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The 3D structure analysis of SARS-CoV S1 protein reveals a link to influenza virus neuraminidase and implications for drug and antibody discovery

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Abstract

The spike protein of SARS-associated coronavirus (SARS-CoV) is an important target for anti-SARS drug discovery. Its S1 domain is responsible for receptor binding and SARS-CoV entry into cells. In this study, we constructed a rational 3D model for S1 domain of SARS-CoV spike protein by fold recognition and molecular modeling techniques. We found that there is a structure similarity between S1 protein and influenza virus neuraminidase. Our analyses suggest that the existing anti-influenza virus inhibitors and anti-neuraminidase antibody could be used as a starting point for designing anti-SARS drugs, vaccines and antibodies. Interestingly, our prediction for antibody is consistent with a recently experimental discovery of anti-SARS antibody.
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1. Introduction

There is an urgent need for effective antiviral therapy when the reemergence of the highly contagious SARS-CoV infection happens. Chou et al. [1] studied the interaction of SARS-associated coronavirus (SARS-CoV) main proteinase with two ligands. Jenwitheesuk and Samudrala [2] identified some inhibitors of the SARS-CoV proteinase. Xiong et al. [3] is screening possible 73 inhibitors of SARS-CoV 3CL proteinase. Zhang and Yap [4] explored the binding mechanism of SARS-CoV main proteinase.

It is known that SARS-CoV has the same structure proteins as three previously known groups of coronaviruses: spike glycoprotein (S), membrane protein (M), envelope protein (E) and nucleocapsid protein (N). All these proteins can be used as targets for anti-SARS drug development in principle. Among these structure proteins, S protein is a type I transmembrane glycoprotein including two functional domains S1 and S2, which are conserved among coronaviruses. S1 is responsible for the binding with its receptor angiotensin-converting enzyme 2 (ACE2) on host cells

and defines the host range of the virus [5]. The goal of this study is to construct a rational 3D model of S1, to identify noncanonical interactions in the structure of S1, possible inhibitors and antibodies, hence to provide important information for anti-SARS drug, vaccine and antibody discovery.

2. Materials and methods

The sequence of spike protein was downloaded from GenBank (NP_828851). Liu et al. [6] found that the region 75-609 of SARS-CoV S protein matches to the conserved coronavirus S1 domain PF01600 in HMM database and the region 641-1247 matches to conserved coronavirus S2 domain PF01601 in HMM database. In previous study, we have predicted the structure of SARS-CoV S2 protein [7]. Here, we used the same method 3D Jury system [8] to predict the 3D structure of SARS-CoV S1 protein based on the domain (residues 75-609) mentioned above. The proteins with sufficiently high 3D score were used as templates to construct 3D models of S1 by MODELLER program [9]. The quality of 3D model was evaluated by ProQ program [10] and finally validated with the PROCHECK

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                            EEEEEE          EEEE       EEEEEEE      EEEEE      H
SARS_S1_75-335    --TFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFEL
1IJQ_A_1-265      IAYLFFTNRHEVRKMTLDRSEYTSLIPNLRNVVALDTEVASNRIY-WSDLSQRMICSTQL
                       :  .       : :  :* :.:: .      :::.:  *  *   *      *. :*
                  H               EEEEE      HHHEE  EEEE            EEEEEEEEE
SARS_S1_75-335    CDNPFFAVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDG
1IJQ_A_1-265      DRAHGVSSYDTVISRDIQAPDGLAVDWIHSNIYWTDSVLGTVSVADTKGVKRKTLFRENG
                     .:  ..: ::        . *.:  .. *  : *:  : . .: *  ::. .: .::*
                  EEEEEE               HHHHHHHHHHHHHHHHHHHHHHEE          HHH
SARS_S1_75-335    FLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAAAY
1IJQ_A_1-265      SKPRAIVVDPVHGFMYWTDWGTPAKIKKGGLNGVDIYSLVTENIQWPNGITLDLLSGRLY
                     :*::. .    ..  .. *      *:.:* .: :   :. . :   *.  *
                  HH     EEEEEEE      EEEEEE    HHHEEEEEEEE   EEE      EEEEE
SARS_S1_75-335    FVGYLKPTTFMLKYDENG--TITDAVDCSQNPLAELKCSVKSFEIDKGIYQTSNFRVVPS
1IJQ_A_1-265      WVDSKLHSISSIDVNGGNRKTILEDEKRLAHPFSLAVFEDKVFWTDIINEAIFSANRLTG
                  :*.      :     :. : ..  ** :  .   :*::    . * *   *     . . :..


                  EEEEEE        HHHHH
SARS_S1_75-335    GDVVRFPNITNLCPFGEVFNATKFP-
1IJQ_A_1-265      SDVNLLAENLLSPEDMVLFHNLTQPR
                  .**  :.:           :*:  . *
```
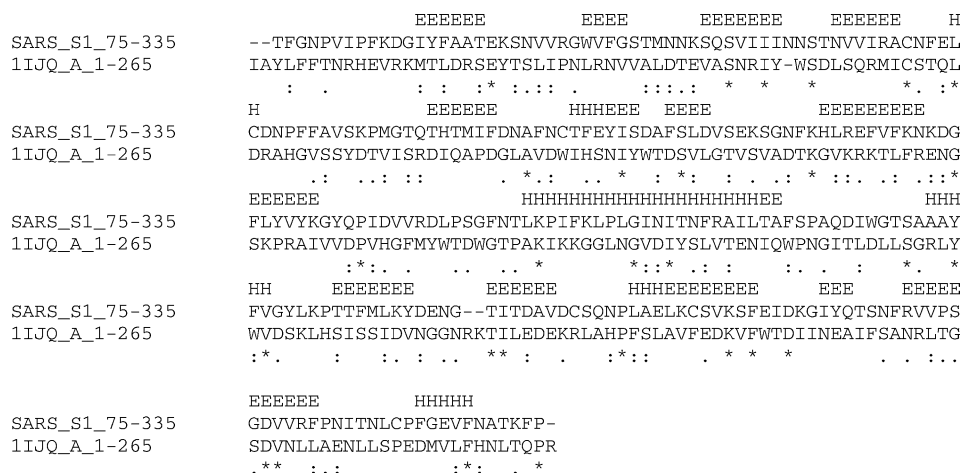
Fig. 1. The sequence alignment between SARS-CoV S1 and template 1ijq with ClustalW. The secondary structure of S1 predicted by PsiPred is also included.

program [11]. The best model was used for further analyses. Specifically, NCI program [12] was used to identify non-canonical interactions in protein structures. VAST (http://www.ncbi.nlm.nih.gov/Structure/VAST/vastsearch.html), DALI (http://www.ebi.ac.uk/dali/) and CE [13] programs were employed to search the structure neighbors of S1 protein. The structural comparison was performed by LGA [14]. The visualization of 3D structure was generated by PROTEINEXPLORER (http://www.proteinexplorer.org).

3. Results and discussion

Fold prediction by meta-server (3D Jury) revealed that top three significant hits (3D score > 50) for S1 protein are as below: 1loq_A (Orotidine monophosphate decarboxylase (lyase), 3D score 154, threading server PCONS2), 1ijq (low-density lipoprotein receptor (lipid transport), 3D score 125, threading server PCONS2) and 2bbk_H (Methylamine dehydrogenase (electron transport), 3D score 118, threading server PCONS2). Using them as templates the corresponding

Fig. 3. Non-canonical interactions in the structure of S1 (75-609). The residue pairs involved are: Trp171 and Phe179, Leu209 and Trp171, represented by blue ball-stick (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

Fig. 2. 3D model for S1 (75-609), which is basically composed of one long α helix and six three-stranded β-sheets arranged in a propeller fashion.
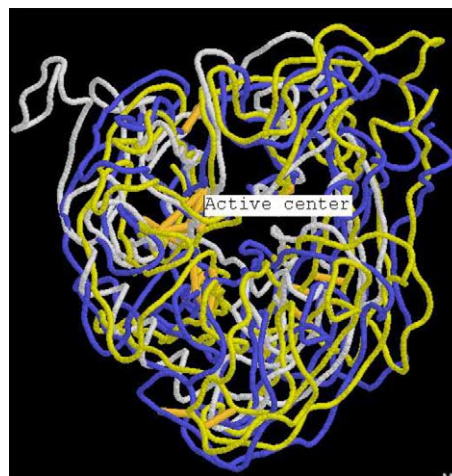
Fig. 4. The superposition of S1 (white) with its structure neighbors: 1INY (neuraminidase from influenza A virus, blue) and 1B9T (neuraminidase from influenza B virus, yellow) (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

```
                                  #                                        #####
SARS-COV_S1     1 FGNPVIPFK------------------DGIYFAATEK------SNVVR-GWVFGSTMN 34
1INY           46 DECRFYALSQGTTIRGKHSNGTIHDRSQYRALISWPLSSPPTVYNSRVECIGWSSTSCHD 105
1B9T_A         50 ECRHFALTHYAAQPGGYYN-GTRKDRNKLRHLVSVKLGKIPTVENSIFHMAAWS-GSACH 107

                       # #                                        ######
SARS-COV_S1    35 NK-SQSVIIINNS--TN--VVIRAC-NFELC----DNPFFAVSKPMGTQTHTMIFDNAFN 83
1INY          106 G-KTRMSICISGPN-NNASAVIWYN--RRPV----TEINTWARNILRTQESECVCH--NG 155
1B9T_A        108 --DGREWTYIGVDGPDNDALVKIKYGEAYTD-----TYHSYAHNILRTQESACNCIGGDC 160

                    ####   ##                                        #####
SARS-COV_S1    84 CTFEYISD--AFSLDVS----------EKS---------------GNFKHLREFVFKNK 115
1INY          156 VCPVVFTDGSATGPAETRIYYFKEGKILK------WEPLA------GTAKHIEECSCYGE 204
1B9T_A        161 --YLMITDGSAS---GISKCRFL-----KIREGRIIKEI----LPTGRVEHTEECTCGFA 206

                     ####       #
SARS-COV_S1   116 D-GFLYVYKGYQP---IDVVRDLPS------GFNTLKPIFKLPLGI-------------- 151
1INY          205 RAEITCTCRDNWQGSNRPVIRIDPV-----AMTHTSQYICSPVLTDNPRPNDPTVGKC-- 256
1B9T_A        207 SNKTIECACRDNSYT---AKRPFVKLNVETDTAEIRLMCTKTYLDTPRPDDGSIAGPCES 263

SARS-COV_S1   152 --------------------------------------------------NITNFRAIL 160
1INY          257 NDPYPGNNNNGVKGFSYLD-GVNTWLGRTISIALRSGYEMLKVPNALTDDKSKPTQGQTI 315
1B9T_A        264 NGDKWLGGIKGGFVHQRMASKIGRWYSRTMSKTNRMGMELYVRYDGDPWTDSDALTLSGV 323

                     ####
SARS-COV_S1   161 --TAFSPAQDIWGTSA----------AAYFVGYL-----------K----PTTFMLKYDE 193
1INY          316 --VLNTDWSGYSGSFMDYWAEGECYRACFYVELIRGRPKEDKVWWT----SNSIVSMCSS 369
1B9T_A        324 MVSIEEPGWYSFGFEIKDKKCDVPCIGIEMVH-----------DGGKGDTWHSAATAIYCL 372
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Fig. 5. The structure alignment between S1 and its structure neighbors: 1INY (neuraminidase from influenza A virus) and 1B9T (neuraminidase from influenza B virus). The bold residues indicate conservative residues. The residues marked as '#' make contact with inhibitors.

3D models of S1 were generated and the quality of protein model was evaluated by ProQ program. The results are as follows: 1loq (ProQ-LG = 0.969, ProQ-MX = 0.055), 1ijq (ProQ-LG = 1.955, ProQ-MX = 0.101), 2bbk (ProQ-LG = 0.877, ProQ-MX = 0.057). So the 'correct' model (the cutoffs for 'correct model' are ProQ-LG > 1.5 or ProQ-MX > 0.1) for S1 protein is the model built on template1ijq. The sequence alignment between template 1ijq and S1 with ClustalW [15] and the secondary structures of S1 predicted by PsiPred v2.3 [16] are displayed in Fig. 1. Thus, the 3D model of S1 is basically composed of one long α helix and six three-stranded β-sheets arranged in a propeller fashion (among them are a couple of sma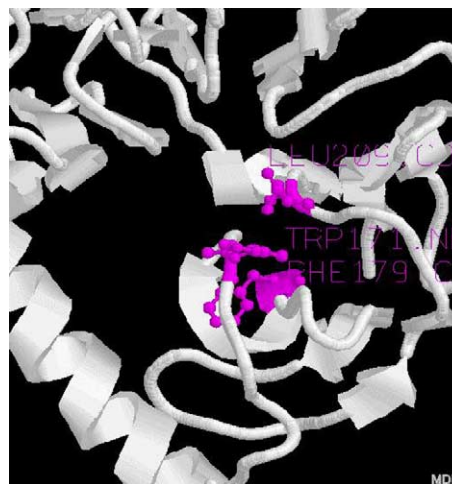ll helices with 3–5 residues only) (Fig. 2), comparable to the structure of its template (low-density lipoprotein receptor): consisting of six four-stranded β-sheets arranged in a propeller fashion. It is not difficult to understand such a structure similarity in terms of their functions, the low-density lipoprotein receptor is responsible for the binding and entry into cells of lipoprotein ligands [17], while SARS-CoV S1 is also responsible for receptor binding and SARS-CoV entry into cells [5].

The non-canonical interactions in S1 protein structure were identified by NCI program and the result shows that there are three pairs of main chain-side chain interactions: Trp 171 (donor) and Phe 179 (acceptor), Ala 210 (donor) and Phe 29 (acceptor), and Leu 209 (donor) and Trp 171 (acceptor). Among these interactions, Trp171 accept one N−H···π bond from Leu209 and donates one N−H···π bond to Phe179, which forms a sandwich-like interaction, as existed in human rac1 [18] and SARS-CoV main protease [4]. These noncanonical bindings fix the helix Trp171 locate to the two β-sheets Phe179 and Leu209 locate, hence stabilizes the structure of S1 protein, but probably reduces the active site cavity for ligand binding (Fig. 3). Indeed, the non-canonical interactions have been shown to be important for the stability of protein structure [19–21] and ligand recognition [22].

In order to extract more information from the predicted structure of S1 protein, we employed VAST, DALI and CE

Fig. 6. (A) The interactions between S1 (white cartoon) and Epana inhibitor (yellow spacefill) from influenza A virus neuraminidase, the binding pockets are represented by blue ball-stick. (B) The chemical structure of Epana inhibitor (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).
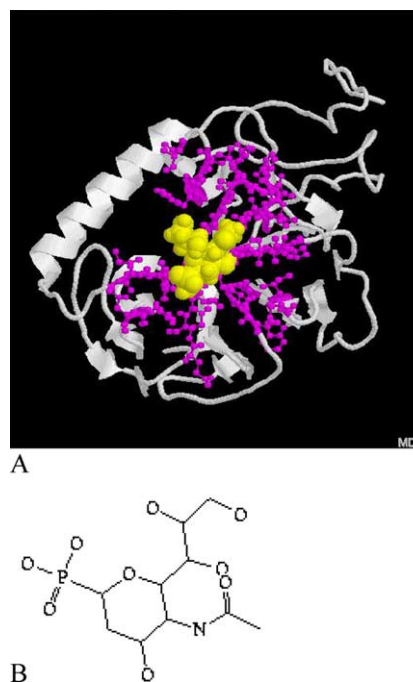
Fig. 7. (A) The interactions between S1 (white cartoon) and RAI inhibitor (yellow spacefill) from influenza B virus neuraminidase, the binding pockets are represented by blue ball-stick. (B) The chemical structure of RAI inhibitor (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

programs to search its structure neighbors and found two interesting neighbors: 1INY (neuraminidase from influenza A virus) and 1B9T (neuraminidase from influenza B virus). In fact, both type A and B neraminidases are composed of six four-stranded anti-parallel β-sheets arranged in a propeller fashion [23,24] and resemble the structure of S1 stated above: basically composed of one long α helix and six three-stranded β-sheets arranged in a propeller fashion. The common feature of these structures is that they share the same active center, as seen in their superpositions (Fig. 4). The structure alignment between 1INY, 1B9T and S1 is shown in Fig. 5. The result demonstrates that there are a number of similar structure patterns showing conservative residues (bold representation). Indeed, their functions are also similar: neuraminidase is an influenza virus glyco-protein embedded in the viral envelope, its function is to facilitate the release of progeny virions from infected cells to other cells [23,24], while SARS-CoV S1 is also responsible for the entry of SARS-CoV into cells, as stated above.

Naturally, such a structure similarity between SARS-CoV S1 protein and influenza virus neuraminidase leads us to consider the possible application of neuraminidase inhibitors to SARS therapy. For example, Epana and RAI inhibitors have been shown to exhibit great potency for type A/B influenza viruses [23,24]. Figs. 6 and 7 shows the structures of docking the two inhibitors from influenza virus

```
VH chain
80R          EVQLVQSGGGVVQPGKSLRLSCAASGFAFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
1NMC_B_Nc10  QVQLQQSGAELVKPGASVRMSCKASGYTFTNYNMYWVKQSPGQGLEWIGIFYPGNGDTSY
             :*** ***. :*:** *:*:** ***::*:.* *:**:*:**:****:.::  ....:. *

80R          ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRSYY-----LDYWGQGTLVTV
1NMC_B_Nc10  NQKFKDKATLTADKSSNTAYMQLSSLTSEDSAVYYCARSGGSYRYDGGFDYWGQGTTVTV
              :..*.: *:: *:*.** *:*:.** :**:*******. . *    :******* ***

80R          SS
1NMC_B_Nc10  SS
             **

VL chain
80R          -TTLTQSPATLSLSPGERATLSCRASQSVRSNLAWYQQKPGQAPRPLIYDASTRATGIPD
1NMC_C_Nc10  DIELTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQNPDGTVKLLIYYTSNLHSEVPS
              ***:.::** * *:*.*:*****.: . * ****:*. : : *** :*.  : :*.

80R          RFSGSGSGTDFTLTISRLEPEDFAVYYCQQRSNWPPTFGQGTKVEVK--
1NMC_C_Nc10  RFSGSGSGTDYSLTISNLEQEDIATYFCQQDFTLPFTFGGGTKLEIRDY
             **********::****.** **:*.*:***   . * *** ***:*::

VH chain
80R          EVQLVQSGGGVVQPGKSLRLSCAASGFAFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
1NCA_H_Nc41  QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTNTGEPTY
             ::******  : :**::::::** ***::*:.*.*:*:**:*****:*:. *. : .: *

80R          ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR---DRSYYLDYWGQGTLVTVSS
1NCA_H_Nc41  GEEFKGRFAFSLETSASTANLQINNLKNEDTATFFCARGEDNFGSLSDYWGQGTTVTVSS
             .:..****::* :.* .* **:*.*: ****.::*** . :  . ******* *****

VL chain
80R          -TTLTQSPATLSLSPGERATLSCRASQSVRSNLAWYQQKPGQAPRPLIYDASTRATGIPD
1NCA_L_Nc41  DIVMTQSPKFMSTSVGDRVTITCKASQDVSTAVVWYQQKPGQSPKLLIYWASTRHIGVPD
              .:**** :*  * *:*.*:::***.* : :.*********:*: *** **** *:**

80R          RFSGSGSGTDFTLTISRLEPEDFAVYYCQQRSNWPPTFGQGTKVEVK
1NCA_L_Nc41  RFAGSGSGTDYTLTISSVQAEDLALYYCQQHYSPPWTFGGGTKLEIK
             **:*******:***** ::.**:*:*****:  . * *** ***:*:*
```

Fig. 8. The sequence alignment between anti-SARS-CoV-S1 human monoclonal antibody 80R and influenza virus anti-neuraminidase antibodies Nc10/Nc41.

to SARS-CoV S1 protein. It can be seen that these compounds can bind to the active center of S1 in the same way as they did in neuraminidase, the residues that make contact with the inhibitors (RMSD ≤ 5) are marked as '#' in Fig. 5 and represented by blue ball-stick in Figs. 6 and 7, among them are 10 conservative residues.

Furthermore, we also speculate that influenza virus anti-neuraminidase antibody could be applied to SARS-CoV S1 protein after appropriate modification, such as, Nc10 and Nc41 anti-influenza virus neuraminidase antibodies [25,26]. Indeed, a very recent publication confirmed our speculation, Sui et al. [27] identified an anti-S1 human monoclonal antibody 80R, which potently neutralizes SARS-CoV infection and efficiently inhibits syncytia formation through blocking of S1 binding to its receptor angiotensin-converting enzyme 2 (ACE2) on host cells. The sequence alignments between Nc10/Nc41 and 80R show more than 50% identity (Fig. 8).

In summary, our modeling exercise on S1 protein find a structure similarity between SARS-CoV S1 protein and influenza virus neuraminidase. In fact, there are clinically similar symptoms for influenza virus and SARS-CoV, both can cause a fever, cough, pains, pneumonia and death [28]. Moreover, our study suggests that the influenza virus neuraminidase inhibitors and anti-neuraminidase antibody could be a starting point for screening anti-SARS drug, vaccine and antibody.

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