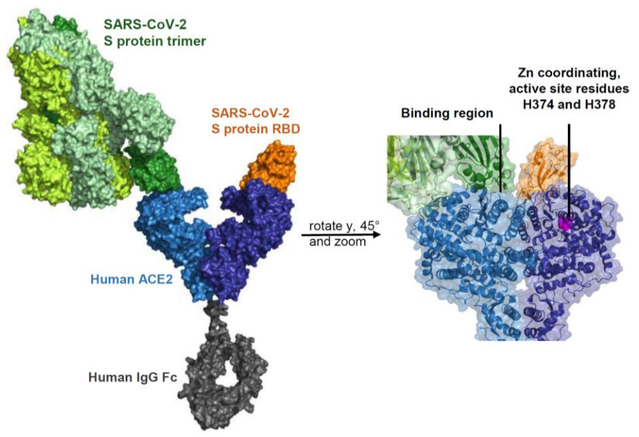
Highly efficient inhibition of SARS-CoV-2 entry by a biologically unique ACE2-IgG4-Fc fusion protein with a stabilized hinge region

Hristo L. Svilenov*[1], Julia Sacherl*[2], Alwin Reiter*[5], Lisa Wolff[2], Cho-Chin Cheng[2,4], Marcel Stern[3,4], Frank-Peter Wachs[5], Nicole Simonavicius[5], Susanne Pippig[5], Florian Wolschin[5], Oliver T. Keppler[3,4], Johannes Buchner[1], Carsten Brockmeyer[5], Ulrike Protzer[2,4]

*Equal contribution; [1]Department of Chemistry, Technical University of Munich, Garching, Germany; [2]Institute of Virology, Technical University of Munich / Helmholtz Zentrum Munich, Munich, Germany; [3]Max von Pettenkofer Institute & Gene Center, Virology, LMU Munich, Munich, Germany; [4]German Center for Infection Research, Munich partner site, Munich, Germany; [5]Formycon AG, Martinsried/Planegg, Germany

Introduction

Severe acute respiratory syndrome (SARS)-like coronavirus 2 (SARS-CoV-2) and other human pathogenic coronaviruses use the angiotensin-converting enzyme 2 (ACE2) to enter human host cells.[1] ACE2-Fc fusion proteins composed of the human immunoglobulin G (IgG) fragment crystallizable (Fc) part fused to the extracellular domain of ACE2 have been suggested as a high-priority treatment option for COVID-19[2], however bear the risk of unwanted Fc-receptor activation and antibody-dependent disease enhancement. We have designed an innovative ACE2-IgG4-Fc fusion protein as a potent SARS-CoV-2 entry blocker.[3] First, the ACE2-IgG4-Fc fusion protein acts as a highly efficient virus trap by targeting the entire receptor-binding domain (RBD) of SARS-CoV-2. Second, ACE2-IgG4-Fc cleaves angiotensin II to angiotensin 1-7, potentially providing additional protection to the lung and cardiovascular system. Third, the risk of antibody-dependent disease enhancement is minimized by using the IgG4 Fc region.



Materials and methods

ACE2-Fc construct design

Constr.	ACE2 sequence (active site mutation)	IgG isotype (hinge region modification)
1	Q18-G732 (no)	IgG4 (S228P)
2	Q18-S740 (no)	IgG4 (S228P)
3	Q18-G732 (H374N, H378N)	IgG4 (S228P)
4	Q18-S740 (H374N, H378N)	IgG4 (S228P)
5	Q18-G732 (no)	IgG1 (truncated)
6	Q18-S740 (no)	IgG1 (truncated)
7	Q18-G732 (H374N, H378N)	IgG1 (truncated)
8	Q18-S740 (H374N, H378N)	IgG1 (truncated)

Protein expression and purification – Transient expression in HEK293 cells. Protein A followed by size-exclusion chromatography.

Circular dichroism (CD) – J-1500 spectropolarimeter (Jasco).

Size-exclusion chromatography with multi-angle light scattering (SEC-MALS) – Shimadzu HPLC, Superdex 200 Increase 10/300 GL column and HELEOS II detector (Wyatt Technology).

Enzymatic activity – Cleavage of a peptidyl-4-methylcoumaryl-7-amide (MCA) and fluorimetric detection of the free MCA. Assay kit from Abcam (Cat.No. ab273297).

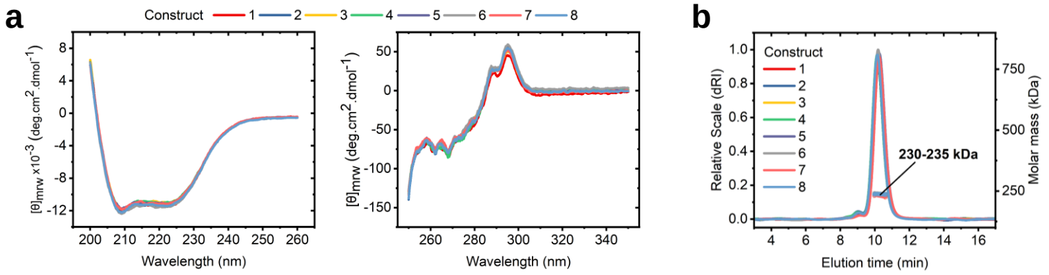
Surface plasmon resonance (SPR) – Biacore X100 (Cytiva), Biotin CAPture kit (Cytiva), captured SARS-CoV-2 RBD with an AviTag™.

ELISA - ACE2:SARS-CoV-2 spike S1 inhibitor screening assay kit (BPS Bioscience; Cat.No. 79945).

Virus neutralization assays - SARS-CoV-2-GFP[4] is based on the original Wuhan SARS-CoV-2 isolate (GenBank accession MT108784). SARS-CoV-2-Jan (EPI_ISL_582134), SARS-CoV-2-April (EPI_ISL_466888), and SARS-CoV (AY291315.1) were isolated from patient material in Germany. Vero E6 cells were used in the assays.

Results

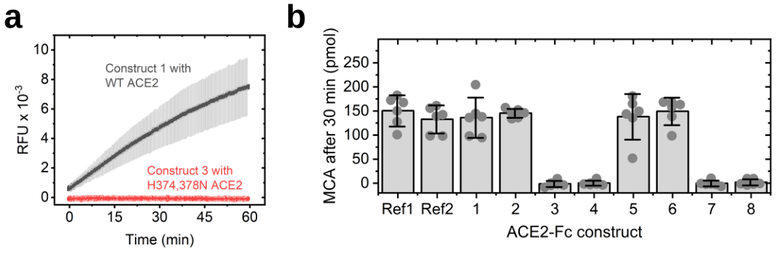
1 Structural characterization



a. The eight ACE2-Fc proteins have identical far- and near-UV CD spectra.

b. SEC-MALS shows that the ACE2-Fc molecules exist as homodimers.

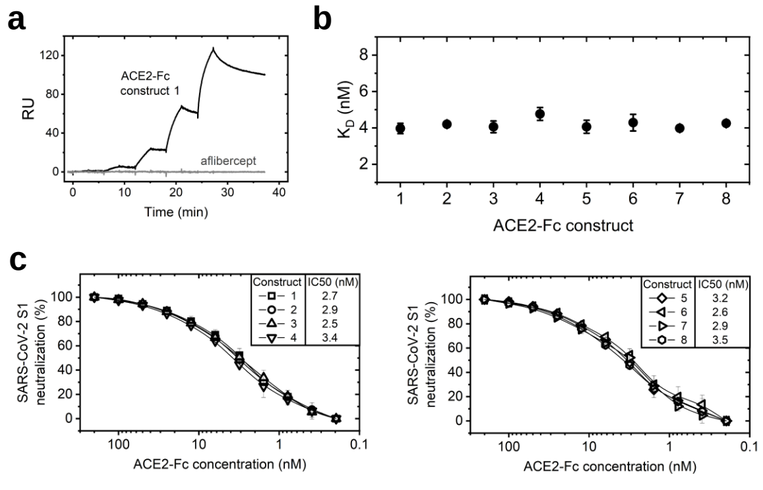
2 Enzymatic activity



a. The H374,378N mutation abolishes ACE2-Fc enzymatic activity.

b. All ACE2-Fc constructs with wild-type (WT) ACE2 are enzymatically active. Ref1 and Re2 are commercial ACE2-IgG1-Fc proteins.

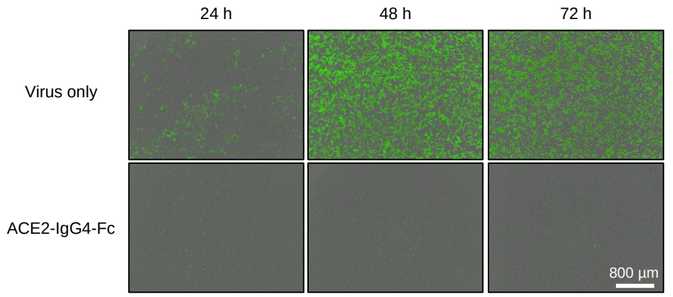
3 Binding to SARS-CoV-2 spike proteins



a, **b.** ACE-Fc proteins bind to the RBD of SARS-CoV-2 with high affinity in SPR experiments.

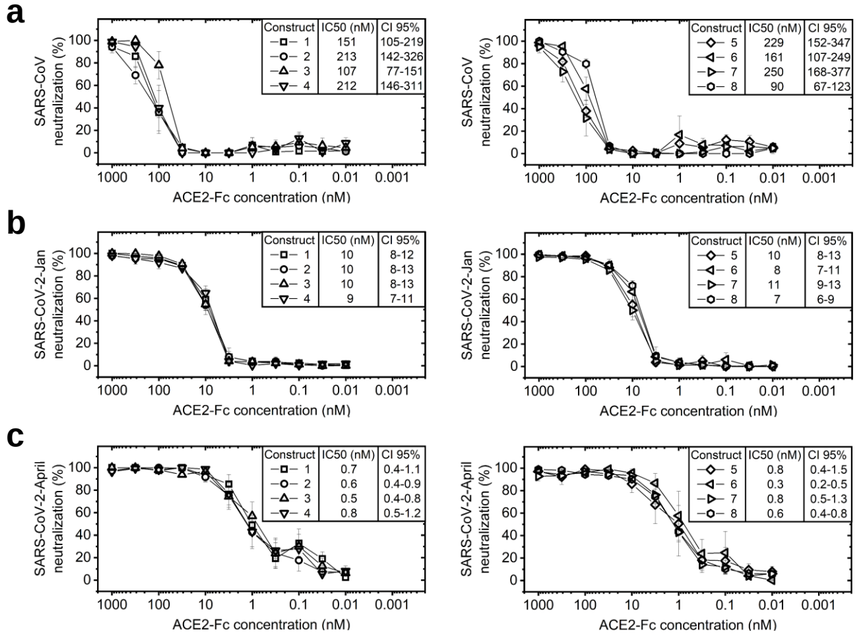
b. ELISA shows that ACE2-Fc proteins bind with high affinity to the spike protein of SARS-CoV-2.

4 Inhibition of SARS-CoV-2-GFP cell entry



Pre-incubation with the ACE2-IgG4-Fc inhibits SARS-CoV-2-GFP replication in Vero E6 cells (MOI = 0.3).

5 Virus neutralization of SARS-CoV and SARS-CoV-2 strains



ACE2-Fc proteins neutralize:

a. SARS-CoV with IC50 between 90 and 250 nM (MOI = 0.03).

b. SARS-CoV-2-Jan with IC50 between 7 and 11 nM (MOI = 0.03).

c. SARS-CoV-2-April with IC50 between 0.3 and 0.8 nM (MOI = 0.03).

Conclusions

- **ACE2-IgG4-Fc proteins have favorable biophysical and pharmaceutical characteristics.**
- **ACE2-Fc molecules with WT ACE2 preserve enzymatic activity.**
- **All ACE2-Fc proteins bind to SARS-CoV-2 spike proteins with high affinity.**
- **ACE2-IgG4-Fc efficiently inhibits SARS-CoV-2-GFP cell entry.**
- **ACE2-Fc proteins neutralize SARS-CoV and different SARS-CoV-2 strains.**

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References

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