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SARS-CoV-2 spike glycoprotein-binding proteins expressed by upper respiratory tract bacteria may prevent severe viral infection

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Abstract

SARS-CoV-2 has become a major global challenge. The virus infects host cells using its spike glycoprotein and has significantly higher infectivity and mortality rates among the aged population. Here, based on bioinformatic analysis, I provide evidence that some members of the upper respiratory tract (URT) commensal bacteria express viral spike glycoprotein-binding proteins. Based on this analysis and available data showing a decline in the population of these bacteria in the elderly, I propose that some URT commensal bacteria hamper SARS-CoV-2 infectivity and that a decline in the population of these bacteria contributes to the severity of infection. Further studies should provide a better understanding of the interaction of URT bacteria and SARS-CoV-2, which may lead to new therapeutic approaches.

1 Introduction

2 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus
3 disease 2019 (COVID-19), has led to many fatalities among humans and huge economical loss
4 around the globe. Thus, there is an urgent need for developing therapeutics to prevent or treat
5 infected individuals. The genome of the virus shows more than 80% identity with severe acute
6 respiratory syndrome coronavirus (SARS-CoV) and bat coronavirus (BatCoV) [1,2]. The high
7 level of identity suggests that SARS-CoV-2 potentially uses a similar mechanism as that of
8 SARS-CoV to infect host cells. It has been demonstrated that the trimeric spike glycoprotein (S-
9 protein) of SARS-CoV is primed by cellular proteases and is cleaved to S1 and S2 subunits [3–5].
10 S1 protein interacts with the host cell receptor angiotensin-converting enzyme 2 (ACE2) [6,7] and
11 S2 mediates fusion of viral particles to cellular membranes [8,9]. A similar mechanism of viral
12 entry to host cells for SARS-CoV-2 has been proposed based on biochemical studies [10]. It has
13 been shown that the SARS-CoV-2 spike glycoprotein initially interacts with the serine protease
14 TMPRSS2 [10]. This interaction was shown to be essential for viral entry into cells and infectivity,
15 and an inhibitor of TMPRSS2 blocked viral entry [10]. Based on these findings, it is suggested
16 that the SARS-CoV-2 uses host TMPRSS2 for S-protein priming. This leads to the interaction of
17 the spike glycoprotein receptor binding domain (RBD) with ACE2 on the surface of host cells [10].
18 Structural studies have revealed the interaction of RBD of the S1 subunit of SARS-CoV-2 with
19 ACE2 protease domain (ACE2-PD) [11,12]. These findings have provided the knowledge for
20 designing therapeutic molecules to block the viral entry process and developing vaccines.

21 Since the emergence of SARS-CoV about 19 years ago, in 2002, efforts to develop a vaccine or
22 an effective therapeutic based on the structure of viral spike glycoprotein and the interaction of
23 viral proteins with different host cell proteins have not been successful. Therefore, there is a need
24 for considering alternative therapeutic interventions. A process, the understanding of which can
25 help in developing new medications, is the interaction of the virus with the upper respiratory tract
26 (URT) commensal microbiota. Emerging evidence has confirmed the interaction of different
27 viruses with commensal microbiota and how this interaction regulates infection and immune
28 response [13–16]. More recently, it has been reported that there is an association between URT
29 microbiota and SARS-CoV-2 infection [17]. It was found that a high population of certain group of
30 bacteria including members of proteobacteria is associated with less severe SARS-CoV-2
31 infection. Interestingly, the occurrence of this population of bacteria decreased linearly with age,
32 suggesting a correlation of these bacteria with susceptibility to COVID-19 [17].

Here, I briefly review available evidence in support of the interaction of URT microbiota with influenza virus. I used bioinformatics to test potential interaction of SARS-CoV-2 with URT commensal bacteria. I demonstrate that some bacteria, whose populations in the URT are reduced due to aging, do produce membrane or secretory proteins with putative binding domains for the spike glycoprotein of SARS-CoV-2. Thus, I hypothesize that the composition of the URT commensal microbiota is essential for reducing the severity of the disease caused by SARS-CoV-2. This proposal offers new preventive therapeutic approaches to avoid severe disease, which will significantly reduce the burden on global economy and health.

The interaction of bacteria and influenza virus. The primary source of the interaction of viral particles and bacteria is the viral particle surface proteins. For the purpose of this work, which is respiratory viruses, I focus on evidence in support of the interaction of influenza virus surface glycoproteins and bacteria. The viral particles consist of two major surface glycoproteins: haemagglutinin (HA) and neuraminidase (NA) [18,19]. Available data suggest that the interactions of URT bacteria with HA or NA either promote viral/bacterial infection or suppress viral infection (Figure 1) [13,15].

The NA protein is shown to have a role at different stages of viral life cycle [20], which can induce bacterial infections. Firstly, enzymatic activity of NA cleaves the host cell surface sialic acids helping receptor recognition by HA and viral entry [21,22]. Secondly, it is suggested that the cleavage of sialic acid by enzymatic activity of NA allows the newly synthesized viral particles to be detached from cells and released [22]. Finally, the NA sialidase activity is shown to cleave the sialic acid from mucin, the glycoprotein constituent of the mucus. This activity of NA allows the virus to abolish the ability of URT to trap viral particles [23]. The cleavage of sialic acid from the surface of host cells and mucins can promote subsequent bacterial infection in at least two different ways (Figure 1). Removal of sialic acid from mucins will scrap their ability to trap inhaled bacteria in the URT, which leads to the accumulation of bacteria in lung and secondary infections (Figure 1A). Alternatively, cleavage of sialic acid from cell surface will expose cryptic receptors that can be identified by inhaled pathogenic bacteria [24] (Figure 1B). On the other hand, it is shown that entry and fusion of virus to host cells requires the activity of the host cell surface proteases to cleave HA to two subunits HA1 and HA2 [25]. This cleavage is essential for viral infectivity because it exposes the fusion peptide, which initiates viral fusion and entry to cells [25]. It is shown that protease of some of the nasal bacteria including those of *Staphylococcus*, a Gram-positive genus of bacteria, can enhance infectivity by proteolytic activation of HA [26].

1 While most available data suggest that the interaction of viral and URT bacteria promotes
2 infectivity of one and/or the other, there are evidence that the interactions of viral and URT
3 bacteria can suppress influenza virus infectivity in different ways. Firstly, the Gram-positive
4 *Enterococcus faecium* bacterium is shown to directly trap viral particles preventing viral infection
5 *in vitro* [27]. Secondly, it was found that the LPS derived from commensal microbiota binds to the
6 viral particles and changes their morphology. Consequently, it can decrease stability of the virus
7 and potentially its infectivity [28]. Finally, the commensal bacteria *Staphylococcus epidermidis*, a
8 Gram-positive bacterium, produces an extracellular matrix-binding protein, which stably binds to
9 viral particles and blocks viral infection [29] (Figure 1C).

10 As discussed above, there are emerging evidence suggesting suppression of influenza virus
11 infection by bacteria. Additionally, it is shown that a peptidoglycan produced by the Gram-
12 positive bacterium *Bacillus subtilis*, a member of phylum *Firmicutes*, can abrogate infectivity of
13 SARS-CoV and Middle Eastern respiratory syndrome coronavirus (MERS-CoV) [30]. Thus,
14 although the interaction of SARS-CoV-2 and URT commensal microbiota should yet be
15 investigated, it is reasonable to assume such an interaction plays a role in pathogenesis of the
16 virus.

17 **The composition of URT commensal microbiota changes due to aging.** To investigate if
18 available data support a role of URT microbiota in the SARS-CoV-2 infectivity, I evaluated
19 available literature to test if there is a correlation between the composition of URT bacteria and
20 the higher infectivity and mortality rates in elderly. Different phyla of bacteria have been identified
21 in URT [31–36]. In general, it is shown that members of the bacterial phylum *Firmicutes*, most of
22 which are Gram-positive like *Staphylococcus aureus* and *Staphylococcus epidermidis*, are
23 predominant [33,36]. Other prevalent bacterial phyla are *Actinobacteria* and *Proteobacteria*,
24 which are Gram-positive and Gram-negative, respectively [33,36]. Based on available data, it
25 appears that the population of some bacterial phyla decreases due to aging, while those of some
26 other phyla increases [36]. Specifically, it appears that the overall population of *Proteobacteria* in
27 the URT decreases upon aging [36]. A decline in population of *Proteobacteria* was also reported
28 due to smoking [34]. On the other hand, it is observed that the severity of SARS-CoV-2 infection
29 and its mortality rate are higher in aged population [37] and may increase due to smoking [38]. It
30 is shown that SARS-CoV-19 can actively replicate in the URT tissue [39]. Therefore, it is possible
31 that a decrease in members of *Proteobacteria* increases the susceptibility of URT or lower
32 respiratory tract (LRT) to viral infection and replication leading to severe disease.

1 **Proteobacteria secrete homologues of TMPRSS2.** Next, I investigated if members of
2 *Proteobacteria* express proteins with the potential ability to interact with SARS-CoV-2 spike
3 glycoprotein. I first tested if these bacteria express a homologue of human TMPRSS2, whose
4 peptidase activity is essential for viral entry. I used blast analysis and identified peptidase S1-
5 domain containing proteins and serine peptidases in members of *Proteobacteria*. Multiple
6 sequence analysis (Supplementary Figure 1) revealed that the proposed catalytic triad of
7 TMPRSS2 [40], which consists of His296, Asp345, and Ser441, and the amino acid residues
8 around this catalytic triad are highly conserved among all proteins (Figure 2A). Using SignalP5.0
9 server [41], I found (with more than 70% probability) that the putative bacterial peptidases contain
10 a secretory signal peptide (Sec/SPI) at the N-terminus. The signal peptidase I (SPI) is responsible
11 for the release of signal peptide, which is essential for transport of protein to the outer membrane
12 and its secretion in Gram-negative bacteria [42]. To test if the highly conserved His, Asp, and Ser
13 in bacterial peptidases form a catalytic triad, I used Phyre2 server [43] and predicted (> 90%
14 accuracy for 90% of the amino acid residues) the structure of a peptidase S1-domain containing
15 protein from *Gammaprotiobacteria bacterium* (Gene ID: A3E01_06460) (Figure 2B). The results
16 show that the highly conserved His, Asp, and Ser form a catalytic triad.

17

18 **Proteobacteria secrete homologues of the ACE2-PD.** Next, I used amino acid blast analysis to
19 identify bacterial homologues of human ACE2. The results revealed the presence of peptidyl
20 peptidases in members of *Proteobacteria* phylum with high similarity to the ACE2 peptidase
21 domain (ACE2-PD). Multiple sequence alignment suggests that the bacterial peptidyl peptidases
22 have 30-50% identity at the amino acid level with human ACE2-PD (Supplementary Figure 2). I
23 used the SignalP5.0 server [41] and tested if bacterial ACE2-PD-like peptidyl peptidase has a
24 secretory signal peptide. The results revealed (with more than 99% probability) the presence of
25 the secretory lipoprotein signal peptide (secretory (Sec)/signal peptidase II (SPII)) in the N-
26 terminus. This signal peptide plays a role in secretion of lipoproteins in gram negative bacterium
27 [42,44]. Subsequently, I predicted the structure of a peptidyl peptidase from *Proteobacteria*
28 *bacterium* (Gene ID: DIU56_02030). The structure of protein (97% of the amino acid residues,
29 except the first 27 amino acid residues at the N-terminus) was predicted with more than 90%
30 accuracy using Phyre 2 server [43] (Figure 3A). The predicted amino acid residues that may
31 interact with SARS-CoV-2 spike glycoprotein receptor binding domain (RBD) are shown (Figure
32 3A). I aligned the predicted structure of bacterial peptidyl peptidase with that of human ACE2-PD
33 (Figure 3B), as determined previously using electron microscopy [11]. The results revealed
34 potential interaction between bacterial ACE2-PD-like peptidyl peptidase and the RBD of spike

glycoprotein. Although these bioinformatics data are limited and further experimental data are required, the results suggest that secretory peptidases homologue of TMPRSS2 and ACE2-PD are produced by *Proteobacteria* and may interact with the RBD of the SARS-CoV-2 spike glycoprotein. This interaction can potentially block viral infectivity like the effect observed by soluble form of human ACE2 and a wide range of orthologues of ACE2 from different species. It is shown that a soluble form of human ACE2 [45] and 17 orthologues of ACE2 from different species [46] can inhibit SARS-CoV-2 infection.

8

Proteobacteria express membrane or secretory glycan binding proteins. Another possible interaction can occur between secreted lectins from bacteria and glycans on the surface of the viral spike glycoprotein. To test this possibility, I first investigated if there is a C-type-like lectin present in any member of the URT commensal bacteria. I tested the presence of this specific lectin because it is known that in humans it can recognize different glycans and activates the immune response [47]. Additionally, it has been shown that in response to MERS-CoV infection the C-type receptor pathway is activated, suggesting interaction of this lectin with the virus spike glycoprotein [48]. Therefore, using the blast analysis of the amino acid sequence, I investigated the presence of C-type-like lectins and found that different members of *Proteobacteria* and *Gammaproteobacteria* express putative C-type-like lectins (Table 1). Additionally, bioinformatics analysis led to the identification of putative lectin leg-B domain-containing and ricin B-type lectin domain-containing proteins. All these proteins are either localized in the membrane or have a secretory signal peptide, with more than 98% probability as predicted using SignalP 5.0 server [41].

23

24 Discussion and hypothesis

Systematic analysis of published data between December 2019 and March 2020 suggest that although children are as susceptible as the elderly in getting infected with SARS-CoV-2, they show milder symptoms and less severe disease [49–51]. Additionally, PCR analysis of samples obtained from patients in different age groups suggest very similar viral loads among infected individuals with different age. However, the number of patients increases significantly with increasing age [52], which is consistent with the general observation of a less severe disease in children. It has been speculated that certain risk factors are associated with more severe infection among the elderly [53]. It has been reported that the expression of host cell entry proteins like ACE2 and TMPRSS2 does not increase with age and cannot explain a more severe disease in

1 the elderly. Different risk factors have been proposed to contribute to the increase in severity of
2 the disease due to aging [53–55]. Some of these risk factors are higher expression of genes
3 associated with cell adhesion and oxytocin signalling, lower expression of genes involved in
4 mitochondrial translation and mitosis, decrease in total alveolar macrophages,
5 immunosenescence, and metabolic diseases. These studies highlight the importance of
6 understanding different mechanisms by which children are protected against severe disease.

7 A very recent study of healthy and infected individuals in the Netherlands suggests that
8 the composition of URT microbiota is associated with SARS-CoV-2 infection and that this
9 association is age-dependent [17]. Based on these data and the reported data with other
10 respiratory viruses, it is evident that the interaction of commensal bacteria with viral particles or
11 host cells plays an important role in the pathogenesis of the virus [13–16]. Bacterial components
12 may interact with virus particles and/or the virus-infected eukaryotic cells displaying viral proteins
13 on their surface. While in many cases it is reported that such interactions can promote bacterial
14 infection, there is evidence demonstrating that the commensal bacteria can hamper viral
15 infectivity. Therefore, I investigated the possibility that a decline in the population of some URT
16 commensal bacteria due to aging, increases the SARS-CoV-2 infectivity and mortality rates
17 among the aged population. Firstly, my bioinformatic analysis to identify bacterial proteins with
18 potential ability to interact with SARS-CoV-2 spike glycoprotein, revealed two possible group of
19 proteins from members of *Proteobacteria*: (i) secretory peptidases with similarity to human
20 TMPRSS2 and the ACE2-PD and (ii) transmembrane or secretory lectins. Secondly, available
21 data regarding the change in population of different URT commensal bacteria suggest that
22 generally the population of *Proteobacteria* decreases due to aging. Finally, it is known that the
23 SARS-CoV-2 infectivity and mortality levels increase in elderly. Based on these data together, I
24 put forward the following hypothesis: a decline in the population of certain URT commensal
25 bacteria, such as members of *Proteobacteria*, abolishes the ability of the upper respiratory
26 system to trap viral particles (Figure 4). Consequently, the rate of infectivity and replication of the
27 virus increases, which is a positive contributing factor to the severity of the COVID-19 disease. It
28 should be noted that other alternative mechanisms are possible. For example, it is conceivable
29 that some metabolites or peptides released by URT bacteria bind to the viral spike glycoprotein
30 and block its interaction with host cell receptors. Alternatively, the interaction of viral particles with
31 a cell-wall protein of a bacteria leads to translocation and accumulation of the bacteria in the
32 lower respiratory tract and formation of secondary bacterial infection.

33 Future studies will provide a better understanding of the interaction between the URT commensal
34 bacteria and SARS-CoV-2. These findings may lead to the discovery of new antiviral molecules

or therapeutic approaches based on manipulating the URT bacteria, as it is suggested in the case of other diseases [56–58]. I speculate that it might be possible to manipulate the population of URT commensal microbiota or to identify bacterial spike glycoprotein-binding proteins or metabolites to trap viral particles at the URT, block viral entry, and reduce viral replication.

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Table 1. Bioinformatics analyses suggest the presence of glycoprotein-binding proteins in members of the URT commensal bacteria.

Lectin domain	Bacteria	Gene ID	Localization
C-type	Gammaproteobacteria bacterium	CMQ88_02965	Transmembrane
C-type	Gammaproteobacteria bacterium	A9Q81_23845	Signal peptide (Sec/SPI)
C-type	Proteobacteria bacterium	CMK59_07605	Lipoprotein signal peptide (Sec/SPII)
C-type	Rhodobacteraceae bacterium	CML44_00865	Signal peptide (Sec/SPI)
Ricin B-type	Gammaproteobacteria bacterium	DEQ32_10900	Signal peptide (Sec/SPI)
Ricin B-type	Alphaproteobacteria bacterium	DEP10_04400	Signal peptide (Sec/SPI)
LegB	Gammaproteobacteria bacterium	CBC55_05095	Transmembrane

The proteins were identified using blast analysis and the presence of secretory signal peptide was determined, with more than 98% probability, using SignalP-5.0 server [41].

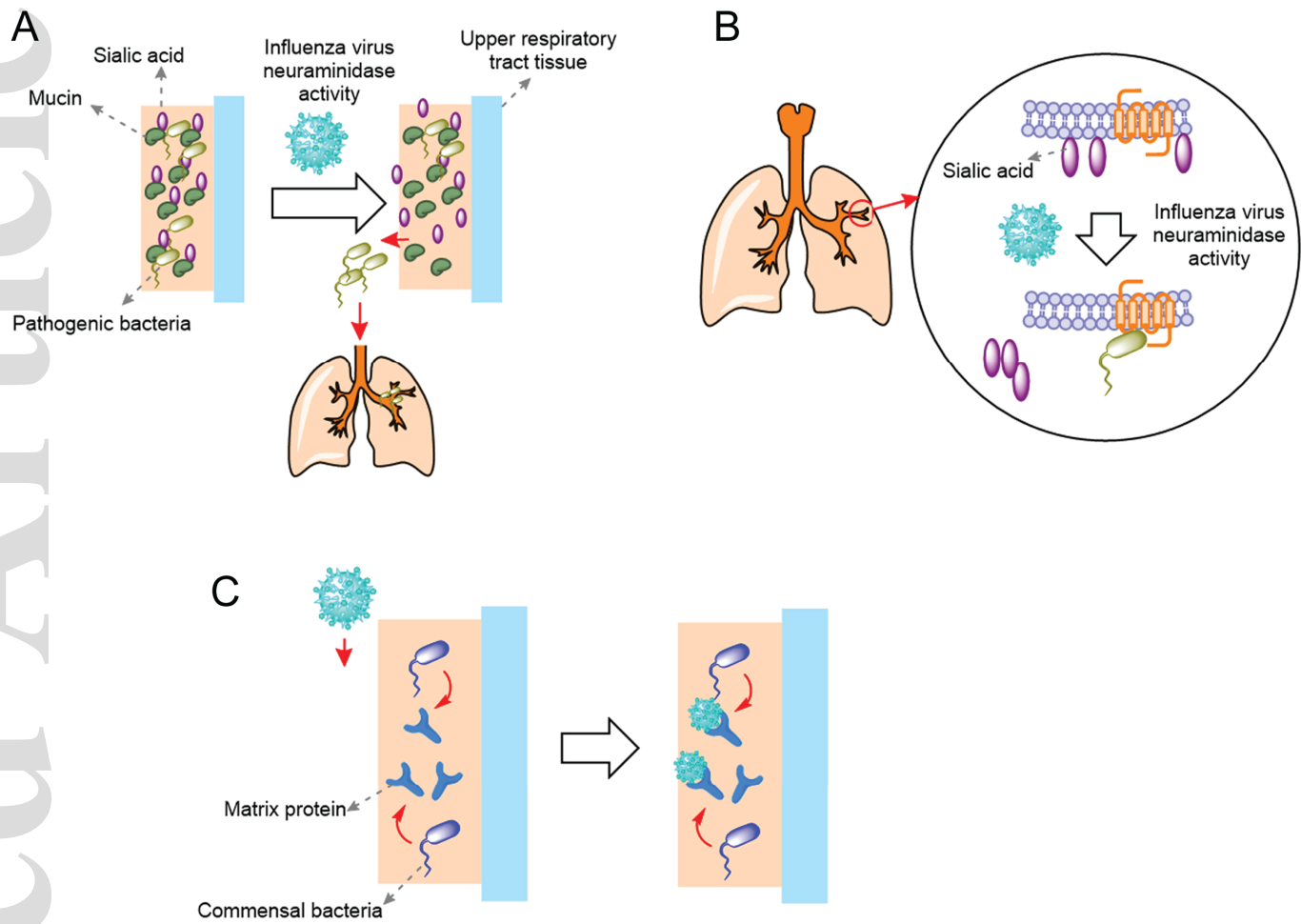
Figure 1. Interaction of influenza virus with commensal bacteria. (A) Influenza virus NA cleaves sialic acid from mucins allowing some of the pathogenic bacteria trapped in the upper respiratory tract (URT) to be released to the lungs and cause secondary infection. (B) Influenza virus NA cleaves the sialic acid from cell surface exposing the receptors that can be identified by pathogenic bacteria. (C) The commensal bacteria in the upper respiratory tract release matrix proteins, which trap viral particles and abolish viral infectivity rate.

Figure 2. Members of Proteobacteria express homologues of TMPRSS2. (A) Multiple sequence alignment shows that the catalytic triad of human TMPRSS2, which consists of His, Asp, and Ser, is highly conserved. The amino acid numbering is for peptidase S1 domain-containing protein from *Gammaproteobacteria bacterium* (ID: A0A1G0HZN5). From Top to bottom: human TMPRSS2; Peptidase S1 domain-containing protein from *Gammaproteobacteria bacterium*; Peptidase S1 domain-containing protein from *Gammaproteobacteria bacterium*; Serine protease from *Proteobacteria bacterium*; and Peptidase S1 domain-containing protein from *Gammaproteobacteria bacterium*. (B) The predicted structure of a peptidase S1 domain-containing protein from *Gammaproteobacteria bacterium* (ID: A0A1G0HZN5) confirms that the highly conserved His96, Asp141, and Ser240 form the catalytic site.

Figure 3. Members of proteobacteria express a peptidyl peptidase with similarity to the peptidase domain (PD) of human ACE2. (A) The predicted structure of peptidyl peptidase from *Proteobacteria bacterium*. The structure was predicted using Phyre2 server. The amino acid residues of the putative binding site of the spike glycoprotein are numbered. (B) Alignment of the predicted structure of the bacterial ACE2-PD-like peptidyl peptidase (ochre) with the 3D structure of ACE2-PD (cyan) in complex with the receptor binding domain (RBD) of the SARS-CoV-2 spike glycoprotein (purple). (C) Possible interactions between amino acid residues of bacterial ACE2-PD-like peptidyl peptidase (orange) and those of RBD of the SARS-CoV-2 (Pink).

Figure 4. Upper respiratory tract (URT) commensal bacteria can hamper SARS-CoV-2 replication and infection. In healthy individuals, who show no symptom of COVID-19 disease, the spike glycoprotein-binding proteins expressed by URT commensal bacteria trap viral particles. Consequently, the viral replication rate abolishes. Due to aging or other conditions the population of the URT commensal bacteria, which produce the spike glycoprotein-binding proteins, reduces. As a result, either viral particles easily reach lower respiratory tract causing severe disease (path 1) or viral particles infect the URT tissue (path 2), which increases replication rate and viral load. The resulting viral particles reach the lower respiratory tract and cause severe disease.

Figure 1



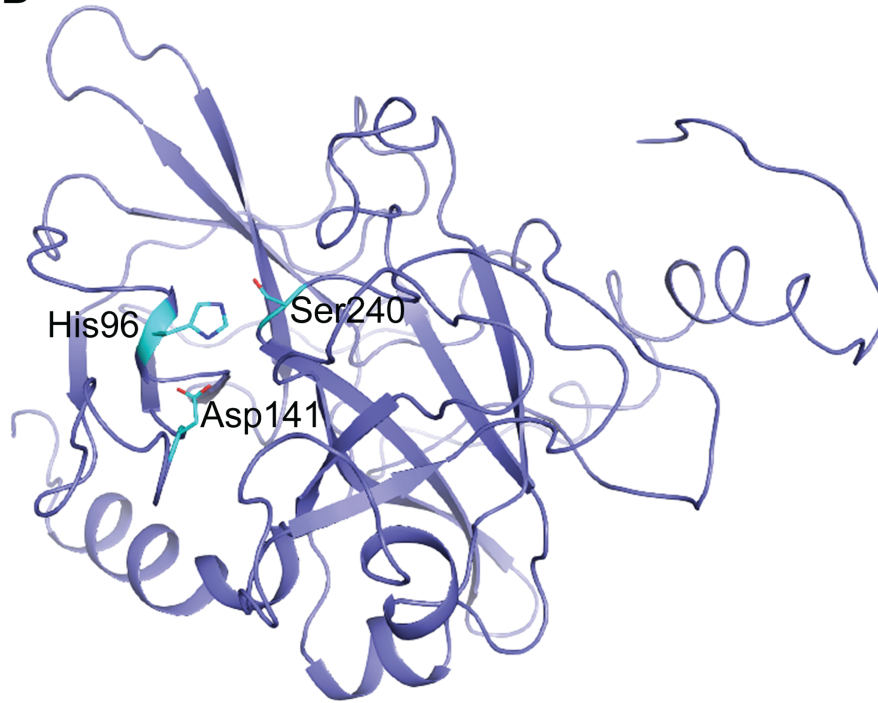
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Figure 2

A

					His96		Asp141		Ser240
SP	O15393	TMPS2			W V I T A A H C V		D I A L M K L		C Q G D S G G P L
TR	A0A1G0HZN5	A			W V T A A H C V		D I A L L E L		C Q G D S G G P L
TR	A0A3D0SRN6	A			W V T A A H C V		D I A L L E L		C Q G D S G G P L
TR	A0A4V2BBY4	A			W V L T A A H C V		D Y A L I R L		C Q G D S G G P L
TR	A0A496V4Z3	A			W L T A A H C I		D I A L L Q L		C V G D S G G P L
consensus					W V T A A H C V		D I A L L E L		C Q G D S G G P L

B



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Figure 3

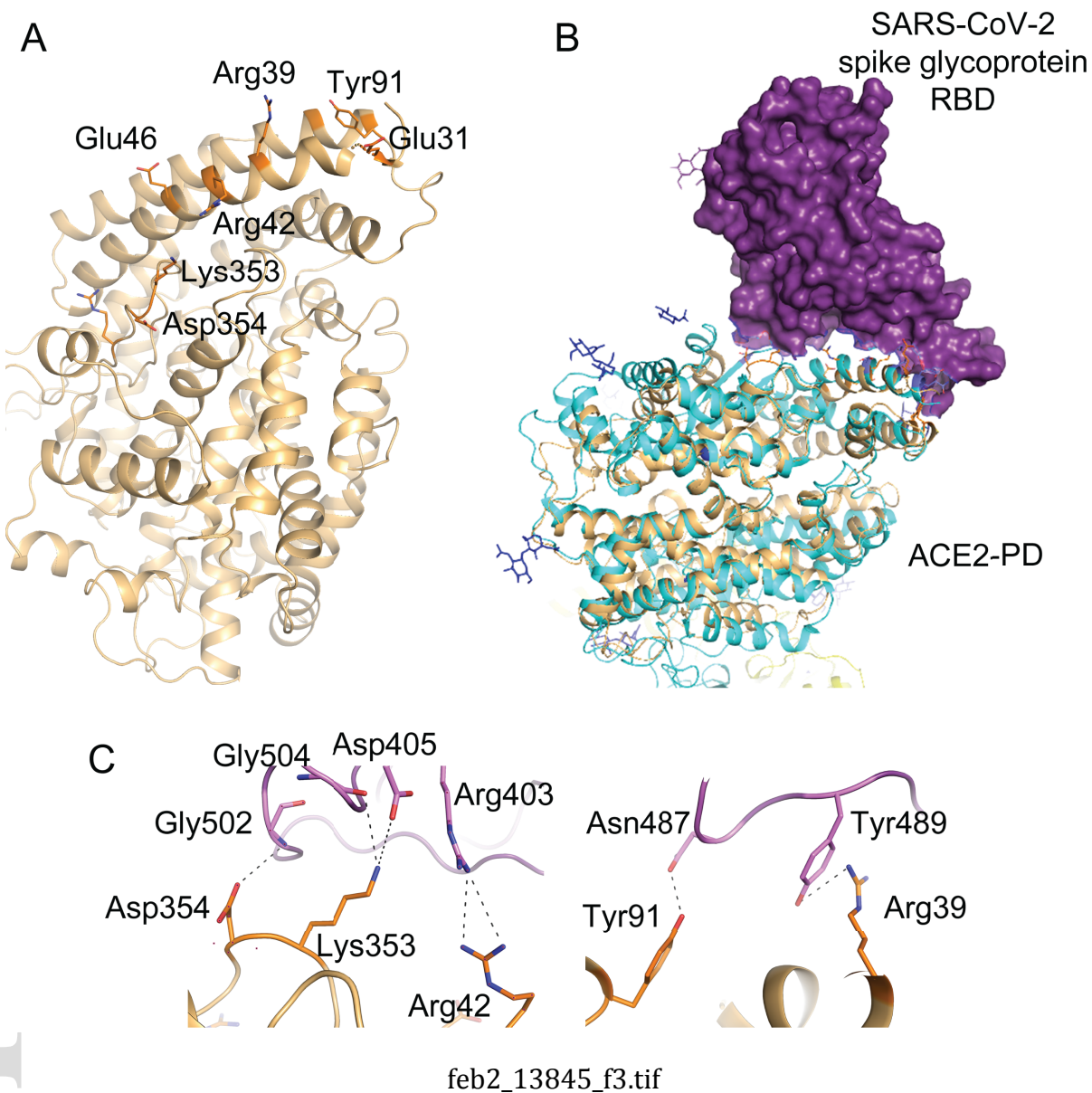
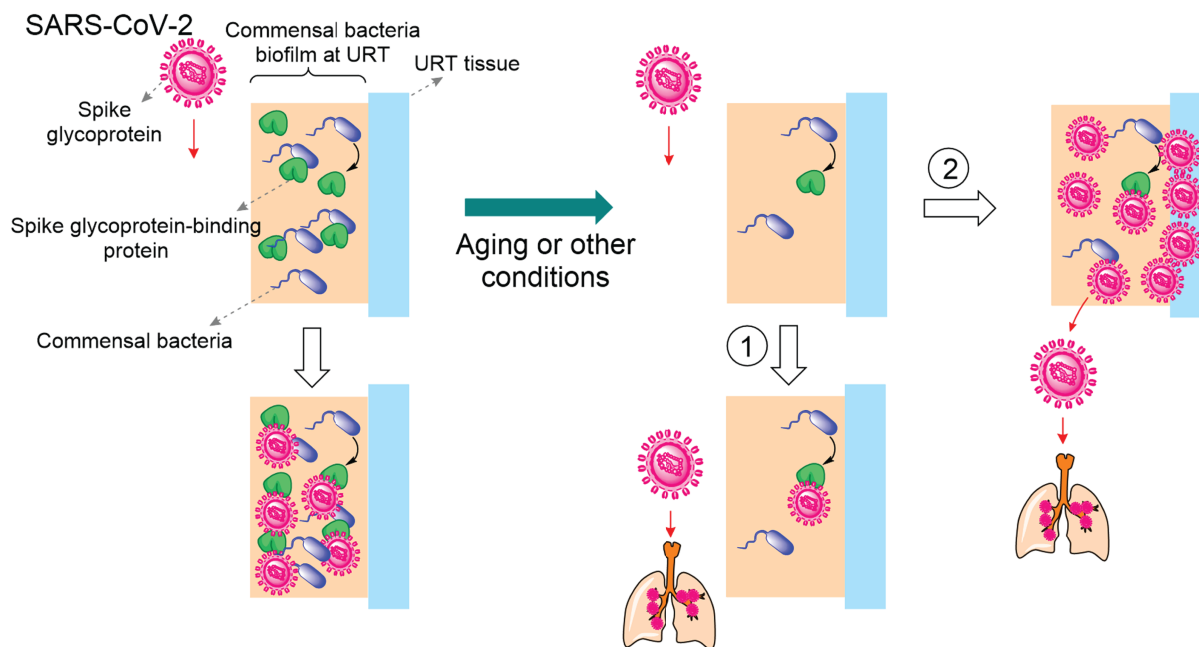


Figure 4



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