

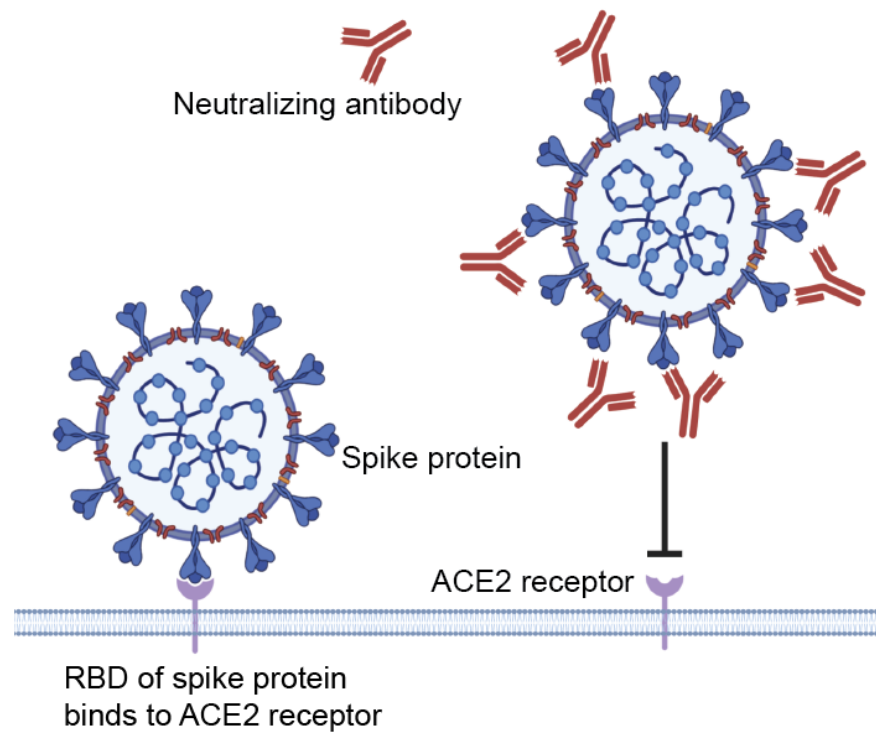
Highly potent pan-sarbecovirus neutralizing antibodies induced by serial cross-clade immunization

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Programme in Emerging Infectious Diseases

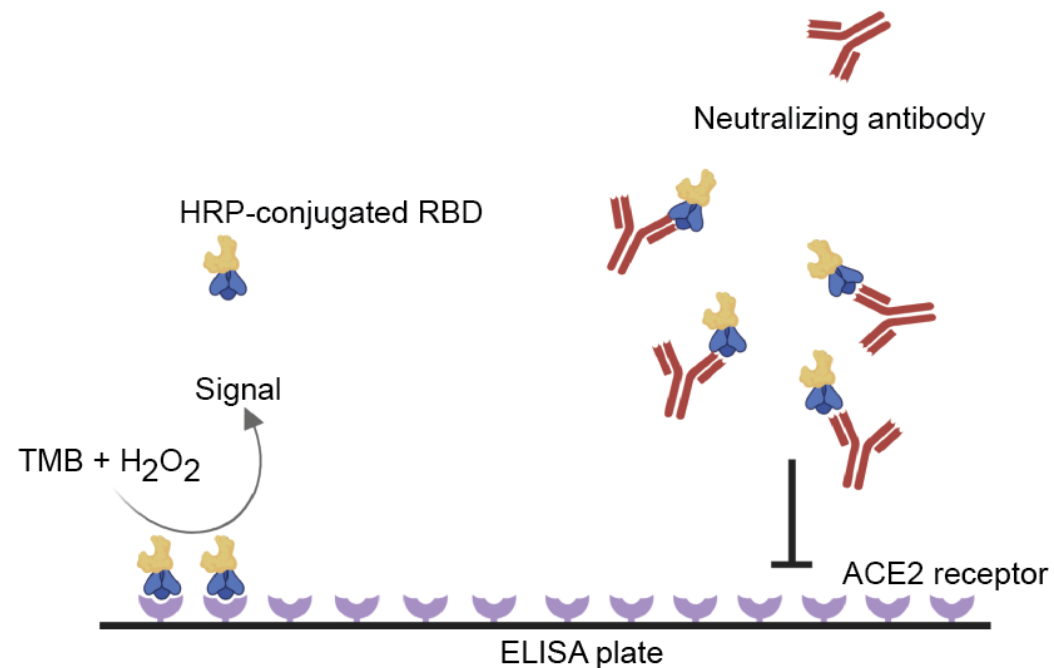
- **The assay platform:** multiplex surrogate virus neutralization test (sVNT)
- **The cohort:** SARS survivors in Singapore who received the BNT162b2 vaccine
- **The finding:** strong pan-sarbecovirus NAbs against all ACE2-binding sarbecoviruses tested
- **The mechanism:** synergy of multiple neutralizing epitopes vs immunodominant cross-clade neutralizing epitopes
- **The implication:** serial cross-clade immunization is a promising/powerful approach to induce pan-sarbecovirus NAbs

The principle of sVNT

a Virus Neutralization Test (VNT)

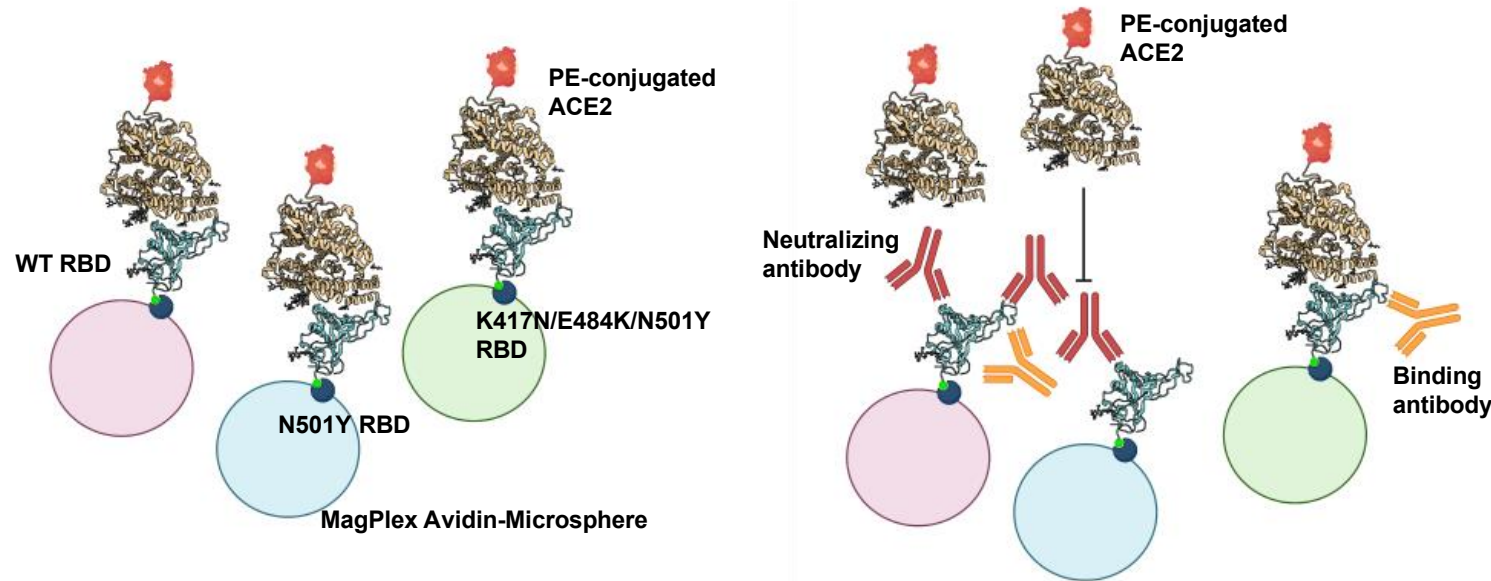


b surrogate Virus Neutralization Test (sVNT)



Tan et al. Nat Biotech (2020)

Multiplex sVNT

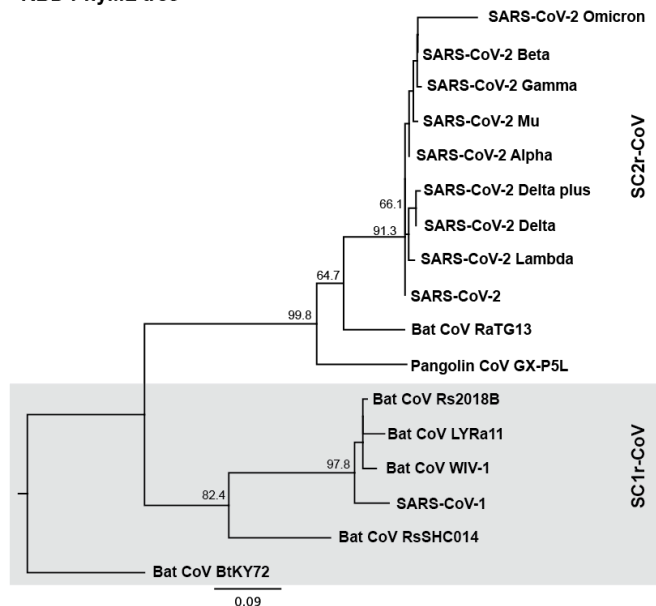


- Reversing the liquid-solid phase configuration: RBD on beads and PE-ACE2 in liquid
- Use of biotinylated RBD to achieve uniform coating in a multiplex system
- Presence of equimolar RBDs creates “in-tube competition”



ACE2-binding sarbecovirus RBDs (16 plex sVNT)

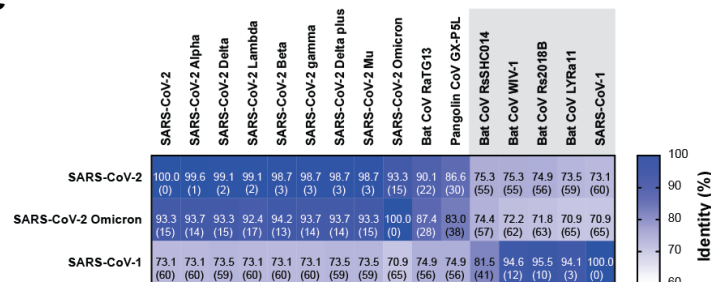
RBD PhyML tree



RBD alignment

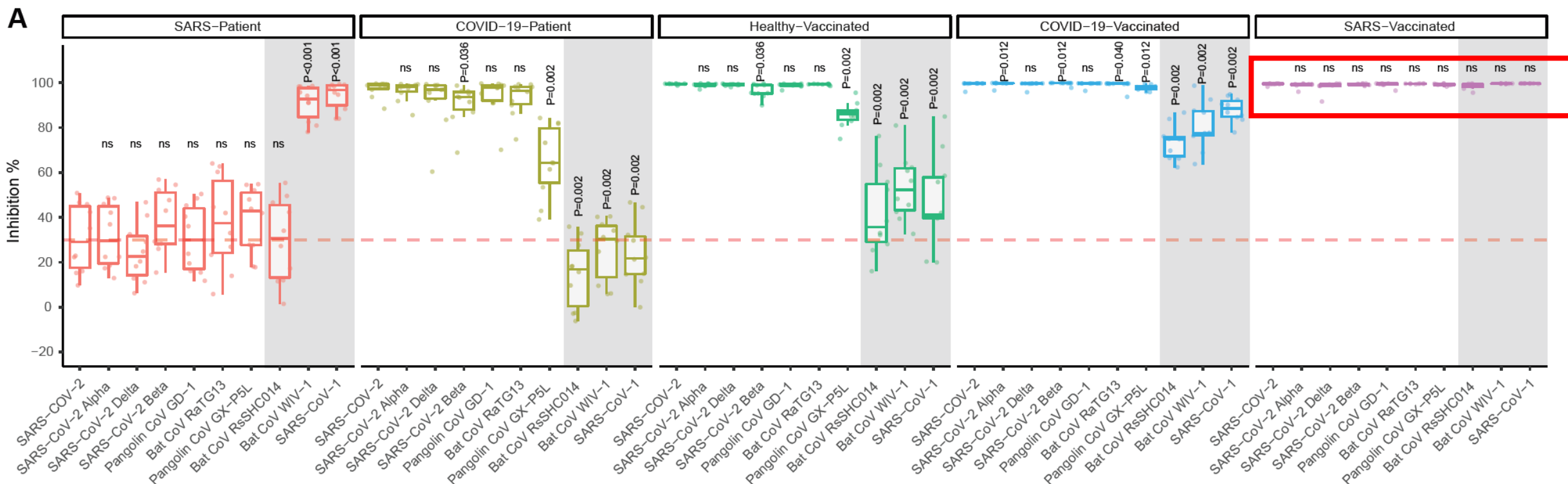
SARS-CoV-2	RVQPTESIVRFPNITNLCPPGEVFNATRFASVYAWNKRKISNCVADYSVLNYSASFSTFKCYGVSPTKLNLCFTNVIAD	80
SARS-CoV-2 Alpha	80
SARS-CoV-2 Delta	80
SARS-CoV-2 Beta	80
SARS-CoV-2 Gamma	80
SARS-CoV-2 Delta plus	80
SARS-CoV-2 Lambda	80
SARS-CoV-2 Mu	80
SARS-CoV-2 OmicronD.....L.P.F.....	80
Bat CoV RaTG13D.....T.....	80
Pangolin CoV GX-P5LI.....SK.....T.....	80
Bat CoV Rs2018BA.SKEV.....T.P.....E.....T.....A.....S.....	80
Bat CoV LYRa11S.SKEV.....T.P.....E.....T.....AI.....S.....	80
Bat CoV RsSHC014A.SKEV.....T.P.....E.....I.....T.....A.....S.....	80
Bat CoV WIV-1A.SKEV.....T.P.....E.....T.....A.....S.....	80
SARS-CoV-1V.SGDV.....K.P.....E.K.....TF.....A.....	80
SARS-CoV-2	SFVIRGDEVRCQIAPGQTGKIADYNYKLPPDFTGCVIAWNSNNLDSKVGNGNYLYRLFRKSNLKPFFERDISTEIYQAGST	160
SARS-CoV-2 Alpha	160
SARS-CoV-2 DeltaR.....K.....	160
SARS-CoV-2 BetaN.....	160
SARS-CoV-2 GammaT.....	160
SARS-CoV-2 Delta plusN.....R.....K.....	160
SARS-CoV-2 LambdaR.....	160
SARS-CoV-2 Mu	160
SARS-CoV-2 OmicronN.....K.....S.....NK.....	160
Bat CoV RaTG13T.....KHI.A.E.F.....A.....K.....	160
Pangolin CoV GX-P5LVK.....V.....VKQ.ALT.....G.....K.....	160
Bat CoV Rs2018BVK.D.....V.....M.....L.....TR.I.ATST.....K.SL.HGK.R.....NVFPSPDGK	160
Bat CoV LYRa11VK.D.....V.....M.....L.....TR.I.ATSS.....F.K.SL.HGK.R.....NVFPSPDGK	160
Bat CoV RsSHC014VK.D.....V.....L.....L.....T.SK..STS.....WV.R.K.N.Y.....L.ND..SP.GQ	160
Bat CoV WIV-1VK.D.....V.....L.....TR.I.ATQT.....K.SL.HGK.R.....NVFPSPDGK	160
SARS-CoV-1VK.D.....V.....M.....L.....TR.I.ATST.....K.YL.HGK.R.....NVFPSPDGK	160
SARS-CoV-2	PCNGVEGFNCYFPLQSYGFQPTNGVGYPYRVVLSFELLHAPATVCGPKKSTNLVRNKCYNF	223
SARS-CoV-2 AlphaY.....	223
SARS-CoV-2 Delta	223
SARS-CoV-2 BetaK.....Y.....	223
SARS-CoV-2 GammaK.....Y.....	223
SARS-CoV-2 Delta plus	223
SARS-CoV-2 LambdaS.....	223
SARS-CoV-2 MuK.....R.....S.R.....Y.....H.....	223
SARS-CoV-2 OmicronA.....R.....S.R.....Y.....H.....	223
Bat CoV RaTG13QT.L.....Y.YR.....Y.D.....H.....N.....	223
Pangolin CoV GX-P5LQV.L.....Y.ER.....H.T.N.....F.....NG.....L.T.....D.....	223
Bat CoV Rs2018BTP-PA.....W.ND.....FT.....I.....N.....L.D.I.....Q.....	222
Bat CoV LYRa11TP-PA.....W.ND.....YT.....I.....N.....L.D.IT.Q.....	222
Bat CoV RsSHC014S.SA-V.P.....N.RP.....FT.A.....H.....N.....L.D.I.....Q.....	222
Bat CoV WIV-1TP-PA.....W.ND.....YI.....I.....N.....L.D.I.....Q.....	222
SARS-CoV-1TP-PAL.....W.ND.....YT.T.I.....N.....L.D.I.....Q.....	222

C



- $N = 8$
- Recovered from SARS-CoV-1 in 2003
- Some still have good NAbs against SARS-CoV-1, but no cross-NAbs against SARS-CoV-2
- Received 1 or 2 doses of BNT162b2 mRNA vaccine
- Blood samples were taken 21-62 days after the first dose vaccination

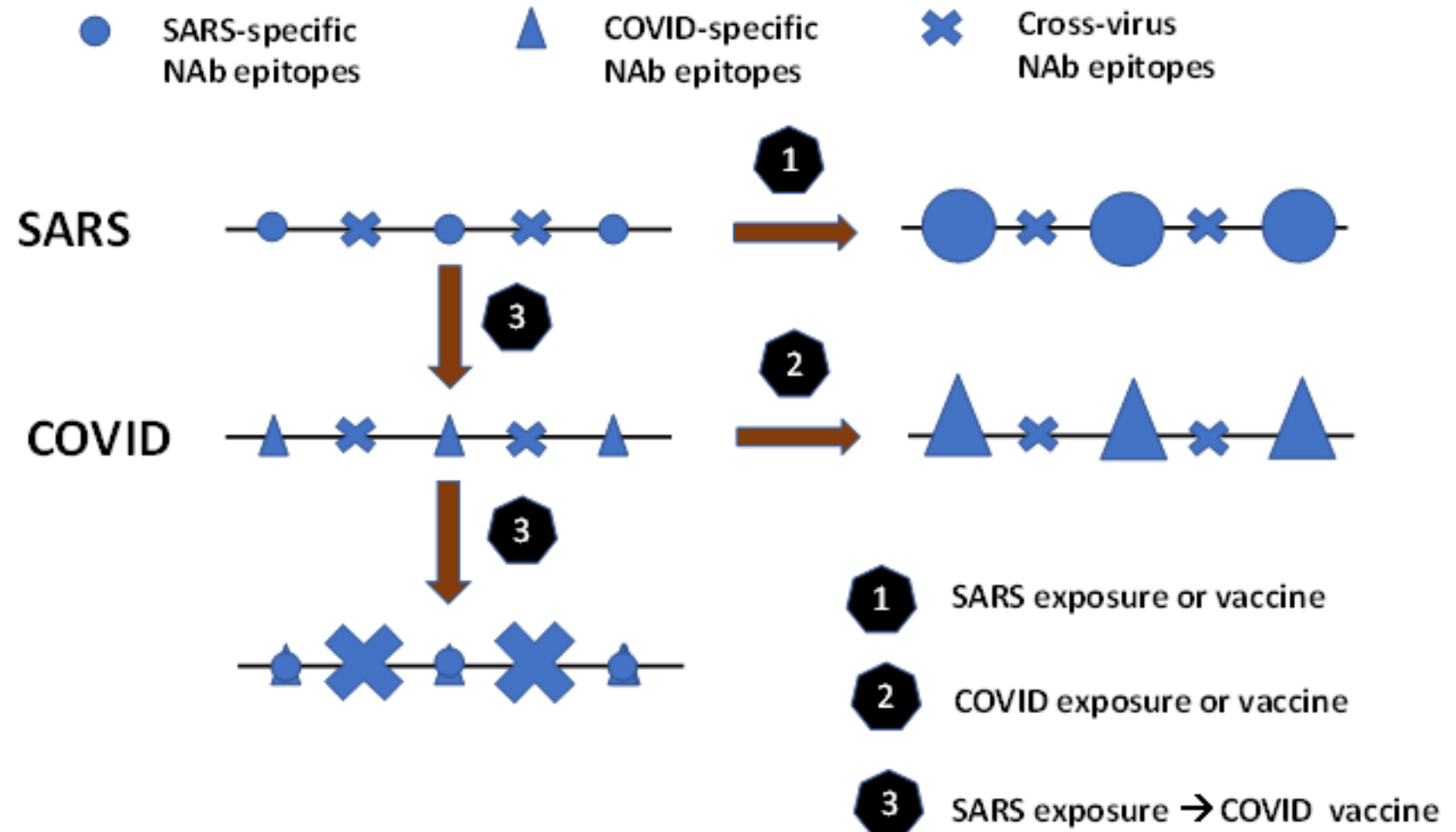
NAbs against 10 sarbecoviruses



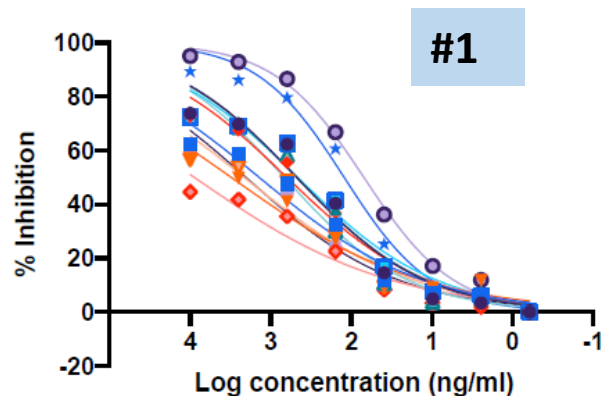
Tan et al. NEJM (2021)



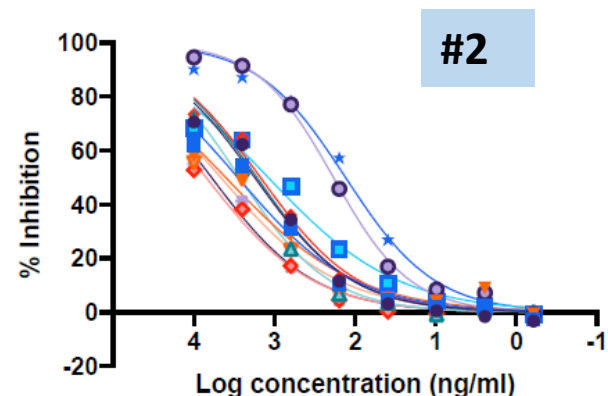
Proposed mechanism



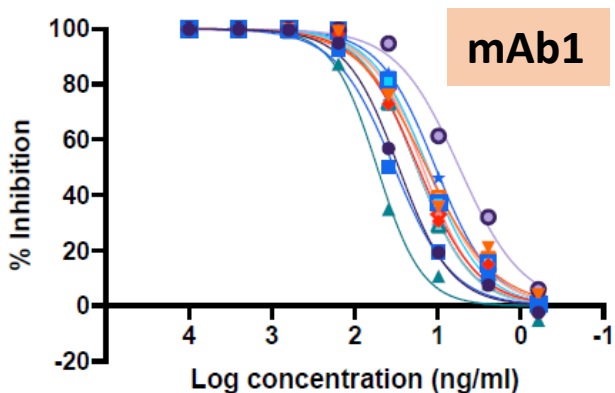
Comparison of pan-sarbecovirus NAb (mAb)



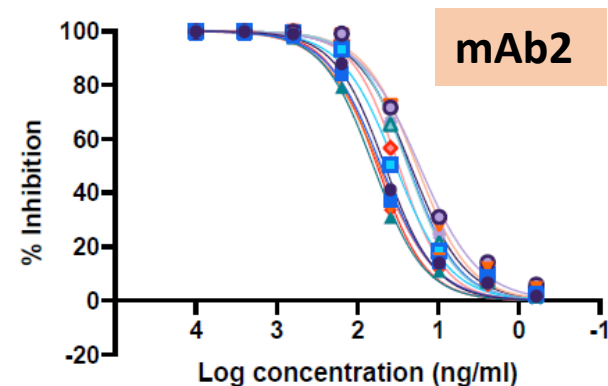
- SARS-CoV-2
- Alpha
- ▲ Delta
- ▼ Beta
- ◆ Gamma
- RaTG13
- GX-P5L
- ▲ Rs2018B
- ▼ LYRa11
- ◆ RsSHC014
- WIV1
- ★ SARS-CoV-1



- SARS-CoV-2
- Alpha
- ▲ Delta
- ▼ Beta
- ◆ Gamma
- RaTG13
- GX-P5L
- ▲ Rs2018B
- ▼ LYRa11
- ◆ RsSHC014
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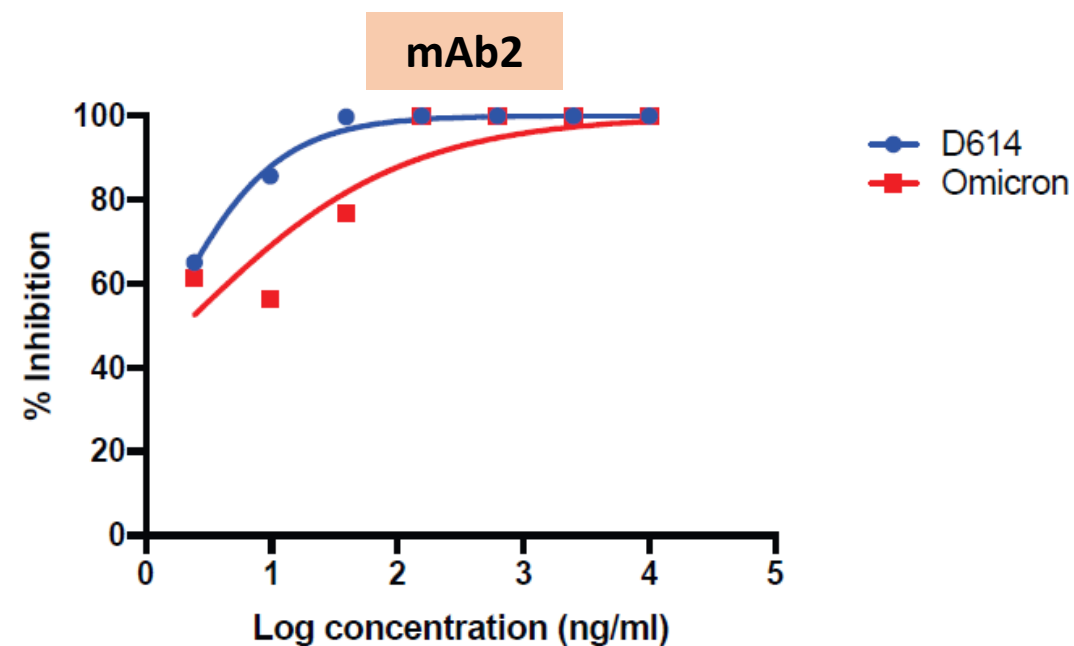
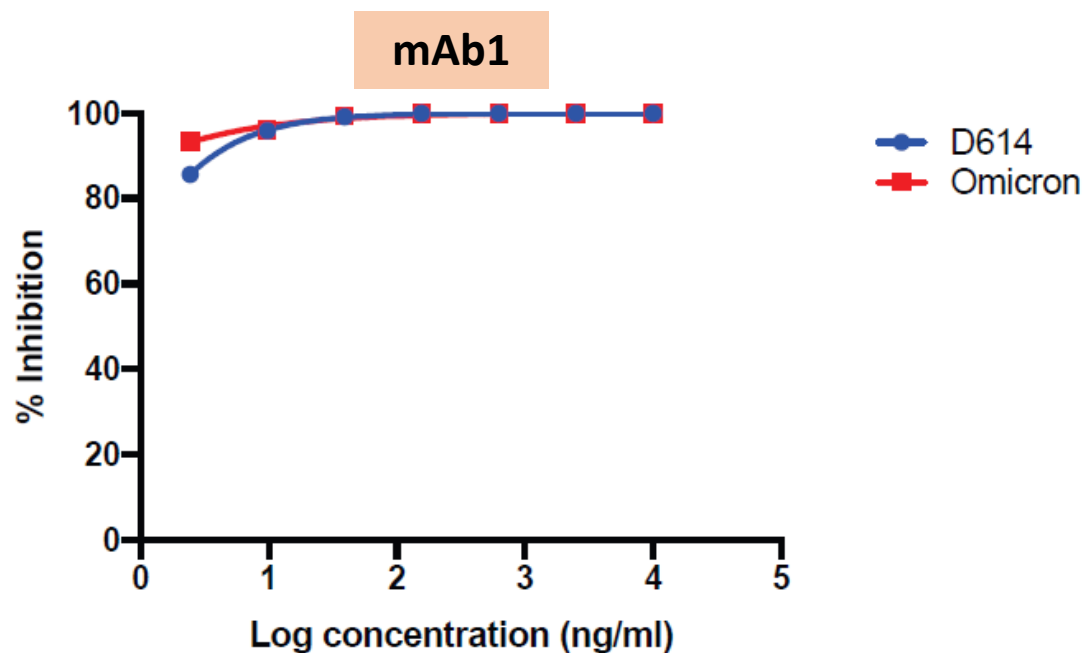


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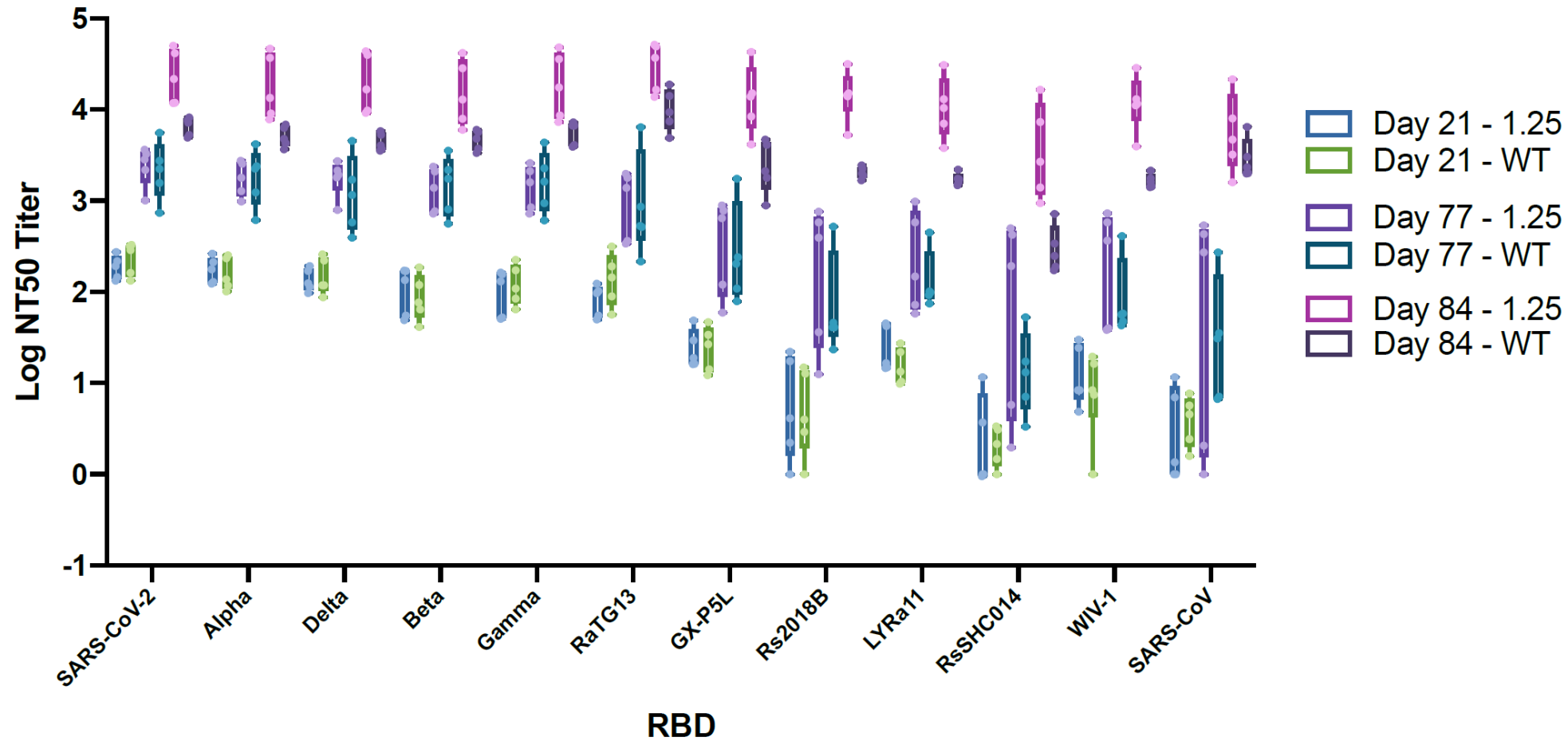


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- ▼ LYRa11
- ◆ RsSHC014
- WIV1
- ★ SARS-CoV-1

Highly potent against Omicron



- Designed a “consensus” Spike protein from all known Clade-1 SARS-like CoVs (mainly from bats)
- Produced in-house trimeric Spike with PP mutations
- Immunized mice with approved human vaccines in Singapore (BNT162b2, mRNA-1273, CoronaVac) using pre-determined doses by the vaccine producers
- Boosted with two versions of Spike proteins (in experimental adjuvant):
C1.25 = the consensus; WT = the human SARS-CoV-1 Spike
- All groups produced pan-sarbecovirus NAbs



Acknowledgments

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