395 | ASN_B388 | ARG_B357 | LYS_B378 | CY5_B337 |
| PHE_B342a | PHE_B396 | SER_B514 | LYS_B378 | CY5_B379 |
| ILE_B358 | ALA_B397 | CY5_B384 |
| ILE_B363 | VAL_B433 | CY5_B391 |
| TYR_B365 | ILE_B434 | CY5_B432 |
| LEU_B368 | VAL_B511 | GLY_B525 |
| TYR_B369 | VAL_B512 | GLY_B526 |
| ILE_B377 | LEU_B513 | PRO_B527 |
| LEU_B387 | VAL_B524 |
| LEU_B390 |

| ILE_A312 | ALA_B771 | THR_A320 | ASN_B764 | ARG_A646 | ASP_A614 | PRO_A665 |
| TYR_A313 | VAL_B772 | GLN_A314 | THR_B768 | GLY_B733 | ASP_B775 | GLY_B667 |
| LEU_A611 | VAL_B861 | SER_A596 | ARG_B765 | PRO_B862 |
| ALA_A647 | LEU_B864 | GLN_A613 | PRO_B863 |
| ILE_A666 | THR_B761 |

*a Green= residues predicted by dogsitescorer and sitefinder MOE
*b Yellow blocked= residues predicted by dogsitescorer, sitefinder MOE, and Depth
*c Black= residues predicted by dogsitescorer only
*d Blue= residues predicted by dogsitescorer and Depth
3.2 Molecular docking

Interaction of ligand to binding pocket provides the molecular basis for the activity of drug, therefore analyzing of binding pocket is the most important aspect in the discovery of new medicines. To test the ligand interaction with the binding pocket, molecular docking were performed using nafamostat, nelfinavir, chloroquin diphosphate as ligands[10],[11],[12]. The nafamostat binds strong pair of hydrogen bonds with P_0 followed by P_5, P_3, P_10, and P_6 throughout the docking simulation, whereas nelfinavir ligands bound remains the same on P_0, P_3, P_5 (Table 3). π-π interactions are only shown on the P_0 and P_1. The present study showed that hydrophobicity properties of the pocket in the S-Glycoprotein influence the ligand-protein binding and binding free energy using molecular docking (Table 3). This finding is consistent with our study showing specificity and directionality of hydrogen bonds in the hydrophobic pockets[18].

We showed chloroquine diphosphate has some interactions in the hydrophobic pocket of SARS-Cov-2 S-glycoprotein(Table 3, Figure 3), though chloroquine diphosphate is a hydrophilic drug [19]. This might be due the ratio of polar higher than nonpolar residues, but not significant different (Table 1). In fact, hydrophilic drugs have weak interactions and low entrapment efficiency in drug transport [19].

| Pocket | Ligand                 | Binding Free Energy (kcal/mol) | Hydrogen Bond | pi-pi interaction | Ionic interaction |
|--------|------------------------|-------------------------------|---------------|------------------|-------------------|
| 0      | Nafamostat             | -13,4518                      | 5             | 1                | 0                 |
|        | Nelfinavir             | -13,7167                      | 2             | 0                | 0                 |
|        | Chloroquin diphosphate | -11,2096                      | 1             | 2                | 1                 |
| 3      | Nafamostat             | -13,8193                      | 3             | 0                | 0                 |
|        | Nelfinavir             | -17,8084                      | 2             | 0                | 0                 |
|        | Chloroquin diphosphate | -12,6073                      | 1             | 0                | 0                 |
| 4      | Nafamostat             | -10,5998                      | 0             | 0                | 0                 |
|        | Nelfinavir             | -12,2938                      | 0             | 0                | 0                 |
|        | Chloroquin diphosphate | -10,7605                      | 1             | 0                | 0                 |
| 5      | Nafamostat             | -13,0684                      | 1             | 0                | 1                 |
|        | Nelfinavir             | -14,0162                      | 3             | 0                | 0                 |
|        | Chloroquin diphosphate | -12,7057                      | 2             | 0                | 0                 |
| 6      | Nafamostat             | -12,1221                      | 1             | 0                | 0                 |
|        | Nelfinavir             | -16,1730                      | 0             | 0                | 0                 |
|        | Chloroquin diphosphate | -11,6616                      | 0             | 0                | 0                 |
| 10     | Nafamostat             | -13,9993                      | 1             | 1                | 1                 |
|        | Nelfinavir             | -14,3682                      | 0             | 0                | 0                 |
|        | Chloroquin diphosphate | -12,4754                      | 1             | 0                | 0                 |

The present study determined that nafamostat and nelfinavir had ligand-protein interaction with almost all pockets of SARS-Cov-2 S-glycoprotein(Table 3, Figure 3). Sebastian (2014) showed that hydrogen bond are considered as the main facilitators of protein-ligand interaction [20]. We then, searched for a chemical structural using the PubChem database for its properties. Both nafamostat and nelfinavir have high number of hydrogen acceptor and donor, suggesting stronger protein-ligand interactions due to its properties. Thus, both hydrophobicity of pocket and hydrogen donor/acceptor play important role in facilitating complex ligand-protein interaction.
Figure 3. Interaction ligand nafamostat, nelfinavir, and chloroquine diphosphate with a) P_0, b) P_3, and c) P_5 of SARS-Cov-2 S-glycoprotein. , , , , and are shown as hydrogen bond from side chain acceptor, hydrogen bond from side chain donor, hydrogen bond from backbone acceptor, hydrogen bond from backbone donor, metal contact receptor, pi-pi (arene) interaction, respectively.

4. Conclusion
In conclusion, we have identified hydrophobic pocket (P_0, P_3, and P_5) are suitable for binding pocket in both hydrophobic and hydrophilic ligand, nafamostat, nelfinavir, and chloroquine diphosphate. These findings provide a potential insight in design of anti-SARS-Cov-2.

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