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Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody

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Group 3

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Lab background

- Vir Biotechnology Inc.
  - Humabs BioMed SA in Switzerland[1]

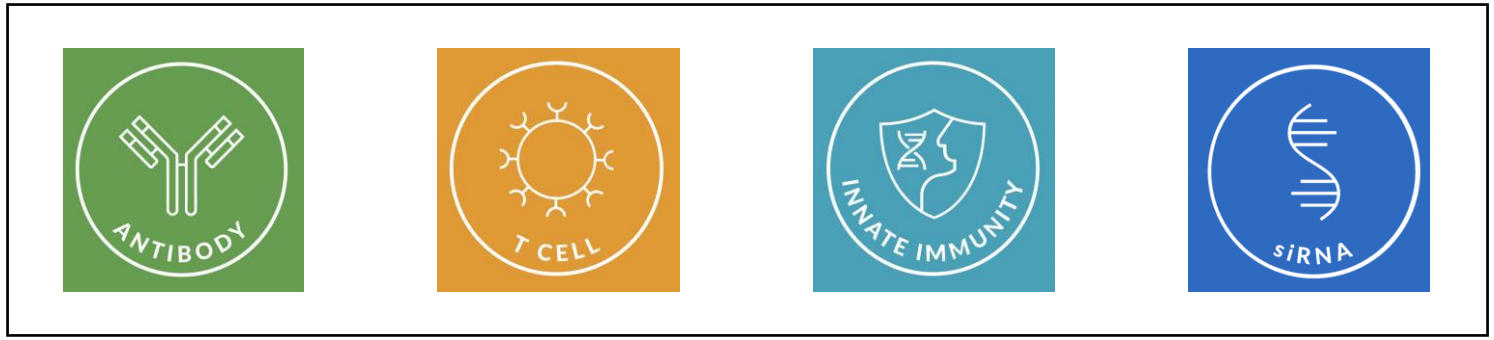


Image from Vir Biotechnology, 2021.

- Senior PI's:
  - **David Veesler**, PhD., Associate Professor of Biochemistry at the University of Washington School of Medicine.
  - **Davide Corti**, PhD., Senior Vice President of Antibody Research at Vir.

Monoclonal antibody (mAb) candidates for SARS-CoV-2

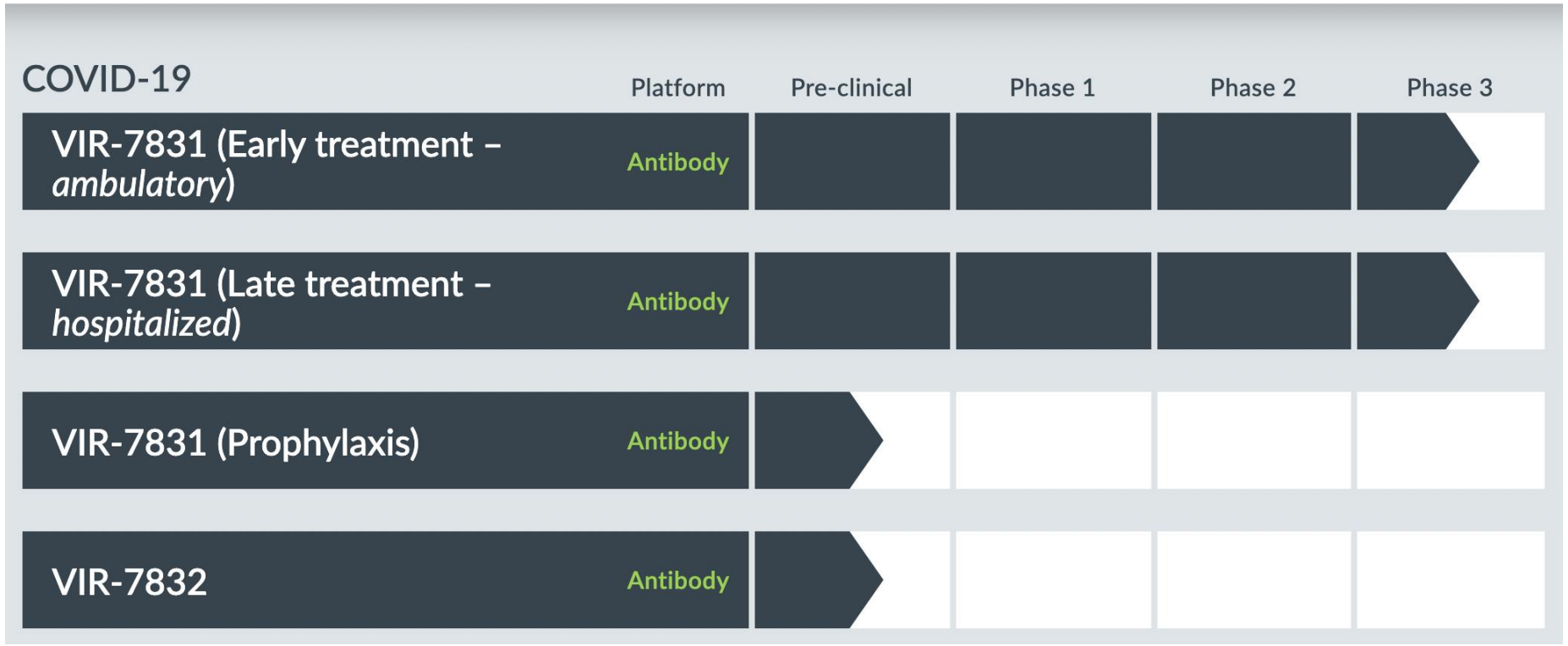
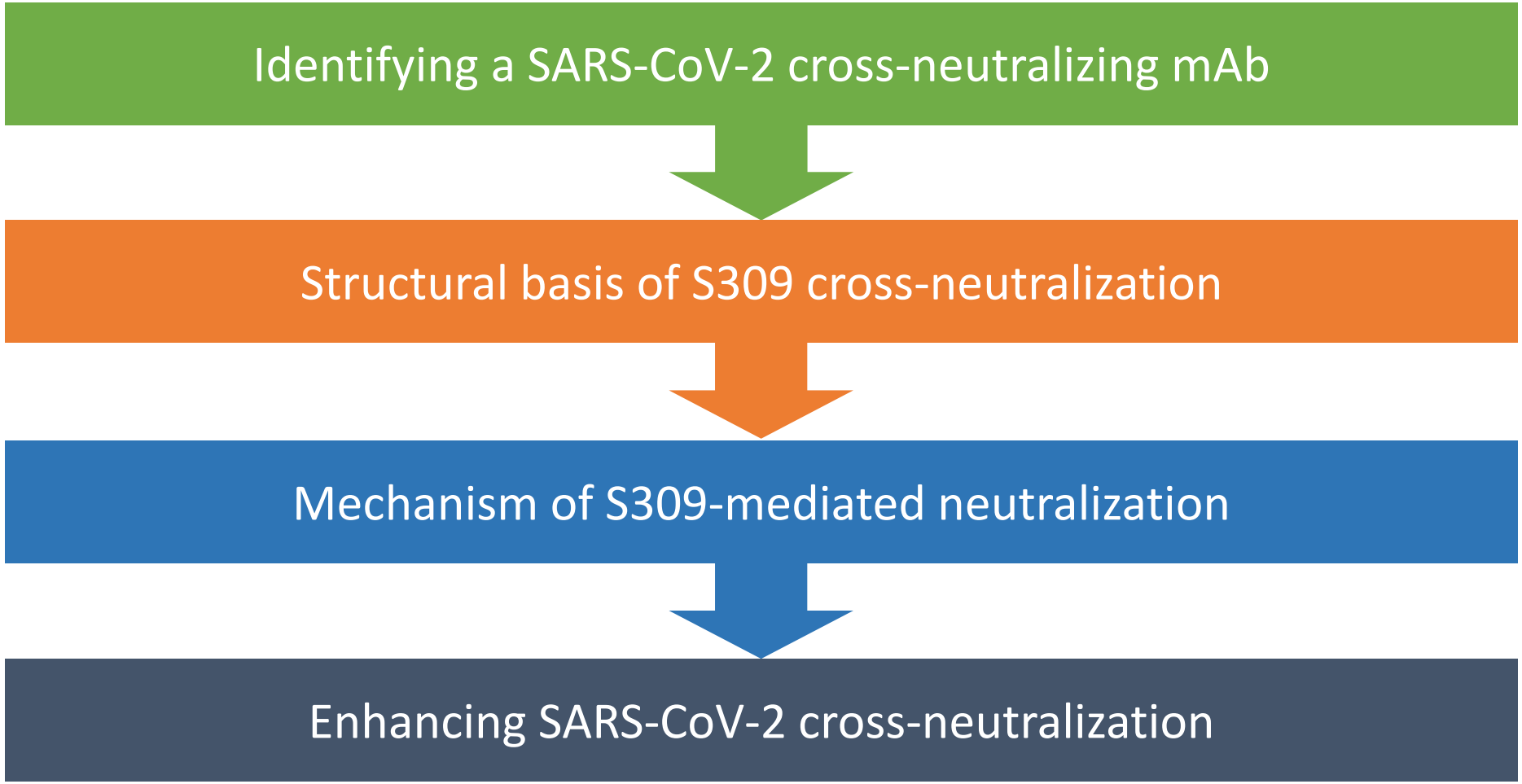


Image from Vir Biotechnology, 2021.

Outline of the results



Identifying a SARS-CoV-2 cross-neutralizing mAb

- Evaluated **25** mAbs for cross-reactivity to **SARS-CoV-2**[1]
- **19** mAbs identified in the 2004 screen[1]
- **6** recombinant IgG-LS antibodies[1]

mAb	VH (per cent identity)	HCDR3 sequence	VL (per cent identity)	SARS-CoV	SARS-CoV-2	Binding
S110	VH3-30 (96.88)	AKDRFQFARSWYGDYFDY	VK2-30 (96.60)	+	+	RBD and non-RBD
S124	VH2-26 (98.28)	ARINTAAYDYDSTTFDI	VK1-39 (98.57)	+	+	RBD
S109	VH3-23 (93.75)	ARLESATQPLGYYFYGMDV	VL3-25 (97.85)	+	–	RBD
S111	VH3-30 (95.14)	ARDIRHLIVVVSDMDV	VK2-30 (98.30)	+	–	RBD
S127	VH3-30 (96.53)	AKDLFGYCRSTSCESLDD	VK1-9 (98.92)	+	–	RBD
S215	VH3-30 (90.28))	ARETRHYSHGLNWFDP	VK3-15 (98.92)	+	–	RBD
S217	VH3-49 (95.58)	SWIHRIVS	VK1-33 (98.21)	+	–	RBD
S218	VH3-30 (93.40)	ARDVKGHIVVMTSLDY	VK2-30 (97.62)	+	–	RBD
S219	VH1-58(92.01)	AAEMATIQNYYYYYGMDV	VK1-39 (95.34)	+	–	RBD
S222	VH1-2 (91.67)	ARGDVPVGTGWVFDF	VK1-39 (92.47)	+	–	RBD
S223	VH3-30 (95.14)	ATVSVEGYTSGWYLGTLDF	VK3-15 (98.21)	+	–	RBD
S224	VH1-18 (90.97)	ARQSHSTRGGWHFSP	VK1-39 (95.70)	+	–	RBD
S225	VH3-9 (96.18)	AKDISLVFWSVNPPRNGMDV	VK1-39 (98.57)	+	–	RBD
S226	VH3-30 (89.61)	ARDSSWQSTGWPINWFDR	VK3-11 (96.11)	+	–	RBD
S227	VH3-23 (95.14)	ASPLRNYGDLLY	VK1-5 (96.06)	+	–	RBD
S228	VH3-30 (96.53)	ARDLQMRVVVVSNFDY	VK2D-30 (99.32)	+	–	RBD
S230	VH3-30 (90.97)	VTQRDNSRDYFPHYFHDMDV	VK2-30 (97.62)	+	–	RBD
S231	VH3-30 (90.62)	ARDDNLDRHWPLRLGGY	VK2-30 (94.56)	+	–	RBD
S237	VH3-21 (96.53)	ARGFERYYFDS	VL1-44 (96.84)	+	–	RBD
S309	VH1-18 (97.22)	ARDYTRGAWFGESLIGGFDN	VK3-20 (97.52)	+	+	RBD
S315	VH3-7 (97.92)	ARDLWWNDQAHYYGMDV	VL3-25 (97.57)	+	+	RBD
S303	VH3-23 (90.28)	ARERDDIFPMGLNAFDI	VK1-5 (97.49)	+	+	RBD
S304	VH3-13 (97.89)	ARGDSSGYYYYFDY	VK1-39 (93.55)	+	+	RBD
S306	VH1-18 (95.49)	ASDYFDSSGYYHSFDY	VK3-11 (98.92)	+	+	Non-RBD
S310	VH1-69 (92.71)	ATRTYDSSGYRPYYYGLDV	VL2-23 (97.57)	+	+	Non-RBD

VH and VL per cent identity refers to V gene segment identity compared to germline (as per the International Immunogenetics Information System (http://www.imgt.org/)).

Image from Pinto et al., 2020

Enzyme-linked immunosorbent assay (ELISA)

- SARS-CoV RBD[1]
- SARS-CoV-2 RBD[1]
- SARS-CoV S glycoprotein[1]
- SARS-CoV-2 S glycoprotein[1]
- HCoV-OC43 S glycoprotein[1]
- MERS-CoV S glycoprotein[1]

Indirect ELISA

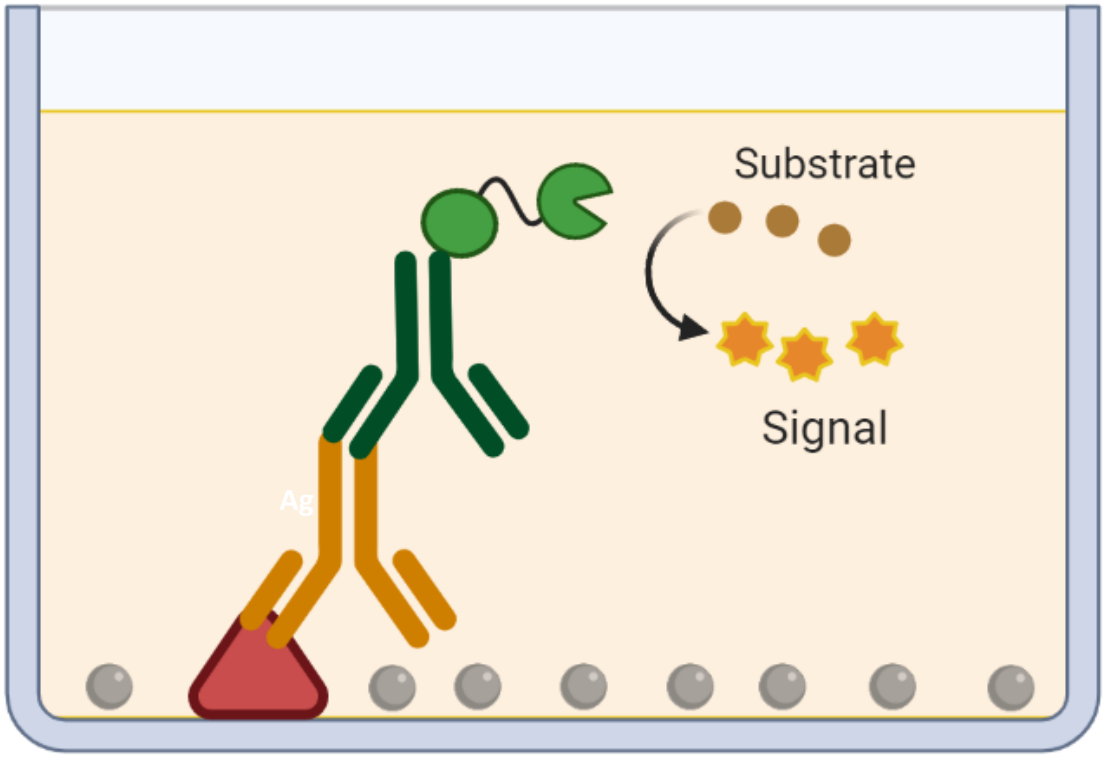


Image created using BioRender

ELISA results

- **None** of the mAbs bound to the **MERS-CoV** or **HCoV-OC43** S glycoprotein[1]
- **Four** mAbs bound to the RBD of both **SARS-CoV** and **SARS-CoV-2**[1]
  - S309
  - S304
  - S303
  - S315

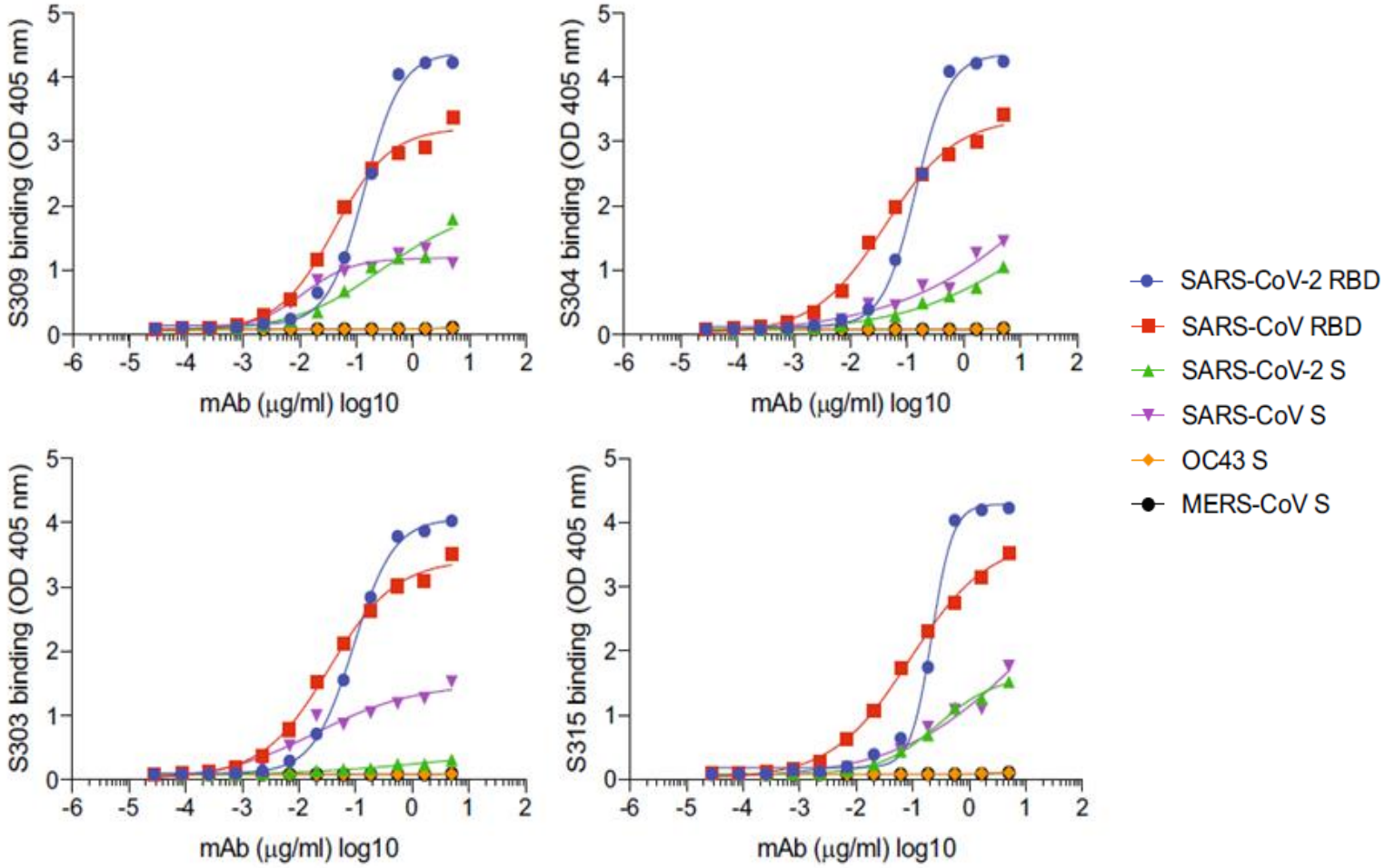


Image from Pinto et al., 2020

Biolayer interferometry

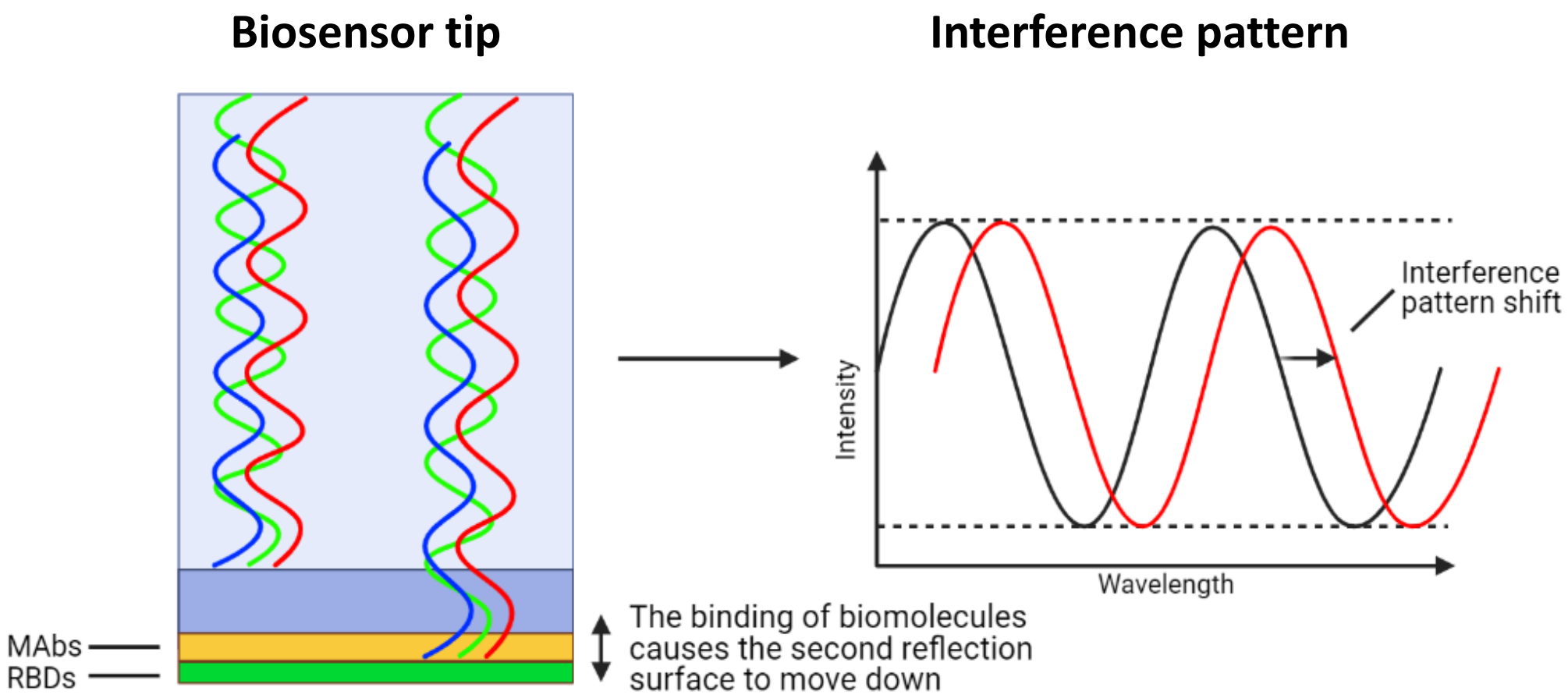


Image created using BioRender, adapted from 2Bind molecular interactions, 2020

Biolayer interferometry results

- S309, S303, S304 and S315 bound to the RBD of **SARS-CoV** and **SARS-CoV-2** with nano- to sub-picomolar affinities[1]
- **S309** bound to the RBD of **SARS-CoV-2** with the highest affinity[1]

mAb	KD (SARS-CoV)	KD (SARS-CoV-2)
S309	KD $<1.0 \times 10^{-12}$M	KD $<1.0 \times 10^{-12}$M
S303	KD $<1.0 \times 10^{-12}$M	KD 2.36×10^{-8}M
S304	KD 8.69×10^{-10}M	KD 4.58×10^{-9}M
S315	KD $<1.0 \times 10^{-12}$M	KD 8.11×10^{-10}M

Adapted from Pinto et al., 2020

Neutralization assay

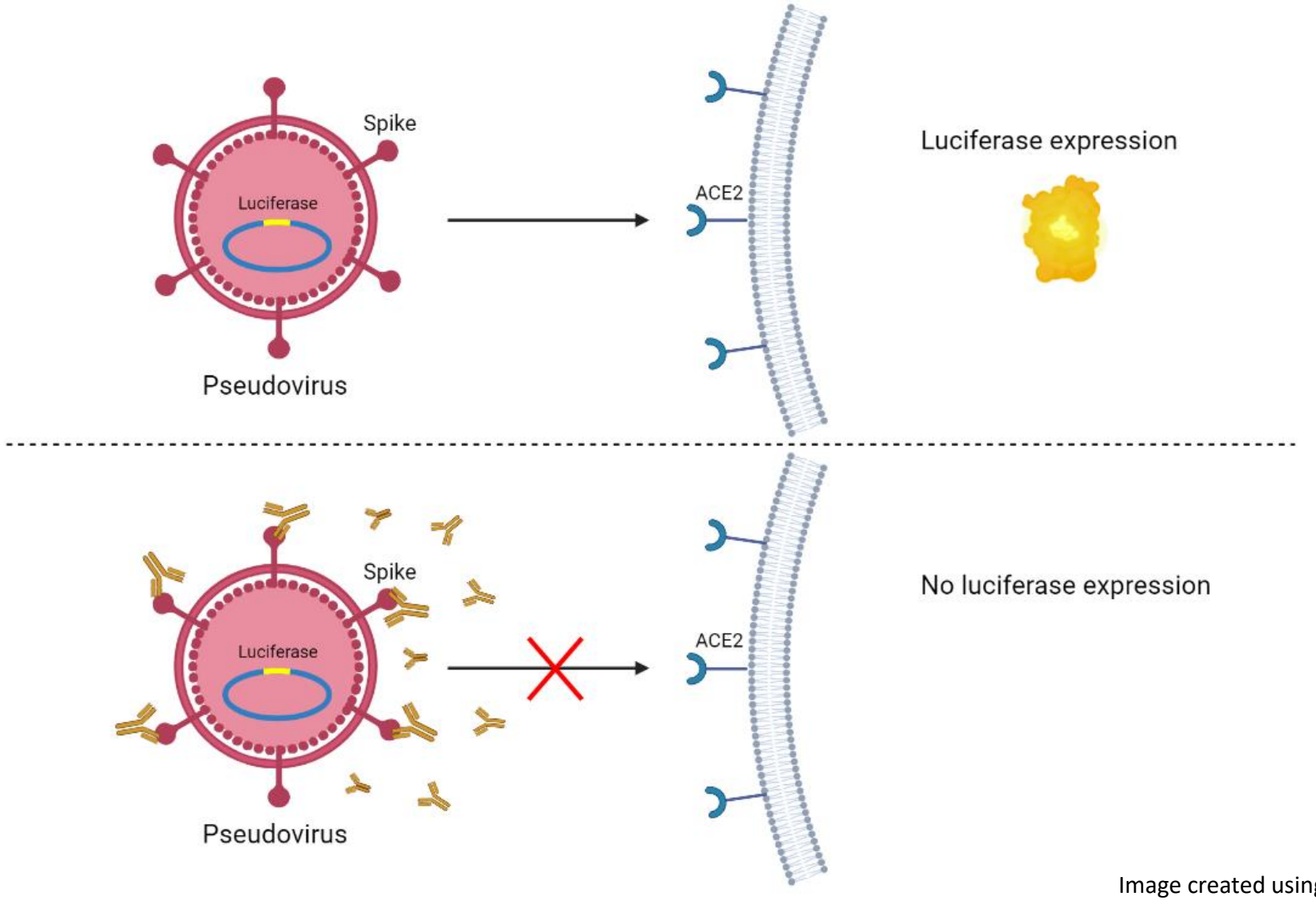


Image created using BioRender, adapted from Berthold, 2020

Neutralization assay results

- **S315** & **S304**
  - **Weakly neutralized** both SARS-CoV-MLV and SARS-CoV-2-MLV[1]
- **S303**
  - **Neutralized** SARS-CoV-MLV but not SARS-CoV-2-MLV[1]
- **S309**
  - **Potently neutralized** both SARS-CoV-MLV and SARS-CoV-2-MLV[1]

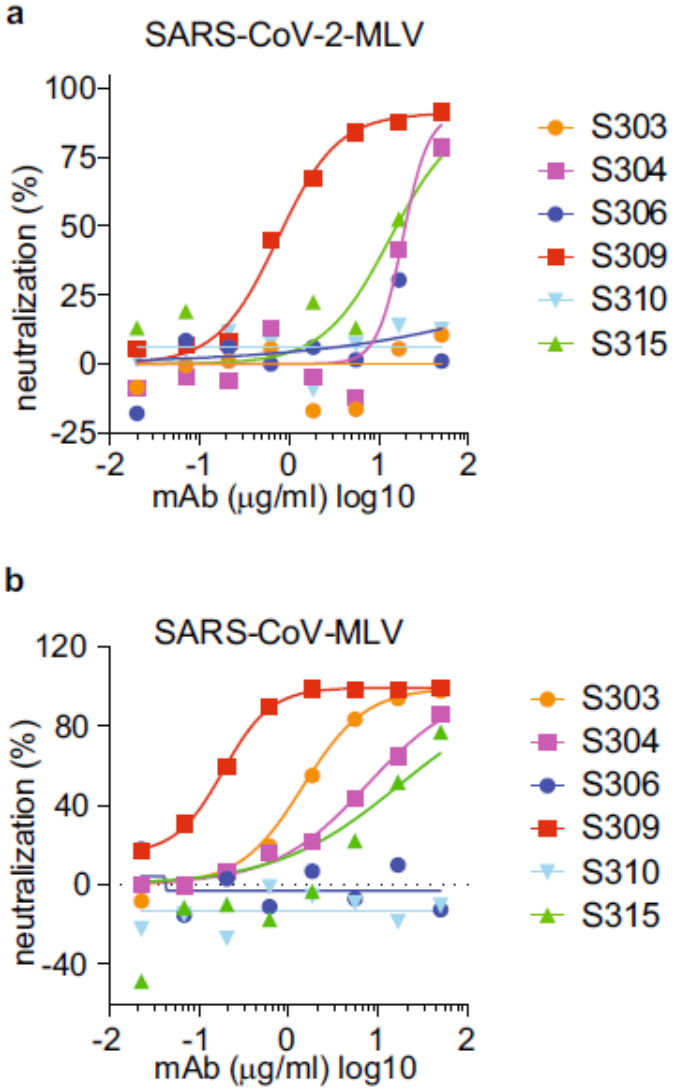


Image from Pinto et al., 2020

Structural basis of S309 cross-neutralization

- Characterized the Complex between:
  - **S309** Fab fragment[1]
  - Ectodomain trimer of SARS-CoV-2 **S glycoprotein**[1]
- Cryo-electron microscopy (Cryo-EM)
  - Structural molecular technique[2]

Image from Cryo-EM Facility, n.d

Cryo-EM structures of the complex

- A: **Partially open** state[1]
- B: **Closed** state[1]
- S309 recognizes an **epitope** on the SARS-CoV-2 S^{B} [1]
- **Stoichiometric binding** of Fab to the S glycoprotein trimer [1]

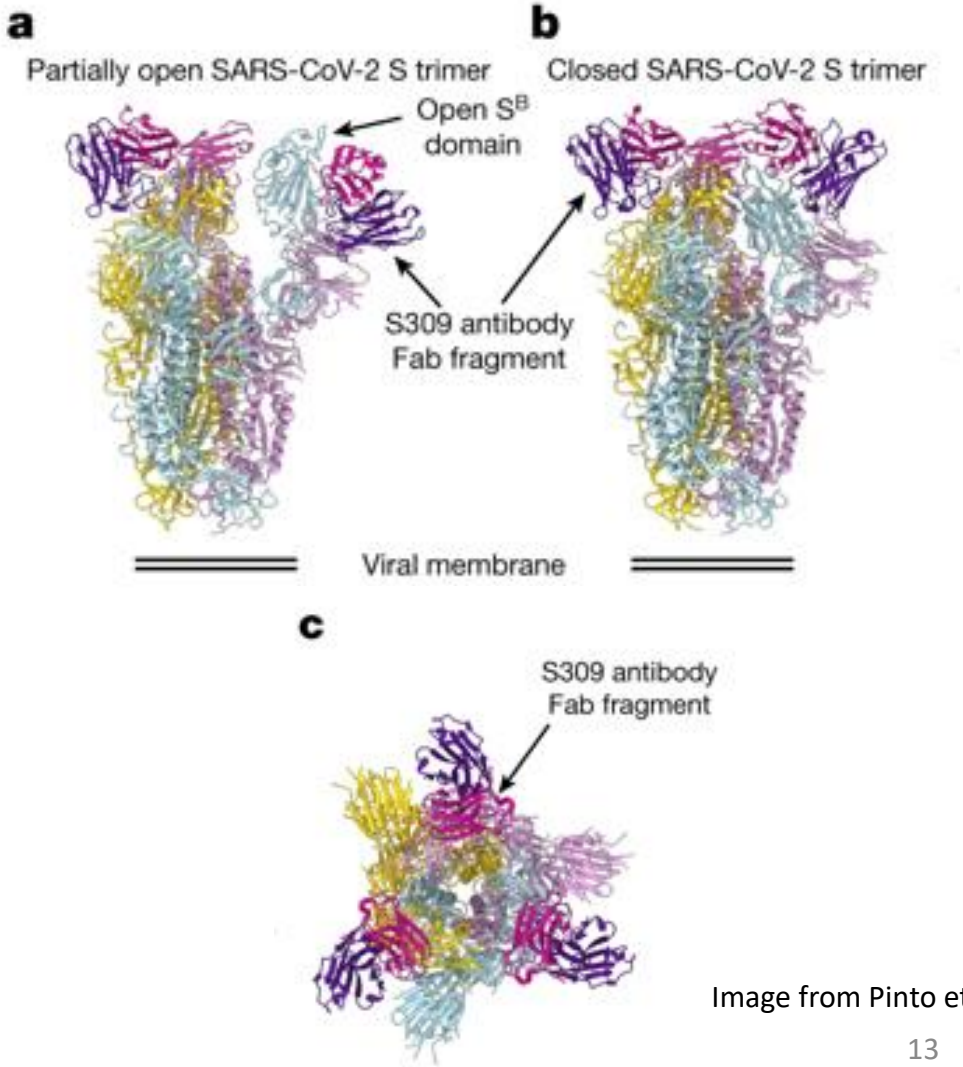


Image from Pinto et al., 2020

Close-up view of the S309 epitope

- **D:** Core fucose & glycan at N343[1]
  - Core fucosylation[3]
- **E:** CDRH3 sitting atop the S^B helix[1]
  - Antigen recognition and binding[4]
- Both shows **selected residues** involved in the **interactions**[1]

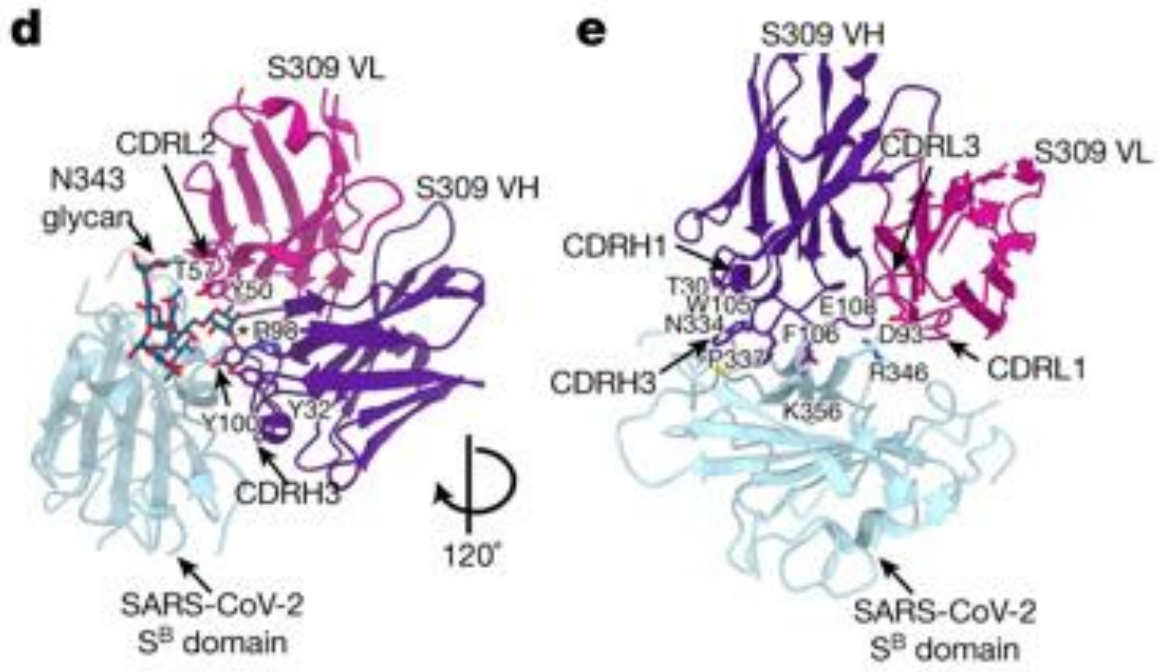


Image from Pinto et al., 2020

Highly conserved S309 epitope

- **17 out of 22** residues of the epitope are **strictly conserved**[1]
- Explained S309 cross-reactivity between SARS-CoV-2 & SARS-CoV[1]
- S309 could neutralize potentially all SARS-CoV-2 and many other zoonotic sarbecoviruses[1]

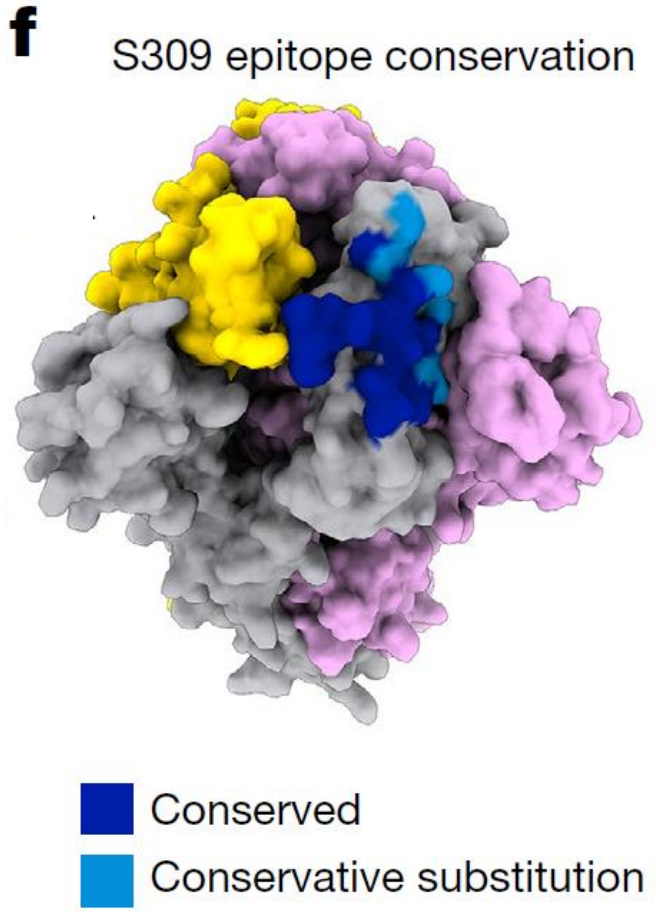


Image from Pinto et al., 2020

Mechanism of S309-mediated neutralization of SARS-CoV-2

- Cryo-EM structure of S309 bound to SARS-CoV-2 S glycoprotein, with SARS-CoV-2 S^B in complex with **ACE2**[1]
- **Fab** engages an epitope distinct from the receptor-binding motif[1]
- **Fab** would not clash with **ACE2** upon binding to S glycoprotein[1]

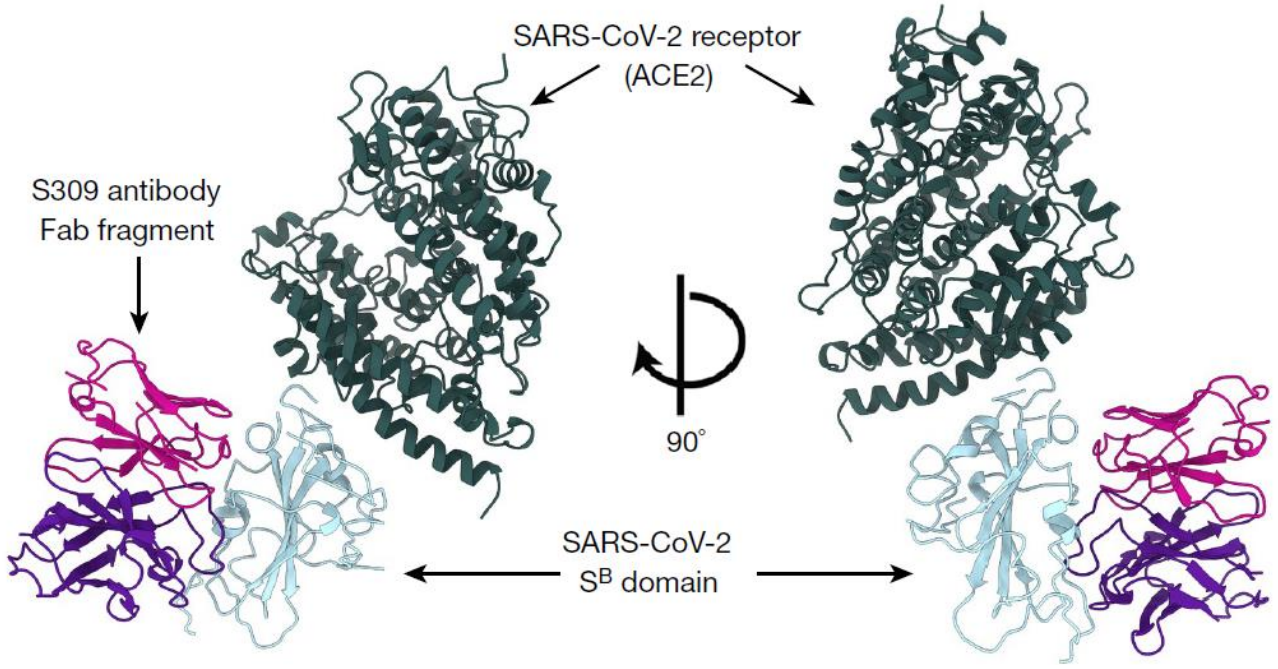
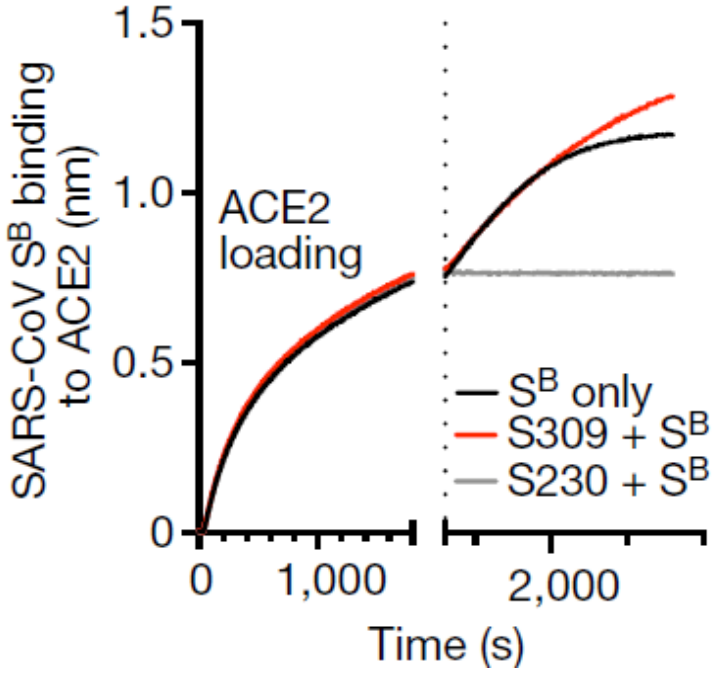


Image from Pinto et al., 2020

S309 engagement with SARS-CoV-2



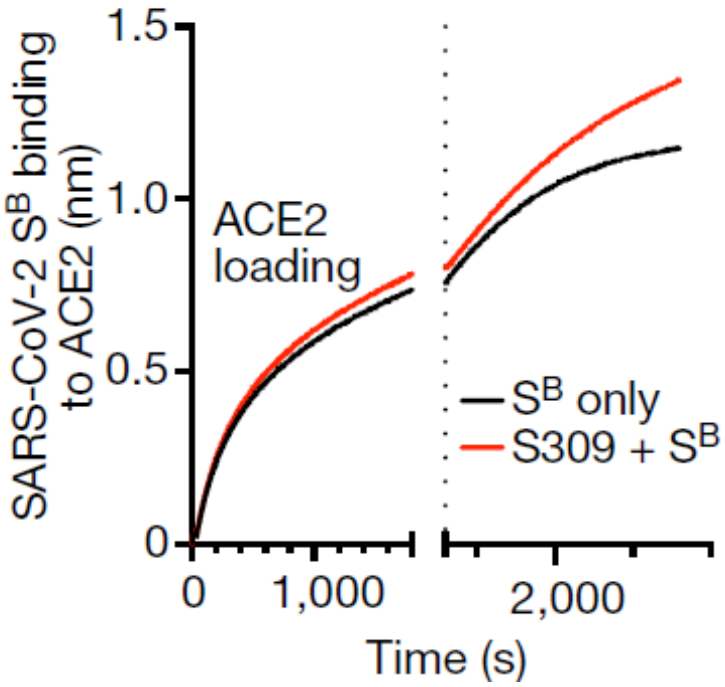


Image from Pinto et al., 2020

Results confirm the absence of competition between S309 and ACE2 for binding to the SARS-CoV-2 S glycoprotein[1].

S309-mediated neutralization

- Similar potencies for IgG and Fab[1]
- Other IgG-specific **bivalent mechanisms** may contribute to the ability of S309 to fully neutralize pseudovirions[1]:
  - **S-glycoprotein trimer cross-linking**
  - **Steric hindrance**
  - **Aggregation of virions**

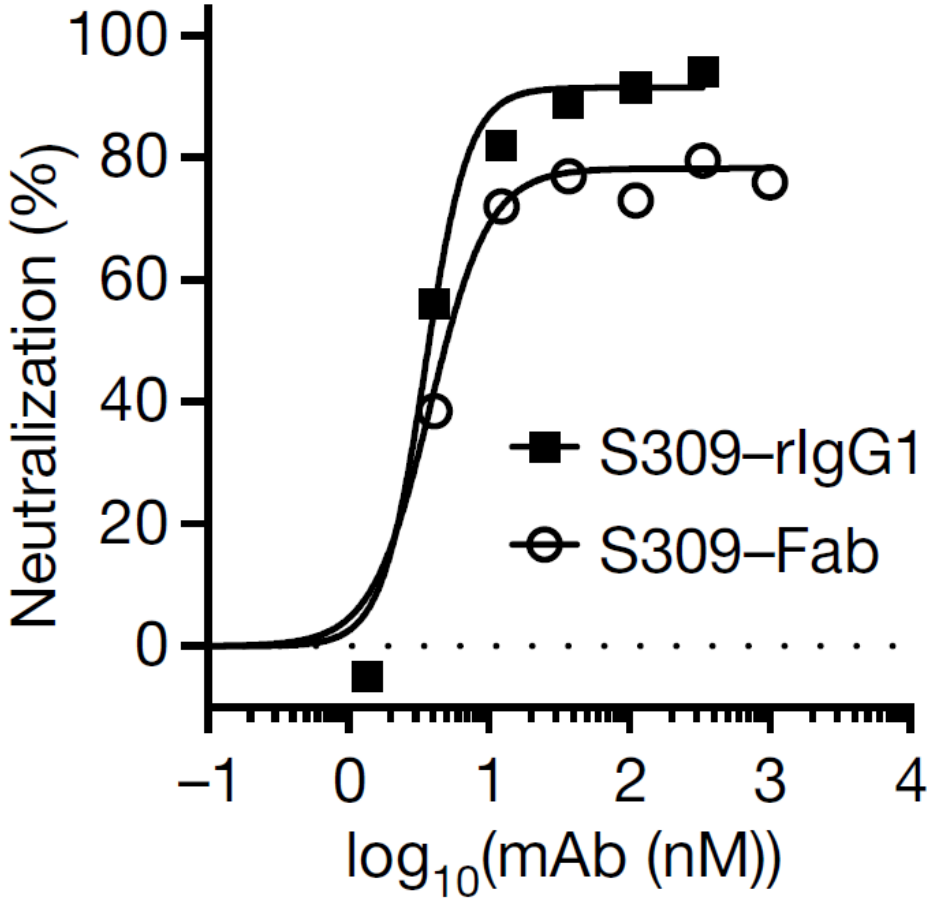


Image from Pinto et al., 2020

Fc-dependent effector mechanisms

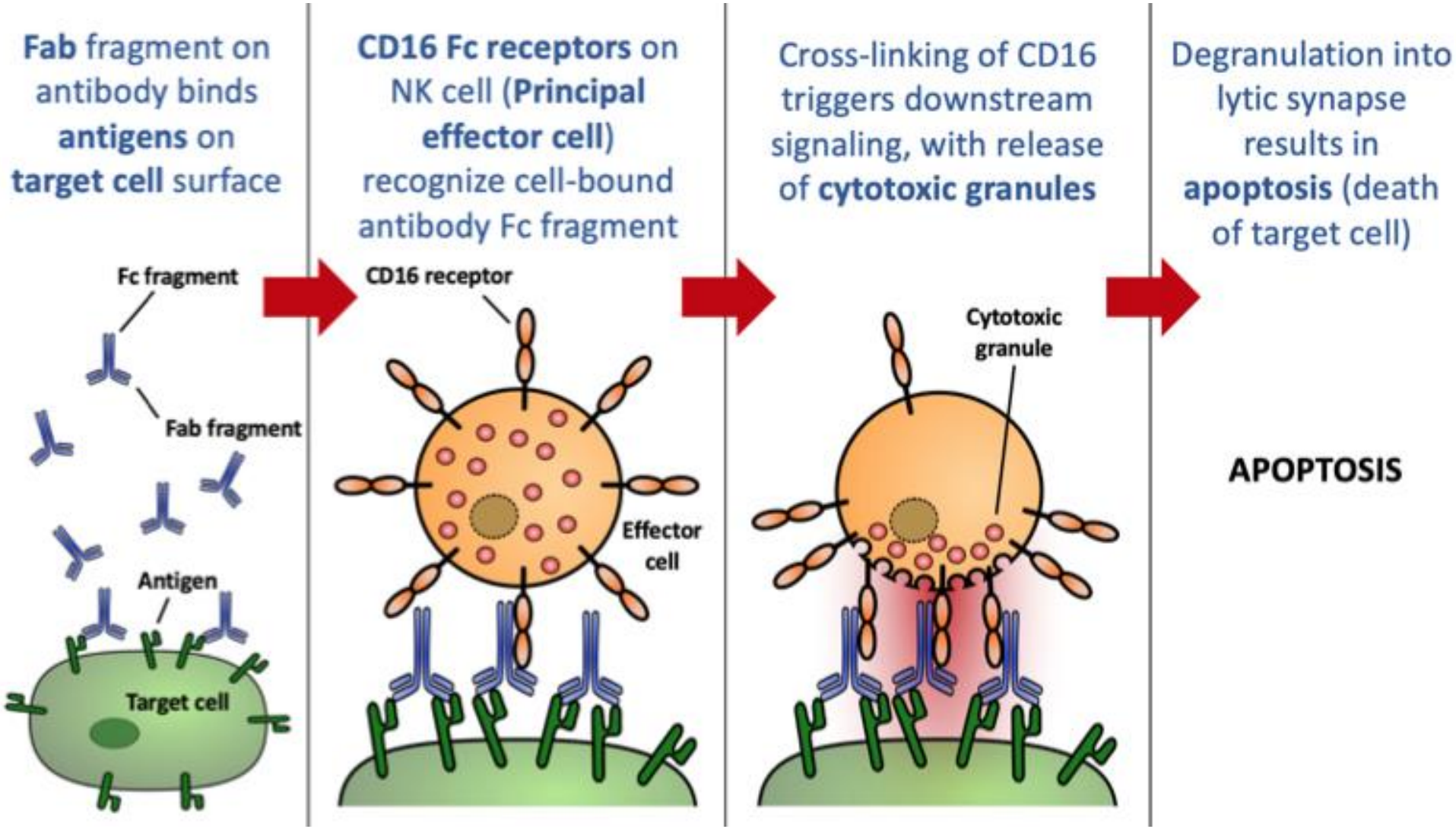


Image created by A. Nagi using BioRender

Antibody-dependent cellular cytotoxicity assay

- Efficient S309 and S306 mediated ADCC of SARS-CoV-2 S-glycoprotein-transfected cells[1]
- Other mAbs show limited or no activity[1]
- Distinct binding orientations or positioning of mAb Fc fragment might be key[1]

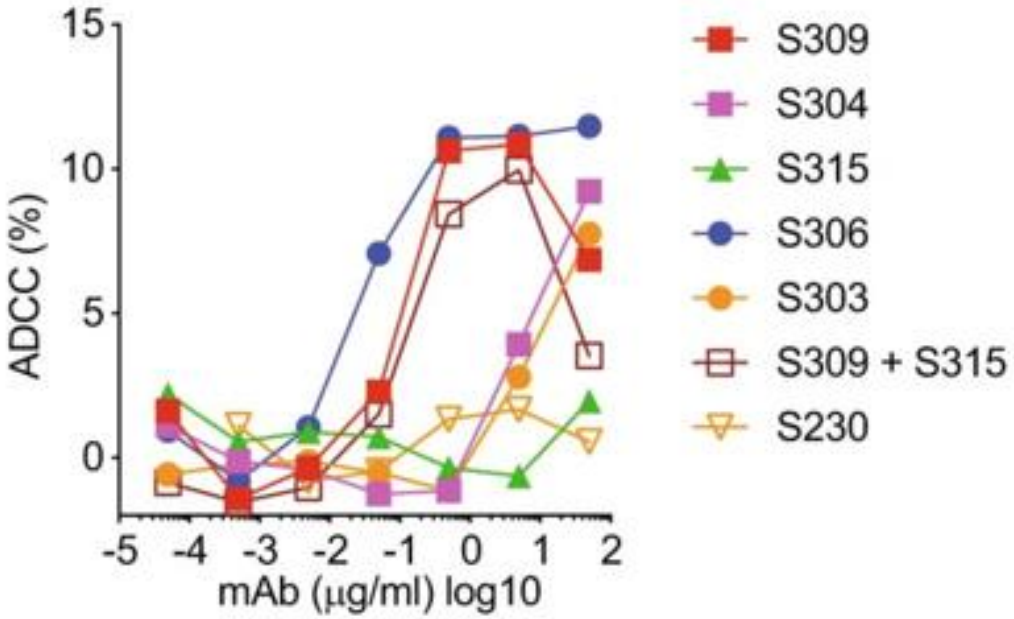


Image from Pinto et al., 2020

Further exploring Fc mediated effector function

Target cells transfected with fluorescent labels; phagocytosis determined by **flow cytometry**[1]**.**

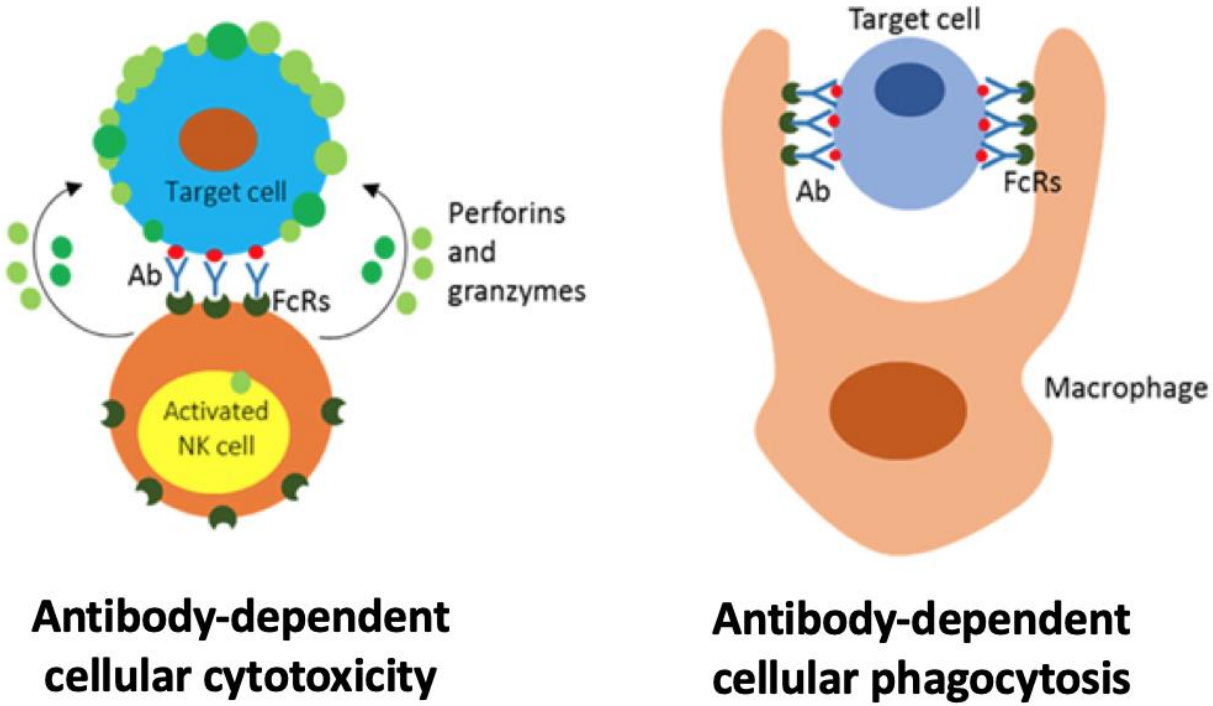


Image created by A. Nagi using BioRender, adapted from Weiner et al., 2010.

Antibody-dependent cellular phagocytosis assay

- mAbs **S309** and **S306** showed the strongest ADCP response[1]
- **S309** may leverage additional protective mechanisms in *vivo*[1]

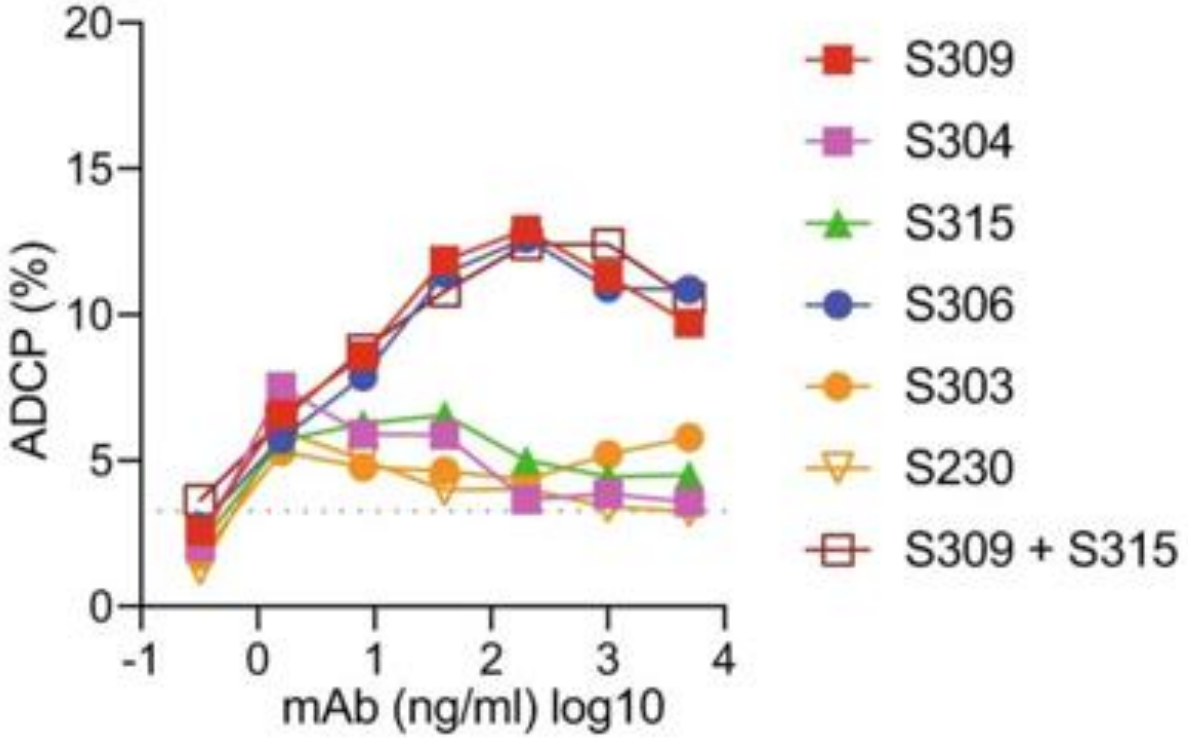
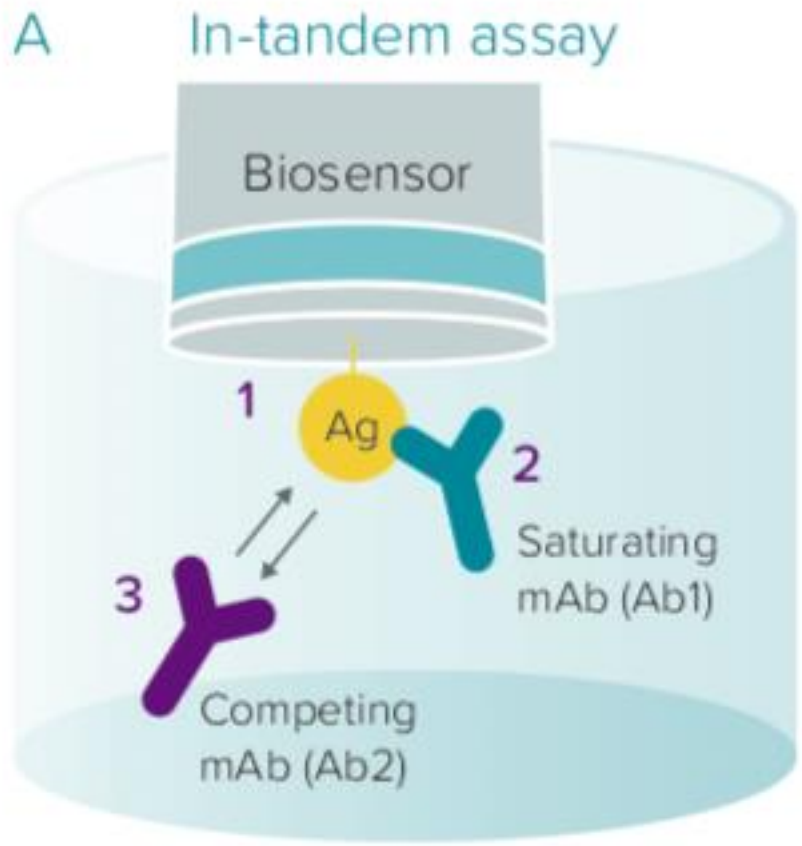
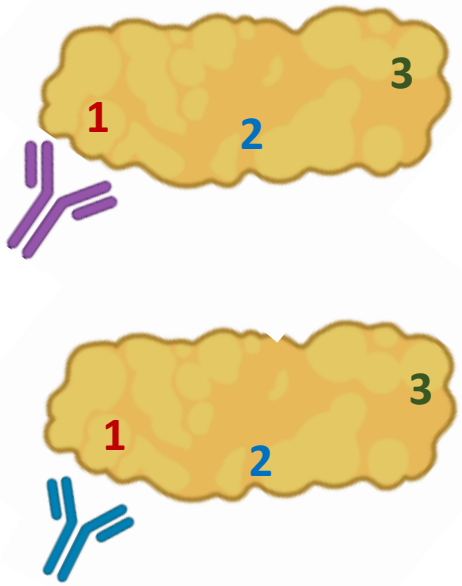


Image from Pinto et al., 2020

BLI-based epitope binning



Competing mAbs;
both placed in 1 bin



Non-competing mAbs;
each in its own bin

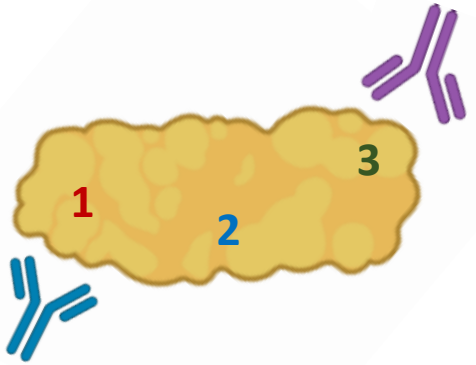


Image from Takkar et al, 2020

Adapted from Takkar et al, 2020 using BioRender

BLI-based competition of mAbs; SARS-CoV

- **4 distinct epitopes** of the S^B domain of SARS-CoV[1]

- I: S110, S230 & S227[1]
- II: S315[1]
- III: S124[1]
- IV: S309, S109, S303[1]

Bridged together by S124

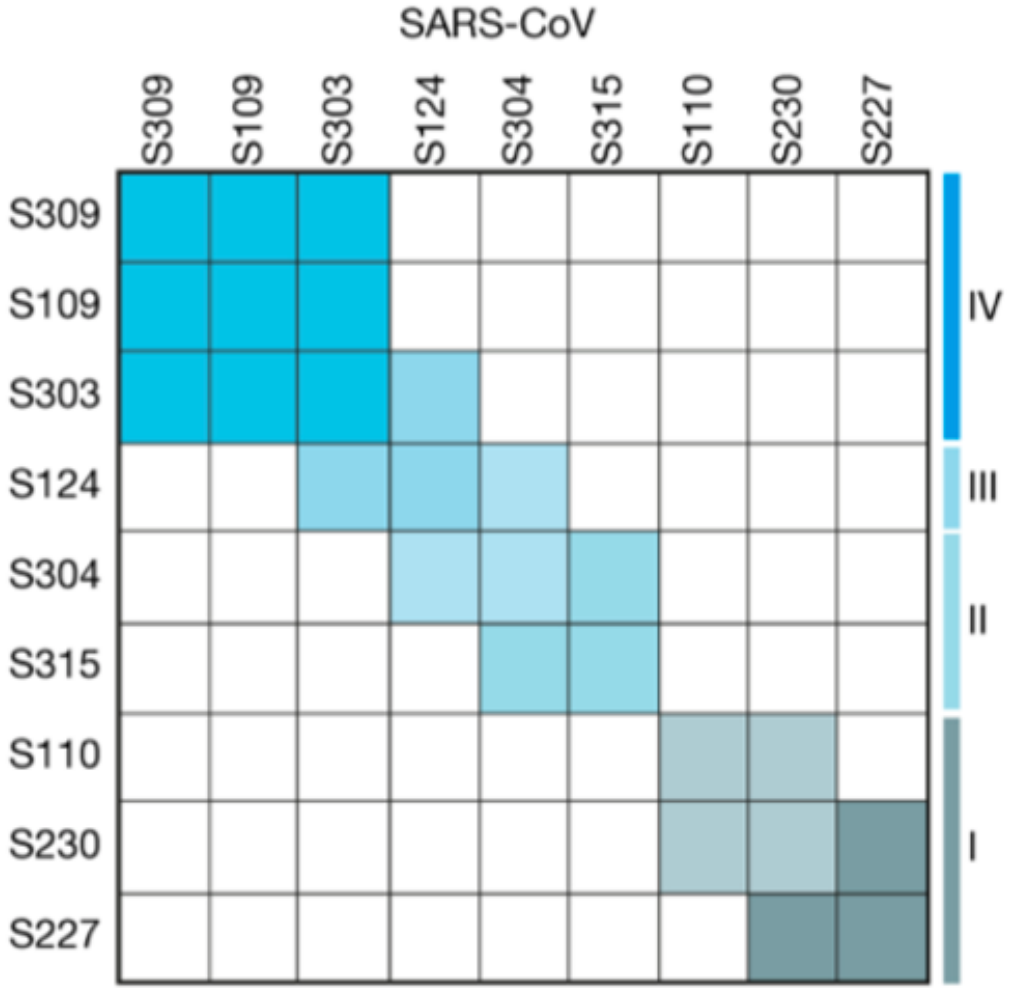


Image from Pinto et al., 2020

BLI-based competition of mAbs; SARS-CoV-2

- **Cross neutralizing mAbs**; bind to S^B domain of SARS-CoV and SARS-CoV-2[1]

- **IV**: S309 & S303[1]
- **II & III**: S304 & S315[1]

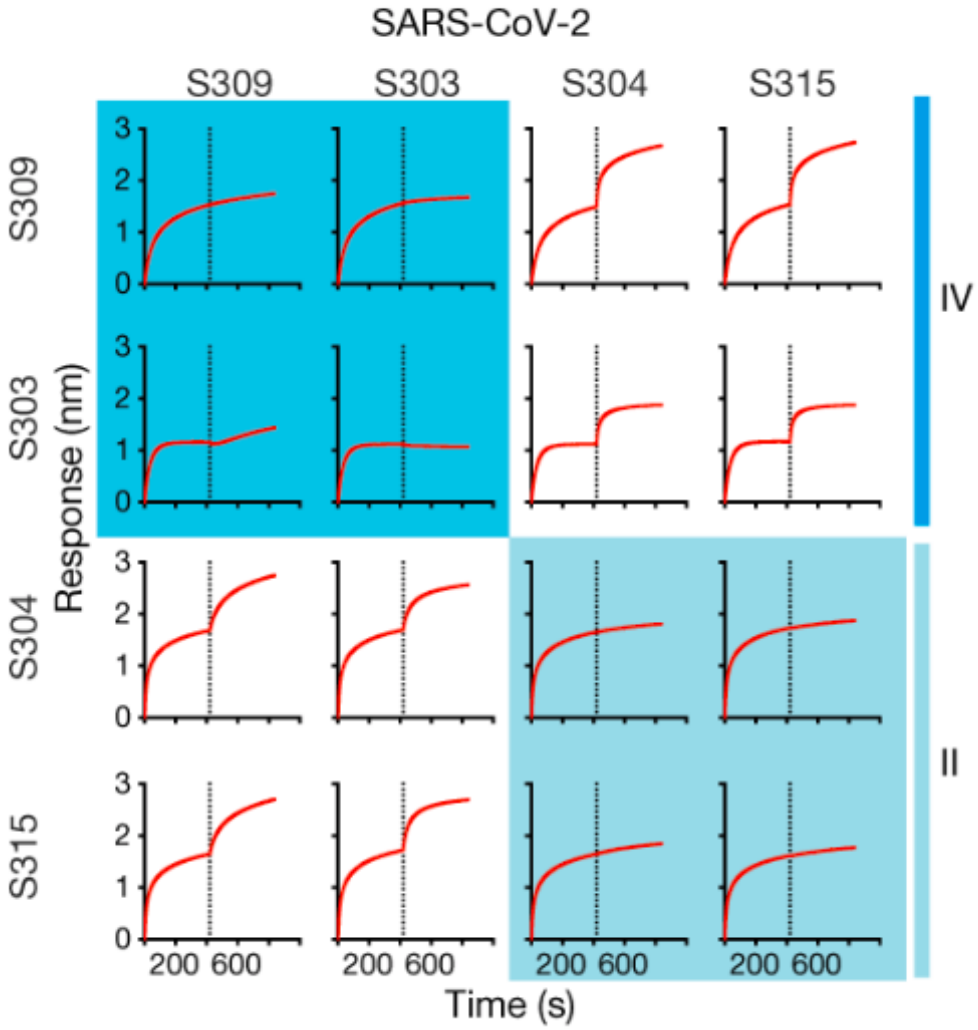


Image from Pinto et al., 2020

mAb cocktails enhance SARS-CoV-2 neutralization

- **pseudovirus neutralization assays[1]**

- **S309**: high neutralization potency[1]
- **S315**: weak neutralization potency[1]
- **S309 & S315**: strongest neutralization potency[1]

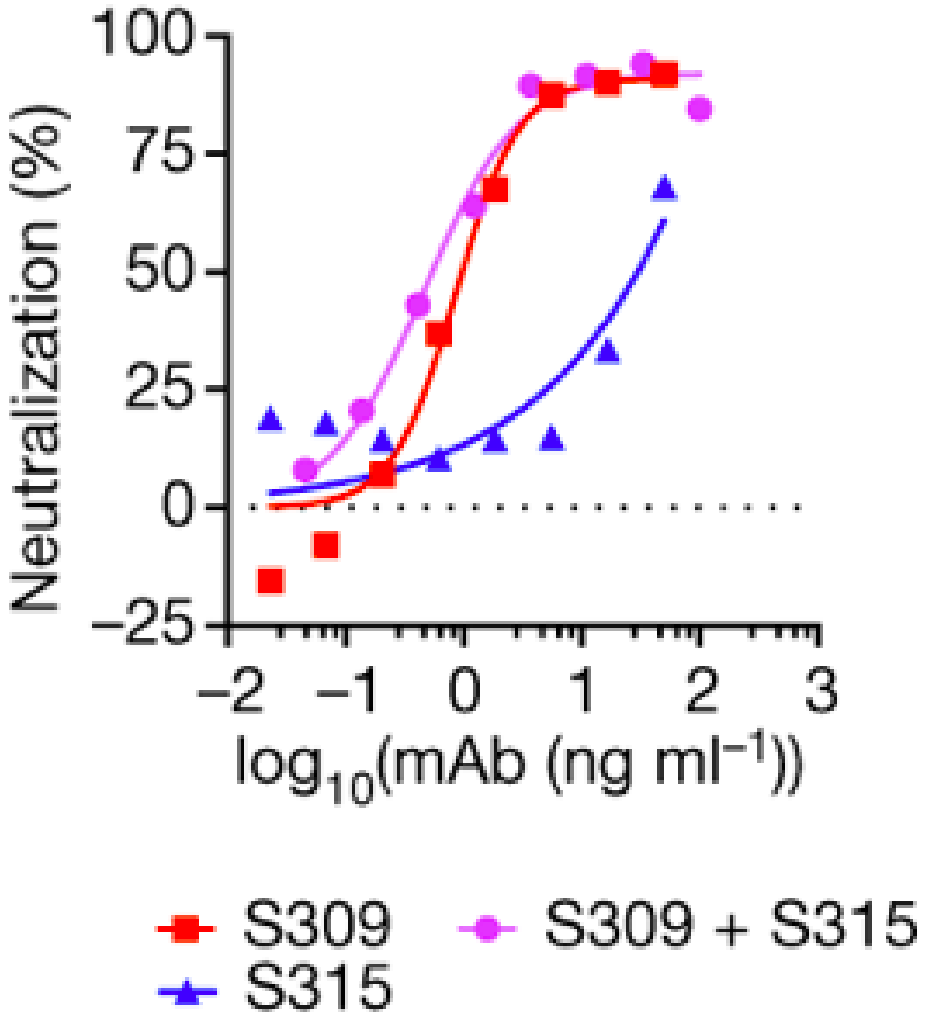


Image from Pinto et al., 2020

Critical Appraisals

What the study did well:

- Simple and effective study design
- Showed cross-neutralization ability of S309

Criticisms:

- Could have used an *in vivo* model to test S309
- More focus on IgG-specific bivalent mechanisms

Future Implications

U.S.: Regeneron Pharmaceuticals

Image from Industry Week, 2020

Canada: Eli Lilly and AbCellera Biologics

Image from NPR, 2020

Conclusion

- **S309 Properties**
  - Ability to neutralize S glycoprotein of SARS-CoV-2
  - Shows broad neutralization activity across multiple sarbecoviruses
  - Can recruit effector mechanism such as ADCC and ADCP
  - Shows increased neutralization in combination with weak neutralizing mAbs

- VIR-7831 & VIR-7832 are S309 based mAbs in clinical trials!

References

1. Pinto, D., Park, Y., Beltramello, M., Walls, A. C., Tortorici, M. A., Bianchi, S., . . . Corti, D. (2020). Cross-neutralization of sars-cov-2 by a human monoclonal SARS-CoV antibody. Nature, 583(7815), 290-295. doi:10.1038/s41586-020-2349-y
2. Carroni, M., & Saibil, H. R. (2016). Cryo electron microscopy to determine the structure of macromolecular complexes. Methods, 95, 78–85. https://doi.org/10.1016/j.ymeth.2015.11.023
3. Fernández-Quintero, M. L., Kraml, J., Georges, G., & Liedl, K. R. (2019). CDR-H3 loop ensemble in solution–conformational selection upon antibody binding. MAbs, 11(6), 1077–1088. https://doi.org/10.1080/19420862.2019.1618676
4. Hwang, H., Jeong, H. K., Lee, H. K., Park, G. W., Lee, J. Y., Lee, S. Y., Kang, Y. M., An, H. J., Kang, J. G., Ko, J. H., Kim, J. Y., & Yoo, J. S. (2020). Machine Learning Classifies Core and Outer Fucosylation of N-Glycoproteins Using Mass Spectrometry. Scientific Reports, 10(1), 1–10. https://doi.org/10.1038/s41598-019-57274-1

Image References

Vir Biotechnology. (2021). *A new era.* VIR. Retrieved February 24, 2021, from https://www.vir.bio/pipeline/

2Bind molecular interactions. (2020, November 19). Biolayer Interferometry: Label-free kinetics without fluidics. Retrieved February 17, 2021, from https://2bind.com/bli/

Berthold. (2020, December 09). Pseudovirus neutralization assays in SARS-CoV-2 research - Berthold Technologies. Retrieved February 17, 2021, from https://www.berthold.com/en/bioanalytic/solutions-sars-cov-2-covid-19-research/pseudovirus-neutralization-assays-in-sars-cov-2-research/

Cryo-EM Facility. (n.d.). Retrieved February 28, 2021, from https://www.utep.edu/science/chemistry/Facilities/cryo-em-facility.html

Takkar, R. et al. (2020). Cross-competition or epitope binning assays on the Octet HTX system. *ForteBio,* 1-20, https://www.fortebio.com/sites/default/files/en/assets/app-note/cross-competition-or-epitope-binning-assays-on-octet-htx-system.pdf

Presse-France, Agence (Nov. 23, 2020). US Approves Regeneron Antibody Treatment Given to Trump. *IndustryWeek*. https://www.industryweek.com/covid19/article/21148471/us-approves-regeneron-antibody-treatment-given-to-trump

Harris, Richard (Nov. 10, 2020). FDA OKs Eli Lilly COVID-19 Drug, But Supplies Will Be Limited. NPR. https://www.npr.org/sections/health-shots/2020/11/10/933444237/fda-oks-eli-lilly-covid-19-drug-but-supplies-will-be-limited

Weiner, L. M., Surana, R., & Wang, S. (2010). Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nature Reviews Immunology, 10(5), 317-327.