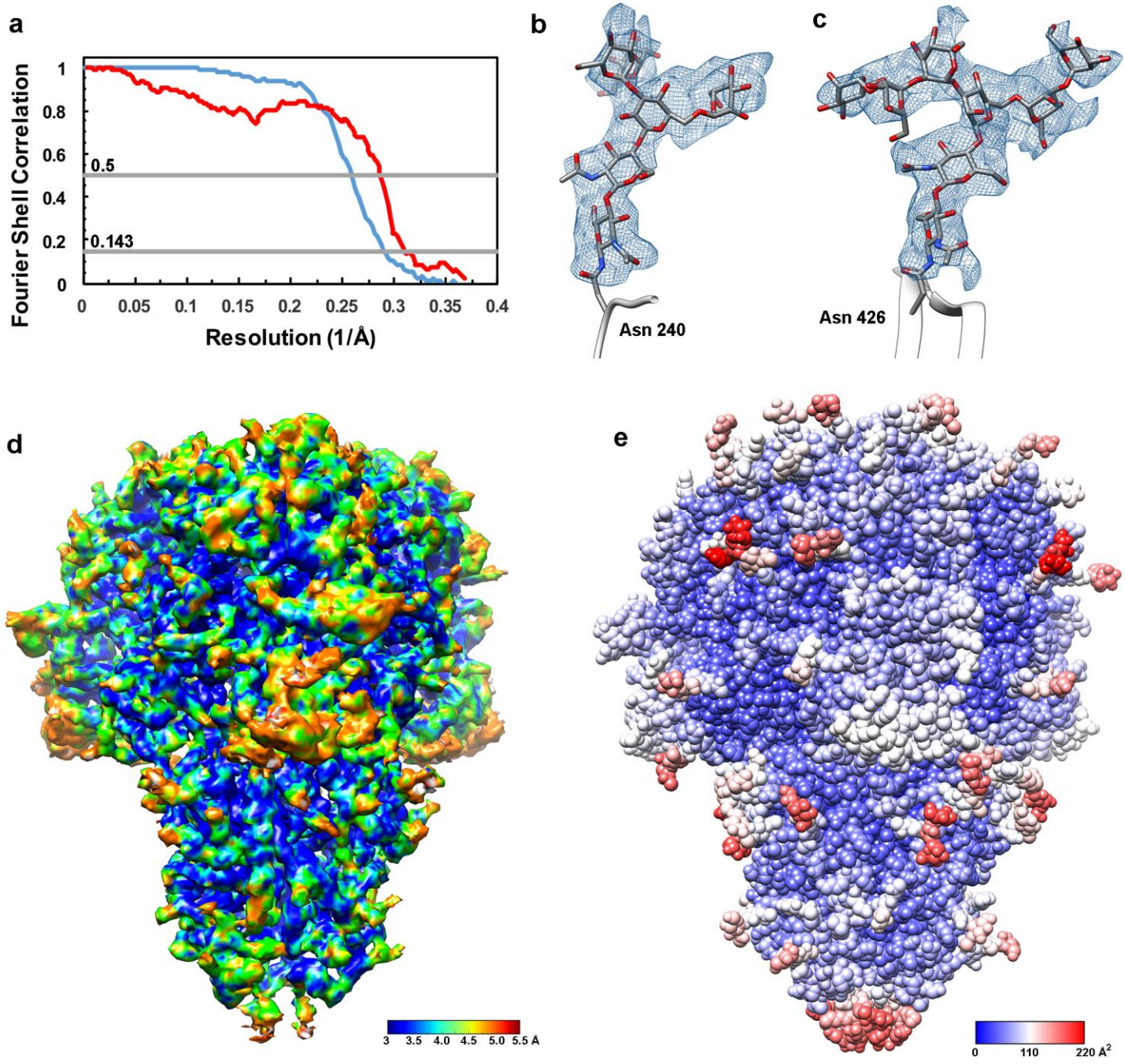
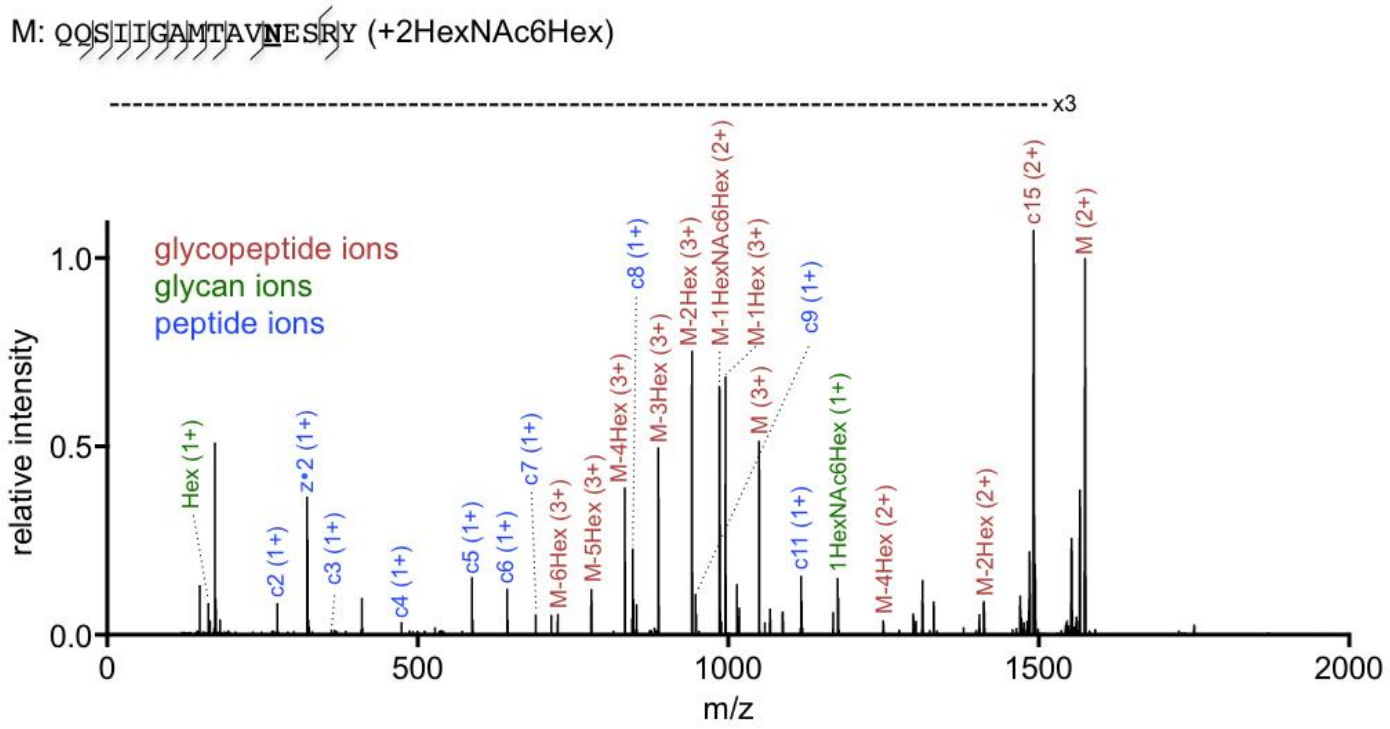


Supplementary Figure 1

Cryo-EM analysis of the HCoV-NL63 S trimer.

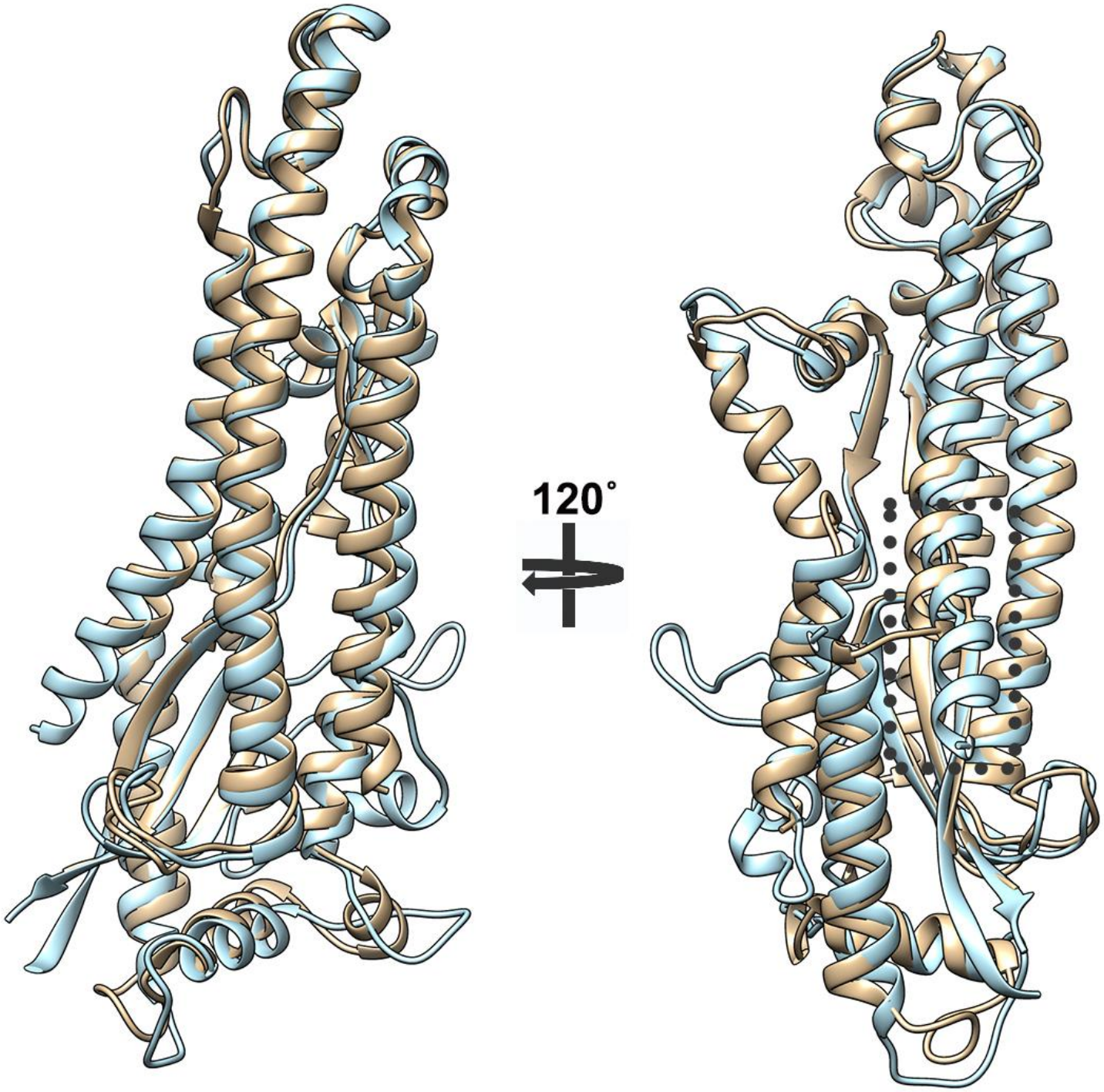
a, Gold-standard (blue) and model/map (red) Fourier shell correlation (FSC) curves. The resolution was determined to 3.4 Å. The 0.143 and 0.5 cut-off values are indicated by horizontal grey bars. **b,** The glycan linked to Asn 240 is rendered as ball and sticks and the corresponding region of the cryoEM map is shown as a blue mesh. **c,** The glycan linked to Asn 426 is rendered as ball and sticks and the corresponding region of the cryoEM map is shown as a blue mesh. In panels (**b-c**), carbon, nitrogen and oxygen atoms are colored grey, blue and red, respectively. **d,** HCoV-NL63 S cryoEM map colored according to local resolution. **e,** HCoV-NL63 S atomic model colored according to refined B factors.



Supplementary Figure 2

Characterization of the HCoV-NL63 S glycans by using mass spectrometry.

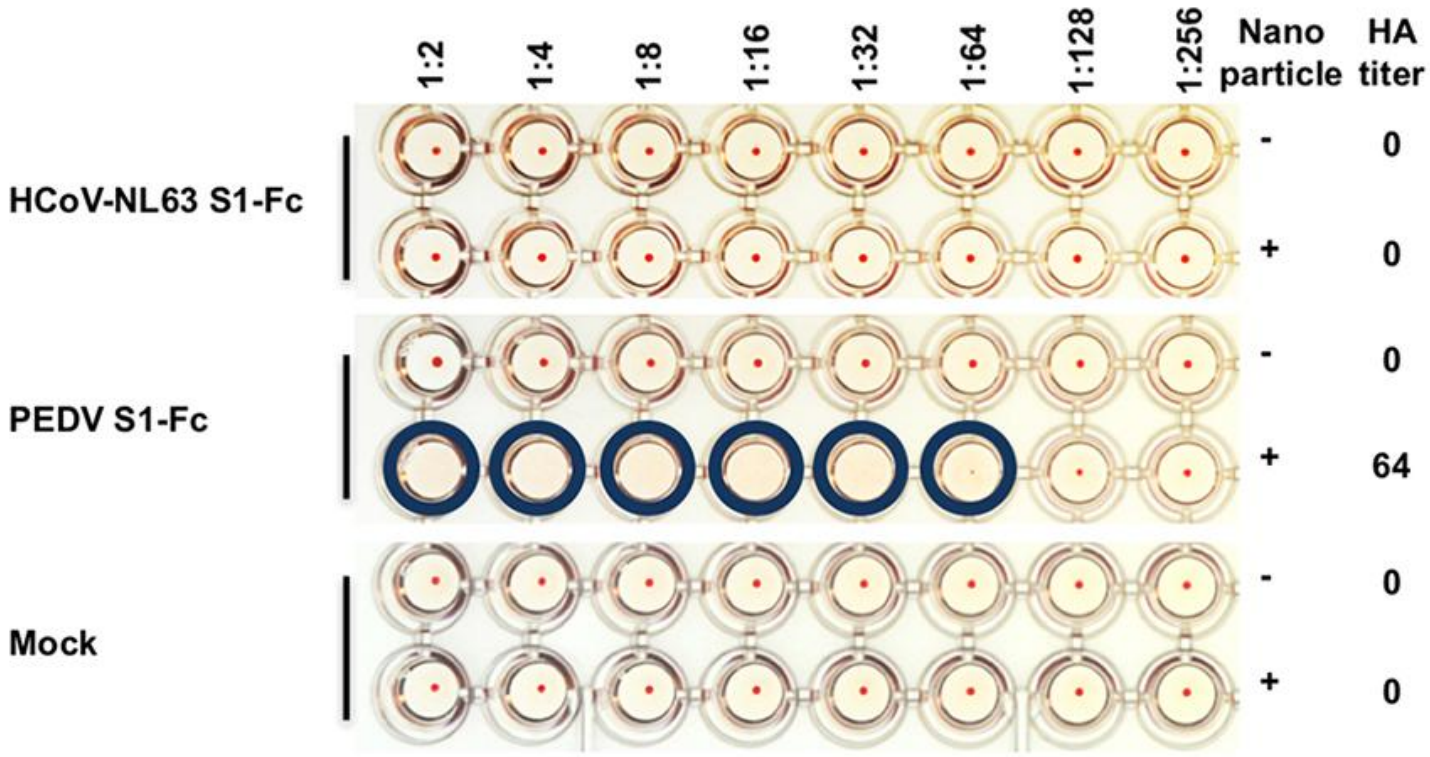
Tandem MS EThcD spectrum of a 3+ glycopeptide with HexNAc(2)Hex(6) attached to Asn 699 of the HCoV-NL63 S glycoprotein digested with chymotrypsin. The relative intensity normalized to the most intense ion is plotted against mass-to-charge ratio. The peaks under the horizontal dashed line are multiplied by 3 for visualization. "M" denotes the molecular ion. The charge state of the fragment ions is indicated in brackets. Ions relating to the glycopeptide, glycan and peptide fragments are colored red, green and blue, respectively. In this example of a glycopeptide identification the matched fragment ions define a large part of the peptide sequence and also provide detailed information about the glycan composition.



Supplementary Figure 3

Structural similarity of coronavirus fusion machineries.

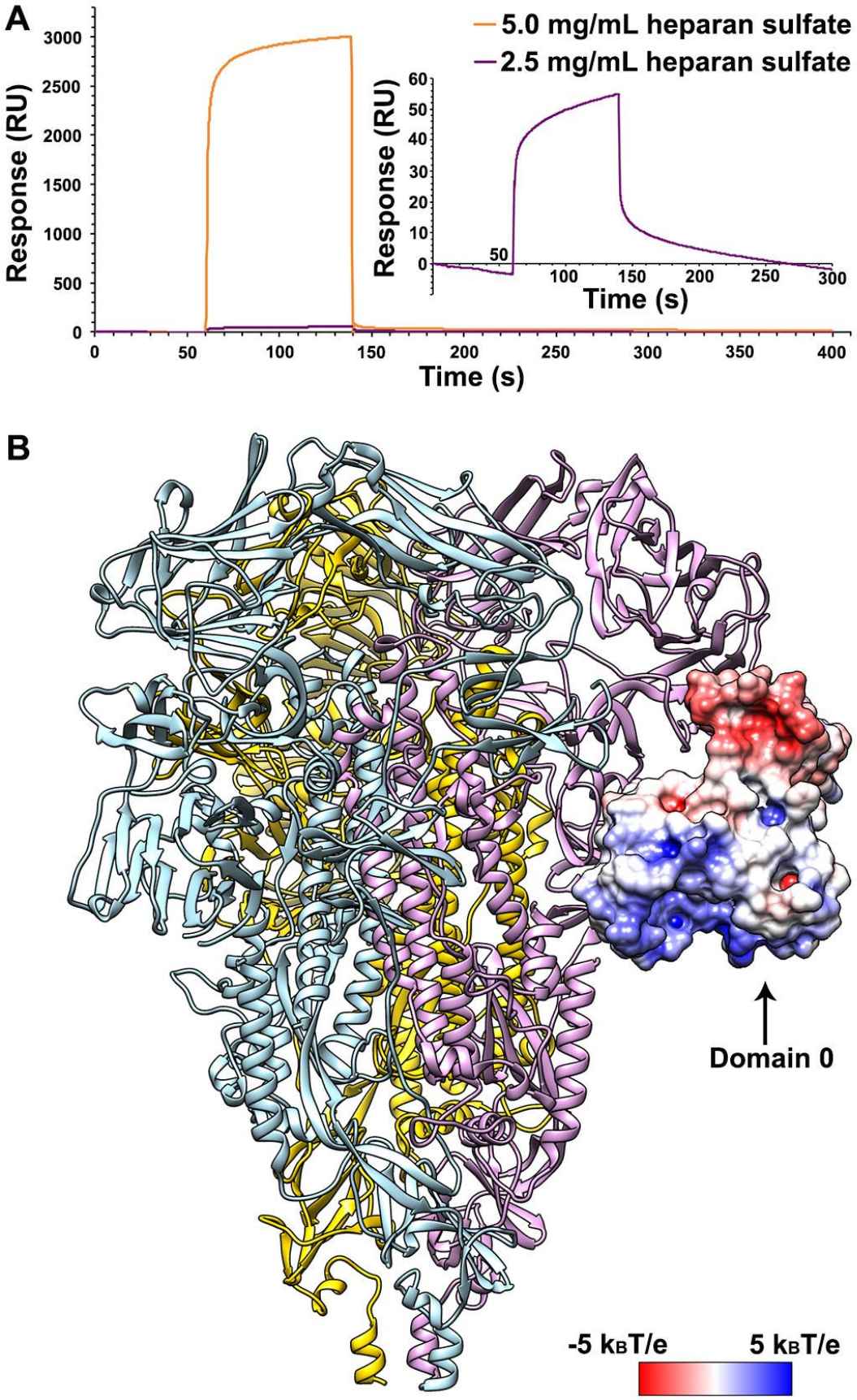
Ribbon diagram of the HCoV-NL63 (blue) and MHV (tan) S_2 fusion subunits. The dashed box highlights the two extra helical turns present in the S protein HR1 region of α-coronaviruses but not β-coronaviruses.



Supplementary Figure 4

The HCoV-NL63 S_1 subunit does not bind sialic acid.

Binding of sialic acid by the HCoV-NL63 S_1 subunit (N-terminally fused to human IgG Fc) was assessed by probing the hemagglutination of human eryhthrocytes. The porcine epidemic diarrhea coronavirus S_1 subunit was used as a positive control. Mock indicates the absence of coronavirus S_1 subunit (negative control). The assays were performed using either free S_1-Fc or nanoparticle-displaying S_1-Fc to increase the avidity for sialic acid on the erythrocyte surface. Wells showing hemagglutination are circled.


A
5.0 mg/mL heparan sulfate
2.5 mg/mL heparan sulfate
Response (RU)
Time (s)
B
Domain 0
-5 kBT/e
5 kBT/e

Supplementary Figure 5

HCoV-NL63 binds heparan sulfate.

a, Surface plasmon resonance sensorgram showing binding of heparan sulfate to HCoV NL63 S. The right panel shows a blow-up view of the sensorgram corresponding to 2.5 mg/mL heparan sulfate. **b,** Ribbon diagram of the HCoV-NL63 S atomic model colored by protomer. Domain 0 is shown in surface representation colored according to its electrostatic surface potential for one protomer. The positively charged patch on its surface could putatively mediate binding to heparan sulfate.