Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors

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The Middle East respiratory syndrome coronavirus (MERS-CoV) causes severe and often lethal respiratory illness in humans, and no vaccines or specific treatments are available. Infections are initiated via binding of the MERS-CoV spike (S) glycoprotein to sialosides and dipeptidyl-peptidase 4 (the attachment and entry receptors, respectively). To understand MERS-CoV engagement of sialylated receptors, we determined the cryo-EM structures of S in complex with 5-N-acetyl neuraminic acid, 5-N-glycolyl neuraminic acid, sialyl-Lewisα, α2,3-sialyl-N-acetyl-lactosamine and α2,6-sialyl-N-acetyl-lactosamine at 2.7–3.0 Å resolution. We show that recognition occurs via a conserved groove that is essential for MERS-CoV S-mediated attachment to sialosides and entry into human airway epithelial cells. Our data illuminate MERS-CoV S sialoside specificity and suggest that selectivity for α2,3-linked over α2,6-linked receptors results from enhanced interactions with the former class of oligosaccharides. This study provides a structural framework explaining MERS-CoV attachment to sialoside receptors and identifies a site of potential vulnerability to inhibitors of viral entry.

MERS-CoV is an enveloped Nidovirus decorated with homotrimers of the spike (S) glycoprotein that mediates entry into host cells. S is the major antigen present at the viral surface and is the target of neutralizing antibodies during infection as well as the focus of vaccine design. Recent cryo-EM structures of MERS-CoV and related coronavirus S ectodomain trimers provided snapshots of this key protein in prefusion and postfusion conformations, receptor-bound states and in complex with neutralizing antibodies. MERS-CoV S is composed of an N-terminal S1 subunit, which is folded as four domains (A–D) and mediates attachment to dipeptidyl-peptidase 4 (DPP4, the host receptor) and is processed at the onset of membrane fusion. Both cleavage sites, designated S2′, are found upstream of the fusion peptide in the S2 subunit and is processed at the onset of membrane fusion. Both cleavage sites participate in enhancing viral entry and modulating host range and cell tropism.

Sialic acids (derivatives of neuraminic acid) are ubiquitous carbohydrates found as terminal residues on glycoproteins and glycolipids decorating the surface of eukaryotic cells. Neuraminic acid modifications, along with the formation of specific glycosidic linkages, result in a wide chemical variety of sialoglycoconjugates across cell types, tissues and animal species. As a result, differential sialoside recognition can profoundly impact the zoonotic transmission, tropism and virulence of many viruses. For example, a few amino acid substitutions in influenza virus hemagglutinins account for the preference switch from avian enteric tract to human respiratory tract sialylated receptors.

MERS-CoV primarily infects human lung epithelial cells upon interacting with DPP4. Crystal structures of the MERS-CoV S domain B in complex with the DPP4 ectodomain and cryo-EM structures of the MERS-CoV S ectodomain trimers, have furthered our understanding of the mechanism of DPP4 engagement. In addition to attachment to DPP4, we recently showed that MERS-CoV infection of human airway epithelial cells involves low-affinity interactions with sialosides, using the S glycoprotein domain A, as depletion of sialic acid from the cell surface dampened viral entry. A binding preference was found for α2,3-linked over α2,6-linked sialosides, and these interactions were hindered by 9-O-acetylation or 5-N-glycolylation of the terminal neuraminic acid. Furthermore, we found that MERS-CoV S sialylated receptors are abundant in the camel nasal respiratory epithelium and the human lung alveoli, which coincides with DPP4 expression and the sites of MERS-CoV replication in these mammals. As sialoside modifications, linkages and distribution vary among and within host species, the selectivity of MERS-CoV S for certain sialoside glycoconjugates may provide a determinant of host and tissue tropism of this zoonotic pathogen. These findings provided a plausible rationale for explaining that equine DPP4 could support MERS-CoV infection of cultured cells, although horses were found to be resistant...
to experimental MERS-CoV infection (despite the presence of DPP4 in their respiratory tract)34–36. Although sialoside attachment appears to be a key step modulating MERS-CoV infection, the location of the sialoside-binding site, the interactions involved in ligand recognition and the molecular basis for receptor specificity remain unknown. This information is crucial for understanding viral tropism and infectivity, assessing the zoonotic potential of MERS-CoV and related coronaviruses, and providing a blueprint for the design of coronavirus inhibitors.

To understand the structural basis of MERS-CoV attachment to and specificity for host sialosides, we determined cryo-EM structures of the S glycoprotein ectodomain trimer in complex with 5-N-acetyl neuraminic acid (Neu5Ac), 5-N-glycolyl neuraminic acid (Neu5Gc), sialyl-Lewis^x^ (sLe^x^), α2,3-sialyl-N-acetyl-lactosamine (2,3-SLN) and α2,6-sialyl-N-acetyl-lactosamine (2,6-SLN) at 2.7, 3.0, 2.7, and 2.9 Å resolution, respectively. We demonstrate that the receptor binds in a groove located at the surface of domain A that is distinct from the 9-O-acetyl sialoside-engagement site identified for HCoV-OC43 and related β-1 coronavirus S glycoproteins8,9,19. We further show that the residues involved in Neu5Ac recognition are conserved across MERS-CoV isolates and essential for MERS-CoV S-mediated hemagglutination of human erythrocytes and entry into human airway epithelial cells. Our data rationalize MERS-CoV S attachment to neuraminic acids that are not 9-O-acetylated or 5-N-glycolylated and suggest an explanation for the selectivity for α2,3-linked over α2,6-linked sialosides. This study provides a structural framework for understanding MERS-CoV S sialoglycan receptor engagement and specificity, illuminates host range and cell tropism, and identifies a site of vulnerability accessible to neutralizing antibodies and small-molecule inhibitors.

**Results**

A sialoside-binding site in the MERS-CoV S glycoprotein. To identify the MERS-CoV S sialoside-recognition site, we determined a cryo-EM structure of the MERS-CoV S 2P stabilized ectodomain trimer14 incubated with 100 mM Neu5Ac at 2.7 Å resolution (Fig. 1a, Extended Data Figs. 1a and 2a–g and Table 1). The presence of the sialoside stabilized a large fraction of MERS-CoV S trimers in the closed state, with all three DPP4-binding B domains adopting a down conformation, obeying three-fold symmetry. We did not detect major conformational changes compared to closed protomers of the LCA60 Fab-bound MERS-CoV S structure29 (Cα r.m.s. deviation (r.m.s.d.) of 1.35 Å for 1,145 aligned residues). The resolution estimate of our map is supported by the detection of ordered water molecules44 interacting with the S glycoprotein (Extended Data Fig. 1f,g) and by the observation of folic acid bound to S, as previously identified using X-ray crystallography and mass spectrometry of isolated domain A14. The density is equally well resolved for the ligand and the surrounding S amino acid residues, allowing unambiguous identification of the binding site and docking of Neu5Ac in the map (Extended Data Fig. 1d). The ligand interacts with a groove located at the periphery of domain A that is distinct from the HCoV-OC43 S 9-O-acetyl sialoside-binding site19 (Figs. 1a–e and 2a–c). The absence of N-linked glycans in the immediate vicinity of the binding groove probably facilitates unobstructed engagement of sialosides on a glycan array14. The structure suggests the MERS-CoV S sialoside-binding site is required for S-mediated viral attachment and entry. To evaluate the importance of the interactions identified by cryo-EM for sialoside recognition, we substituted with alanine individual MERS-CoV S amino acid residues interacting with Neu5Ac and assessed the effect on domain A-mediated hemagglutination of human erythrocytes. As previously described, the wild-type MERS-CoV S domain A fused to human immunoglobulin Fc only promoted hemagglutination of human erythrocytes when multimerized on lumazine synthase nanoparticles28, indicative of enhancement of low-affinity interactions via avidity (Fig. 3a and Extended Data Fig. 5a). Complete inhibition of hemagglutination was observed, however, for each binding site mutant tested, that is, F39A, H91A, I132A, S133A and R307A (Fig. 3a and Extended Data Fig. 5a). These results validated the structural observations by

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demonstrating that recognition of Neu5Ac via these residues is necessary for MERS-CoV S-mediated attachment to sialosides.

To further assess the functional relevance of the identified interactions, we interrogated the impact of individual domain A substitutions on MERS-CoV S-mediated entry into target cells. We used the murine leukemia virus platform with a luciferase reporter for quantifying entry of particles pseudotyped with either wild-type or mutant MERS-CoV S into human airway Calu-3 cells. The S133A and R307A substitutions abrogated entry of pseudotyped particles due to disruption of the aforementioned electrostatic interactions involving the Neu5Ac C1 carboxylate and glycerol side chain, respectively (Fig. 3b and Extended Data Fig. 5b). The F39A and H91A mutants showed reduced infectivity of the pseudotyped particles by more than 90%, probably due to disruption of the ligand-binding groove and loss of favorable van der Waals or electrostatic interactions (Fig. 3b and Extended Data Fig. 5b). These findings demonstrate the key role of the identified residues for interacting with Neu5Ac in the context of a full-length, membrane-embedded MERS-CoV S glycoprotein and show that attachment to sialoside receptors using the binding site identified by cryo-EM is essential for promoting MERS-CoV S-mediated entry into human airway Calu-3 cells. Furthermore, as sialidase treatment of Calu-3 cells or mutations of the S sialoside-recognition site abrogated MERS-CoV S-mediated entry, it is likely that sialoside attachment either precedes DPP4 engagement or occurs simultaneously.

MERS-CoV S specificity for α2,3-linked sialoside receptors. We previously showed that the MERS-CoV S domain A preferentially interacts with α2,3-linked, compared to α2,6-linked, sialosides, with sulfated sLeX being the preferred binder. To understand the architectural principles underlying this specificity, we determined three cryo-EM structures of MERS-CoV S in the presence of 50 mM sLeX (Extended Data Figs. 1c and 6 and Table 1), 70 mM 2,3-SLN (Extended Data Figs. 1d and 7, and Table 1) or 70 mM 2,6-SLN (Extended Data Figs. 1e and 8 and Table 1) at 2.7, 2.7 and 2.9 Å resolution, respectively.

The cryo-EM reconstruction obtained for MERS-CoV S in complex with sLeX resolves the terminal Neu5Ac linked to galactose, via a
2,3-glycosidic bond adopting a cis configuration, as well as the N-acetyl glucosamine (GlcNAc) and fucose rings at lower contour level due to conformational heterogeneity of the ligand (Fig. 4a,b). Engagement of the Neu5Ac moiety of the sLeX involves identical interactions to those observed in our structure of MERS-CoV S in complex with Neu5Ac (Fig. 1c–e). This finding, and the detection of a galactose moiety linked to the oxygen at position 2 of Neu5Ac, validate our docking of the ligand in the cryo-EM density. The sLeX trisaccharide core (Neu5Ac(α2–3)galactose(β1–4)GlcNAc) adopts a roughly linear conformation when bound to MERS-CoV S (Fig. 4a,b), as is the case for influenza virus hemagglutinins in complex with α2,3-sialosides29,31,44.

The MERS-CoV S structure in complex with 2,3-SLN shows a very similar pose of the ligand to that observed for sLeX, although the GlcNAc is even more conformationally heterogeneous in the trisaccharide compared to sLeX and was therefore not modeled (Fig. 4c,d). The cryo-EM map obtained for MERS-CoV S bound to 2,6-SLN resolves the Neu5Ac and galactose moieties, linked via a 2,6-glycosidic bond adopting a cis configuration, and the GlcNAc ring at lower contour level due to conformational variability of the ligand (Fig. 4e,f). The 2,6-SLN has a U-shaped conformation, comparable to reports on influenza virus hemagglutinins29,30, that positions the GlcNAc moiety away from the binding groove and the rest of the S glycoprotein (Fig. 4e,f).

The cryo-EM structures suggest an explanation for the finding that sulfated sLeX is the preferred MERS-CoV S sialoside ligand34. Although the sLeX conformational dynamic limited its resolvability, the galactose and the GlcNAc moieties appear to be positioned within contact distance of the MERS-CoV S domain A due to the ligand conformation (Fig. 4a–d,g,h). Furthermore, sulfation of the GlcNAc hydroxyl group at position 6 could putatively allow
formation of electrostatic interactions with the Gln304 side chain or neighboring residues (Fig. 4g,h), as described for influenza virus A/Vietnam/1194/2004 hemagglutinin. The bent architecture of 2,6-SLN, however, orients the GlcNAc away from the binding site, hindering contacts with S (which appear limited to the Neu5Ac and galactose moieties). We therefore hypothesize that the trend of preferential attachment to α2,3 over α2,6-sialosides results from enhanced interactions with the former type of ligand, leading MERS-CoV to target cell types presenting such sialylated receptors at their surface.

Discussion

We show here that sialosides bind to MERS-CoV S using a site distinct from the one observed for HCoV-OC43 S in complex with 9-O-acetyl sialylated receptors, despite the fact that the bound ligands are only separated by a few ångstroms from each other when superimposing the two structures. Although the β-sandwich architecture of domain A is conserved among all coronaviruses, distinct variable loops are grafted onto it, and neither the MERS-CoV S nor the HCoV-OC43 S sialoside-binding site is conserved in the infectious bronchitis virus S glycoprotein, which is known to engage α2,3-sialosides at the surface of avian host cells. These findings suggest coronavirus S glycoproteins have independently evolved the ability to recognize sialosides using domain A, similar to their ability to engage different proteinaceous receptors via modifications of domain B. Our results therefore set coronavirus S glycoproteins apart from influenza virus A hemagglutinins, which recognize the Neu5Ac moiety through a largely conserved binding site29,30.
We hypothesize that coronaviruses that utilize both an entry receptor and an attachment receptor for infection, such as MERS-CoV or transmissible gastroenteritis virus, have more evolutionary freedom with respect to their ability to attach to sialoglycans than viruses only using an entry receptor. This could have led to the independent loss and/or acquisition of a sialoside-recognition function as an adaption to new hosts upon cross-species transmission of distinct viruses. The ability to interact with sialo-glycoconjugates might be a recent MERS-CoV S acquisition, as neither HKU4 S nor HKU5 S could hemagglutinate human erythrocytes. This modular evolutionary plasticity underlies coronavirus cross-species transmission, switching of host cell types and viral fitness.

We previously suggested that MERS-CoV S interacts with sialoside receptors through lower-affinity interactions than influenza A/California/04/2009 hemagglutinin (containing the T200A and E227A substitutions), as multimerization with a lumazine synthase ectodomain-Fc construct but not of a dimeric hemagglutinin E227A substitutions), as multimerization with a lumazine synthase.

Although MERS-CoV S is densely decorated with N-linked glycans, the sialoside-recognition site is unobstructed, a possible requirement for receptor engagement. This finding is in marked contrast with the conformational masking and/or glycan shielding of the domain B entry receptor-binding motifs observed for several coronavirus S glycoproteins, including MERS-CoV S.13,15,18,20,21. We recently identified an antibody-blocking domain A-mediated hemagglutination of erythrocytes with in vitro neutralizing activity of MERS-CoV S pseudovirions and partial in vivo prophylactic protection of mice challenged with a lethal dose of MERS-CoV.49. These findings, along with our mutagenesis data, indicate that the sialoside-binding groove identified here represents a key site of vulnerability to inhibitors of MERS-CoV infection that could be targeted for the future development of therapeutics.

Online content
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**Methods**

**Cell lines.** HEK293F is a cell line adapted to grow in suspension (Life Technologies). HEK293T/17 cells (ATCC, catalog no. CRL-11268) and human HepG-2 cells (National Institutes of Biomedical Innovation, Health and Nutrition, catalog no. RBR0403) are adherent cells. Cell lines were not authenticated or tested for mycoplasma contamination.

**Protein expression and purification.** The MERS-CoV S 2P ectodomain was produced in 500 ml HEK293F cells grown in suspension using FreeStyle 293G expression medium (Life Technologies) at 37 °C in a humidified 8% (vol/vol) CO₂ incubator rotating at 130 r.p.m. The cultures were transfected using 293fectin (ThermoFisher Scientific) with cells grown to a density of one million cells per ml and cultivated for three days. The supernatants were harvested and cells resuspended for another three days, yielding two harvests. Clarified supernatants were purified using a 5 ml Cobalt affinity column (Takara). Purified protein was concentrated and flash-frozen in Tris-saline (50 mM Tris, pH 8.0, 150 mM NaCl) before cryo-EM analysis.

Purified protein was oxidized using a 12% (wt/vol) SDS–PAGE gel under reducing conditions and stained with GelCodeBlue stain reagent (Thermo Scientific).

**Cryo-electron microscopy sample preparation and data collection.** Incubation of 1 μM MERS-CoV S 2P stabilized ectodomain trimer with 100 mM Neu5Ac (Millipore 110138), 100 mM Neu5Gc (Millipore 362000), 70 mM 2,3-SLN (Carbosynth OS04058), 70 mM 2,3-SLN (Carbosynth OS06484) or 70 mM 2,6-SLN (Carbosynth OS09313) was performed overnight at 4 °C. We used 100 mM Neu5Ac as based on our previous work on HCoV-OCA43 S 55 and work on influenza virus 56, whereas the lower concentrations used for larger ligands were determined empirically based on the contrast of cryo-EM micrographs. A 3 μl volume of 0.16 mg/ml MERS S-sialoglycan complex was loaded onto a freshly glow-discharged (30 s at 20 mA) lacy carbon grid with a thin layer of evaporated continuous carbon before plunge freezing using a vitrobot MarkIV (ThermoFisher Scientific) using a blot force of ~1 and 2.5 s blot time at 100% humidity and 25 °C. Data were acquired using an FEITitan Krios transmission electron microscope operated at 300 kV and equipped with a Gatan K2 Summit direct detector and Gatan Quantum GIF energy filter, operated in zero-loss mode with a slit width of 20 eV. Automated data collection was carried out using Leginon 57 at a nominal magnification of ×10,000 and a pixel size of 0.525 Å. The dose rate was adjusted to 8 counts per pixel per second, and each video was acquired in super-resolution mode fractionated in 50 frames of 200 ms.

**Cryo-electron microscopy data processing.** Video frame alignment, estimation of the microscope contrast-transfer function parameters, particle picking and classification without refining angles and shifts using a soft mask delineating the ligand-binding site and with a tau value of 60. Final 3D refinements were carried out using non-uniform refinement in cryoSPARC. Local resolution estimation, filtering and sharpening were carried out using cryoSPARC. Local resolution estimation is based on the gold-standard Fourier shell correlation (FSC) of 0.143 criterion and Fourier shell correlation curves were corrected for the effects of soft mask by high-resolution noise substitution 56.

**Model building.** We used UCSF Chimera 57 to fit the MERS-CoV S atomic model (PDB 6NBR) into the cryo-EM map followed by subsequent manually rebuilding using Coot 58. N-linked glycans were hand-built into the density where visible, and then refined and adjusted using Rosetta 6. Glycan refinement relied on a dedicated Rosetta protocol which makes physically realistic geometricss based on prior knowledge of saccharide chemical properties 59, and was aided by using both sharpened and unsharpened maps. The final models were analyzed using MolProbity 60, EMRinger 61, Privatize 62 and PISA 63. Figures were generated using UCSF ChimeraX 64.

**Pseudovirus entry assay.** Murine leukemia virus pseudotyped with MERS-CoV S was prepared as previously described 65. HEK293T/17 cells were co-transfected with a full-length MERS-CoV S or MERS-CoV S mutant encoding plasmid, a murine leukemia virus Gag-Pol packaging construct and the murine leukemia virus transfer vector encoding a luciferase reporter using the Lipofectamine 2000 or 3000 transfection reagent (Life Technologies) according to the manufacturer’s instructions. Cells were incubated for 5–12 h at 37 °C and 8% CO₂ with transfection medium. Cells were then washed with DMEM twice and DMEM containing 10% (vol/vol) FBS was added for 72 h. The supernatants were then harvested and filtered through 0.45 μm membranes before concentration with a 30 kDa centrifugal concentrator membrane (Amicon).

Calu-3 cells grown in DMEM containing 10% (vol/vol) FBS and 1% (vol/vol) PenStrep were plated into 96-well plates following trypsinization at a density of ~3.5 × 10⁵ cells per ml and allowed to grow for 48 h at 37 °C with 8% (vol/vol) CO₂. The cells were washed three times with DMEM before addition of 40 μl of MERS-CoV S or MERS-CoV S mutant pseudovirus for 2 h at 37 °C with 8% (vol/vol) CO₂. Following the 2 h incubation, 40 μl of (vol/vol) FBS and 2% (vol/vol) PenStrep was added to the wells and the plate was incubated for 72 h. ONE-Glo EX was added in volume (~75 μl to account for evaporation) and the luciferase counts were read on a Varioskan plate reader.

**Western blotting.** SDS–PAGE (4%) loading buffer was added to all concentrated pseudovirus samples. The samples were run on a 4–15% (wt/vol) gradient Tris-glycine gel (BioRad) and transferred to PVDF membranes based on a previously described protocol. Anti-MERS CoV S purified 1.67 primary antibody 66 (1:500 dilution) and an Alexa Fluor 680-conjugated goat-anti-human secondary antibody (1:40,000 dilution, Jackson Laboratory) were used for western blotting. A Li-COR processor was used to develop images.

**Hemagglutination assay.** High-sensitivity nanoparticle-based hemagglutination assays were performed as previously described 67. Briefly, wild-type or mutant MERS-CoV S domain A-Fc (starting at 6.77 μg per well) was coated with protein A-fused lumazine synthase nanoparticles at a 0.6:1 molar ratio for 30 min on ice. Mixtures were subsequently serially diluted (twofold dilutions) and mixed with human erythrocytes (0.5% (wt/vol) in phosphate buffered saline) in V-bottomed, 96-well plates. Hemagglutination was scored after 2 h of incubation at 4 °C.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The cryo-EM maps (sharpened and unsharpened) and atomic models have been deposited to the EMDB and wwPDB under accession numbers EMDB-20542 and PDB 6Q04 (MERS-CoV S + Neu5Ac), EMDB-20829 (MERS-CoV S + Neu5Gc), EMDB-20543 and PDB 6Q65 (MERS-CoV S + sLeX), EMDB-20544 and PDB 6Q66 (MERS-CoV S + 2,3-SLN) and EMDB-20545 and PDB 6Q67 (MERS-CoV S + 2,6-SLN). Source Data are available online for Extended Data Fig. 5.

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**Author contributions**

B.-J.B., F.D. and D.V. supervised the research. Y.-J.P., A.C.W., Z.W., M.M.S., W.L., M.A.T., B.-J.B. and D.V. designed the experiments. A.C.W. and M.M.S. expressed and purified the MERS-CoV S ectodomain. Y.-J.P. and Z.W. performed cryo-EM sample preparation and data collection. Y.-J.P., Z.W. and D.V. processed the cryo-EM data. Y.-J.P., Z.W., F.D. and D.V. built and refined the atomic models. A.C.W. and Z.W. performed S mutagenesis. A.C.W. carried out the pseudovirus assays. W.L. carried out the hemagglutination assays. Y.-J.P., A.C.W., W.L., B.-J.B. and D.V. analyzed the data. Y.-J.P., A.C.W. and D.V. prepared the manuscript with input from all authors.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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Extended Data Fig. 1 | Chemical structures of the sialosides used for structural studies. a, Neu5Ac. b, Neu5Gc. c, sLeX. d, 2,3-SLN. e, 2,6-SLN.
Extended Data Fig. 2 | CryoEM analysis of the MERS-CoV S glycoprotein in complex with Neu5Ac at 2.7 Å resolution. a,b, Representative electron micrograph (a) and class averages (b) for the Neu5Ac-bound MERS-CoV S structure. c, Gold-standard (blue) and map/model (red) Fourier shell correlation curves. The 0.143 and 0.5 cutoffs are indicated by horizontal dashed lines. d, Local resolution map calculated using cryoSPARC. The Neu5Ac ligand is estimated to be resolved at 3Å resolution. e–g, Representative cryoEM densities shown as blue mesh with the corresponding atomic model rendered as sticks colored grey, blue and red for carbon, nitrogen and oxygen atoms, respectively. Dashed bonds indicate hydrogen bonds with ordered water molecules.
Extended Data Fig. 3 | Structural basis for the MERS-CoV S selectivity for neuraminic acids that are not 9-O-acetylated or 5-N-glycolylated.

a, Superimposition of the 5-N-acetyl,9-O-acetyl neuraminic acid α-methyl glycoside (9-O-Ac-Neu5Ac, green) from the HCoV-OC43 S holo structure (PDB 6NZK) to the bound Neu5Ac from the MERS-CoV S/Neu5Ac complex structure suggests the MERS-CoV S sialoside-binding site could not sterically accommodate the 9-O-acetyl group in this conformation. The distances between the 9-O-acetyl group and His91 or Ala92 are indicated.

b, The MERS-CoV S sialoside-binding site rendered as a ribbon diagram with the side chains of key surrounding residues shown. Neu5Ac is rendered with the corresponding region of cryoEM density from the MERS-CoV S/Neu5Ac complex structure (low-pass filtered to 3Å and scaled to the MERS-CoV S/Neu5Gc map) shown as a blue mesh contoured at 6σ.

c–f, The cryoEM structure of MERS-CoV S in complex with Neu5Gc shows weaker density for the sialoside (blue mesh contoured at 6σ), indicating poor steric and/or chemical accommodation of the 5-N-glycolyl hydroxyl group in the hydrophobic pocket defined by Phe39, Phe101, Ile131 and Ile132. In panels (c–e) Neu5Gc was modeled based on the porcine rotavirus CRW-8 VP8* holo structure (PDB 3TAY) which corresponds to the conformation observed in 1 out of 8 structures available in the protein data bank with a Neu5Gc analogue. In panel f, Neu5Gc was modeled based on the rhesus rotavirus VP8* holo structure (PDB 3TB0) which is the only structure in the protein data bank featuring Neu5Gc with the 5-N-glycolyl group in an alternate orientation. The distance between the 5-N-glycolyl hydroxyl group and Gln36 is indicated.
Extended Data Fig. 4 | CryoEM analysis of the MERS-CoV S glycoprotein in complex with Neu5Gc at 3.0 Å resolution. a,b, Representative electron micrograph (a) and class averages (b) for the Neu5Gc-bound MERS-CoV S structure. c, Gold-standard Fourier shell correlation curve. The 0.143 cut-off is indicated by horizontal dashed lines. d, Representative density shown as blue mesh with the corresponding atomic model rendered as sticks colored grey, blue and red for carbon, nitrogen and oxygen atoms, respectively.
Extended Data Fig. 5 | SDS-PAGE and Western blot analyses. **a**, SDS-PAGE analysis of purified wild type or mutants MERS-CoV S domain A fused to human immunoglobulin Fc. Two micrograms of each protein was loaded. **b**, Western-blot analysis of murine leukemia viral particles pseudotyped with wild type or mutants MERS-CoV S using an anti-MERS-CoV S\(_1\) polyclonal antibody. Uncropped blot image is available as source data.
Extended Data Fig. 6 | CryoEM analysis of the MERS-CoV S glycoprotein in complex with sLeX at 2.7 Å resolution. **a, b.** Representative electron micrograph (**a**) and class averages (**b**) for the sLeX-bound MERS-CoV S structure. **c.** Gold-standard (blue) and map/model (red) Fourier shell correlation curves. The 0.143 and 0.5 cutoffs are indicated by horizontal dashed lines. **d.** Representative density shown as blue mesh with the corresponding atomic model rendered as sticks colored grey, blue and red for carbon, nitrogen and oxygen atoms, respectively.
Extended Data Fig. 7 | CryoEM analysis of the MERS-CoV S glycoprotein in complex with 2,3-SLN at 2.7 Å resolution. a, b, Representative electron micrograph (a) and class averages (b) for the 2,3-SLN-bound MERS-CoV S structure. c, Gold-standard (blue) and map/model (red) Fourier shell correlation curves. The 0.143 and 0.5 cutoffs are indicated by horizontal dashed lines. d, Representative density shown as blue mesh with the corresponding atomic model rendered as sticks colored grey, blue and red for carbon, nitrogen and oxygen atoms, respectively.
Extended Data Fig. 8 | CryoEM analysis of the MERS-CoV S glycoprotein in complex with 2,6-SLN at 2.9 Å resolution. a, b. Representative electron micrograph (a) and class averages (b) for the 2,6-SLN-bound MERS-CoV S structure. c. Gold-standard (blue) and map/model (red) Fourier shell correlation curves. The 0.143 and 0.5 cutoffs are indicated by horizontal dashed lines. d. Representative density shown as blue mesh with the corresponding atomic model rendered as sticks colored grey, blue and red for carbon, nitrogen and oxygen atoms, respectively.
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The cryoEM maps (sharpened and unsharpened) and atomic models have been deposited to the EMDB and PDB with accession numbers EMD-20542 and PDB 6Q04 (MERS-CoV S + NeuSAC), EMD-20829 (MERS-CoV S + NeuS6c), EMD-20543 and PDB 6Q05 (MERS-CoV S + sLeX), EMD-20544 and PDB 6Q06 (MERS-CoV S + 2,3-SLN), and EMD-20545 and PDB 6Q07 (MERS-CoV S + 2,6-SLN).
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