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Involvement of sialoglycans in SARS-COV-2 infection: opportunities and challenges for glyco-based inhibitors

Sakonwan Kuhaudomlarp^{1,2,*}, Anne Imberty³

¹Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand;

²Center for Excellence in Protein and Enzyme Technology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand;

³Univ. Grenoble Alpes, CNRS, CERMAV, 38000 Grenoble, France

* Corresponding author: sakonwan.kuh@mahidol.ac.th.

Abstract

Viral infections have been the causes of global pandemics, including the ongoing coronavirus disease 2019, which prompted the investigation into the infection mechanisms to find treatment and aid the vaccine design. Betacoronaviruses use spike glycoprotein on their surface to bind to host receptors, aiding their host attachment and cell fusion. Protein-glycan interaction has been implicated in the viral entry mechanism of many viruses and has recently been shown in SARS-CoV-2. Here, we reviewed the current knowledge on protein-glycan interactions that facilitate SARS-CoV-2 host entry, with special interest in sialoglycans present on both the virions and host cell surfaces. We also analyze how such information provides opportunities and challenges in glyco-based inhibitors.

Introduction

The emergence of an ongoing global pandemic coronavirus disease 2019 (COVID-19) has socio-economically devastated the human population. Coronaviruses are members of a large kingdom of viruses, biological agents that infect and propagate only in cells of living organisms and have been proposed as the most abundant biological entity on the planet (1). Before the current pandemic, viruses were implicated in several infectious diseases, especially in recurring outbreaks that affect the global human population. Due to the involvement of viruses in human diseases, structures, genomic architectures of viruses, and the mechanism viruses use to infect and propagate in human cells are of great research interest.

COVID-19 is caused by severe respiratory syndrome coronavirus 2 (SARS-CoV-2), which uses its surface, multidomain glycoprotein (spike protein), as a host attachment factor via an interaction between its receptor binding domain (RBD) and angiotensin-converting enzyme 2 (ACE2) of the human host (2). Since then, many of the receptors/co-receptors have been identified. A recent review (3) demonstrated that ACE2 alone is insufficient to explain the tissue/host tropism and the variation in the pathogenicity in different hosts. As in many infectious processes, the host/pathogen interaction does not involve only protein-protein contacts; glycans' role should be taken into account. Cell surface glycosylation includes post-

translational modification of proteins in the form of *N*- and *O*-glycans, and glycolipids inserted in membranes (4). The host machinery synthesizes viral glycoproteins subjected to glycosylation, via post-translation modification to maintain the biological functions of the viral proteins (5).

During the interaction, receptors called lectins bind specifically to glycans on the other partners (6). Lectins are carbohydrate-binding proteins produced by many pathogenic agents. For example, bacterial lectins from *Pseudomonas aeruginosa* are secreted as soluble or membrane-associated proteins, mediate biofilm formation and attachment to host cells and bind to glycans on the host cell surface (7–9). On the other hand, lectins are part of mammals' innate system and can bind to glycans present on pathogens (10). Similar to bacterial lectins, viral surface proteins can facilitate host attachment by interacting with glycans on the surface of host cells. Sialoglycans, glycans containing sialic acids, are found prevalently on the vertebrate cell surface and have been demonstrated as receptors of several viral surface proteins and define the preferential infection of the target host, tissues or cell types (tropism) (11).

Structures of viral surface proteins provide insight into the location of host receptor binding sites and distinct protein conformations that enable their interaction with host receptors. Due to the rather large size and multimeric nature of viral surface proteins, Cryo-EM has been used as a major tool in elucidating the structures of spike glycoprotein from SARS-CoV (12), MERS-CoV (13) and SARS-CoV-2 (14–16). The Cryo-EM structures provide valuable information that enables the design of many neutralizing

antibodies (17) and ACE2 receptor decoys, as comprehensively reviewed in (18). Moreover, the structural context of escape mutations arising in variants of concerns can be mapped (19–21) allowing conserved epitopes to be identified and facilitating the design of pan-coronavirus antibodies and vaccines (22). X-ray crystallography has also provided high-resolution detail of the interaction between ACE2 and isolated receptor-binding domain (RBD) (23–25), complementing the information from cryo-EM structures. With regards to protein-glycan interaction in viral infections, several structures of spike glycoprotein from SARS-CoV-2 and related coronaviruses in complex with their target host glycans, which will be discussed later in this review, pinpoint the glycan binding epitope that can be targeted by glyco-based inhibitor.

Given the importance of glycans as attachment factors for many pathogens, including SARS-CoV-2, this review focuses on the function of glycans, particularly sialoglycans, as the host attachment factor of SARS-CoV2. Since glycans have profound implications on infection dynamics, the possible strategies for developing glyco-derived therapeutics are also explored in the present review.

Roles of host glycans in viral entry

Glycans exist as conjugates of proteins (glycoprotein and proteoglycans) and lipids (glycolipids) with myriad associated glycans containing diverse monosaccharides connected by glycosidic linkages. Sialic acid, a nine-carbon acidic monosaccharide presented at the termini of many glycoproteins and glycolipids, has been implicated as a

receptor for many pathogens (26). Various natural modifications occur in sialic acids, such as those commonly found in humans are 5-*N*-acetyl-neuraminic acid (Neu5Ac) and 5-*N*-acetyl-9-*O*-acetyl neuraminic acid

(Neu5,9Ac₂) (4) (Figure 1A and B). Sialic acids are often linked to glycan chains on glycoconjugates via an α -linkage to carbon C2 (27); Figure 1C and D display some examples which will be discussed later.

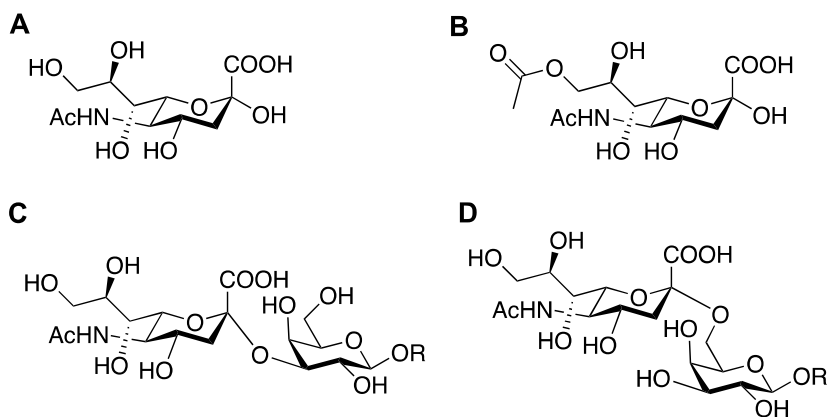


Figure 1. Sialic acid structures. A. Neu5Ac. B. Neu5,9Ac₂. C. α 2,3-Neu5Ac linked to galactose, D. α 2,6-Neu5Ac linked to galactose. Neu5Ac = 5-*N*-acetyl-neuraminic acid, Neu5,9Ac₂ = 5-*N*-acetyl,9-*O*-acetyl-neuraminic acid.

A well-investigated example of sialic acid involvement in viral infection is the interaction between homotrimeric hemagglutinin (HA, Figure 2A) of influenza viruses and terminal sialic acids of glycoproteins and glycolipids found on the host cell surface, which facilitate viral attachment and membrane fusion (28). HAs of human influenza viruses display specificity towards α 2,6-Neu5Ac linked to galactose (29), the structure which is prevalently found in the human upper respiratory tract (30, 31). Structural comparison between HAs of human and avian influenza viruses reveals that Q226L mutation located in sialoglycan binding site contributes to human HA preference for α 2,6-linked over α 2,3-linked sialoglycans, the latter of which are found prevalently in avian. (Figure 2B and C) (32, 33) A primary specificity towards α 2,6-sialoglycan may be an indicator of viral adaptation to human hosts. Other animal viruses also use sialic acids as a

point of entry for the infection, such as rotavirus (34, 35), and adenoviruses (36),

Betacoronaviruses, the sub-family of coronaviruses that include SARS-COV-2, were previously known to exploit glycans as attachment factors through an interaction with the homotrimeric spike glycoprotein (Figure 3A). Spike glycoprotein from human coronavirus OC43 (HCoV-OC43) used sialoglycan containing Neu5,9Ac₂ as a main host attachment factor to facilitate viral entry (37). The Middle East respiratory Syndrome Coronavirus (MERS-CoV) spike glycoprotein binds to sialoglycan as a co-receptor in addition to its primary interaction with cell surface protein receptor dipeptidyl peptidase 4 (DPP4), as evidenced by a decrease but unabridged viral infectivity upon depletion of sialic acids on the host cell surface (38). Cryo-EM-derived structures from the viruses mentioned above in complex with sialoglycans

revealed the location of the sialoglycan binding site in the N-terminal domain (NTD) of spike protein, which displays β -sandwich

galectin-like architecture that is conserved in all coronaviruses (13, 37, 39), (Figure 3B-D).

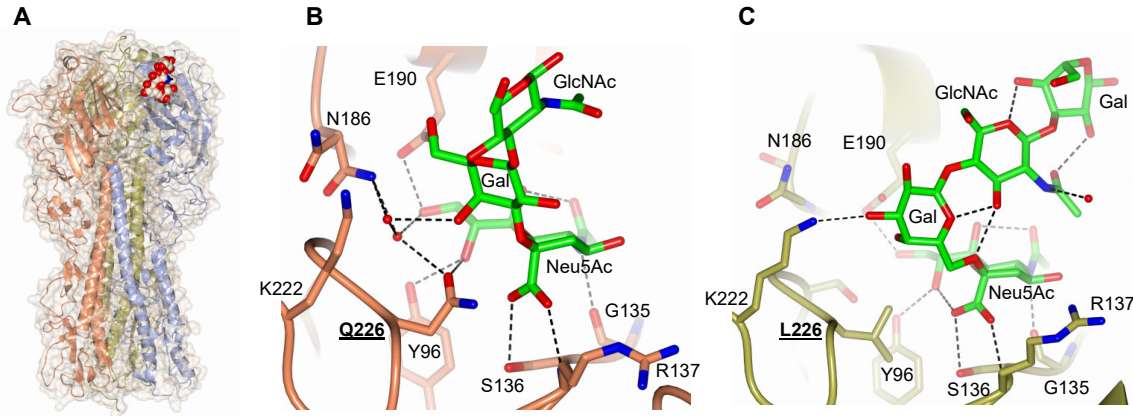


Figure 2. Hemagglutinin structures from influenza viruses in complex with sialic acid-containing glycans. **A.** Homotrimeric structure of hemagglutinin. Sialic acid ligand is shown as sphere. **B.** Avian receptor analog containing α 2,3-sialoglycan in complex with avian influenza virus (PDB: 2WR2). **C.** Human receptor analog containing α 2,6-sialoglycan in complex with human influenza virus (PDB: 2WR7).

The glycan binding capability was a subject of debate for SARS-CoV-2 variants of concerns (alpha, beta, delta and omicron) since the amino acids constituting the glycan binding site in NTD are somewhat divergent from those in other coronaviruses (40). In the first structural models of spike proteins obtained by electron microscopy (14), the conformation of the loops surrounding the putative glycan binding site could not be determined, due to intrinsic flexibility, preventing the identification of a clear binding pocket, even with the help of extensive molecular dynamics study (41). However, a lateral flow device utilizing gold nanoparticles functionalized with Neu5Ac demonstrated rapid detection of SARS-CoV-2 through interaction with the spike protein (42). Recently a saturation transfer difference (STD) NMR experiment demonstrated that the isolated NTD domain of SARS-CoV-2 spike interacted with both α 2,3 and α 2,6

sialoglycans. Such finding established NTD as the Neu5Ac recognizing domain (43). A recent study also showed a preferred recognition of α 2,6-sialylactose over α 2,3 linkage by S1 domain of spike protein and removal of sialic acid from ACE2 attenuated the interaction between ACE2 and S1 (44). Extensive molecular dynamics simulation also concluded the possibility of Neu5Ac to interact with NTD, albeit with several possible orientations and short residence time, in agreement with limited affinity, at least for monosaccharides (45). The role of NTD was further corroborated by universal saturation transferred analysis (uSTA). The location of the binding pocket of NTD interacting with sialic acid was obtained by cryo-EM (Figure 3D and G) (40) However, this latter study also concluded that only the original strain (Wuhan one) presents an active binding site on the NTD, and that all subsequent variants

contained mutations resulting in losing the ability to bind to Neu5Ac.

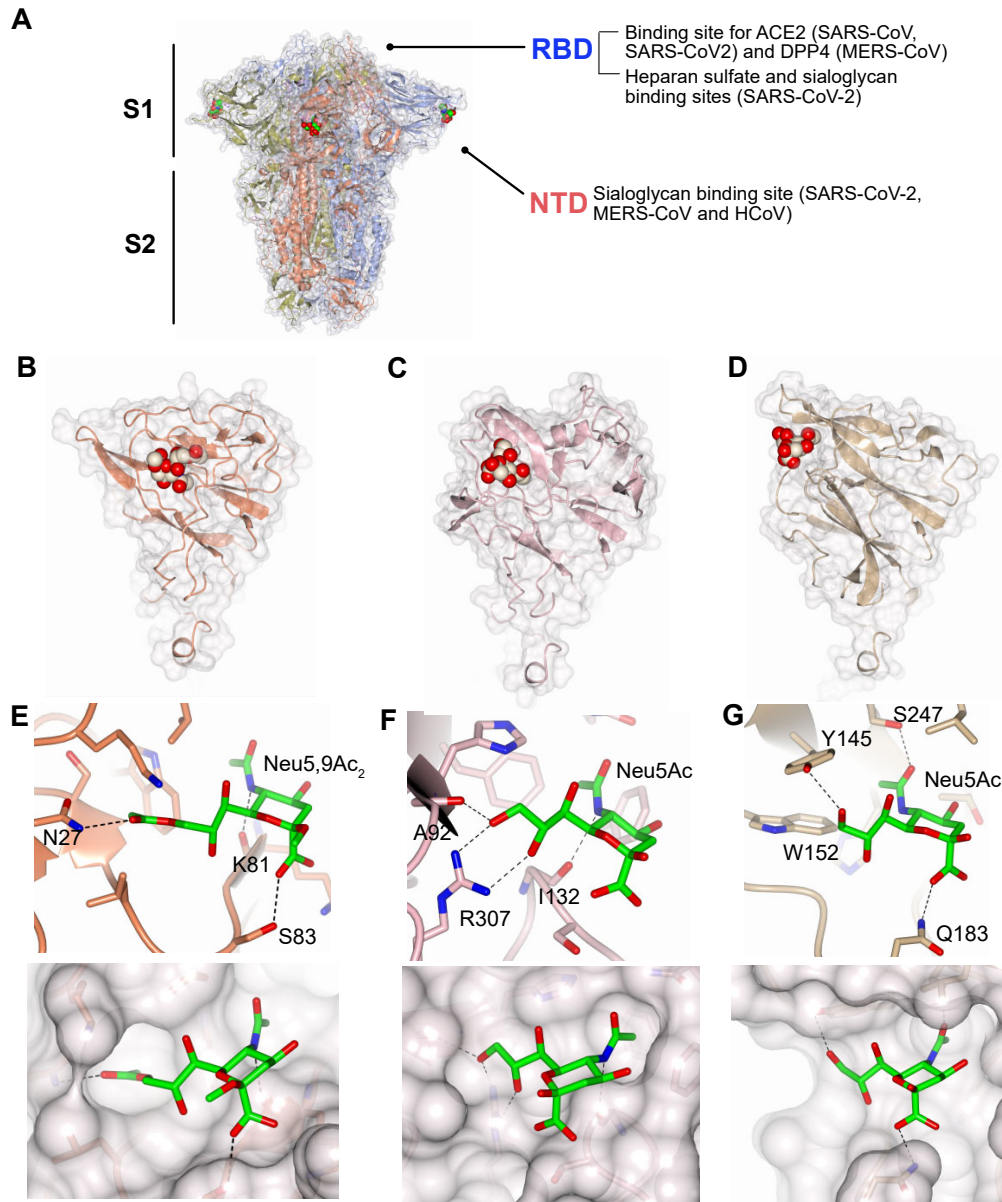


Figure 3. Structure of betacoronavirus in complex with sialoglycans. **A.** Overall structure of homotrimeric spike glycoprotein and receptor binding sites. N-terminal domain (NTD) and receptor-binding domain (RBD) are indicated in circles. **B.** NTD of spike protein from human coronavirus OC43 in complex with Neu5,9Ac₂ (PDB: 6NZK). **C.** NTD of MERS-CoV in complex with Neu5Ac (PDB: 6Q04). **D.** NTD of SARS-CoV-2 Wuhan strain in complex with Neu5Ac (PDB: 7QUR). Sialoglycans are shown as sphere. **E-F.** Sialoglycan binding sites in NTD of spike protein from HCoV-OC43, MERS-CoV and SARS-CoV-2, respectively. The lower panels show the surface topology of sialoglycan binding sites.

In stark contrast to a highly conserved sialic acid binding site in HAs of influenza viruses, the topology and amino acid residues involved in sialoglycan recognition vary greatly among betacoronaviruses (Figure 3E-G), suggesting that the glycan binding sites may have evolved independently. Preference for Neu5,9Ac₂ by HCoV-OC43 and SARS-CoV-2 spike-NTD is reflected in the topology of the sialic acid binding groove, which has a pocket (in HCoV-OC43) or an extended surface shape (in SARS-CoV-2) to accommodate 9-*O*-acetyl group in Neu5,9Ac₂ (Figure 3E and G lower panels), whilst steric hindrance near C9-OH of sialic acid may restrict the binding of MERS-CoV to Neu5Ac (Figure 3F, lower panel). MERS-CoV spike binds preferentially to α 2,3-linked sialoglycan found prevalently in the lower respiratory tract of humans (13, 38), whilst NTD from SARS-CoV-2 original strain interacted with either α 2,3/ α 2,6-Neu5Ac linked to *N*-acetylglucosamine, with an additional binding contribution from Gal residue (40), emphasizing the importance of glycan structure in defining tissue tropism within a human host and distinct glycan usages among betacoronaviruses.

A conservation in glycan binding ability in viral surface proteins may be driven by an interplay of two factors; 1) the requirement of glycan as a primary viral entry factor and 2) the reversibility of the interaction that allows the virus progeny to be released, avoid off-target and/or decoy glycans presented on host cells. The requirement of sialoglycan as a primary viral entry factor and the presence of a receptor-destroying enzyme (hemagglutinin esterase) in influenza virus and HCoV-OC43 provide selective pressure to retain glycan binding sites and ensure the

facile release of virus progeny. In contrast, there might be less selection pressure to retain the glycan binding ability in viruses that use glycans as auxiliary receptors and lack of a receptor-destroying enzyme (i.e. MERS-CoV and SARS-CoV-2, which may subsequently lead to the loss of glycan binding ability in these viruses.

In addition to NTD, RBD, which primarily interacts with ACE2, has also been identified as a sialic acid binding site through computational modelling, and the interaction between sialic acid and RBD was subsequently confirmed by mass spectrometry and SPR (46). RBD has been reported to interact with sialoglycan of gangliosides potentially through the ionic interactions between the positively charged groove in RBD and the negatively charged sialic acids (47). Despite the lack of structural evidence for RBD-glycan interaction, molecular docking has been performed to demonstrate that monosialylated ganglioside (GM1) and blood group A type 1 antigen can be accommodated in a cleft at N370 in RBD (48). Interestingly, molecular dynamics demonstrated that the cleft at N370 is firmly occupied by N-glycan from RBD when the spike protein is in closed state, so the loss of N-glycan at this position vacates the cleft for accommodation of other host glycans. (49) Interaction between sialoglycans and the SARS-CoV-2 spike glycoprotein has now been confirmed via various biophysical methods, as summarized in Table 1.

Besides the direct interaction between SARS-CoV-2 spike protein and host sialoglycan, indirect interaction between spike protein and the host surface sialic acid has also been proposed. Histones released during neutrophil extracellular traps (NETs) were

proposed to bridge between the spike-S2 domain-containing negatively charged amino acids and the sialic acid on the host cell surfaces and enhance ACE2-dependent viral entry and cell-to-cell fusion (50).

Despite the identification of sialoglycan as an interacting partner of spike-NTD, a search for the identity of sialoglycan-containing glycoconjugates that interact with spike protein is still ongoing. Sialoglycan-containing gangliosides (GM1 and GM2) interact with the spike protein with a monomeric affinity of 100-200 μ M. The depletion of sialic acid on the cell surface markedly reduces SARS-CoV-2 pseudotyped viral entry, suggesting that sialoglycan-containing glycolipids' role as co-receptors for viral entry (47). Alternatively, sialoglycan decorated on ACE2 may serve as a binding partner of the spike protein since alteration of sialylation on ACE2 negatively impacted SARS-CoV-2 viral entry (51). Interaction between spike protein and sialylated glycoconjugates may aid the spread and infectivity of SARS-CoV-2 in sialic acid-rich tissues such as the central nervous system, emphasizing the importance of sialic acid as a key determinant for tissue tropism in SARS-CoV-2 infection (52).

In addition to sialoglycans, heparan sulfate, a highly negatively charged polysaccharide attached to extracellular proteoglycans (53), has been shown to engage in a long positively charged channel spanning RBD, NTD and furin cleavage site and such interaction is necessary for ACE2 binding and viral infection (54). Thus, drugs targeting heparan sulfate-dependent viral entry mechanism (55) or heparan mimetics (56) could be therapeutic alternatives for COVID19 treatment. The roles of spike-heparan sulphate interaction in SARS-CoV-2 infection has been

comprehensively reviewed elsewhere (57) and will not be further discussed in this review.

Role of glycans on spike protein in viral entry

SARS-CoV-2 spike glycoproteins consist of putative 22 *N*- (58, 59) and 4 *O*-glycosylation sites (60). The construct of recombinant spike protein and the choice of producer cell line impact the glycosylation pattern (61). The importance of the viral spike glycans in the viral entry is emphasized in a study showing that the introduction of *N*-linked glycosyltransferase inhibitor to SARS-CoV-2-transfected cells inhibited the addition of *N*-glycan on spike protein which reduced the binding affinity of the spike to ACE2 by 5.6-fold and led to approximately 50% reduction in the entry by pseudotyped-virus bearing SARS-CoV-2 spike protein (62).

The roles of the spike glycans have been proposed as; 1) a glycan shield to escape from the host immune system (60, 63). Mutations arising in the variant of concern, such as an additional *N*-glycan site arising in the Gamma variant, may increase the glycan shielding on the surface of spike glycoprotein (64), 2) stabilizing favorable "up" conformation of RBD, which is required for ACE2 interaction (65), and 3) target ligands for host attachment and viral entry through interaction with host lectins. Sialic acid-binding immunoglobulin-type of lectins (Siglec) are animal lectins that recognize sialic acid-containing glycans and facilitate SARS-CoV-2 viral entry in an ACE2-dependent manner. However, single-cell transcriptomic data revealed a limited co-expression of SIGLEC1 and ACE2 on the same cell types, suggesting that SIGLEC1 facilitates SARS-

CoV-2 entry in *trans*-infection where SARS-CoV-2 adheres to SIGLEC-1-bearing cells such as macrophage and dendritic cells, which are not actively being infected by the virus but mediate transfer of the virus to ACE2-containing cells (66, 67).

In addition to sialic acid-binding lectins, C-type lectin receptors (CLRs) that recognize mannose-containing glycans also facilitate viral infection. DC-SIGN/CD-209, L-SIGN/CD-209L have been reported as attachment receptors that facilitate the infection of SARS-CoV-2 in ACE2-containing cells (67, 68). The interaction between spike glycan and the lectins occurs between glycans on N149 located on spike-NTD (69, 70). The average affinity of the interaction between C-type lectins (DC-SIGN, L-SIGN, MGL and Langerin) and glycan on spike protein have been evaluated by surface plasmon resonance (SPR) to be in the low micromolar range (71).

Glycan-targeted therapeutic candidates for COVID-19

Due to the importance of glycans in the interaction of SARS-COV-2 with human cells, interfering with this process is a promising anti-infectious strategy. It has therefore been proposed that the biosynthesis of *O*- and *N*-glycans can be altered by inhibitors that would affect not only the interaction of S-glycoprotein with host receptors but also its folding and conformation (51, 72). Preliminary results have been obtained in cell culture, and the resulting virions demonstrated lower infectious grades than normal ones. Inhibiting the glucosides necessary for processing of *N*-glycans by iminosugars resulted in the inhibition of viral replication (73). Suppression of *N*-glycosylation by genetic

means, RNA interference or inhibitors resulted in fewer virions with decreased infectivity (74, 75). Several of these compounds are approved drugs for some metabolic diseases, so a repurposing strategy is possible. It has to be considered that such compounds alter the glycosylation of host cells as well, but due to the very fast virus replication, the glycosylation of virions should be comparatively more affected.

Another strategy relies on the ability of glycomimetics to compete in the host-pathogen interactions. Glycomimetics are molecules that mimic structures and binding mode of glycans towards lectins and are considered potential anti-pathogenic agents. Such an approach has been used widely as an alternative or complement to antibiotic treatment and is considered a valid strategy to fight against multi-resistant pathogens (76). For example, in the case of *P. aeruginosa*, monovalent glycol-compounds but also multivalent ones and noncarbohydrate glycomimetics targeting these lectins were rationally designed based on the structural information the glycan binding sites (77–79). Due to the multimeric nature of lectins, multivalent glycomimetics have been designed to bind simultaneously to multiple glycan binding sites, resulting in an enhanced binding affinity in the nM range (80, 81). Recently, a novel class of non-carbohydrate glycomimetics have also been discovered through *in silico* and biophysical screenings, providing a new scaffold for developing new glycomimetics as an anti-attachment therapeutic combating bacterial infection (82).

The S-glycoprotein is covered with a variety of glycans, including oligomannose type. They were considered of high interest in HIV due to their abundance in gp120 (83).

Glycomimetics targeting mannose-specific human C-types lectin that were developed for HIV or other infections have been "relocated" against COVID and showed some interesting preliminary results. Several works proposed glycomimetics targeting DC-SIGN and L-SIGN as therapeutics for SARS-CoV-2 treatment. Cramer *et al* identified a new class of DC-SIGN binders through biophysical screens. They employed chemical synthesis to construct a potent multivalent glycomimetic that binds to DC-SIGN and inhibits the *trans*-infection of ACE2-positive cells (84). A known multivalent glycomimetics antagonist of DC-SIGN in HIV infection (85) was tested against SARS-CoV-2. It has the IC₅₀ value in a similar range to the apparent K_D for spike binding and the low M range of the antagonist. It was able to abrogate the infection of pseudotyped-virus-bearing spike protein (71)

A group of sialomimetic compounds targeting SIGLEC1 have been designed *in silico*. One of the sialoside analogs shows a potent IC₅₀ of 0.38 μ M against murine and human SIGLEC-1 and selectively targets SIGLEC-1 both *in vitro* and in an animal model (86). Agonists of Siglec-9 demonstrated effectiveness against inflammation related to covid, but through a downstream effect (87). Targeting host lectins by glycomimetics may disrupt their intrinsic biological functions; thus, using these compounds at high concentrations may not be practical for SARS-CoV-2 treatment. Therefore, glycomimetics targeting the spike protein may be an alternative option for SARS-CoV-2 neutralization. Multivalent 9-*O*-acetylated sialic acid glycocluster grafted on porphyrin tetramer was demonstrated to potently bind to S1 subunit of spike protein (IC₅₀ \sim 1 μ M). They exhibited anti-attachment and neutralization activity in a cell-based viral

infectivity assay (88). Based on the available 3D structures and characterization of sialoglycan properties of SARS-CoV-2, rational design and *in silico* drug screening may be used to develop new classes of glycomimetics that can interfere with SARS-CoV-2 attachment and subsequent viral entry.

Future challenges and conclusion

The functions of glycan-mediating infection by SARS-CoV-2 can be summarized as 1) host glycan interacts with spike protein and acts as a co-receptor on ACE2-containing cells, which enhance viral attachment and entry via *cis*-infection, 2) host lectins interact with spike glycans and facilitate the transmission of the virus via *cis*- and *trans*-infection and 3) indirect interaction between host glycan and spike protein (Figure 4). Identification of these interactions raises questions on the interplay among these roles and their combined effect on viral entry and pathogenesis. The function of host lectins has been demonstrated mainly in *trans*-infection, but the expression of these lectins on ACE2-containing cells heavily influenced the neutralizing activity of anti-RBD monoclonal antibodies both in *cis* and *trans*-infection (67). An antagonistic effect on spike-ACE2 interaction has been reported for two human lectins, Clec4g and CD209c. Clec4g has been hypothesized to interact with complex GlcNAc-terminated N-glycan on N343 located close to the RBD-ACE2 interface, thus directly interfering with RBD-ACE2 binding, while CD209c engaged multiple GlcNAc-terminated glycan sites on the spike protein. The two identified lectins illustrate the role of host lectins as an antiviral response, providing alternative therapeutic candidates for COVID19 treatment (89). The binding of

sialoglycans to spike-NTD did not interfere with ACE2-RBD interaction (40), suggesting that host sialoglycans and ACE2 may engage SARS-CoV-2 spikes during viral attachment, but whether the interaction with host sialoglycan enhances or interferes with ACE2

binding are still unknown. An in-depth understanding of the connections between various viral entry pathways may enable the design of new therapeutics or formulation of combined existing drugs that simultaneously target multiple pathways.

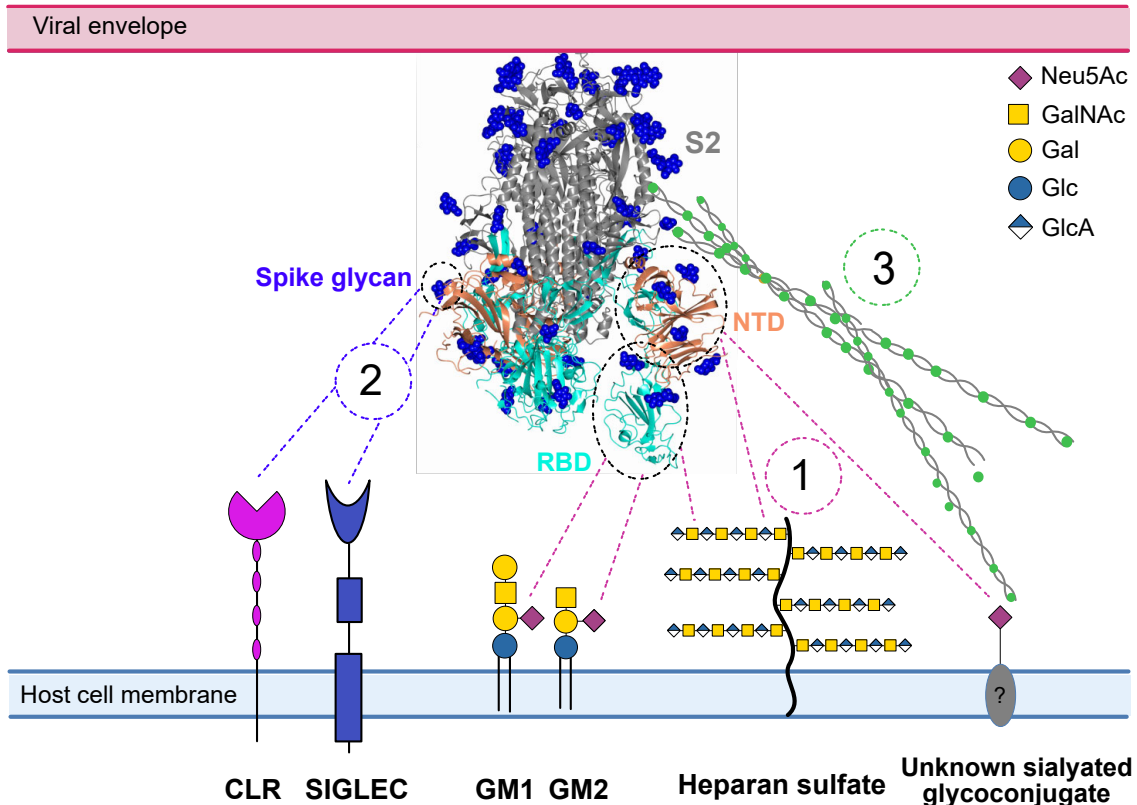


Figure 4. Summary of glycan roles in SARS-CoV-2 infection. 1 Direct interaction between host glycans and spike protein (pinkdotted lines) 2. Direct interaction between spike glycans and host lectins (blue dotted lines). 3. Indirect interaction between host sialylated glycoconjugate with S2 spike, mediated by histones in neutrophil extracellular traps (NETs).

A discrepancy in the identification of sialoglycan binding sites also exists. Sialoglycan-containing glycolipids were first proposed to interact with spike-RBD (47), while a latter report showed that anti-RBD antibodies did not interfere with this interaction (40). Such discrepancies may arise from differences in protein preparation and

biophysical techniques used for affinity determination. Thus, when comparing glycan binding affinities and specificities across multiple experiments using different biophysical techniques, care must be taken.

At the end of the betacoronavirus replication cycle, spike proteins accumulate on

the surface of infected cells and may initiate fusion between infected cells and adjacent uninfected ACE2-containing cells, leading to syncytia formation. Sialic acid receptors mediate syncytia development during murine coronavirus (JHM) and human MERS-CoV infection (90). It is unknown whether sialoglycans are also involved in syncytia formation during SARS-CoV-2 infection. Since syncytia formation is not abrogated in the SARS-CoV-2 variant of concerns (91) in which the glycan binding ability is lost, the interaction between sialoglycan and NTD may not contribute significantly to cell-to-cell fusion. In contrast, the glycosylation pattern on the spike and its alteration in variants of concern could impact syncytia formation. GALNT1-mediated *O*-glycosylation at T678, immediately adjacent to the furin cleavage site, is dependent on the presence of P681 and the *O*-glycosylation is important for spike protein maturation (92), and mutations at this position in alpha (P681H) and delta (P681R) variants result in a reduction in *O*-glycosylation and an increase in furin cleavage, which enhanced syncytia formation (93).

A characterization of glycan binding capability of spike-NTD from betacoronaviruses of non-human hosts revealed that the spike-NTDs engaged uncharacterized glycan-conjugated molecules

in vitro and depletion of sialic acids from host cells enhanced viral entry, suggesting that SARS-related coronaviruses retained glycan-binding in their NTDs. However, such interaction did not contribute to viral entry (94). Understanding glycobiology in non-human hosts and the biology of SARS-related viruses will shed light on the function of these conserved galectin-like NTDs in defining host tropism, cross-species transmission and viral infectivity. It will aid early detection and prevention of spill events that may cause future pandemics.

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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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