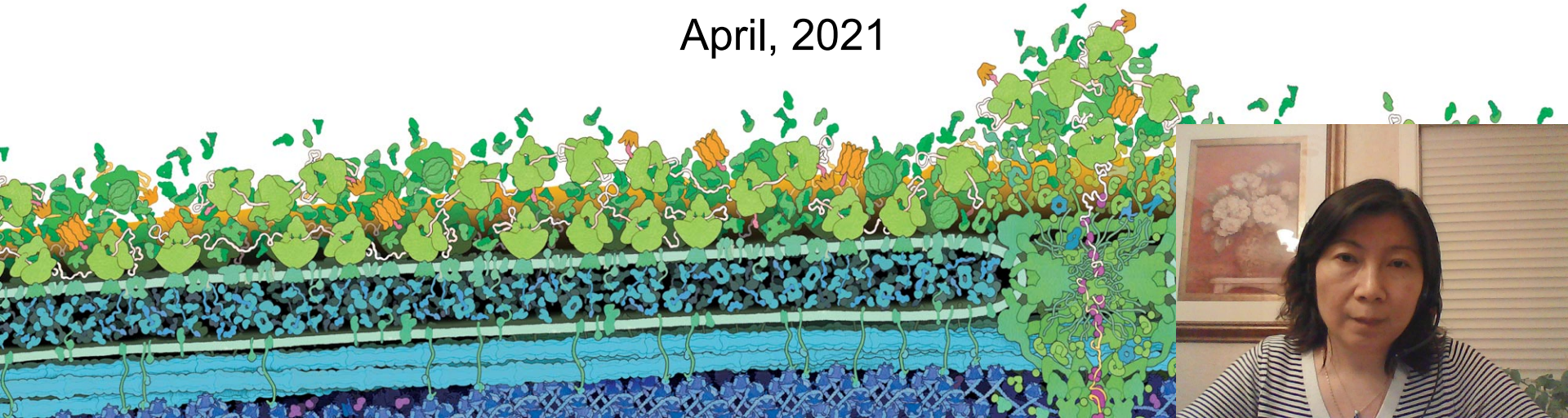
SARS-CoV-2 Bioassays

Jey Cheng, Ph.D

April, 2021

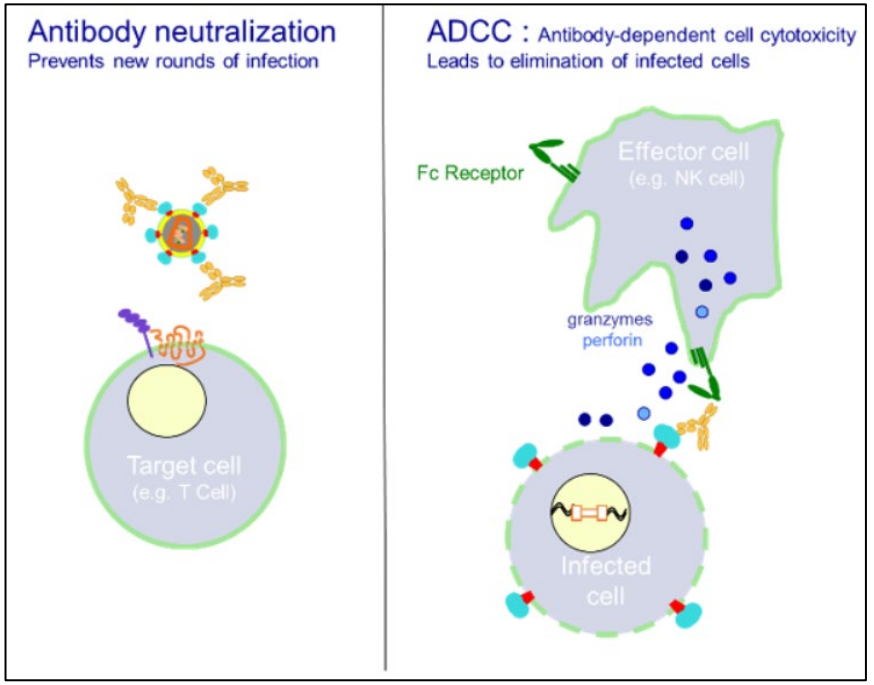


Background

Antibodies produced from viral vaccines have been reported to provide protection against viral infection through two major mechanisms.

1. Neutralizing antibodies prevent new rounds of infection by preventing viral binding and entry of host cells.

2. Antibodies can mediate ADCC or ADCP that results in the clearance of infected cells by immune effector cells.



Fred Hutchinson Cancer Research Center

Dual MoA for Many SARS-CoV-2 Antibodies

The Fc-mediated effector functions of a potent SARS-CoV-2 neutralizing antibody, SC31, isolated from an early convalescent COVID-19 patient, are essential for the optimal therapeutic efficacy of the antibody

Conrad E.Z. Chan[1]*, Shirley G.K. Seah[1], De Hoe Chye[1], Shane Massey[2], Maricela Torres[2], Angeline P.C. Lim[1], Ste
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MTX-COVAB, a human-derived antibody with potent neutralizing activity against SARS-CoV-2 infection *in vitro* and in a hamster model of COVID-19

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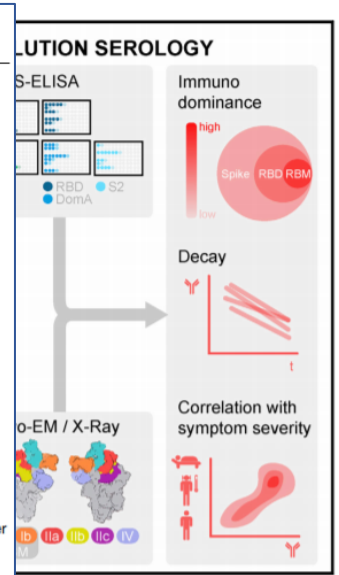
Applied Microbiology, Helmholtz Centre fo

Cell

Article

Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology

Graphical Abstract



Authors

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In Brief

Serological analyses of ~650 SARS-CoV-2-exposed individuals show that 90% of the serum or plasma neutralizing activity targets the virus receptor-binding domain, with structural insights revealing how distinct types of neutralizing antibodies targeting the ACE2-binding site dominate the immune response against SARS-CoV-2 spike.

PLOS ONE

RESEARCH ARTICLE

Presence of antibody-dependent cellular cytotoxicity (ADCC) against SARS-CoV-2 in COVID-19 plasma

For Yue Tso[1,2], Salum J. Lidenge[1,2,3,4], Lisa K. Poppe[1,2], Phoebe B. Peña[1,2], Sara R. Privatt[1,2], Sydney J. Bennett[1,2], John R. Ngowi[3], Julius Mwaiselage[3,4], Michael Belshan[1,5], Jacob A. Siedlik[6], Morgan A. Raine[5], Juan B. Ochoa[7], Julia Garcia-Diaz[8], Bobby Nossaman[8], Lyndsey Buckner[8], W. Mark Roberts[8], Matthew J. Dean[9], Augusto C. Ochoa[9,10], John T. West[1,11], Charles Wood[1,2,11]*

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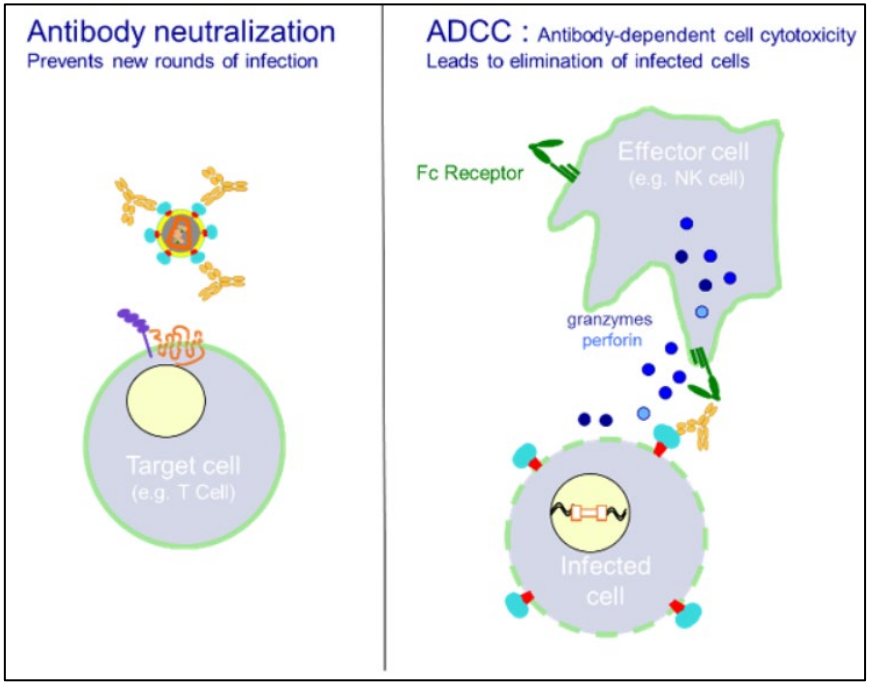
Outline

Two SARS-CoV-2 Bioassays are developed to address the two major Mechanisms of Actions for SARS-CoV-2 antibodies.

1. SARS-CoV-2 HiBiT-PsVLP Assay for neutralization activity

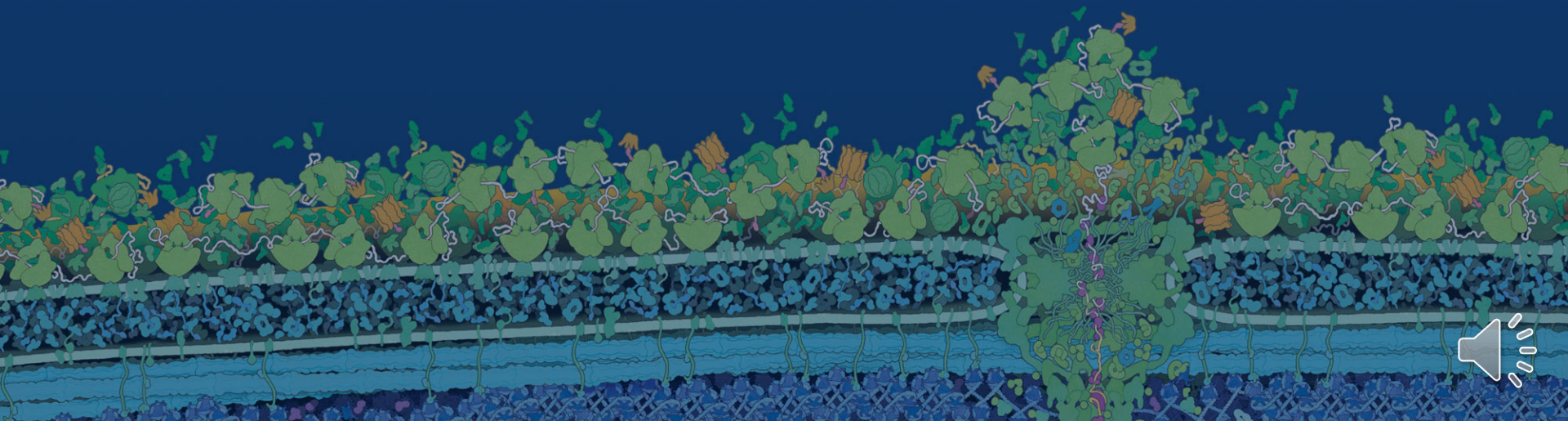
2. ADCC/ADCP Reporter Bioassays for antibody Fc effector function



Dr. Julie Overbaugh
Fred Hutchinson Cancer Research Center

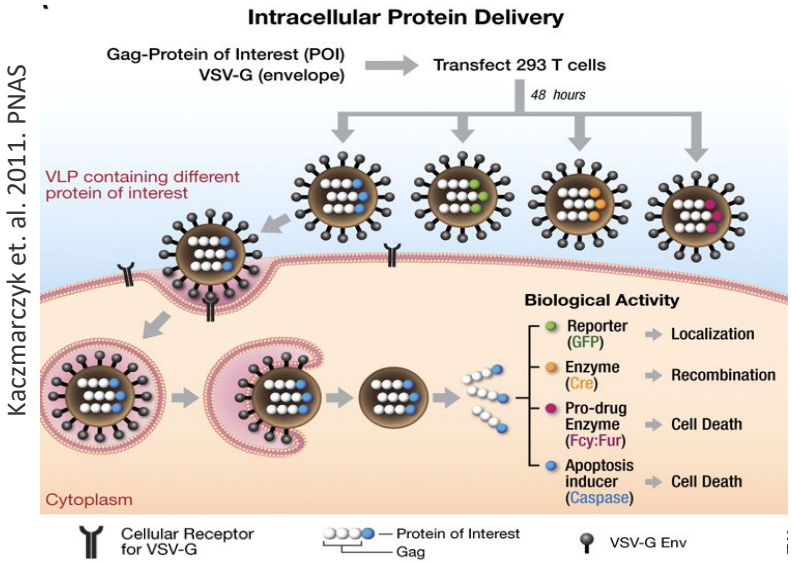
Promega

SARS-CoV-2 HiBiT-PsVLP Assay to Measure the Blocking Activity for Small Molecule Inhibitors and Neutralizing Antibodies



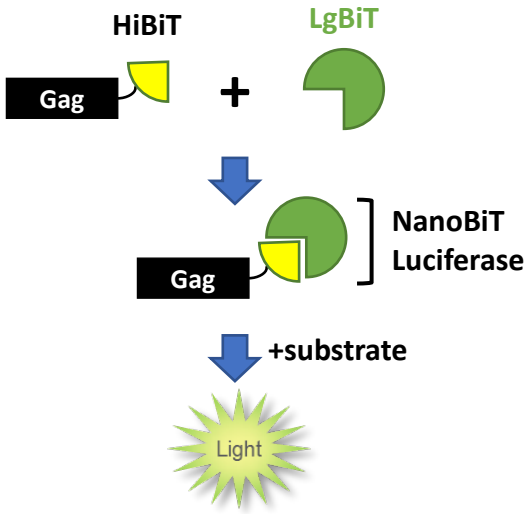
Pseudotyped Virus-Like Particles (PsVLPs)

Virus-like particles (VLPs): non-replicating nanostructures comprised of viral structural proteins and a lipid envelope; VLPs are non-infectious because they contain no viral genetic material



Kaczmarczyk et. al. 2011. PNAS

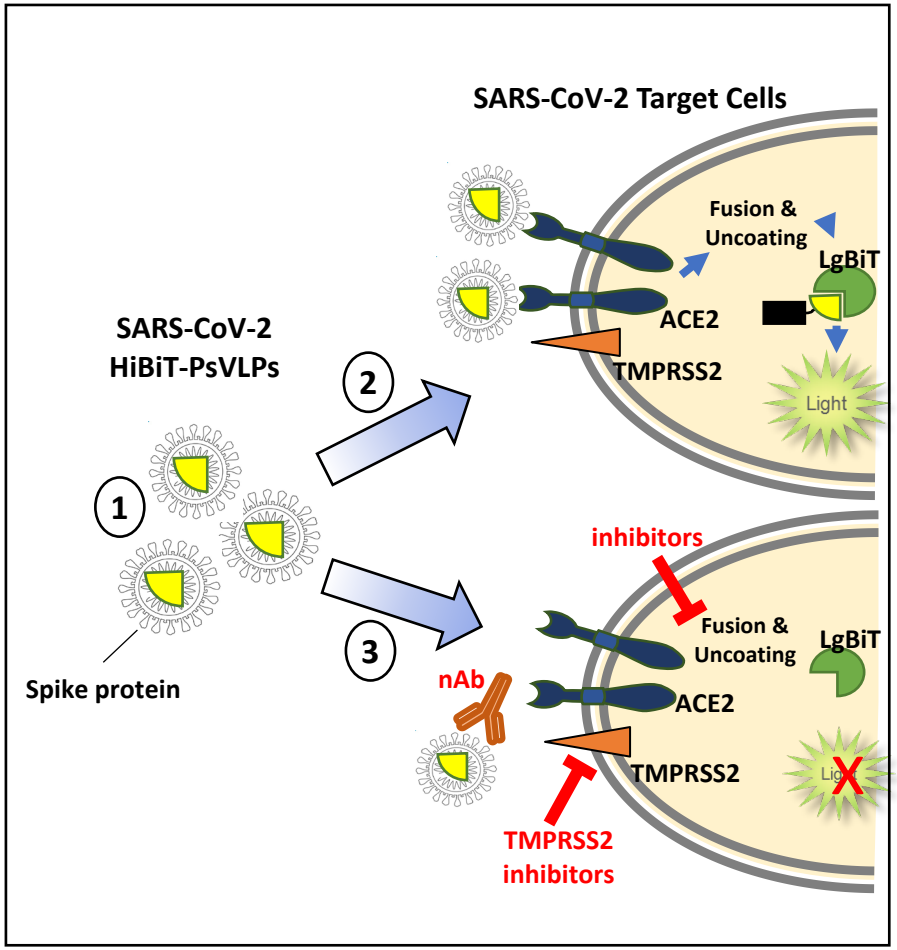
HiBiT Protein Tagging Technology



- HIV-1 Gag polyprotein can self-assemble into VLPs in the absence of other viral factors
- Gag VLPs can be pseudotyped (PsVLPs) with heterologous viral envelope proteins to permit cellular entry

Goal: Generate a safe, rapid, and quantitative assay to monitor SARS-CoV-2 entry using HiBiT-tagged PsVLPs

SARS-CoV-2 HiBiT-PsVLP Assay Design



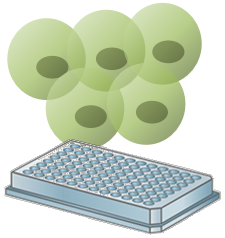
Assay Design:

1. HiBiT-tagged VLPs pseudotyped with SARS-CoV-2 Spike protein are added to SARS-CoV-2 Target Cells
   - HiBiT is packaged inside the PsVLPs
2. In the absence of inhibitors or neutralizing antibodies (nAbs), SARS-CoV-2 HiBiT-PsVLPs bind to target cells via Spike/ACE2 interaction and undergo membrane fusion mediated by cellular proteases. HiBiT is released into target cells and binds to LgBiT to generate a luminescent signal in the presence of substrate.
3. In the presence of inhibitors or nAbs of SARS-CoV-2 entry, the entry/fusion processes of PsVLPs are blocked, thereby preventing HiBiT release. No luminescent signal is produced.

SARS-CoV-2 HiBiT-PsVLP Assay

Assay Workflow

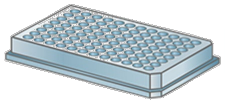
Plate SARS-CoV-2 Target Cells



Pre-treat with cell-targeted inhibitor(s)

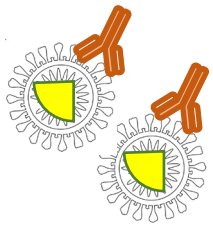
Add live cell detection Reagent

1 hour



3 hours

Measure Luminescence



15 mins

Add PsVLPs to target cells

Prepare HiBiT-PsVLPs ± Blocking Abs

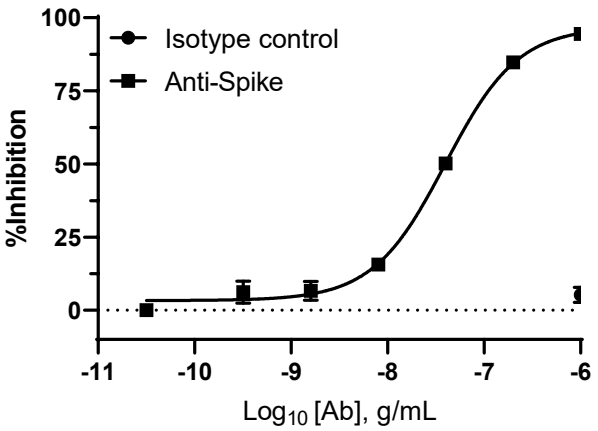
Simple, fast, real-time, quantitative!

Measure the Activity for Neutralizing Antibodies and Small Molecule Inhibitors

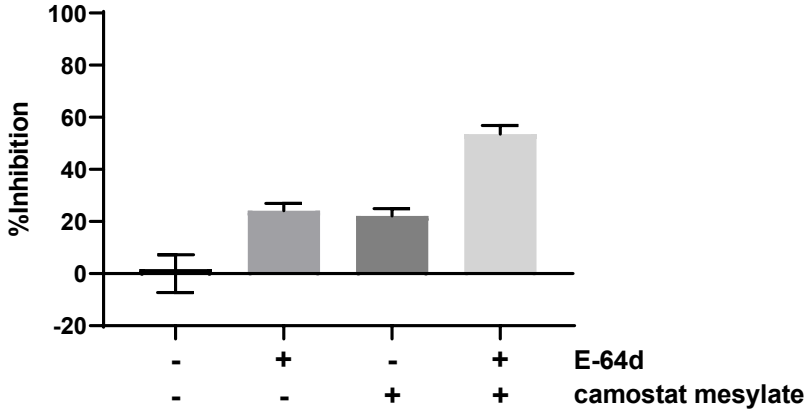
SARS-CoV-2 PsVLP Entry

- is inhibited by anti-spike neutralization antibody
- Is inhibited by protease inhibitors

Anti-Spike neutralization antibody



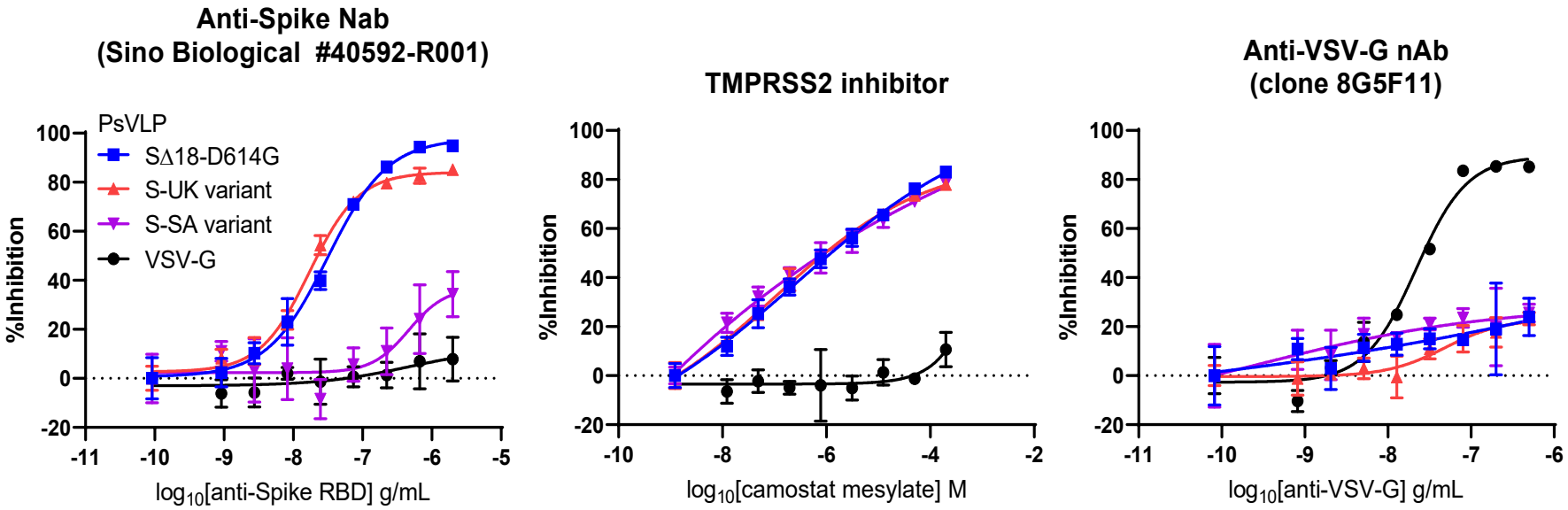
protease inhibitors



- **E-64d =** Cathepsin inhibitor – targets the endosomal entry pathway
- **Camostat mesylate =** TMPRSS2 inhibitor – targets the cell surface entry pathway

Evaluating the Neutralization Activities against SARS-CoV-2 Spike Variants

- SA variant is resistant to neutralization by the anti-Spike-RBD Ab tested
- TMPRSS2 inhibitor efficiently reduced the entry for all SARS-CoV-2 HiBiT-PsVLP variants



Three SARS-CoV-2 PsVLP carrying spike proteins with D614G mutation, major mutations reported for UK variant or South African variant were produced and tested in the assay.

Summary of SARS-CoV-2 HiBiT-PsVLP Assay

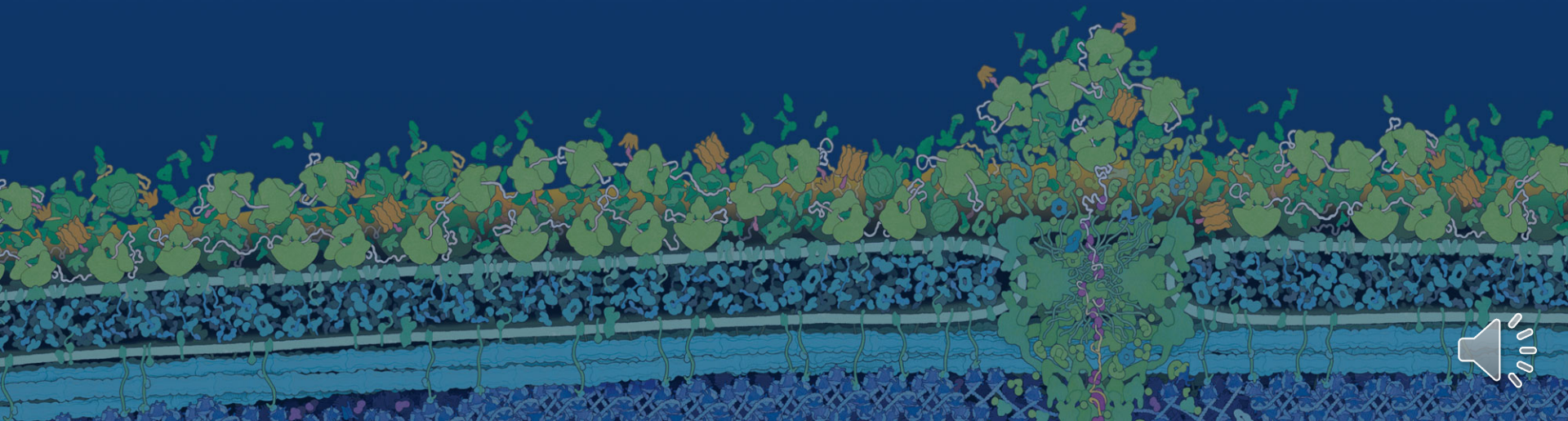
1. Increased biosafety
   - HiBiT-PsVLPs are non-replicating
   - No viral genome present

2. Simple and rapid
   - No gene expression steps required for assay readout
   - Monitor viral entry in live cells in real time

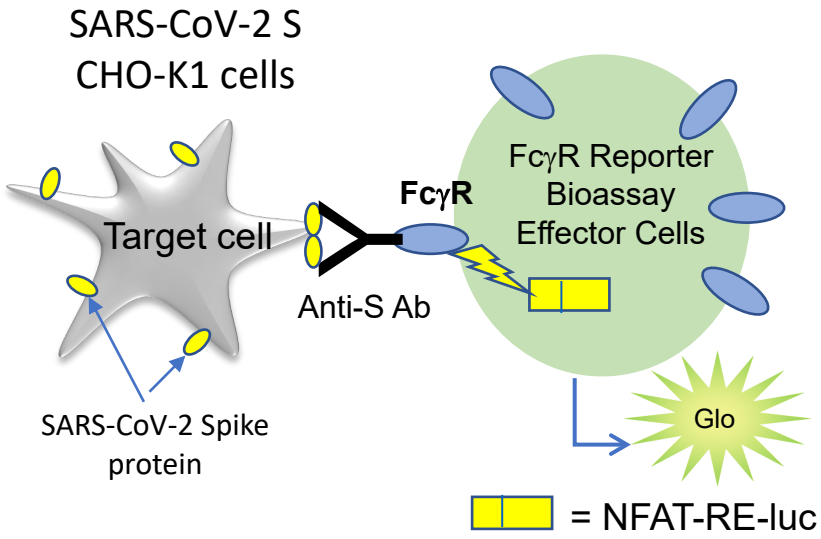
3. PsVLPs and Target Cells offered in Thaw-and-Use format
   - No need to generate live virus or pseudovirus
   - No need to culture cells

4. Quantitative assay readout

ADCC/ADCP Reporter Bioassays to Measure the Fc Function for SARS-CoV-2 Antibodies



Reporter Bioassays to Measure Fc Function (ADCC, ADCP) for SARS-CoV-2 Antibodies



1) Anti-spike antibody binds to S protein on target cells and FcγR* on FcγR Reporter Bioassay Effector Cells simultaneously.

2) It leads to the activation of FcγR receptor and luciferase activation in the reporter effector Cells.

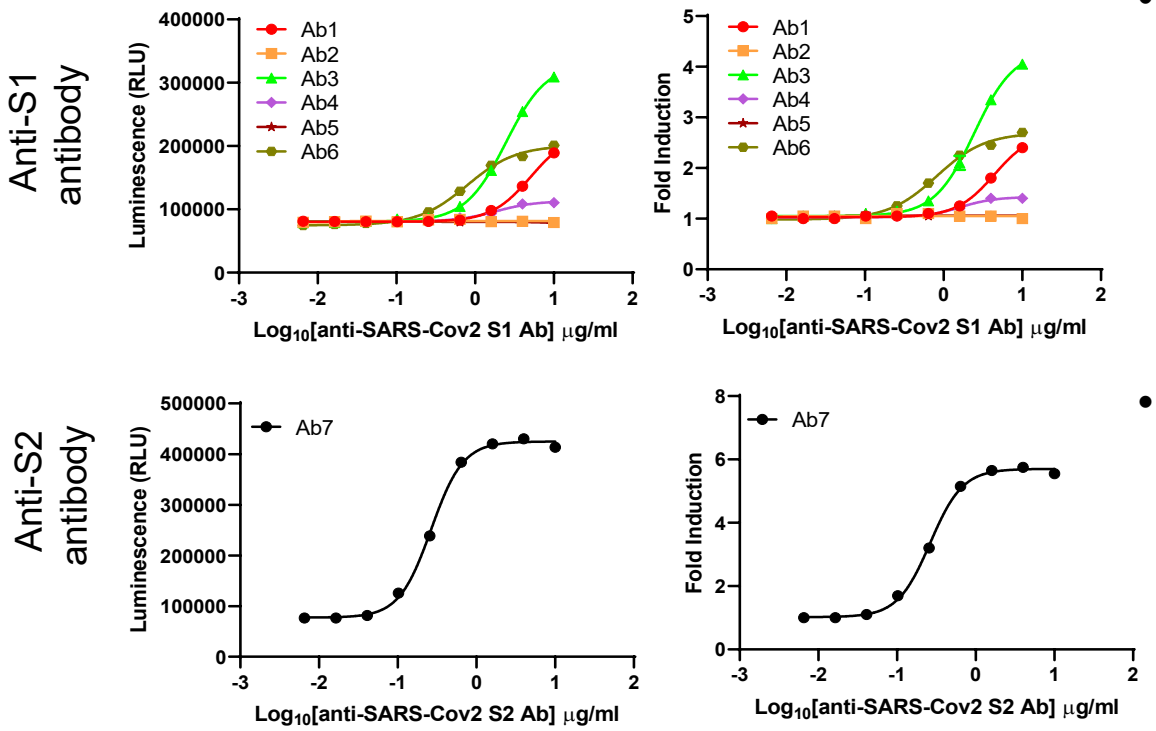
*ADCC Reporter Bioassay: FcγRIIIa
*ADCP THP-1 Reporter Bioassay: FcγRIIa, FcγRI

Commercial SARS-CoV-2 Antibodies Evaluated

Test Ab	anti-SARS-CoV-2 S Ab	vendor	CAT#	IgG isotype	Specificity	Neutralization Activity	Reference
1	Clone 105-9	Biolegend	938501	Human IgG1	S1	Nab, ND50: 0.2-0.8 µg/mL	1
2	Clone 415-6	Biolegend	938601	Human IgG1	RBD	No	1
3	Clone 414-1	Biolegend	938701	Human IgG1	RBD	Nab, ND50: 0.03-0.12 µg/mL	1
4	Clone 414-2	Active motif	91349	Human IgG1	RBD	Nab, ND50 =28.58 nM	1
5	Clone CR3022	Absolute Antibody	Ab01680	Human IgG1	RBD	Nab	2
6	anti-Spike Ab	ACRO Biosystems	SAD-S35	Human IgG1	RBD	Nab, IC50 =1.47 µg/mL	NA
7	anti-Spike S2 Ab	Sino Biological	40590-D001	mouse/ human IgG1	S2	No	NA

References:
1. Wan J, *et al. bioRxiv*. 2020 doi: https://doi.org/10.1101/2020.05.19.104117
2. Meulen, J *et al*. PLoS Med. 2006 Jul; 3(7): e237 PMID:16796401

ADCC Reporter Activities for anti-SARS-CoV-2 Spike Antibodies

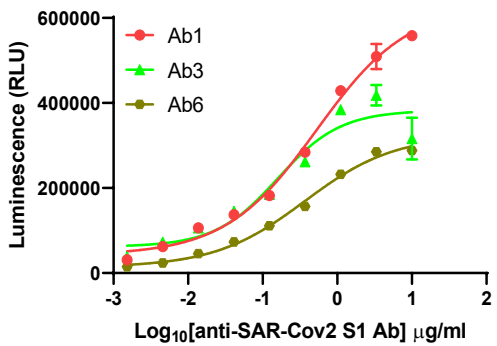


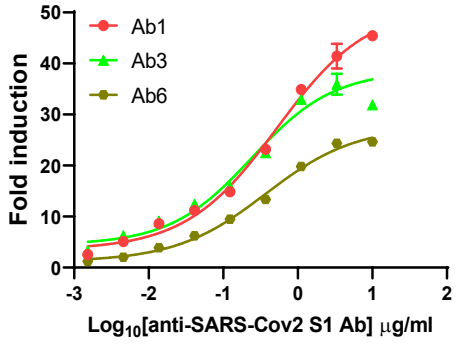
- Seven anti-SARS-CoV-2 S antibodies were tested in ADCC Reporter Bioassay using SARS-CoV-2 S CHO-K1 target cells.
- Four antibodies, Ab1, 3, 6 and 7 showed positive ADCC activity.

	Ab 1	Ab 2	Ab 3	Ab 4	Ab 5	Ab 6	Ab 7
EC50,mg/ml	~4.4	NA	~2.5	1.7	NA	0.8	0.3
fold	2.7	1.1	4.3	1.4	1.1	2.7	5.7

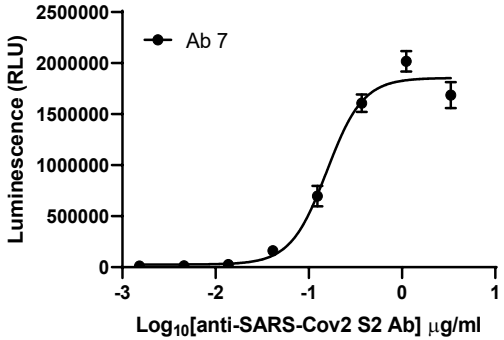
ADCP Reporter Activities for anti-SARS-CoV-2 Spike Antibodies

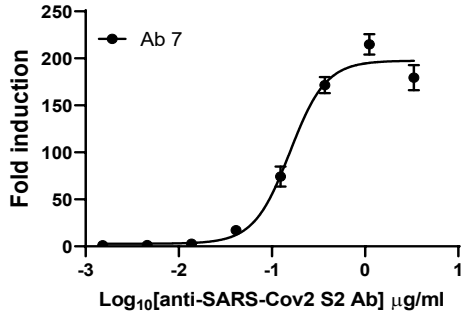
Anti-S1 antibody





Anti-S2 antibody





- Four anti-SARS-CoV-2 S Antibodies, Ab1, 3, 6 and 7 were tested in ADCP THP-1 Reporter Bioassay using SARS-CoV-2 S CHO-K1 target cells.
- All four antibodies tested showed strong ADCP reporter activity.

	Ab 1	Ab 3	Ab 6	Ab 7
EC50, mg/ml	~0.53	~0.16	~0.36	0.15
fold	50	40	27	198

Summary of SARS-CoV-2 ADCC/ADCP Assays

- Can quantitatively measure ADCC and ADCP activity for SARS-CoV-2 Abs.

- Can measure Ab Fc function in the presence of human serum (data not shown), indicating the potential use for patient's samples after vaccine administration.

Thank You

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