Supporting Information for:

The binding of heparin to spike glycoprotein inhibits SARS-CoV-2 infection by three mechanisms

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Data sharing. Data availability statement. All simulation trajectories are available on the BioExcel COVID-19 platform: https://bioexcel-cv19.bsc.es/#/ with the identifiers from MCV1900217 to MCV1900336.

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Other supporting materials for this manuscript include the following:

IFP-model 02, IFP-model 03, IFP-model 05 (enlargement of single pictures of Tab.S6)
Movies S1-S3

In the following contents, a simplified nomenclature is used for the simulated systems in which they are referred to as model01-05 and contain the following components:

| Model | Description |
|-------|-------------|
| Model01 | Spike in closed conformation |
| Model02 | Closed spike + 1 heparin chain |
| Model03 | Closed spike + 3 heparin chains |
| Model04 | Open spike |
| Model05 | Open spike + 3 heparin chains |
1. Supporting Figures

**Fig. S1 Structural convergence of the simulated systems.** Root mean square deviation (RMSD (Å)) versus time (µs) for the four replica MD trajectories of the five simulated systems. The RMSD values for the individual subunits - S_A, S_B and S_C - were calculated for the C-alpha atoms of residues 51-1063 and are shown in blue, pink and magenta, respectively. The RMSD values of the three heparin chains were calculated for all the C, N and O atoms in all monosaccharides and are shown in green, light green and turquoise.
Fig. S2 Conformational fluctuations of the RBD and S1/S2 sites. Root mean square fluctuation (RMSF) (Å) versus residue number of the three subunits of the spike homotrimer for the four replica MD trajectories of the five simulated systems. The RMSF values for the individual subunits - S_A, S_B, and S_C - were calculated for the C-alpha atoms of residues 1-3697 and are shown in blue, pink, and magenta, respectively. The red boxes represent the RBD (residues 330-530) of each subunit, the black arrows highlight the hinge regions (residue 527-529) and the green arrows point to the S1/S2 residues (residues 682-685). For both the boxes and the arrows, the continuous and dashed lines represent the regions interacting or not interacting with heparin, respectively.
Fig. S3 Heparin-spike hydrogen-bonding interactions. Last snapshots from 1 microsecond MD simulations of spike in closed (left) and open (right) conformations with three heparins bound (replica 1) showing the H-bond interactions of heparin with spike subunits $S_B$ and $S_C$ as in close-ups of the side views in Figure 1. The labelled residues shown in sticks and colored as the subunits are involved in H-bond interactions with heparin for more than half of the simulations of the spike in the closed (with 1 and 3 heparin chains) or open states. The $S_A$, $S_B$ and $S_C$ subunits are shown in cartoon representation and colored in white, pink and magenta, respectively while the covalently attached N-glycans are shown in line representation and colored according to the subunits. Heparin is shown as a surface with carbons, oxygens, sulphates and nitrogens colored in green, red, yellow and blue, respectively.
Fig. S4. Mutations in emerging SARS-CoV-2 spike variants do not impact heparin binding. Last snapshots from 1 microsecond MD simulations of emerging spike variants (listed in Uniprot P0DTC2, on 29.09.2021) in closed (upper panel) and open (lower panel) conformations with three heparins bound (replica 1). The S$_\text{B}$ and S$_\text{C}$ subunits of spike are shown as cartoon representation and colored in pink and magenta, respectively. Heparin is shown in stick representation with carbons colored in cyan. The mutated residues are labelled and shown in spheres colored according to their subunits. Although some of the mutations occur near to the heparin-binding residues, none of them interfere significantly with heparin binding. Mutations within the RBD are mainly localized in the receptor binding motif (RBm).
**Fig. S5 Spike N-glycan interactions with heparin.** N-glycans can modulate binding of the spike to heparin by transiently displacing heparin from the spike surface (see Movies S1-S3). The heparin chains were modelled to interact with the K444 and N448 residues that are in close proximity to the ACE2 binding residues in both the closed and open models of spike. The interactions of heparin with the N-122 (left) and N-343 (right) N-glycans are highlighted in the closed and open models of spike, respectively. Visual inspection of the trajectories and H-bond analysis showed that N-glycans may exert a shielding effect, causing a nonspecific, transient and only partial detachment of heparin from spike. The N-glycan attached to residue 122 of the N-terminal domain contributes to the detachment only in the closed conformation, while the N-glycan attached to residue 343 of the RBD is mainly responsible for the detachment of heparin in both the closed and the open conformations. The localization of the spike region considered is indicated by the squares in the insets. The Sβ and Sc subunits are shown as translucent surfaces and cartoons in pink and magenta, respectively. N-122, N-343 and the N-glycans are shown in stick representation with carbons colored in pink/magenta. Heparin chains are shown in stick representation with carbons in light green (left) and cyan (right). Dashed lines indicate the H-bond interactions between the glycans and heparin. As SPR and circular dichroism spectroscopy experiments were done using unfractionated heparins (13.5-15 kDa, ~48 monosaccharides), we cannot exclude that longer heparin chains could maintain the interaction with these residues (1).
Fig. S6 Exposure of the S1/S2 site during the MD simulations. Solvent Accessible Surface Area (SASA) (Å²) of the S1/S2 site versus time (µs) for the four replica MD trajectories of the five simulated systems. The SASA of the S1/S2 site (residues 682-685) in each spike subunit - Sₐ, S₉ and S₇ - is shown in blue, pink and magenta, respectively, and was computed using CPPTRAJ (2).
Fig. S7 Conformational changes of the hinge region during the simulations. Root mean square deviation (RMSD) (Å) of the hinge region versus time (µs) for the four replica MD trajectories of the five simulated systems. The RMSD values were calculated for the C-alpha atoms of residues 527-529 and plotted for the individual subunits - Sα, Sβ and Sγ - in blue, pink and magenta, respectively.
Fig. S8 Effects of heparin binding on the motion of the RBD-hinge region. Results of dihedral Principal Component Analysis (dPCA) calculated for the Phi/Psi angles of the RBD-hinge region (residues 527-529) of the Sc subunit for the four replica MD trajectories of the five simulated systems. PC1 and PC2 are plotted on the x and y axes, respectively. Note that the second replica of model 02 differs from the others because of the interaction between heparin and the N-glycan at N122 and the fourth replica of model 05 differs from the others because of the interaction between heparin and the N-glycan at N343 in this trajectory.
Fig. S9 Exposition of the receptor binding motif (RBm) during the MD simulations. Solvent Accessible Surface Area (SASA) (Å²) of the RBm versus time (µs) for the four replica MD trajectories of the five simulated systems. The SASA is shown for each subunit - Sₐ, Sₐ, and Sₖ - in blue, pink and magenta, respectively, and was calculated using CPPTRAJ (2).
Fig. S10 Heparin binding is compatible with ACE2 binding to spike. (Left) Ternary complex of open spike, heparin and the ACE2-RBD. The complex was obtained by superimposition of the crystal structure of the ACE2-RBD complex (PDBid 6M0J (3)) on the starting system of the open spike in complex with three heparin chains. (Right) Enlargement of the spike-RBm/ACE2-RBD interface. The ACE2-RBD and spike-RBD are shown in pale blue and magenta cartoon representation, respectively. For clarity, heparin is not shown. Residues of the spike RBm involved in the interaction with ACE2 or with heparin are depicted as stick with purple carbons and indicated by red and black labels, respectively.
Fig. S11 Differences in the spike RBD opening motion in the presence of heparin. Essential Dynamics (ED) analysis on the Sc subunit for the four replica MD trajectories of model 04 and model 05. (A) Values of the first four eigenvalues for the up-subunit of model 04 and model 05. (B) Plot of the first normalized eigenvector along the trajectory. (C) Plot of the direction of motion of the first eigenvector on the RBD of the Sc subunit which can be seen to differ between model 04 and model 05. The direction of the fourth replica of model 05 differs from the other replicas because of the interaction between heparin and the N-glycan at N-343 in this trajectory.
Fig. S12 Mapping of antibody-interacting regions on the open spike bound to heparin. Side (left) and top (right) views of the last snapshot from MD simulations of the spike (grey) in the open conformation with three heparin chains bound (replica 1) and three representative structures of human antibodies (hAbs). The selected hAbs (PDBids: 7BZ5 (4) (pale cyan), 7CAH (5) (pale green), 7C2L (6) (pale yellow), are superimposed onto the spike glycoprotein and cover the three main regions targeted: RBm, RBD and NTD, respectively. The proteins are depicted in surface representation, the glycans in line representation, and the heparin chains in stick representation with carbons in green, cyan and light green. No clashes or overlapping motifs between the hAbs and heparin were identified.
3. Tables

Tab. S1 Average root mean square fluctuation (RMSF) (Å) calculated for the C-alpha atoms of the RBD (residues 330-530). The average fluctuation was calculated based on the average fluctuation of the single residues in the RBD. In model 02, the single heparin chains directly bind to the RBD of monomer Sc. The grey boxes represent the subunits interacting with heparin.

| Subunit SA | Subunit SB | Subunit SC | AVG [Å] | ST.DEV. [Å] |
|------------|------------|------------|---------|-------------|
|            | Rep 01     | Rep 02     | Rep 03  | Rep 04      | Rep 01 | Rep 02 | Rep 03 | Rep 04 | Rep 01 | Rep 02 | Rep 03 | Rep 04 | Rep 01 | Rep 02 | Rep 03 | Rep 04 |
| Model 01   | 10.18      | 9.40       | 9.99    | 9.44       | 10.22  | 11.18  | 13.72  | 13.37  | 11.59  | 11.19  | 11.14  | 10.52  | 10.83  | 1.38  |
| Model 02   | 10.23      | 13.93      | 13.80   | 13.65      | 10.68  | 10.69  | 12.14  | 9.91   | 10.10  | 10.14  | 9.51   | 9.60   | 10.45  | 1.71  |
| Model 03   | 9.39       | 8.83       | 9.38    | 9.19       | 8.51   | 9.77   | 8.52   | 12.05  | 10.79  | 10.30  | 9.93   | 10.88  | 9.57   | 1.06  |
| Model 04   | 10.57      | 12.12      | 10.88   | 10.56      | 14.89  | 12.53  | 14.54  | 10.94  | 17.38  | 15.29  | 13.93  | 13.01  | 12.77  | 2.19  |
| Model 05   | 10.09      | 9.80       | 9.27    | 12.10      | 9.18   | 11.25  | 9.45   | 14.18  | 15.57  | 14.54  | 12.05  | 16.88  | 11.65  | 2.67  |

Tab. S2 Average root mean square fluctuation (RMSF) (Å) calculated for the C-alpha atoms of the hinge region (residues 527-529). The average fluctuation was calculated based on the average fluctuation of the single residues in the hinge region. In model 02, the single heparin chain allosterically acts on the hinge region of monomer Sc. The grey boxes represent the subunits interacting with heparin.

| Subunit SA | Subunit SB | Subunit SC | AVG [Å] | ST.DEV. [Å] |
|------------|------------|------------|---------|-------------|
|            | Rep 01     | Rep 02     | Rep 03  | Rep 04      | Rep 01 | Rep 02 | Rep 03 | Rep 04 | Rep 01 | Rep 02 | Rep 03 | Rep 04 | Rep 01 | Rep 02 | Rep 03 | Rep 04 |
| Model 01   | 1.07       | 0.84       | 1.0     | 1.0        | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 0.06  |
| Model 02   | 1.14       | 1.2        | 1.07    | 0.8        | 1.12   | 1.1    | 0.7    | 1.0    | 1.3    | 1.15   | 1.15   | 1.3    | 1.13   | 0.18  |
| Model 03   | 0.94       | 1.0        | 0.94    | 1.0        | 0.9    | 1.07   | 0.7    | 1.07   | 0.97   | 1.07   | 1.04   | 1.0    | 1.0    | 0.10  |
| Model 04   | 1.4        | 1.1        | 1.34    | 1.67       | 1.24   | 1.3    | 1.3    | 1.24   | 1.5    | 1.5    | 1.27   | 1.27   | 1.3    | 0.15  |
| Model 05   | 1.5        | 0.7        | 0.7     | 1.04       | 0.8    | 1.0    | 0.7    | 1.34   | 1.4    | 1.04   | 1.07   | 0.97   | 1.02   | 0.28  |
Tab. S3 Average root mean square fluctuation (Å) calculated for the C-alpha atoms of the S1/S2 site (residues 682-685). The average fluctuation was calculated based on the fluctuation of the single residues in the S1/S2 site. In model 02, the single heparin chain binds the S1/S2 site of monomer SA. The grey boxes represent the subunits interacting with heparin.

|         | Subunit SA |         |         |         | Subunit SB |         |         |         |         | Subunit SC |         |         |         | AVG [Å] | ST.DEV. [Å] |
|---------|------------|---------|---------|---------|------------|---------|---------|---------|---------|------------|---------|---------|---------|---------|-------------|
|         | Rep 01     | Rep 02  | Rep 03  | Rep 04  | Rep 01     | Rep 02  | Rep 03  | Rep 04  | Rep 01  | Rep 02  | Rep 03  | Rep 04  |         |             |
| Model 01| 6.2        | 6.25    | 9.25    | 2.13    | 2.13       | 3.88    | 2.88    | 1.75    | 2       | 2.63     | 1.38    | 4.65    | 2.75    | 2.4     |
| Model 02| 2.87       | 2.08    | 1.55    | 1.2     | 1.2        | 1.78    | 2       | 1.6     | 1.5     | 6        | 2.93    | 4.18    | 2.04    | 1.38    |
| Model 03| 1.78       | 3.18    | 3       | 3.25    | 3.25       | 2.25    | 1.08    | 1.05    | 1.3     | 2.08     | 2.5     | 1.78    | 1.93    | 0.78    |
| Model 04| 3.9        | 3.05    | 3.18    | 3.43    | 3.43       | 3.25    | 3       | 1.58    | 1.55    | 2.45     | 7.7     | 2.35    | 3.11    | 1.71    |
| Model 05| 2.08       | 1.85    | 2.13    | 2.75    | 2.75       | 5.15    | 2.33    | 2.65    | 2.75    | 1.98     | 3.5     | 3.73    | 2.49    | 0.97    |
Tab. S4 Analysis of hydrogen bonds (H-bonds) between S glycoprotein and the heparin chains in model 03 for the four replica MD trajectories using CPPTRAJ (2). Each heparin chain interacts with two adjacent spike subunits, S₁₅-S₀₉, S₀₉-S₀₃ or S₀₃-S₁₅. Residues involved in the interaction are listed separately as residues of the first subunit and of the second subunit. The yellow boxes represent the H-bonds that were stable in more than half the simulations with an occupancy > 50% in the single trajectory considering the 3 heparin chains on each subunit. The H-bonds for model 02 are very similar to these results and thus not reported.

| Spike Residues | Rep 01 Heparin on S₁₅-S₀₉ subunits | Rep 02 Heparin on S₀₉-S₀₃ subunits | Rep 03 Heparin on S₀₃-S₁₅ subunits | Rep 04 Heparin on S₁₅-S₀₉ subunits | SUM |
|----------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----|
| ARG_34         | 1                                 | 1                                 | 1                                 | 2                                 | 2   |
| TYR_36         | 1                                 | 1                                 | 1                                 | 4                                 | 4   |
| LYS_31         | 1                                 | 1                                 | 1                                 | 3                                 | 3   |
| ARG_158        | 1                                 | 1                                 | 2                                 | 3                                 | 3   |
| SER_162        | 1                                 | 1                                 | 2                                 | 2                                 | 2   |
| ASN_164        | 1                                 | 1                                 | 1                                 | 1                                 | 1   |
| ASY_165        | 1                                 | 1                                 |                                   | 3                                 | 3   |
| CYX_166        | 1                                 | 1                                 |                                   | 2                                 | 2   |
| THR_167        | 1                                 | 1                                 | 1                                 | 1                                 | 1   |
| GLU_169        | 1                                 | 1                                 | 1                                 | 4                                 | 4   |
| TYR_170        | 1                                 | 1                                 | 1                                 | 3                                 | 3   |
| VAL_171        | 1                                 | 1                                 | 1                                 | 4                                 | 4   |
| SER_172        | 1                                 | 1                                 | 1                                 | 4                                 | 4   |
| GLN_173        | 1                                 | 1                                 | 1                                 | 8                                 | 8   |
| LEU_176        | 1                                 | 1                                 | 1                                 | 3                                 | 3   |
| ASN_180        | 1                                 | 1                                 |                                   | 2                                 | 2   |
| THR_184        | 1                                 | 1                                 |                                   | 1                                 | 1   |
| ARG_190        | 1                                 | 1                                 |                                   | 3                                 | 3   |
| LYS_206        | 1                                 | 1                                 |                                   | 1                                 | 1   |
| HIE_207        | 1                                 | 1                                 | 1                                 | 4                                 | 4   |
| THR_208        | 1                                 | 1                                 | 1                                 | 4                                 | 4   |
| GLN_211        | 1                                 | 1                                 | 1                                 | 1                                 | 1   |
| PHE_220        | 1                                 | 1                                 | 1                                 | 8                                 | 8   |
| SER_221        | 1                                 | 1                                 | 1                                 | 4                                 | 4   |
| ASP_228        | 1                                 | 1                                 |                                   | 1                                 | 1   |
| LYS_278        | 1                                 | 1                                 | 1                                 | 5                                 | 5   |
| ASN_280        | 1                                 | 1                                 |                                   | 2                                 | 2   |
| THR_284        | 1                                 | 1                                 |                                   | 2                                 | 2   |
| THR_286        | 1                                 | 1                                 | 1                                 | 1                                 | 1   |
| ASP_287        | 1                                 | 1                                 | 1                                 | 7                                 | 7   |
| LYS_300        | 1                                 | 1                                 | 1                                 | 2                                 | 2   |
| THR_306        | 1                                 | 1                                 | 1                                 | 4                                 | 4   |
| THR_602        | 1                                 | 1                                 | 1                                 | 5                                 | 5   |
| ASN_603        | 1                                 | 1                                 | 1                                 | 2                                 | 2   |
| THR_604        | 1                                 | 1                                 |                                   | 1                                 | 1   |
| SER_605        | 1                                 | 1                                 |                                   | 2                                 | 2   |
| ASN_606        | 1                                 | 1                                 | 1                                 | 1                                 | 1   |
| GLN_607        | 1                                 | 1                                 | 1                                 | 1                                 | 1   |
| TYR_674        | 1                                 | 1                                 | 1                                 | 3                                 | 3   |
| GLN_677        | 1                                 | 1                                 | 1                                 | 3                                 | 3   |
| SER_680        | 1                                 | 1                                 | 1                                 | 3                                 | 3   |
| ARG_682        | 1                                 | 1                                 | 1                                 | 8                                 | 8   |
| ARG_683        | 1                                 | 1                                 | 1                                 | 8                                 | 8   |
| ALA_684        | 1                                 | 1                                 |                                   | 2                                 | 2   |
| ARG_685        | 1                                 | 1                                 | 1                                 | 12                                | 12  |
| SER_686        | 1                                 | 1                                 | 1                                 | 7                                 | 7   |
| VAL_687        | 1                                 | 1                                 | 1                                 | 4                                 | 4   |
| SER_689        | 1                                 | 1                                 | 1                                 | 3                                 | 3   |
| GLN_690        | 1                                 | 1                                 |                                   | 3                                 | 3   |
| SER_940        | 1                                 | 1                                 |                                   | 1                                 | 1   |
| Second subunit | ASN_334                           | 1                                 | 2                                 |                                   | 2   |
| LEU_335        | 1                                 | 1                                 | 2                                 |                                   | 2   |
| PHE_338        | 1                                 | 1                                 |                                   | 1                                 | 1   |
| GLY_339        | 1                                 | 1                                 |                                   | 1                                 | 1   |
| GLU_340        | 1                                 | 1                                 |                                   | 1                                 | 1   |
| Amino Acid | Index | Count |
|------------|-------|-------|
| ASN_343    |       |       |
| THR_345    | 1     |       |
| ARG_346    | 1     |       |
| ASN_353    |       |       |
| ARG_355    | 1     |       |
| LYS_356    | 1     |       |
| ARG_357    | 1     |       |
| SER_359    | 1     |       |
| ASN_360    | 1     |       |
| ASP_364    |       |       |
| TYR_365    | 1     |       |
| SER_366    | 1     |       |
| VAL_367    | 1     |       |
| ASN_394    |       |       |
| TYR_396    |       |       |
| LYS_444    |       |       |
| VAL_445    | 1     |       |
| TYR_449    | 1     |       |
| ASN_450    | 1     |       |
| ARG_466    |       |       |
| ARG_509    |       |       |
| LYS_529    |       |       |
| GLN_563    | 1     |       |
| GLN_564    |       |       |
| ARG_577    | 1     |       |
| THR_581    | 1     |       |
Tab. S5 H-bonds analysis between S glycoprotein and the heparin chains in model 05 for the four replica MD trajectories using CPPTRAJ (2). Each heparin chain interacts with two adjacent spike subunits, \(S_A-S_B\), \(S_B-S_C\) or \(S_C-S_A\). Residues involved in the interaction are listed separately as residues of the first subunit and of the second subunit. The yellow boxes represent the H-bonds that were stable in more than half the simulations with an occupancy > 50% in the single trajectory considering the 3 heparin chains on each subunit.

| Spike Residues | Rep 01 | Rep 02 | Rep 03 | Rep 04 | NUM |
|----------------|--------|--------|--------|--------|-----|
|                | Heparin on \(S_A-S_B\) subunits | Heparin on \(S_B-S_C\) subunits | Heparin on \(S_C-S_A\) subunits | Heparin on \(S_A-S_B\) subunits | Heparin on \(S_B-S_C\) subunits | Heparin on \(S_C-S_A\) subunits | Heparin on \(S_A-S_B\) subunits | Heparin on \(S_B-S_C\) subunits | Heparin on \(S_C-S_A\) subunits | SUM |
| First subunit  |        |        |        |        |     |
| ARG_34        | 1      | 1      | 1      | 1      | 4   |
| ASN_125       | 1      | 1      | 1      | 1      | 4   |
| LYS_129       | 1      | 1      | 1      | 2      |     |
| ARG_158       | 1      | 1      | 1      | 3      |     |
| TYR_160       | 1      | 1      |        | 1      |     |
| ALA_163       |        |        |        |        | 0   |
| ASN_164       | 1      | 1      | 1      | 1      | 3   |
| ASN_165       | 1      | 1      | 1      | 1      | 4   |
| CTX_166       | 1      | 1      | 1      | 1      | 5   |
| THR_167       | 1      | 1      | 1      | 4      |     |
| GLU_168       | 1      | 1      | 1      | 6      |     |
| TYR_170       | 1      | 1      |        | 1      |     |
| VAL_171       | 1      | 1      |        | 1      | 4   |
| SER_172       | 1      | 1      |        | 1      | 3   |
| GLN_173       | 1      | 1      | 1      | 1      | 5   |
| LYS_187       | 1      | 1      |        |        | 1   |
| LYS_206       | 1      |        |        |        | 1   |
| HIS_207       | 1      |        |        |        | 2   |
| THR_208       | 0      |        |        |        |     |
| ASN_211       | 1      |        |        |        | 1   |
| GLN_218       | 1      |        |        |        |     |
| PHE_220       | 1      | 1      | 1      | 1      | 5   |
| SER_221       | 1      |        |        |        | 1   |
| LYS_227       | 1      |        |        |        |     |
| ASN_280       | 1      | 1      | 1      | 1      | 8   |
| ASN_282       | 1      | 1      | 1      | 1      | 5   |
| THR_284       | 1      | 1      | 1      | 1      | 6   |
| THR_286       | 1      | 1      | 1      | 1      | 5   |
| ASP_287       | 1      |        |        |        | 1   |
| THR_307       | 1      |        |        |        |     |
| THR_604       | 1      |        |        |        | 1   |
| SER_605       | 1      |        |        |        | 1   |
| ASN_606       | 1      | 1      | 1      | 1      | 6   |
| GLN_607       | 1      | 1      |        | 1      | 2   |
| TYR_674       | 1      |        |        | 1      | 3   |
| ASN_679       | 1      |        |        |        | 1   |
| SER_680       | 1      |        |        |        |     |
| ARG_682       | 1      | 1      | 1      | 1      | 8   |
| ARG_683       | 1      | 1      | 1      | 1      | 11  |
| ALA_684       | 1      |        |        |        | 3   |
| ARG_685       | 1      | 1      | 1      | 1      | 12  |
| SER_686       | 1      | 1      | 1      | 1      | 6   |
| VAL_687       | 1      |        |        |        | 1   |
| ALA_688       | 1      |        |        |        | 1   |
| SER_689       | 1      | 1      | 1      | 1      | 5   |
| GLN_690       | 1      | 1      | 1      | 1      | 4   |
| Second subunit|        |        |        |        |     |
| ASN_334       | 1      |        |        |        | 1   |
| CTX_336       |        | 1      |        |        | 1   |
| PHE_338       | 1      |        |        |        | 1   |
| GLY_339       | 1      |        |        |        | 1   |
| GLU_340       | 1      |        |        |        | 1   |
| THR_345       | 1      | 1      | 1      | 1      | 4   |
| ARG_346       | 1      | 1      | 1      | 1      | 9   |
| SER_349       | 1      |        |        |        | 1   |
| TYR_351       | 1      |        |        |        |     |
| ASN_354       | 1      | 1      | 1      | 1      | 5   |
| ARG_355       | 1      | 1      | 1      | 1      | 4   |
|     | LYS_356 | ARG_357 | SER_359 | ASN_360 | ASP_364 | SER_443 | LYS_444 | VAL_445 | GLY_447 | ASN_448 | TYR_449 | ASN_450 | LEU_452 | ARG_466 | LYS_558 | GLN_564 | ARG_577 | THR_581 |
|-----|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|     | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       | 1       |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
|     |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
Tab. S6 Interaction Fingerprint Analysis (MD-IFP) (7) between heparin and spike residues. In model 02, one heparin chain interacts with residues of Sₐ and Sₖ subunits. In models 03 and 05, each of the three heparin chains interacts with residues of Sₐ-Sₖ or Sₖ-Sₖ or Sₖ-Sₐ subunits. Histograms show the interaction in the first 10 frames, in the last 10 frames and in all the frames in blue, orange and with lines, respectively. Blue and red boxes include the NTD-RBD and S1/S2 domains, respectively. To read the residue name and number, see the attached files IFP-model 02, IFP-model 03, IFP-model 05. Files report the Amber numbering as follows: subunit Sₐ residues 2-1124, subunit Sₖ, residues 1289-2411, subunit Sₖ, residue number 2574-3696 (fasta numbering for all the subunits; residues 13-1139).
Tab. S7 Solvent Accessible Surface Area (SASA) (Å²) of the S1/S2 site (residues 682-685) calculated for the most representative cluster for each trajectory using NACCESS (8) with a probe radius of 1.4 Å.

| SASA S1/S2 Model 01 | subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|---------------------|------------|--------|--------|--------|--------|----------|-------------|
| SASA w/o glycans   | 629.83     | 633.49 | 530.64 | 372.34 | 541.57 | 122.47   |
| SASA w/ glycans    | 566.64     | 633.34 | 530.64 | 328.5  | 514.78 | 131.27   |
| Glycan shielded Area | 63.19     | 0.15   | 0      | 43.84  | 26.795 | 31.85    |

| SASA S1/S2 Model 02 | subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|---------------------|------------|--------|--------|--------|--------|----------|-------------|
| SASA w/o glycans   | 629.66     | 642.73 | 453.62 | 600.37 | 581.95 | 87.14    |
| SASA w/ glycans    | 570.35     | 571.14 | 438.71 | 493.11 | 518.327 | 64.47    |
| SASA w/ glycans + hepa | 281.36   | 315.2  | 239.59 | 239.2  | 268.8375 | 36.70    |
| Heparin shielded Area | 288.99    | 255.94 | 199.12 | 253.91 | 249.49 | 37.23    |

| SASA S1/S2 Model 03 | subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|---------------------|------------|--------|--------|--------|--------|----------|-------------|
| SASA w/o glycans   | 516.39     | 515.64 | 581.44 | 748.69 | 590.54 | 109.85   |
| SASA w/ glycans    | 487.64     | 426.44 | 563.91 | 746.23 | 556.055 | 138.65   |
| SASA w/ glycans + hepa | 334.23   | 255.98 | 391.9  | 526.14 | 377.0625 | 113.93   |
| Heparin shielded Area | 153.41    | 170.46 | 172.01 | 220.09 | 178.9925 | 28.66    |

| SASA S1/S2 Model 04 | subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|---------------------|------------|--------|--------|--------|--------|----------|-------------|
| SASA w/o glycans   | 513.33     | 530.3  | 504.07 | 577.56 | 531.315 | 32.683   |
| SASA w/ glycans    | 513.33     | 530.3  | 384.19 | 577.56 | 501.345 | 82.70    |
| Glycan shielded Area | 0         | 0      | 119.88 | 0      | 29.97 | 59.94    |

| SASA S1/S2 Model 04 | subunit SB | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|---------------------|------------|--------|--------|--------|--------|----------|-------------|
| SASA w/o glycans   | 537.96     | 490.5  | 387.01 | 426.75 | 460.555 | 66.93    |
| SASA w/ glycans    | 468.25     | 490.5  | 387.01 | 323.6  | 417.34 | 76.70    |
| Glycan shielded Area | 69.71      | 0      | 0      | 103.15 | 43.215 | 51.73    |

| SASA S1/S2 Model 04 | subunit SC | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|---------------------|------------|--------|--------|--------|--------|----------|-------------|
| SASA w/o glycans   | 287.33     | 450.2  | 373.27 | 571.4  | 420.55 | 120.57   |
| SASA w/ glycans    | 268.55     | 450.2  | 373.27 | 571.4  | 415.855 | 127.65   |
| Glycan shielded Area | 18.78      | 0      | 0      | 4.695  | 9.39   |          |
| Subunit | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | STD. DEV. [Å²] |
|---------|--------|--------|--------|--------|----------|----------------|
| SASA w/o glycans - hepa | 586.63 | 608.31 | 620.32 | 473.41 | 572.1675 | 67.30 |
| SASA w/ glycans | 586.63 | 607.13 | 620.32 | 473.41 | 571.8725 | 67.09 |
| SASA w/ glycans + hepa | 240.61 | 273.7 | 410.65 | 281.39 | 301.5875 | 74.83 |
| Heparin shielded Area | 346.02 | 333.43 | 209.67 | 192.02 | 270.285 | 80.67 |
| SASA w/o glycans - hepa | 554.89 | 440.05 | 562.46 | 378.39 | 483.9475 | 89.94 |
| SASA w/ glycans | 499.14 | 440.05 | 554.17 | 378.39 | 467.9375 | 75.73 |
| SASA w/ glycans + hepa | 203.26 | 381.9 | 438.86 | 224.4 | 312.105 | 116.16 |
| Heparin shielded Area | 295.88 | 58.15 | 115.31 | 153.99 | 155.8325 | 101.32 |
| SASA w/o glycans - hepa | 481.46 | 424.66 | 559.17 | 537.64 | 500.7325 | 60.37 |
| SASA w/ glycans | 456.98 | 424.66 | 509.52 | 537.64 | 482.2 | 50.88 |
| SASA w/ glycans + hepa | 183.05 | 203.28 | 293.16 | 320.88 | 250.0925 | 67.21 |
| Heparin shielded Area | 273.93 | 221.38 | 216.36 | 216.76 | 232.1075 | 27.97 |
Tab. S8 Furin cleavage assay. Single measurements obtained for the various experimental conditions used to investigate the ability of heparin to inhibit furin cleavage by binding at the spike S1/S2 site.

| Condition                        | Rep 1   | Rep 2   | Rep 3   | Rep 4   | Rep 5   | Rep 6   | Rep 7   | Rep 8   | Rep 9   | Rep 10  | AVG    | ST. DEV. |
|----------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|--------|----------|
| CTRL                             | 0.191   | 0.222   | 0.214   | 0.228   | 0.219   | 0.239   | 0.431   | 0.405   | 0.264   | -       | 0.268  | 0.087    |
| Heparin (10 µM)                  | 0.18    | 0.218   | 0.187   | 0.196   | -       | -       | -       | -       | -       | -       | 0.196  | 0.017    |
| Furin (25ng/well)                | 0.014   | 0.052   | 0.027   | 0.027   | 0.054   | 0.075   | 0.075   | 0.123   | 0.046   | 0.045   | 0.054  | 0.031    |
| Furin (25ng/well) + Heparin (10 µM) | 0.338   | 0.277   | 0.325   | 0.337   | -       | -       | -       | -       | -       | -       | 0.319  | 0.029    |
| Heparin (10 µM) → PBS → Furin (25ng/well) | 0.331   | 0.31    | 0.322   | 0.229   | -       | -       | -       | -       | -       | -       | 0.298  | 0.047    |
| Heparin (10 µM) → Salt (2M NaCl) → Furin (25ng/well) | 0.111   | 0.144   | 0.098   | 0.054   | -       | -       | -       | -       | -       | -       | 0.102  | 0.037    |

Tab. S9 Furin cleavage assay. Single measurements obtained to compare the ability of unfractionated heparin (UFH), low molecular weight heparin (LMWH) and unsulfated K5 polysaccharide (K5) to inhibit the cleavage of the spike S1/S2 site by furin (25 ng/well).

| Condition | Concentration [µM] | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | AVG | ST. DEV. |
|-----------|--------------------|-------|-------|-------|-------|-------|-------|-----|----------|
| UFH       | 0.001              | 0.038 | 0.008 | 0.01  | -     | -     | -     | 0.019 | 0.017    |
|           | 0.01               | 0.137 | 0.135 | 0.207 | 0.17  | 0.074 | 0.046 | 0.13 | 0.06     |
|           | 0.1                | 0.228 | 0.245 | 0.158 | 0.098 | -     | -     | 0.182 | 0.068    |
|           | 1                  | 0.203 | 0.226 | 0.238 | 0.242 | -     | -     | 0.227 | 0.018    |
|           | 10                 | 0.338 | 0.277 | 0.325 | 0.337 | -     | -     | 0.319 | 0.029    |
|           | 100                | 0.32  | 0.328 | 0.317 | -     | -     | -     | 0.317 | 0.006    |
| LMWH      | 0.01               | 0.055 | 0.109 | 0.08  | -     | -     | -     | 0.0813 | 0.027    |
|           | 0.1                | 0.099 | 0.076 | 0.091 | -     | -     | -     | 0.087 | 0.012    |
|           | 1                  | 0.164 | 0.176 | 0.117 | 0.152 | -     | -     | 0.152 | 0.025    |
|           | 10                 | 0.152 | 0.145 | 0.193 | 0.197 | -     | -     | 0.171 | 0.027    |
|           | 100                | 0.255 | 0.301 | 0.279 | -     | -     | -     | 0.278 | 0.023    |
|           | 1000               | 0.293 | 0.287 | 0.288 | -     | -     | -     | 0.289 | 0.003    |
| K5        | 10                 | 0.037 | 0.021 | 0.025 | 0.015 | -     | -     | 0.024 | 0.009    |
Tab. S10 Solvent Accessible Surface Area (SASA) (Å²) of spike residues within the RBm and directly involved in binding with ACE2 (3). For each model, SASA was calculated for the most representative cluster using NACCESS (8) with a probe radius of 1.4 Å.

### SASA RBm Model 01

| subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|------------|--------|--------|--------|--------|----------|--------------|
| SASA w/o glycans | 1535.01 | 1504.43 | 1210.01 | 1260.4 | 1377.4625 | 166.02 |
| SASA w/ glycans | 1311.63 | 1122.36 | 939.32 | 1126.82 | 1125.0325 | 152.01 |
| Glycan shielded Area | 223.38 | 382.07 | 270.69 | 133.58 | 252.43 | 103.46 |

### SASA RBm Model 02

| subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|------------|--------|--------|--------|--------|----------|--------------|
| SASA w/o glycans | 1332.19 | 1435.41 | 1302.66 | 1193.93 | 1316.0475 | 99.33 |
| SASA w/ glycans | 1040.84 | 1039.22 | 781.12 | 740.21 | 900.3475 | 162.16 |
| Glycan shielded Area | 291.35 | 396.19 | 521.54 | 453.72 | 415.7 | 97.46 |

### SASA RBm Model 03

| subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|------------|--------|--------|--------|--------|----------|--------------|
| SASA w/o glycans | 1417.49 | 1206.9 | 1274.83 | 1329.75 | 1307.2425 | 89.03 |
| SASA w/ glycans | 1042.8 | 985.55 | 1200.53 | 1047.38 | 1069.065 | 92.05 |
| Heparin shielded Area | 374.69 | 221.35 | 74.3 | 282.37 | 238.1775 | 126.13 |

### SASA RBm Model 04

| subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|------------|--------|--------|--------|--------|----------|--------------|
| SASA w/o glycans | 1416.74 | 1050.74 | 921.89 | 1412.17 | 1200.385 | 252.73 |
| SASA w/ glycans | 1170.23 | 671.73 | 774.8 | 1188.74 | 951.375 | 266.85 |
| Heparin shielded Area | 1168.11 | 659.13 | 774.8 | 1136.32 | 934.59 | 256.02 |
| Glycan shielded Area | 248.63 | 391.61 | 147.09 | 275.85 | 249.01 | 100.53 |

| subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|------------|--------|--------|--------|--------|----------|--------------|
| SASA w/o glycans | 1009.43 | 993.37 | 1108.68 | 1135.53 | 1061.7525 | 70.85 |
| SASA w/ glycans | 850.01 | 739.51 | 687.97 | 886.77 | 791.065 | 92.95 |
| Heparin shielded Area | 831.2 | 739.51 | 687.97 | 868.53 | 781.8025 | 82.77 |
| Glycan shielded Area | 18.81 | 0 | 0 | 18.24 | 9.2625 | 10.70 |

| subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|------------|--------|--------|--------|--------|----------|--------------|
| SASA w/o glycans | 1266.88 | 1188.41 | 1374.5 | 1259.85 | 1272.41 | 76.74 |
| SASA w/ glycans | 1218.42 | 1000.93 | 1097.99 | 1015.93 | 1083.3175 | 99.66 |
| Heparin shielded Area | 1218.42 | 1000.93 | 1097.99 | 1015.93 | 1083.3175 | 99.66 |
| Glycan shielded Area | 48.46 | 187.48 | 276.51 | 243.92 | 189.0925 | 100.71 |

| subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|------------|--------|--------|--------|--------|----------|--------------|
| SASA w/o glycans | 934.02 | 1023.2 | 1331.32 | 925.07 | 1053.40 | 190.50 |
| SASA w/ glycans | 925.07 | 1023.2 | 1331.32 | 925.07 | 1051.165 | 192.41 |
| Glycan shielded Area | 8.95 | 0 | 0 | 0 | 2.23 | 4.475 |

| subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|------------|--------|--------|--------|--------|----------|--------------|
| SASA w/o glycans | 1294.16 | 1302.6 | 1352.26 | 1294.16 | 1310.795 | 27.92 |
| SASA w/ glycans | 1115.78 | 1302.6 | 1029.59 | 1115.78 | 1140.94 | 115.18 |
| Glycan shielded Area | 178.38 | 0 | 322.67 | 178.38 | 169.86 | 132.10 |

| subunit SA | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|------------|--------|--------|--------|--------|----------|--------------|
| SASA w/o glycans | 1562.47 | 1302.6 | 1546.65 | 1562.47 | 1493.5475 | 127.52 |
| Subunit | Rep 01 | Rep 02 | Rep 03 | Rep 04 | AVG [Å²] | ST.DEV. [Å²] |
|---------|--------|--------|--------|--------|----------|-------------|
| SA      |        |        |        |        |          |             |
| w/o glycans-hepa | SASA | 811.23 | 717.19 | 851.27 | 824.47 | 801.04 | 58.33 |
| w/glycans | SASA | 726.13 | 516.61 | 802.55 | 679.78 | 681.2675 | 120.88 |
| w/glycans + hepa | SASA | 726.13 | 504.51 | 802.55 | 620.42 | 663.4025 | 129.60 |
| Glycan shielded Area | 0 | 12.1 | 0 | 59.36 | 17.865 | 28.25 |
| w/o glycans-hepa | Glycan | 85.1 | 212.68 | 48.72 | 204.05 | 137.6375 | 83.08 |
| w/glycans | Glycan | 778.58 | 786.46 | 981.52 | 1233.6 | 1243.99 | 66.13 |
| w/glycans + hepa | Glycan | 778.58 | 786.46 | 981.52 | 1233.6 | 1243.99 | 66.13 |
| Glycan shielded Area | 0 | 12.43 | 0 | 59.36 | 17.865 | 28.25 |
| w/o glycans-hepa | Subunit | 333.87 | 206.73 | 185.98 | 222.28 | 214.50 | 66.13 |
| w/glycans | Subunit | 1512.39 | 1384.55 | 1370.12 | 1356.64 | 1405.925 | 71.89 |
| w/glycans + hepa | Subunit | 1512.39 | 1384.55 | 1370.12 | 1356.64 | 1405.925 | 71.89 |
| Heparin shielded Area | 0 | 33.35 | 0 | 117.34 | 37.6725 | 55.39 |
| Glycan shielded Area | 0 | 33.35 | 0 | 117.34 | 37.6725 | 55.39 |

SASA RBm Model 05
4. Supporting Movies

**Movie S1. Closed conformation of spike bound to 1 heparin chain.**
The movie shows first the spike structure with a focus on the heparin binding site followed by the MD trajectory for replica 1 (corresponding to all figures of the main text).

**Movie S2. Closed conformation of spike bound to 3 heparin chains.**
The movie shows first the spike structure and the binding modes of the three heparin chains followed by the MD trajectory for replica 1 (corresponding to all figures of the main text).

**Movie S3. Open conformation of spike bound with 3 heparin chains.**
The movie shows first the spike structure and the binding modes of the three heparin chains with a focus on the open RBD followed by the MD trajectory for replica 1 (corresponding to all figures of the main text).
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