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Molecular recognition in the infection, replication, and transmission of COVID-19-causing SARS-CoV-2: an emerging interface of infectious disease, biological chemistry, and nanoscience

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Abstract

A coronavirus (CoV) commonly known as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and causing COVID-19 (coronavirus disease of 2019) has become a pandemic following an outbreak in Wuhan. Although mutations in the SARS-CoV-2 spike glycoprotein (SGP) are obvious from comparative genome studies, the novel infectious nature of the virus, its new varients detected in the UK, and outside and recovery–death ratios of COVID-19 inspired us to review the mechanisms of the infection, replication, release, and transmission of progeny virions and the immune response in the host cell. In addition to the specificity of SARS-CoV-2 binding to angiotensin-converting enzyme 2 receptor and transmembrane protease serine 2, the varied symptoms and severity of the infection by the original and mutated forms of the virus suggest the significance of correlating the host innate and adaptive immunity with the binding of the virus to the mannose receptor via lipopolysaccharides (LPSs), toll-like receptors via LPS/proteins/RNA, and sialic acid (Sia) via hemagglutinin, or sugar-acid segments of glycans. HA-to-Sia binding is considered based on the innate Sia N-acetylneuraminic acid and the acquired Sia N-glycolylneuraminic acid in the epithelial cells and the sialidase/neuraminidase- or esterase-hydrolyzed release and transmission of CoVs. Furthermore, the cytokine storms common to aged humans infected with SARS-CoV-2 and aged macaques infected with SARS-CoV encourage us to articulate the mechanism by which the nuclear capsid protein and RNAs bypass the pattern recognition-induced secretion of interferons (IFNs), which stimulate IFN genes through the Janus-activated kinase-signal transducer and activator of a transcription pathway, leading to the secretion of antiviral proteins such as myxovirus resistance protein A/B. By considering the complexities of the structure, and the infectious nature of the virus and the structures and functions of the molecules involved in CoV infection, replication, and immune response, a new interface among virology, immunology, chemistry, imaging technology, drug delivery, and nanoscience is proposed and will be developed. This interface can be an essential platform for researchers, technologists, and physicians to collaborate and develop vaccines and medicines against COVID-19 and other pandemics in the future.

Introduction

The halo or crown structure of the proteinaceous spike peplomers or glycoproteins (SGPs) detected in a transmission electron microscope image (Fig. 1) is the basis for the name coronavirus (CoV) given to viruses causing a series of respiratory illnesses, including COVID-19 (coronavirus disease of 2019), SARS (severe acute respiratory syndrome), and MERS (Middle East respiratory syndrome). The structure[1], size (80–120 nm)[2], genome[3,4], and RNA-based pathogenesis[5,6] of SARS-CoV-2 resemble those of other CoVs[7]. The highly pathogenic nature of SARS-CoV-2 and its recent genetic varients suggests that the binding

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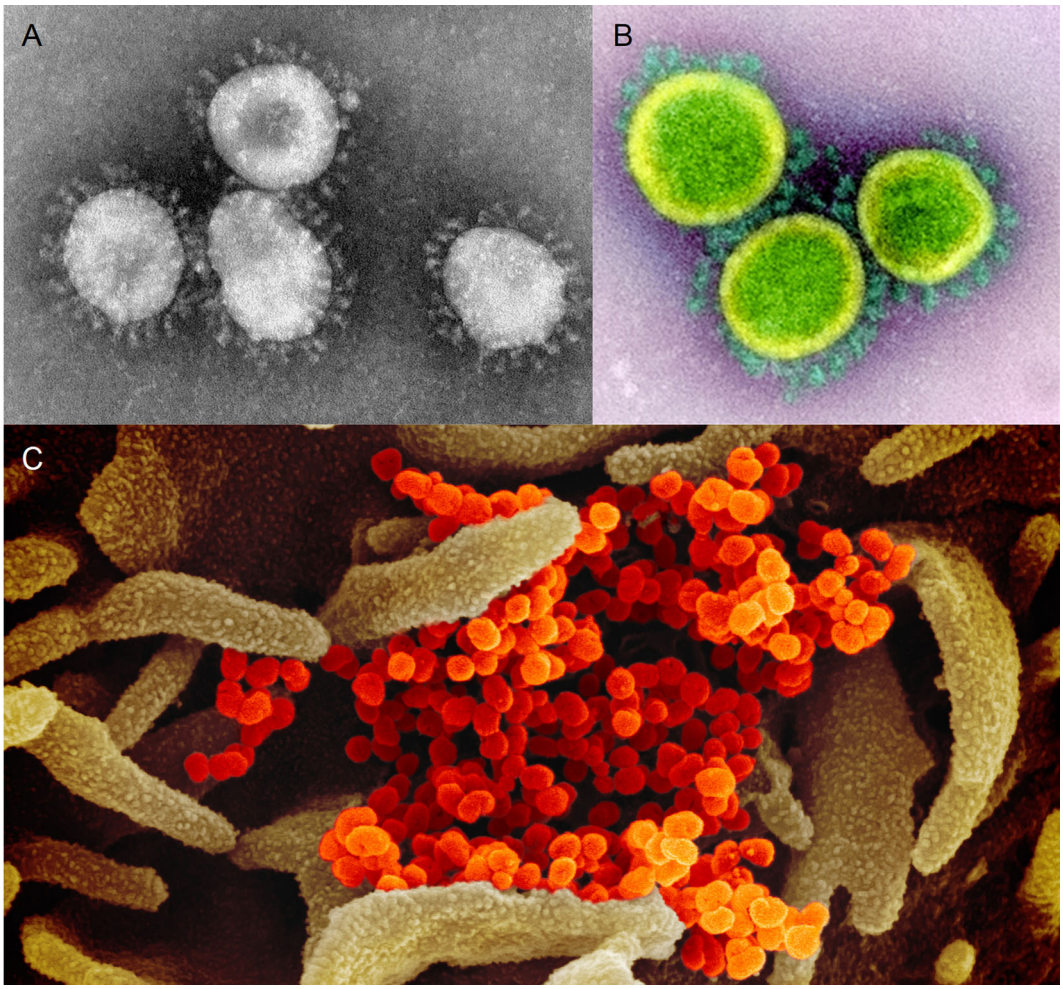


Fig. 1 The structures of CoVs and SARS-CoV2. A Transmission electron microscope (TEM) image of human respiratory alphacoronavirus particles critical for infection with the common cold, bronchiolitis, and pneumonia. **B**, **C** TEM images of SARS-CoV-2. Courtesy of CDC 1975 and NIAID-RML.

affinities of these pathogens are high for a host cell, and it competently bypasses or blocks the cytokine (interferon (IFN))-triggered immune responses of a host cell. Thus, the following fundamental questions related to the tropism, replication, and release/transmission of SARS-CoV-2 face us. How does SARS-CoV-2 acclimate to the specific SGP genes[3,4,7,8] that supplement the virus with a furin cleavage segment (FCS)[1,9] to efficiently recognize angiotensin-converting enzyme 2 receptor (ACE2R)[1,10] and bind to it? Is hemagglutinin (HA) a coreceptor for sialic acid (Sia)-based binding to a host cell? How does neuraminidase (NASe)- or esterase (ES)-based cleavage release a progeny virion? How do nonstructural proteins, the nuclear capsid (NC) and other structural proteins, and RNA bypass the IFN-induced JAK-STAT (Janus-activated kinase-signal transducer and activator of transcription) mechanism and form progeny virions[11–13]? Initially, SARS-CoV-2 was connected to *Rhinolophus affinis* (*R. affinis*), a bat species, because of the 96% sequence similarity between the 29.9 kb RNA of SARS-CoV-2 and the RNA of RaTG13 virus in *R. affinis*[7,8,14]. The similarities between the SGP amino acids of SARS-CoV-2 and Malayan pangolin (*Manis javanica*, *M. javanica*) CoV (pangolin-CoV) attracted further attention to the studies on the chimeric nature of the viral RNA and the origin of SARS-CoV-2[15,16]. Independent of the suggested lineage relationships between RaTG13, SARS-CoV-2, and Pangolin-CoV, investigations have validated the zoonotic evolution of SARS-CoV-2. An emerging and serious concern about COVID-19 is the propagation of SARS-CoV-2 RNA mutations[16–18] in humans or animals, particularly during the development of an effective drug or vaccine against COVID-19.

Following the repeated declarations of emergency in most countries and by the World Health Organization (WHO), the pharmaceutical giants and private and public research organizations in Russia, the United States, the

United Kingdom, India, Japan, China, Italy, Spain, Belgium, Germany, Australia, Singapore, and Israel began developing drugs and vaccines against COVID-19 without neglecting the abilities of an RNA virus to bypass an antiviral drug or modify its own genome to override the innate or adaptive immunity of the host. The early episodes of COVID-19 show similarities of SARS-CoV-2 pathogenesis to influenza viruses, HIV (human immunodeficiency virus), Ebola virus, SARS-CoV, and MERS-CoV. Thus, various antiviral drugs have been tested in COVID-19 patients, such as Tamiflu[19] and favipiravir[20], which are used against common influenza viruses; lopinavir and ritonavir[21], which are used against HIV; and remdesivir[22], which is used against Ebola virus, Marburg virus, Lass virus, Syncytial virus, Nipah virus, Junin virus, Hendra virus, and CoVs causing SARS and MERS. In addition, several clinical tests were conducted with drugs against malaria-causing *Plasmodium falciparum* (*P. falciparum*), such as chloroquine[23], mefloquine[24], hydroxychloroquine (HCQ)[25], artemisinin[26], clindamycin[27], doxycycline[28], and pyrimethamine[29]. The positive and negative outcomes of these tests alarm tropism switching and genetic modification of the virion. The ongoing treatments of a COVID-19 patient includes the suppression of RNA copying by evading exoribonuclease proofreading, for which the blocking of the endolysosomal transport of the virus-encapsulated endosome is under debate[25]. In addition, the positive outcome of remdesivir in inhibiting Ebola virus by mutating its RNA is correlated with the positive response of this drug against the first COVID-19 case in the United States[30]. Nonetheless, the side effects of these drugs, including cardiac malfunction in patients treated with the chloroquine derivatives[31], should be carefully considered during the management of COVID-19.

In addition to the aforementioned tests, molecular-level information (Fig. 2) about the infection, immune response, replication, and transmission of SARS-CoV-2 is inevitable during the development of an effective vaccine or a drug. The complexity of the infection and treatment demands a novel platform at the interface of virology, immunology, drug delivery, genetics, chemistry, materials science, and nanoscience, which will help researchers, physicians, and technologists to collaborate and develop vaccines and medicines against COVID-19. The efficient use of the wealth of information about nanomaterials[32–36], imaging probes[37–41], bioimaging techniques[42–46], vaccine development[47–51], and in vitro and in vivo drug/gene/nanomaterial delivery[52–61] is essential. Nevertheless, the toxicity of nanomaterials[62–66] is a major concern during the consideration of virus mimetic nanoviruses for in vivo applications. This article summarizes the fundamental aspects of the molecular interactions in viral infection and the host immune response and provides future prospects for the aforementioned interface in the fight against the pandemic.

The roles of SGP and ACE2R in viral infection

The membrane-binding proteins of viruses have highly conserved frameworks that are modified according to the available receptors in a host cell. These proteins are β-spirals, similar to those in SGP of SARS-CoV-2[1], or the coiled-coil α-helix, such as those in HA[67] of the influenza virus. We independently consider the roles of SGP-to-ACE2R binding and HA-to-Sia binding (Fig. 2) in SARS-CoV-2 infection. Mechanistically, SARS-CoV-2 infection begins with the recognition of its SGP receptor-binding domain (RBD; Fig. 3) by ACE2R in the host epithelial cells of the respiratory system. The low affinity of ACE2R to its natural ligand is an advantage for the virus. The SGP-to-ACE2R affinity suggests that ACE2Rs in the vascular endothelial cells and myocytes increase the risk and mortality through the systemic transmission of SARS-CoV-2 to vital organs such as the kidneys and the heart[68,69]. This risk factor is correlated with irregular heartbeats and increased levels of troponin I and creatine kinase in acute COVID-19 patients[70]. Similarly, physicians are in the early stage of correlating ACE2R and COVID-19 with an immune overreaction and an inflammation in children, which are similar to Kawasaki disease[71], affecting vital organs such as the liver, heart, and kidneys.

The spike in HCQ prescriptions for use against COVID-19 has produced positive and negative results, as well as different opinions about the action of the drug. Although HCQ shows many side effects, including delayed ventricular repolarization and low pulse rates, the success stories of this drug in COVID-19 patients suggest the drug has bimodal action involving the suppression of glycosylation of ACE2R and SGP and the inhibition of RNA release by attenuating the endolysosomal passage of the virion-encompassed endosomes[31,72]. Thus, HCQ suppresses the release of both SARS-CoV-2 RNA and the progeny virions. Furthermore, HCQ can suppress cytokine production[73] and inhibit matrix metalloproteinases[74]. Nevertheless, the overall outcome for patients treated with HCQ is not positive, which has guided the WHO and many countries to stop recommending this drug for treating COVID-19.

The amino acid residues, particularly five of the six residues critical for SGP-to-ACE2R binding, show a similarity between SARS-CoV-2 and Pangolin-CoV, whereas four of the six critical amino acids in RaTG13 are different[7,8,14]. However, the presence of a polybasic FCS at the S1–S2 boundary of SGP in SARS-CoV-2[1,9], which is not common to Pangolin-CoV or other CoVs, increases the affinity of SGP to ACE2R. The deep canyon formed at the S1–S2 junction of SGP enables the efficient binding of the virus to ACE2R. Following binding, the proteolytic S1–S2-cleaved subunits endow SGP with a 10- to 20-fold greater affinity for ACE2R and a more infectious nature to SARS-CoV-2 than SARS-CoV. In contrast, the trimeric

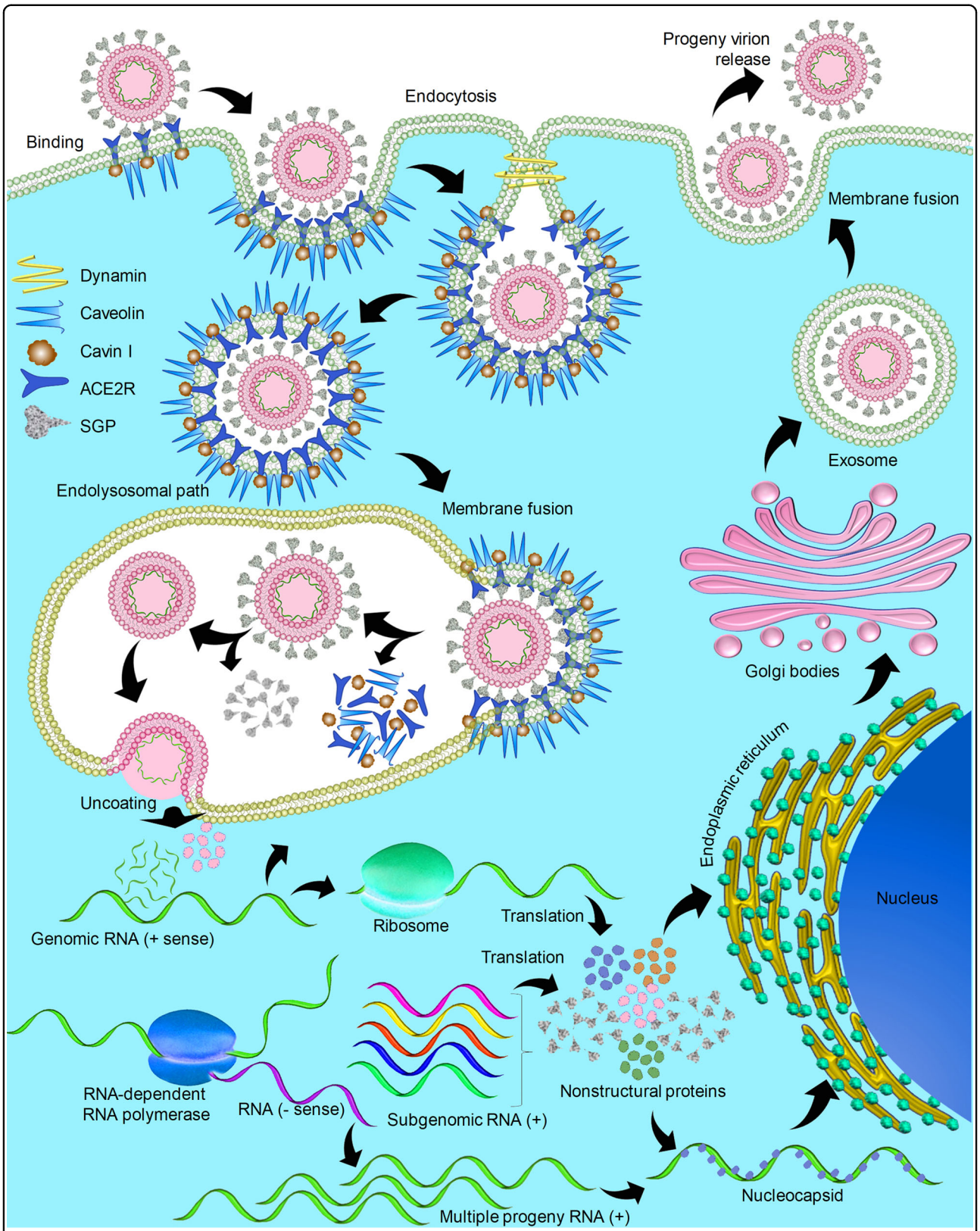


Fig. 2 The lifecycle of SARS-CoV-2 in a human respiratory epithelial cell. A scheme of cell binding, endocytosis, viral uncoating, transcription, translation, replication, and release of SARS-CoV-2.

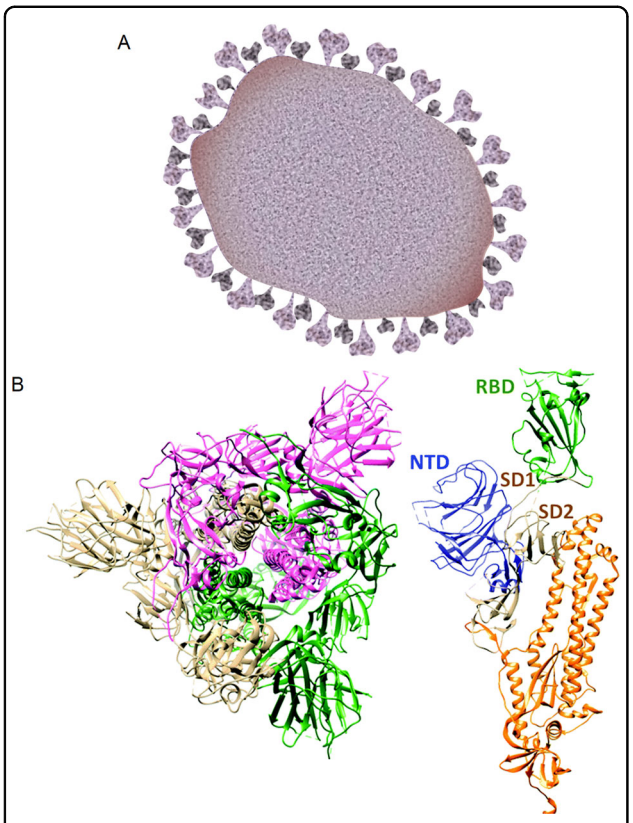


Fig. 3 The vital structures involved in the SARS-CoV-2 infection. **A** A cartoon structure of SARS-CoV-2 showing its SGPs. **B** The SGP and RBD structures of SARS-CoV-2[174]. **B** Copyright of the Royal Society of Chemistry.

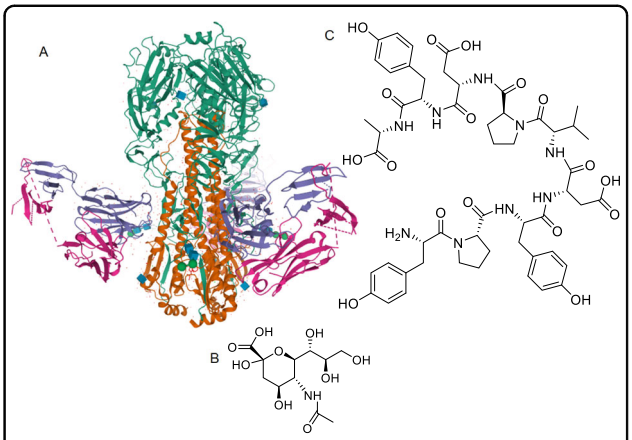


Fig. 4 Molecular structures other than SGP and ACE2R in the binding of SARS-CoV-2 to a host cell. The structures of **A** HA, **B** the Neu5Ac segment of Sia, and **C** the Sia-binding domain of HA. Courtesy of D. Sehnal, A. S. Rose, J. Kovca, S. K. Burley, S. Velankar (2018), RCSB Protein Data Bank 3GBN.

RBD of SARS-CoV is conserved throughout the infection period. The S1 subunit helps SARS-CoV-2 bind to ACE2R, and S2 enables the entry of the virus into a host cell. The free-energy change accompanying an SGP-induced conformational change to an ACE2R increases the infectivity by favoring the binding of another SGP of the same or different virus to a proximal receptor. Indeed, the energy barrier for appropriately destabilizing the host cell membrane and membrane fusion is as high as 42 kcal/mol, which can be supplemented by the low-affinity pseudo or secondary receptors in a host cell, such as heparin sulfate, ceramide derivatives, and HA.

The genes for FCS in SARS-CoV-2 suggest that a mutation occurred in *R. affinis*, *M. javanica*, or humans. Nevertheless, S1–S2 cleavage and the specificity of SGP to ACE2R indicate repeated passages of the SARS-CoV-2 progenitor in host cells expressing ACE2R, such as in humans, pigs, or civets. Thus, we do not rule out the possibility that the progenitor infected a human and repeatedly passed through one or more persons, who may or may not have shown COVID-19 symptoms. To date, there is no evidence to suggest that SARS-CoV mutated to SARS-CoV-2 in a cell or an animal model. Nevertheless, the mutations to SARS-CoV-2 continue to emerge in COVID-19 patients and is likely to continue further through animal and human hosts because, similar to other CoVs, SARS-CoV-2 virus lacks an enzyme for RNA proofreading, and preparations are necessary to deal with more strains that are more aggressively infectious than SARS-CoV-2. Despite the involvement of FCS, SGP, and ACE2R moieties in infection, the roles of Sias and HAs in SARS-CoV-2 and coreceptors such as chemokine receptors and O-linked glycan receptors in host cells should be considered during not only the development of a vaccine or a drug but also selecting foods and supplements to fight COVID-19.

The roles of Sia and HA in the infection and replication

Sia-capped proteoglycans in cells, secreted molecules, and vaccines play key roles in cell repulsion/adhesion, embryogenesis, pathogenesis, and the immune response[75,76]. HA (Fig. 4A), a lectin with ligand-binding domains 1 and 2, is common to CoVs. The binding of a pathogen to a Sia, such as the HA-based binding of CoVs to N-acetylneuraminic acid (Neu5Ac) (Fig. 4B), is the first step of an infection[77,78]. Thus, HA-to-Sia binding is addressed in several experimental and theoretical studies involving HA of influenza viruses[77,79–81]. Domain 1 (Fig. 4C) of HA shows a high affinity for Sia-capped glycans in a host cell[77]. Similarly to HA enrichment in an inactivated influenza vaccine, we hypothesize that one of the aims of COVID-19 management can be the HA-based binding of SARS-CoV-2 to proteoglycans in a host cell. We suggest this strategy by considering the binding of a mutated HA in the avian influenza virus to Neu5Ac in human epithelial cells[75,77,82]. Neu5Ac is human-specific α2–6-linked Sia that caps the outermost parts of proteoglycans in cell

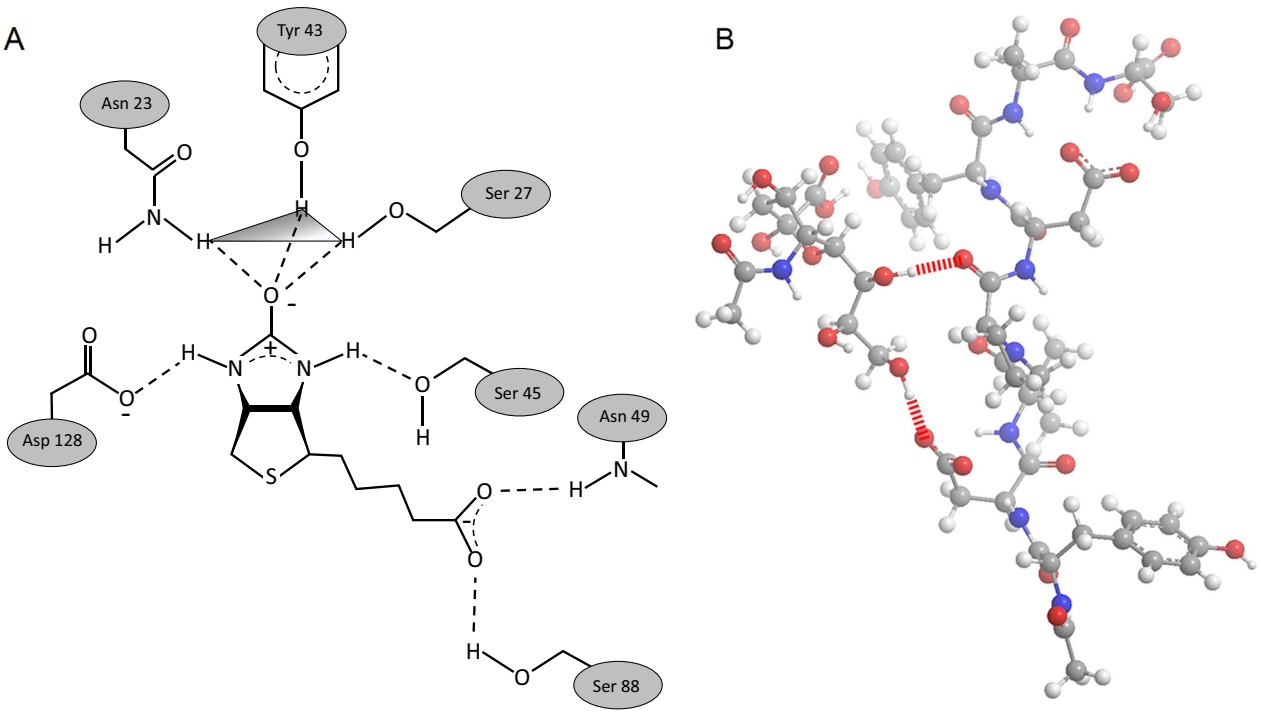


Fig. 5 The molecular basis for the Sia-based infection of SARS-CoV-2. Hydrogen-bonds in complexes formed between **A** biotin and seven amino acids in the biotin-binding domain of streptavidin and **B** the Sia-binding domain of HA and Neu5Ac.

membranes and secretions. Sia derivatives inhibit HA and prevent the attachment of influenza viruses to cells[77]. In contrast, the native HA of a CoV selectively binds to N-glycolylneuraminic acid (Neu5Gc) in a bird and the bird-to-human transmission is inefficient[83]. While several studies have focused on mutations to SGP and SGP-to-ACE2R binding, less attention has been directed to the roles of HA and Neu5Ac in SARS-CoV-2 and the levels of Neu5Ac in the upper respiratory system and the lungs of COVID-19 patients. Nonetheless, the affinity of HA for Neu5Gc should be considered. The presence of HAs and Neu5Ac-selective NAs in CoVs directed our attention to the Sia-bearing glycans, and the pathological and immunological aspects of COVID-19.

Similarly to other pathogens, CoVs express Sia-bearing glycans on their surfaces to deceive sensors that trigger IFN signaling and bypass host innate immunity. Thus, vertebrates modify their own glycans to fight pathogens. To comprehend the HA- and Neu5Ac-based infection of the respiratory epithelial cells by SARS-CoV-2, we compare the structures (Fig. 4) of Neu5Ac and the Sia-binding domain of the HA peptide. The multiple hydroxyl moieties in Neu5Ac, which are hydrogen (H)-bond donors, and the multiple carboxylic and amide moieties in the HA domain, which are H-bond acceptors, can be the thermodynamic regulators of the binding and infection. The strength of the H-bonds formed between HA and Neu5Ac is assessed based on the free-energy change of biotin–streptavidin (B-S) complex formation (Fig. 5A)[84]. Although the net entropy change (−13.7 kcal/mol) does not favor the formation of the B-S) complex, the enthalpic contribution (−32 kcal/mol) stabilizes it[84–86]. In the B-S complex, the enthalpy change involves multiple H-bonds of the ureido and carboxylic groups in biotin to seven amino acids in avidin. Similarly, by considering multiple H-bond donors and acceptors in the HA domain and Neu5Ac, we calculate the H-bond energy of the HA–Neu5Ac complex at 34.3 kcal/mol using the B3LYP/6–31 + G** level of the density functional theory[87]. The initial structures of the HA domain and Neu5Ac were obtained from the Protein Data Bank[88]. In addition, the initial relative positions of the two were assumed from the structures in the data bank. The heat of HA-to-Neu5Ac complexation is not surprising to us by considering the eight H-bonds between α2-3-linked sialyllactose and the eight amino acids in the Sia-binding domain of HA[77]. The appreciably high enthalpy of H-bonding stabilizes the HA–Neu5Ac complex more than it does in the B-S complex, enabling the HA–Neu5Ac complex to form deep potential well that attracts a virus and a progeny virion, which benefit from energy-efficient NA/ES-hydrolyzed cleavage during endocytosis and release.

Although the HA-mediated binding of pathogens to Neu5Ac in epithelial cells is common to many diseases, an important lineage difference between chimpanzees and

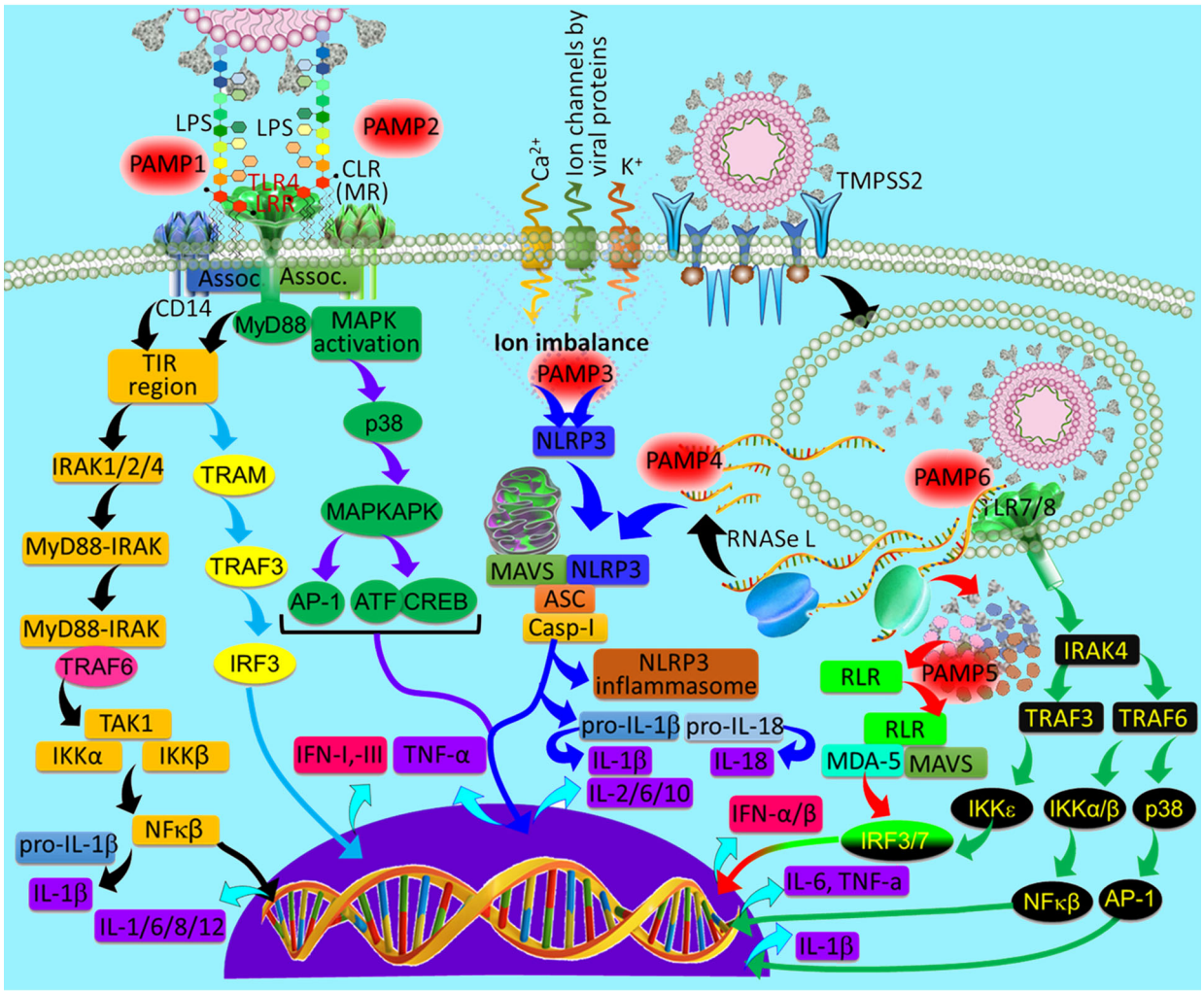
humans, that is, the presence of Ne5Gc in place of Ne5Ac in chimpanzees, is neglected during clinical tests of the antimalarial drugs such as HCQ against SARS-CoV-2. While chimpanzees or bonobos injected with *P. falciparum*-infected blood or exposed to *P. falciparum*-carrying Anopheles mosquitoes do not show the malaria symptoms, *P. falciparum* infects human erythrocytes expressing Neu5Ac and causes life-threatening malaria[89–92]. In contrast, *Plasmodium reichenowi*, which shares a common ancestor with *P. falciparum*, effectively infects apes expressing Neu5Gc in their cell membrane[93]. Thus, in addition to the FCS modification and ACE2R-based binding of SARS-CoV-2 onto human respiratory epithelial cells, lineage-derived Neu5Ac capping of epithelial glycoproteins may be at the center of the human-selective lethal infection of SARS-CoV-2 through HA. Although HCQ suppresses the endosomal escape of SARS-CoV-2, it does not show any relation to Neu5Ac in humans or Neu5Gc in chimpanzees. Nevertheless, the risk factors for COVID-19 may be related to the presence of any Neu5Gc-specific agglutinin in SARS-CoV-2 and a high level of Neu5Gc-capped glycans acquired through the assimilation of animal proteins by the COVID-19 patient. This HA- and Sia-based pathogenesis becomes significant to CoVs bearing an ES and a NASe specific to the α2-3-linked Sias. Although the T and B cells with the α2-6-linked glycans and the macrophages surveilling for the Neu5Ac-bearing pathogens play crucial roles in the immunosuppression of HA-to-Sia selective infections, we suggest that verifying the relationships of the acquired Neu5Gc to various agglutinins, O-linked glycans, and ESes and NASes is important. This verification can be relevant to COVID-19 because of the different infection and mortality rates of the disease in populations with different dietary habits, and lineages.

Pattern recognition, immune response, IFN production, and virus replication

An immune response against SARS-CoV-2, similarly to that against other CoVs and microbes, begins with the activation of a series of pattern recognition receptors (PRRs). During surface binding, internalization, endosomal transport, cytosolic uncoating, RNA polymerization, and translation in a host cell, viral lipids, proteins, and RNA are recognized by various pathogen-associated molecular patterns (PAMPs) in the cell membrane, endosomes, and cytoplasm. This recognition continues throughout the viral lifecycle in a host cell. Figure 6 shows various PAMP- and PRR-associated immune responses in COVID-19, which are analogous to other CoVs. In contrast to a nonenveloped virus that enters a host cell by the endocytic/nonendocytic path[94,95], the enveloped nature of SARS-CoV-2 leads to its preferential endocytic membrane fusion through the recruitment of clathrin/calveolin[96,97]. The mechanisms of infection involving the virological synapse, cell penetration, and transcytosis have yet to be verified. SARS-CoV-2 binds to a host cell through the SGP-TMPSS2-ACE2R (SGP-transmembrane protease serine 2-ACE2R) network[1,8,14,68,69]. In SGP-to-ACE2R binding, the free-energy change of the conformation switching of a single SGP enables membrane fusion[98].

Following the extracellular ACE2R-specific binding using SGP, and the different stages of pattern recognition, the immune responses in COVID-19 are classified according to the activation of PAMPs by various viral patterns (Fig. 6). In general, pattern recognition is initiated by the interactions of surface proteins, genetic materials (single-stranded RNA (ssRNA)/double-stranded DNA (dsRNA)/ssDNA/dsDNA), or uncoated/translated proteins of a pathogen by PRRs, such TLRs (toll-like receptors)[99–103], RLRs (RIG-like receptors)[104–107], NLRs (NOD-like receptors)[108–112], MDA-5 (melanoma differentiation-associated protein 5)[113,114], CLR/MR (C-type lectin-like receptor/mannose receptor)[115,116], and DAI (DNA-dependent activator of IFN-regulatory factors (IRFs))[117,118] in a host cell. For example, the dsRNA of the nonenveloped Reoviridae family of viruses, such as rotavirus, is recognized by TLR3, RIG-I (retinoic acid-inducible gene I), and MDA-5[119–121], whereas the ssRNA of viruses such as SARS-CoV-2, SARS-CoV, MERS-CoV, rhinoviruses, dengue virus, and hepacivirus C are recognized in the endosome by TLR7 and TLR8[122–127]. In contrast, the ssDNA of viruses such as the smallpox virus and the chickenpox or varicella viruses is effectively recognized by TLR9, and RIG-I[128,129]. Indeed, independent of the DNA genome of various viruses, pattern recognition, and IFN induction follow the RNA pathway because of reverse transcription during replication.

Various PRRs and PAMPs involved in SARS-CoV-2 and other pathogens are summarized in Fig. 6 and briefly discussed here. PAMP1 is initiated by the recognition of viral lipopolysaccharide (LPS) by TLR4[130] followed by its association with CD14, leading to the activation of the MyD88-dependent[131] and MyD88-independent[132] pathways and the secretion of several cytokines, including interleukin-1β (IL-1β)/6/8/12, and IFN-α/β/γ. Extracellular PAMP2 is recognized by TLR4[133,134], which associates with CLRs such as MR and activates the complex MAPK downstream signaling. Only a part of the MAPK pathway leading to the secretion of IL-1β/2/6/10/18, and TNF-α is shown in Fig. 6. The cytosolic ion imbalance forms PAMP3[135,136], which is created by ion channel mimicking nonstructural viral proteins[137,138], pathogen-associated Ca^{2+} influx, or K^{+} efflux. PAMP4 is triggered by ssRNA[139,140] released from the endosomes, subgenomic RNAs produced by the RNA-dependent RNA polymerase, or the RNA fragments produced by RNASe-L. PAMP3 and PAMP4 are recognized by NLRP3[135–141], leading to the activation of multiple signaling pathways by Casp-I and the secretion of various ILs, and TNF-α, which are shown in Fig. 6. The NLRP3 inflammasome pathways are excluded here. PAMP5 is a combination



AP : activator protein
ASC : apoptosis-associated speck-like protein containing C-terminal caspase recruitment domain
ATF : activating transcription factor
Casp : caspase
CD : cluster of differentiation
CLR : C-type lectin receptor
CREB: cyclic adenosine monophosphate-response element-binding protein
IFN : interferon
IKK : inhibitory kappa B kinase
IL : interleukin
IRAK : interleukin-1 receptor-associated kinase
IRF : interferon regulatory factors
LPS : lipopolysaccharide
MAPK: mitogen-activated protein kinase
MAPKAPK: MAPK activated protein kinase
MAVS : mitochondrial antiviral-signaling protein
MDA : melanoma differentiation-associated protein
MR : mannose receptor
MyD88: myeloid differentiation primary response 88
NFκβ : nuclear factor-kappa B
NLRP : nucleotide-binding oligomerization domain (NOD)-like receptor pyrin domain containing
PAMP : pathogen associated molecular pattern
RLR : RIG (retinoic acid-inducible gene)-I-like receptor
TAK : transforming growth factor-β-activated kinase
TIR : toll/interleukin-1 receptor
TLR : toll-like receptor
TMPSS2: transmembrane protease, serine 2
TNF : tumor necrosis factor
TRAF : TNF-receptor-associated factor
TRAM: TLR-associated molecule

Fig. 6 The mechanisms of SARS-CoV-2 infection and the corresponding immune responses. A scheme showing various pathways of the extracellular binding, internalization, endosomal transport, and uncoating of SARS-CoV-2 and the corresponding pattern recognition and cytokine production in a host cell.

of viral proteins, including those uncoated and released from endosomes, and those translated by reading the genomic/subgenomic viral RNA. PAMP5 is recognized by RLR[142–144], which associates with MDA-5 and MAVS (mitochondrial antiviral signaling) and activates IRF3/7, leading to the secretion of IFN-α/β. PAMP6 is the endosomal recognition of ssRNA by TLR7/8[124,125,145–147], leading to multiple signaling pathways through TNF receptor-associated factors (IκB kinases and p38), and the secretion of IL-1/6, IFN-α/β, and TNF-α.

IFNs[148–157], a family of immunomodulatory macromolecular ligands or secreted cytokines play an important role in an immune response against SARS-CoV-2. Apart from the important antitumor and antiproliferative roles of IFNs[158,159], these cytokines help fight viral infections by activating innate and adaptive immune responses. SARS-CoV-2 pattern-recognized secretion of IFNs is shown in Fig. 6. On the one hand, pattern recognition followed by downstream signaling pathways produces ILs, IFNs, and TNF-α and on the other hand, antiviral action is triggered in the JAK-STAT transcription pathway, which is by secondary signaling and the activation of IFN receptors in the infected and immune cells[148,149]. Among the 23 widely known members in the IFN family in mammals, 21 are common to the human body. Based on functioning, signaling pathways, and clustering in chromosomes, IFNs are classified into IFN-I, -II, and -III. IFN-I is the largest family, with 19 members for which the genes are clustered on human chromosome 9[150]. Among the 19 members, IFN-α (13 homologs) and IFN-β distributed in essentially all cells play the primary role in the innate response against infections, through the JAK-STAT pathway. IFN-II, also called IFN-γ, with its genes clustered in human chromosome 12[151], is common to immune cells, and its secretion and action are delayed until the immune cells are activated. Thus, IFN-γ is the adaptive cytokine. IFN-III includes three members, IFN-λ1, -λ2, and -λ3, also called ILs 28A, 28B, and 29, with their genes clustered in human chromosome 19[152]. Similar to IFN-I cytokines, IFN-III cytokines are induced by viruses and are secreted by any cell in response to SARS-CoV-2. Thus, IFN-I and IFN-III secreted by the respiratory epithelial cells initiate innate immunomodulation against SARS-CoV-2. The JAK-STAT pathway of secondary signaling is discussed elsewhere[148,149].

Following asymptomatic infection by SARS-CoV-2, a person with a weak immune system becomes sick and suffers from a severe immune overreaction or cytokine storm, leading to an increased respirational distress and fatality[153]. Thus, immune suppressants such as Actemra or tocilizumab are prescribed to COVID-19 patients[159]. Nonetheless, the recovery rate of COVID-19 patients depends on the health condition of the patients. For example, patients younger than 40 years old with diabetes, cardiovascular diseases, impaired renal health, or cancer show a poor recovery rate after COVID-19 infection[160,161]. The relationship between the innate and adaptive immune (IFN) response of host cells, including the production of various proinflammatory cytokines, IFN-stimulated genes, IRFs, and various proteins including myxovirus (Mx) proteins and the ability of the virus to escape the immune response are important to the recovery of a COVID-19 patient. Mutations to the RNA of SARS-CoV-2 when compared with those in Pangolin-CoV, MERS-CoV, SARS-CoV, and RaTG13 may provide clues about turning off or overstimulating the IFN pathway in COVID-19. The cytokine storm in several fatal COVID-19 cases suggests that the virus does not bypass pattern recognition (Fig. 6). Thus, the role of IFN-inducible genes such as ISGs and Mx-A/-B proteins, which are crucial for preventing the formation of progeny virions through both the inactivation of the nucleocapsid (NC) protein and inhibition of the progeny ribonucleoprotein, in the cytokine storm and the progression of COVID-19 is being extensively investigated.

To date, there is no strong experimental correlation between SARS-CoV-2 and any acute respiratory illness in mice or pets. In addition, in contrast to humans, several animals do not show functional IFNs. Thus, the long lineage from a common ancestor of humans and animals should be the basis for a search for a correlation between IFN-inducible Mx genes and COVID-19. In addition to the presence of Mx-inducible genes in humans, the defective Mx genes in mice susceptible to influenza[162] and the IFN-regulated Mx in mice resistant to influenza[163] educate us on the importance of examining how SARS-CoV-2 inactivates the innate IFN-I/III pathways and successfully integrates progeny virions.

Summary and outlook

Despite the auspicious progress of COVID-19 vaccine research and vaccination, the highly infectious nature and mutations of SARS-CoV-2 are warnings of a backbiting annual revival of the virus. Evidence from RNA-sequencing showing the infection mode of SARS-CoV-2 to be SGP-to-ACE2R binding is supported by cleavage at the S1–S2 boundary of the furin segment. On the one hand, ACE2R in the respiratory endothelial cells is involved in the infection, and on the other hand, its presence in the cells of the heart, kidneys, and other vascular systems underscores the relationship of COVID-19 to cardiovascular diseases, cancer, and diabetes. Also, ACE2R-based infection may be related to the Kawasaki disease-mimetic multisystem inflammatory syndrome in children. In addition to ACE2R-based binding, the roles of HA, Neu5Ac(Ge), and NASe/SE in SARS-CoV-2 infection and release cannot be neglected.

The relationship between Sias and HA has attracted much attention since the 1918 influenza (H1N1) pandemic, whereas the details of the binding between the two are the focus of ongoing studies. Enzymes such as NASe, which cleaves the bond between the viral HA and the host Sias[164], likely have roles in the endocytosis of SARS-CoV-2 in the

respiratory epithelial cells and the release of its progeny through the ER pathway. Although the Neu5Ac Sia innate to humans is the target of several pathogens, including CoVs, the negative results for COVID-19 patients treated with several antiviral drugs and vaccines may be related to mutations in not only the FCS motif but also the Neu5Ac-binding segment of HA. If a innate Sia is not the target of the SARS-CoV-2 HA, it is worth considering a connection between the levels of Sias acquired from the diet, such as Neu5Gc, and the severity of COVID-19. Another aspect of Sias in COVID-19 is the escape of the virus from the innate IFN response, which is similarly to other pathogens that express and modify Sias on the cell surface. In addition, the abilities of Sia derivatives to inhibit HA and thwart influenza viruses encourage us to hypothesize that dietary supplements with acidic sugars, such as ascorbic acid, gluconic acid, saccharic acid, and tartaric acid, may have impacts on COVID-19. Although clinical studies are needed to understand whether Neu5Ac, Neu5Gc, other Sias, or sugar acids alleviate the severity of COVID-19, diets rich in sugar acid-rich fruits can always be recommended to a COVID-19 patient. Nevertheless, the stereoselectivity of sugar acids, Sias, and NASe/SE needs further study.

To resolve the preclinical challenges of COVID-19, an interface among chemistry, nanoscience, cell biology, and virology is emerging[165–173]. The primary objective can be the construction of a SARS-CoV-2 mimetic virus nanoparticle (SCoV-MNP; Fig. 7) decorated with SPG, HA, and

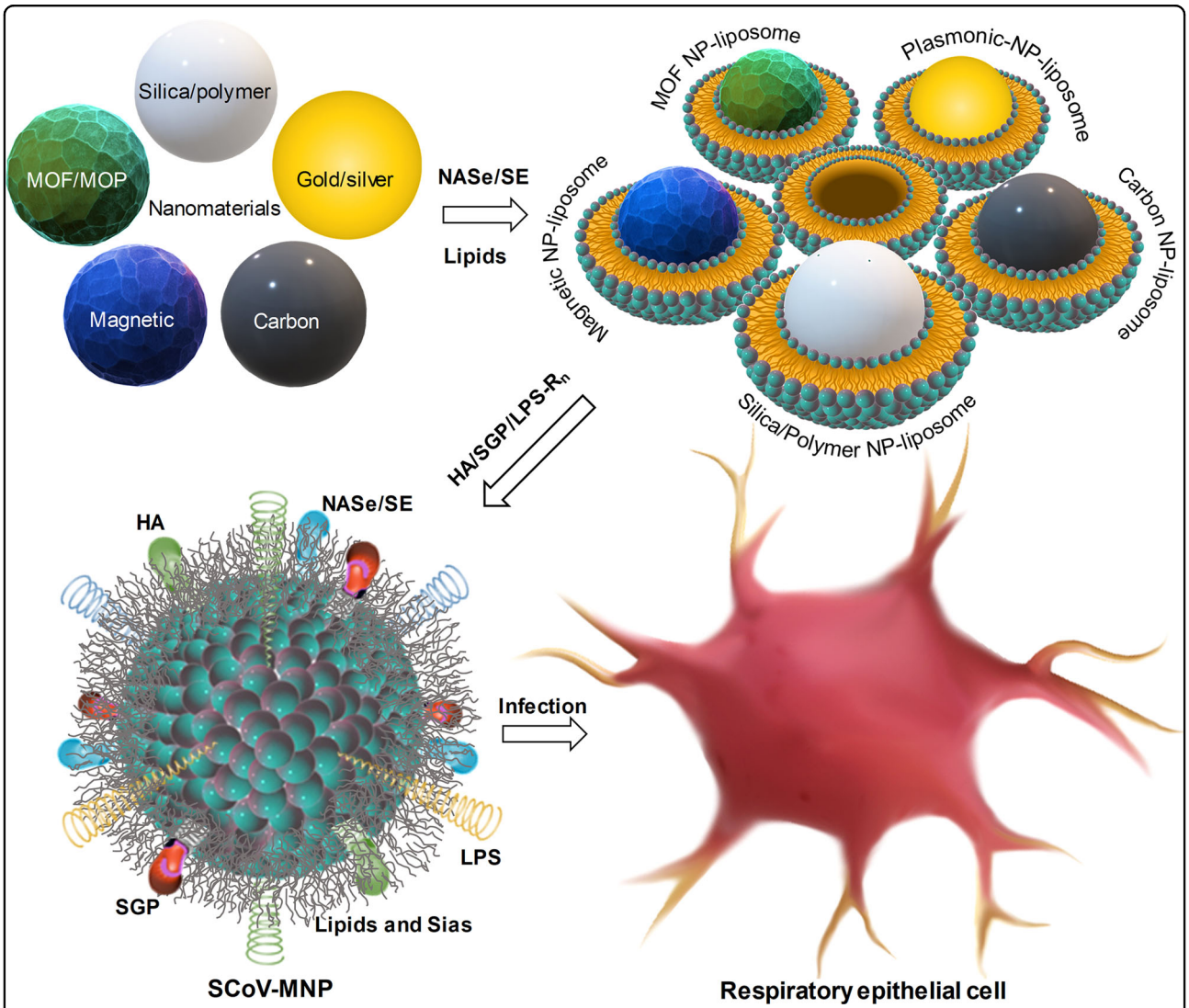


Fig. 7 A scheme showing the prospects of SCoV-MNPs for SARS-CoV-2 mimetic research at the interface of materials science, chemistry, nanoscience, immunology, drug delivery, and medicine. Nanoparticles are prepared with silica, iron oxide, gold, carbon, or metal organic polyhedra (MOPs) or frameworks (MOFs). NASe/ES can be incorporated in liposomes to analyze HA–Neu5Ac cleavage. SGP, HA, LPS, and Sias can be incorporated on the surface of SCoV-MNPs using polyarginine (R_n).

Sias and encompassed by ESs/NASes but without the SARS-CoV-2 RNA. Subsequently, the microspectroscopic correlation of SPG- or HA-based binding of SCoV-MNPs to Sias in human respiratory/lung epithelial cells has helped increase the understanding of the mode of infection. In addition, SCoV-MNPs decorated with proton-buffering molecules will be helpful for revealing the endolysosomal pathway and the viral uncoating processes. Such virus mimetics can be modified into antiviral drugs engaged in the endosomal arrest of SARS-CoV-2. Fluorescence-assisted cell sorting, confocal fluorescence microscopy, super-resolution fluorescence imaging, and Förster resonance energy transfer-microspectroscopy are powerful tools for analyzing the binding, membrane fusion, endocytosis, uncoating, the formation of progeny virions, and the release of SARS-CoV-2. In such studies, fluorescence probes and energy donors can be selected from among organic dyes, fluorescent proteins, or brilliantly luminescent semiconductor quantum dots. These studies will be helpful for evaluating the stereoselectivity of ESs and NASes in SCoV-MNPs to O-linked glycans. In addition, the escape of CoVs from the host innate immune response can be analyzed using SCoV-MNPs decorated with Sias.

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Conflict of interest
The authors declare that they have no conflict of interest.

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