

## Supplementary Information

for

### Extrinsic stabilization of antiviral ACE2-Fc fusion proteins targeting SARS-CoV-2

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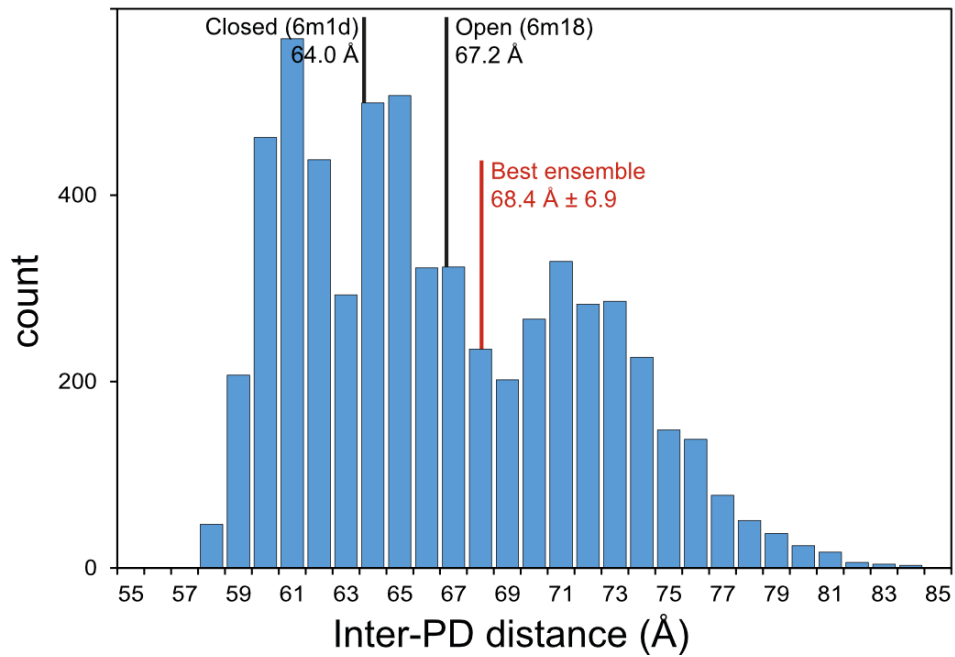
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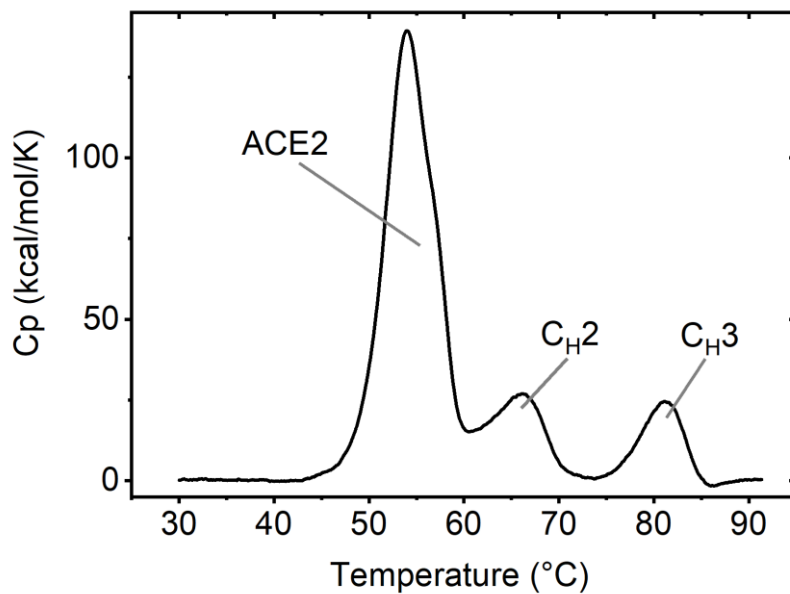
[hristo.svilenov@ugent.be](mailto:hristo.svilenov@ugent.be), <https://orcid.org/0000-0001-5863-9569>

[johannes.buchner@tum.de](mailto:johannes.buchner@tum.de), <https://orcid.org/0000-0003-1282-7737>

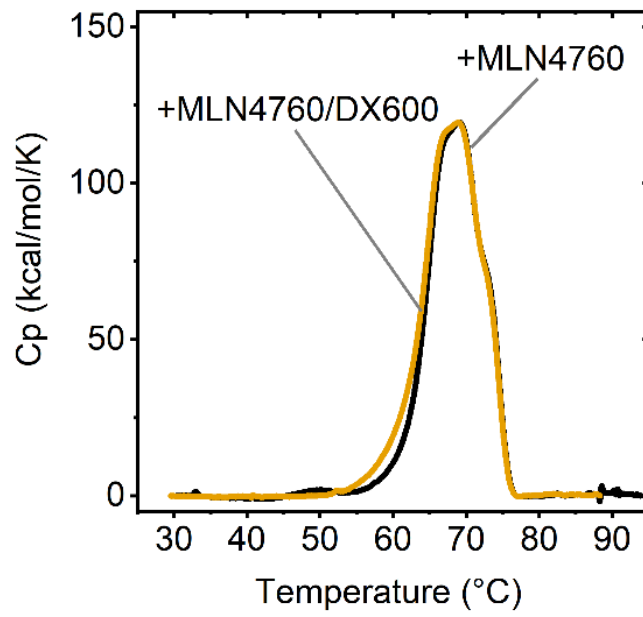
phone: +498928913340; fax: +498928913345



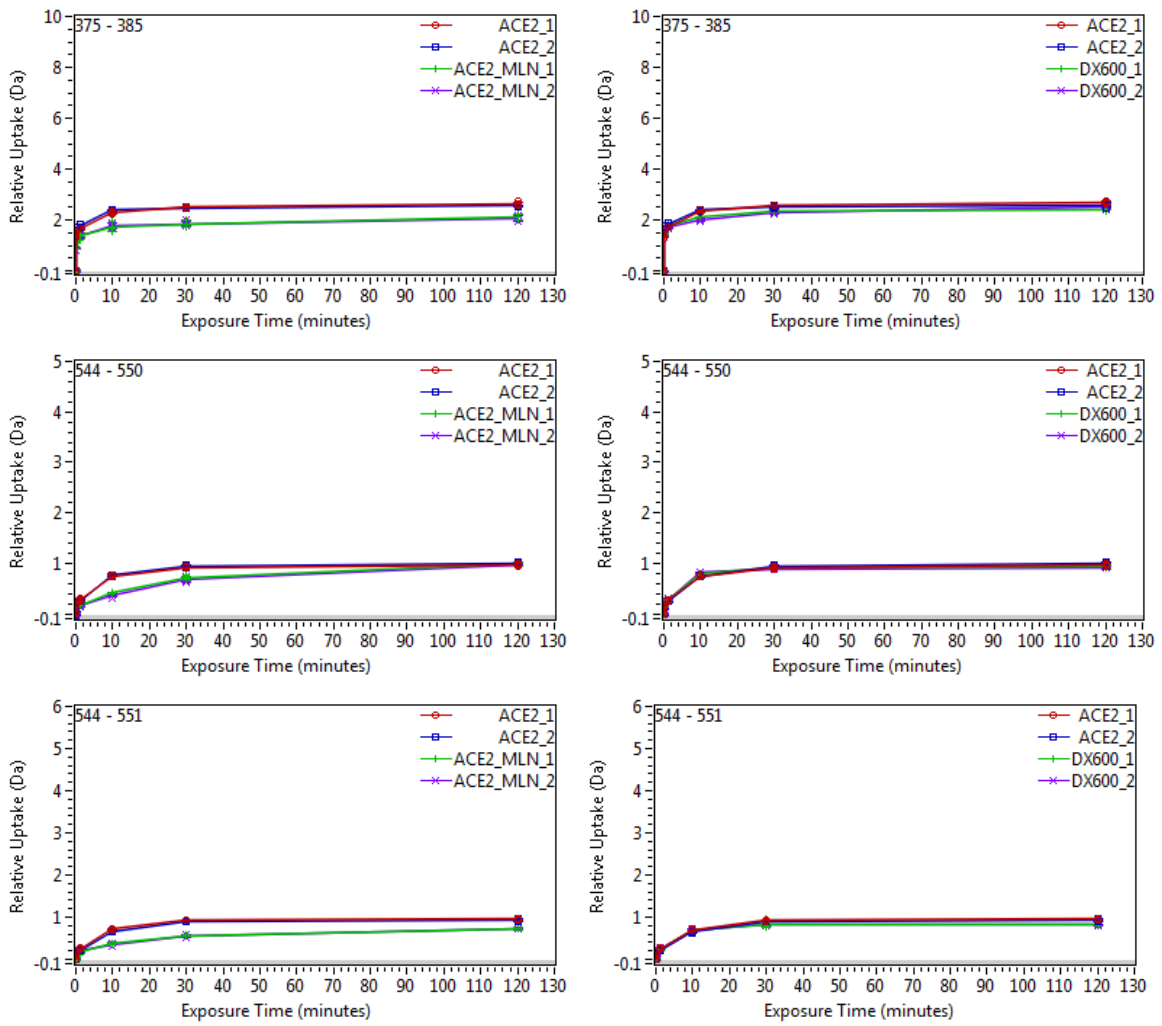
**Supplementary Figure 1.** Distribution of Inter-PD distance from the pool of all ACE2-Fc conformations generated by MD. This pool was used to select a minimum ensemble fitting best the SAXS data, of which the inter-PD distance is indicated (red), as well as for the known structures of ACE2 in the open and closed conformations.



**Supplementary Figure 2.** Differential scanning microcalorimetry analysis of an ACE2-IgG1-Fc.

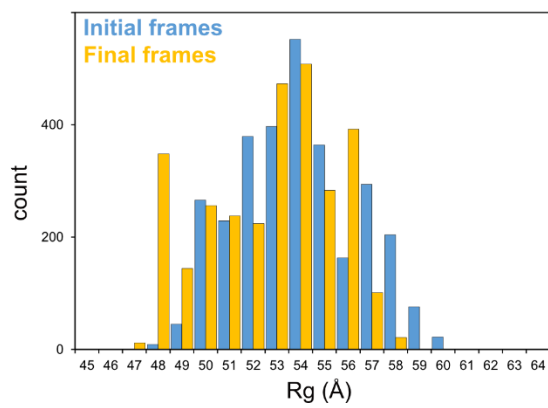


**Supplementary Figure 3.** DSC analysis of 5  $\mu\text{M}$  ACE2-IgG4-Fc in the presence of 50  $\mu\text{M}$  MLN4760 or 50  $\mu\text{M}$  MLN4760 plus 50  $\mu\text{M}$  DX600

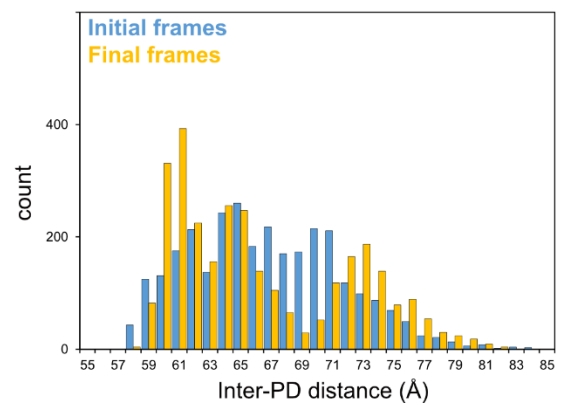


**Supplementary Figure 4.** Deuterium uptake plots of other peptides in the hinge region between the two subdomains of ACE2 PD. The ACE2 indicates ACE2-IgG4-Fc without inhibitors, ACE2\_MLN is ACE2-IgG4-Fc in the presence of MLN4760, DX600 is ACE2-IgG4-Fc in the presence of DX600.

a)



b)



**Supplementary Figure 5.** Comparison of the first (blue) and the final (yellow) half of the MD simulation runs. **a)** R<sub>g</sub> distribution and **b)** inter-PD distance distribution show the same general trend in both subsets.

**Supplementary Table 1.** Overview of the attached glycans and the corresponding protein sites.

Protein Site	Glycan
N36,N529	DGlcNAcb1-4DGlcNAcb1-2DManpa1-6[DGlcNAcb1-2DManpa1-3]DManpb1-4DGlcNAcb1-4DGlcNAca1-OH
N73, N673	DGlcNAcb1-2DManpa1-6[DGalpb1-4DGlcNAcb1-2DManpa1-3]DManpb1-4DGlcNAcb1-4[LFucpa1-3]DGlcNAca1-OH
N86, N415	DGlcNAcb1-2DManpa1-6[DGlcNAcb1-2DManpa1-3]DManpb1-4DGlcNAcb1-4[LFucpa1-3]DGlcNAca1-OH
N305	DGlcNAcb1-2DManpa1-6[DGlcNAcb1-4[DGlcNAcb1-4DGlcNAcb1-2]DManpa1-3]DManpb1-4DGlcNAcb1-4DGlcNAca1-OH
T723	DNeup5Aca2-6[DNeup5Aca2-3DGalpb1-3]DGalpNAca1-OH
N794	DGlcNAcb1-2DManpb1-6DManpb1-4DGlcNAcb1-4[LFucpa1-6]DGlcNAca1-OH

**Supplementary Table 2.** Overview of the properties and associated values used for the MD simulations.

Property	Value
Simulation box dimensions	14 Å minimum distance to box edge
Total number of atoms glycosylated	593412
Total number of water molecules glycosylated	186666
Total number of ions	58
Thermostat	Langevin
Collision frequency in ps <sup>-1</sup>	2
Protein force field	FF14SB
Glycan force field	GLYCAM_06j-1
Water force field	TIP3P
Nonbonded cutoff	8.5 Å