High-throughput biochemical assays for early antiviral discovery

AViDD Open Science Forum

July 17, 2024

Haim Barr, PhD - Senior Staff Scientist

ASAP Biochemical Core

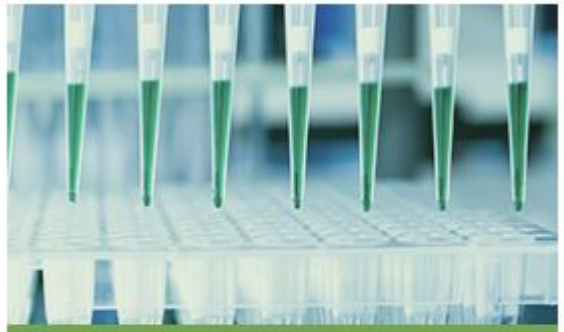
The Nancy and Stephen Grand Israel National Center for Personalized Medicine (G-INCPM)

The Weizmann Institute of Science

The Nancy and Stephen Grand Israel National Center
for Personalized Medicine (G-INCPM)

300,000 Compound Screening Library
Compound logistics
Assay development
Automation
Screening
Orthogonal/Confirmation assays

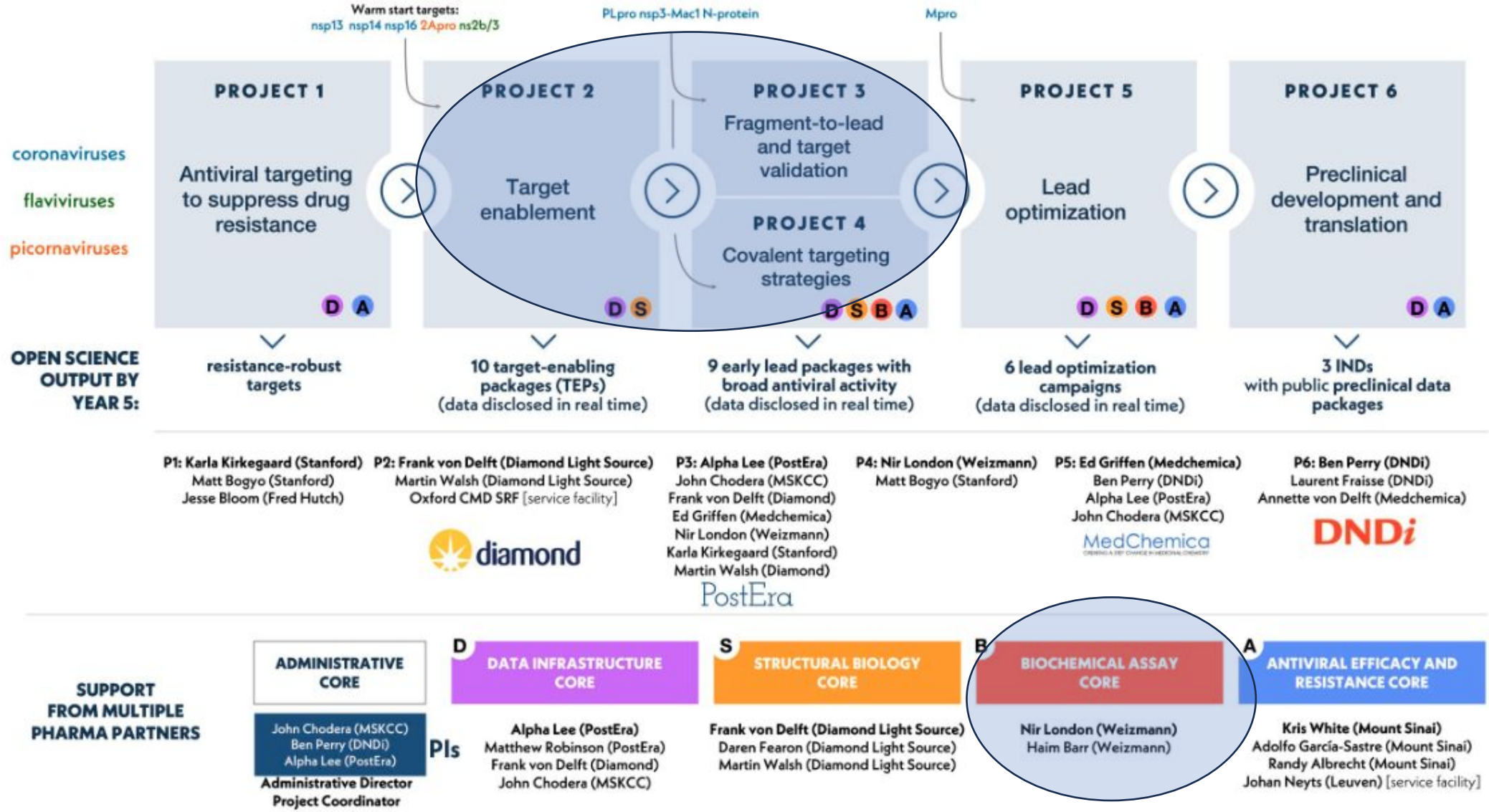
LNP formulation



Maurice and Vivienne Wohl Institute for Drug Discovery

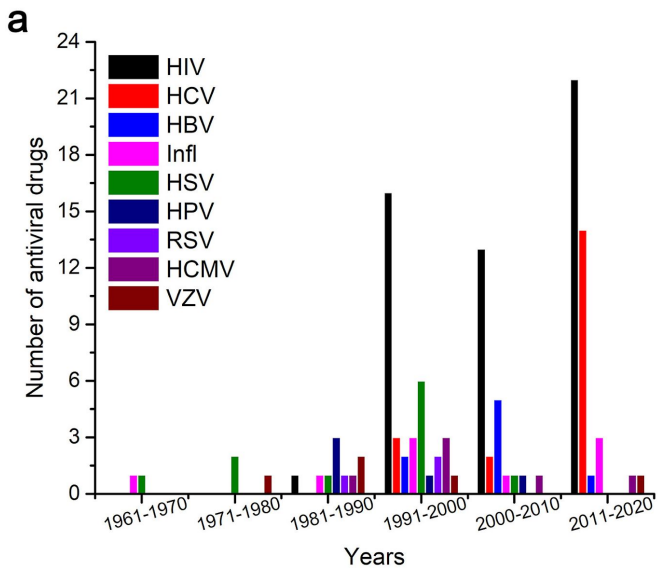
Medicinal Chemistry

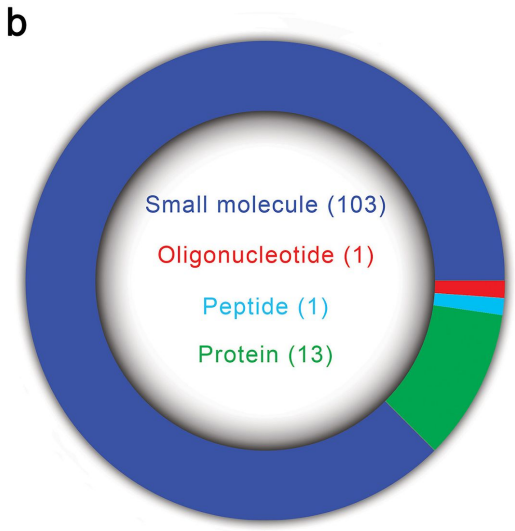
Reagents for screening
Chemical triage
Re-synthesis of screening hits
Analogs for SAR

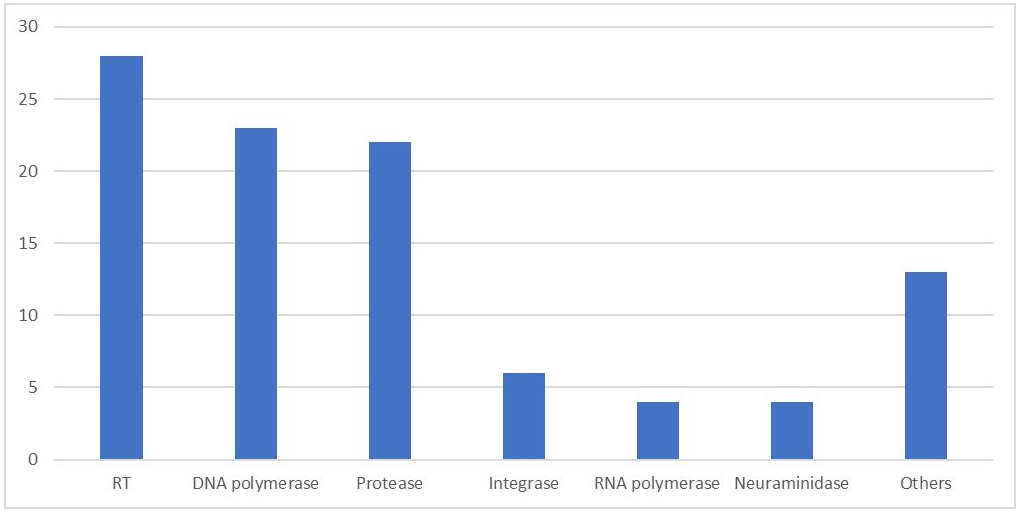


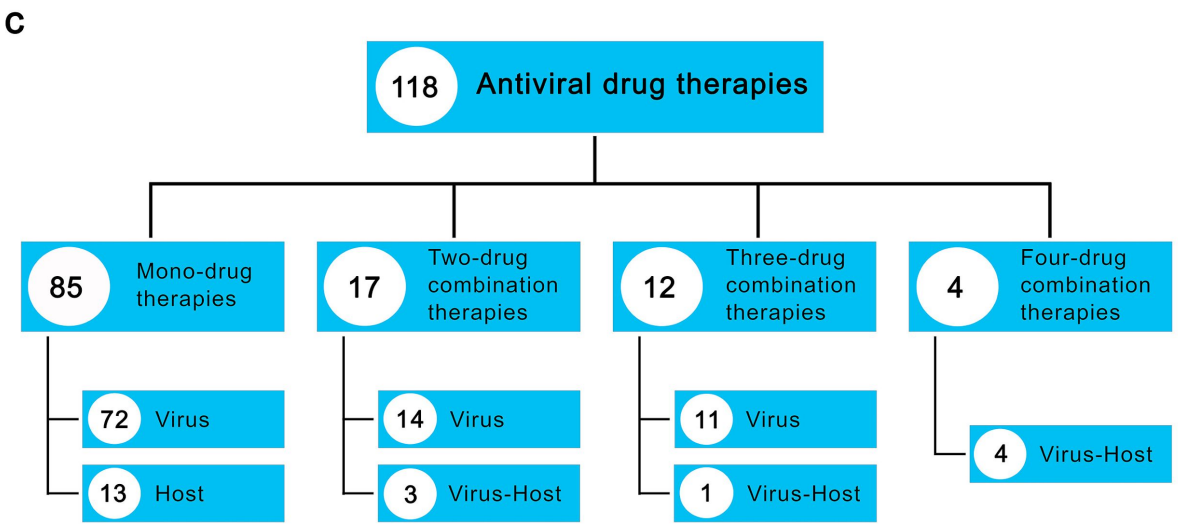
An NIH Antiviral Drug Discovery (AViDD) U19 Center

Clinically approved antivirals (pre SARS-Cov-2)



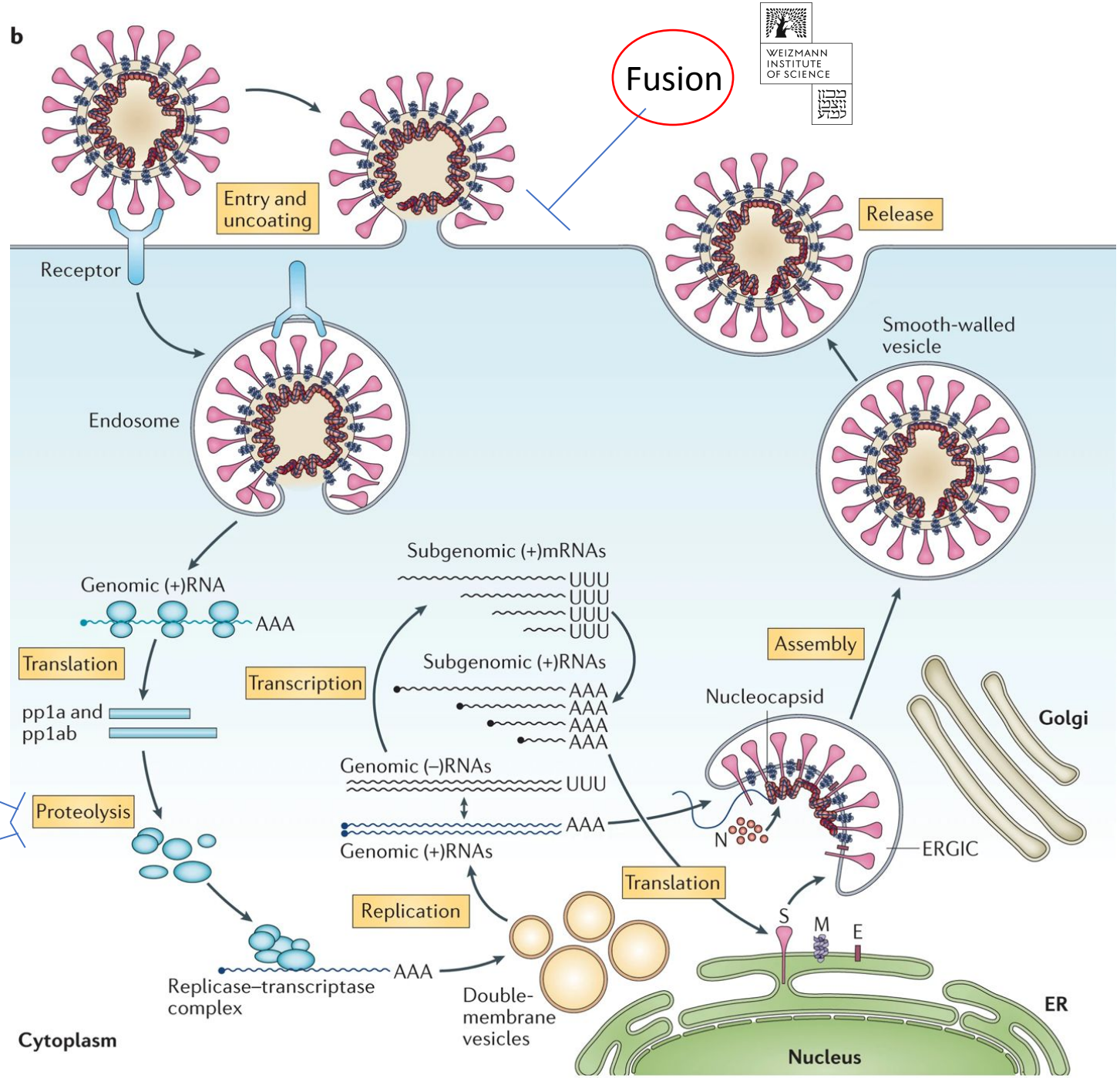






Proteases are good targets for antivirals
Proteases are good enzymes for screening compounds

Dharma Rao Tompa, Aruldoss Immanuel, Srimari Srikanth, Saraboji Kadhirvel, Trends and strategies to combat viral infections: A review on FDA approved antiviral drugs, International Journal of Biological Macromolecules, Volume 172, 2021, Pages 524-541



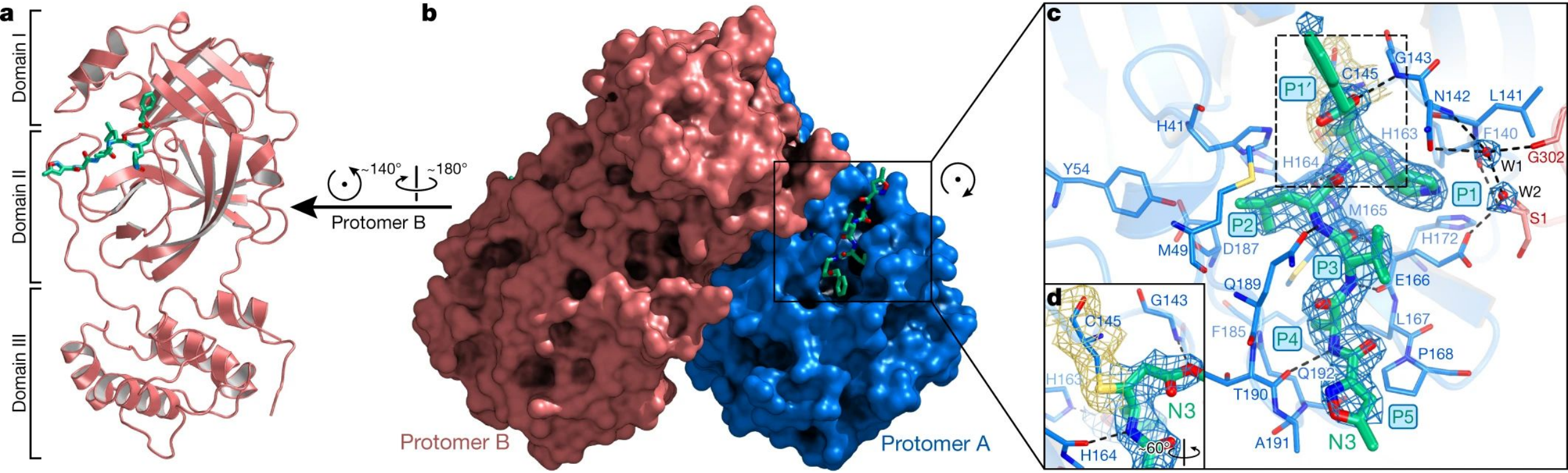
Mpro

PLpro

ASAP
Accessible Antivirals to Prevent Pandemics

de Wit et al. Nat. Rev. Microbiology (2016)

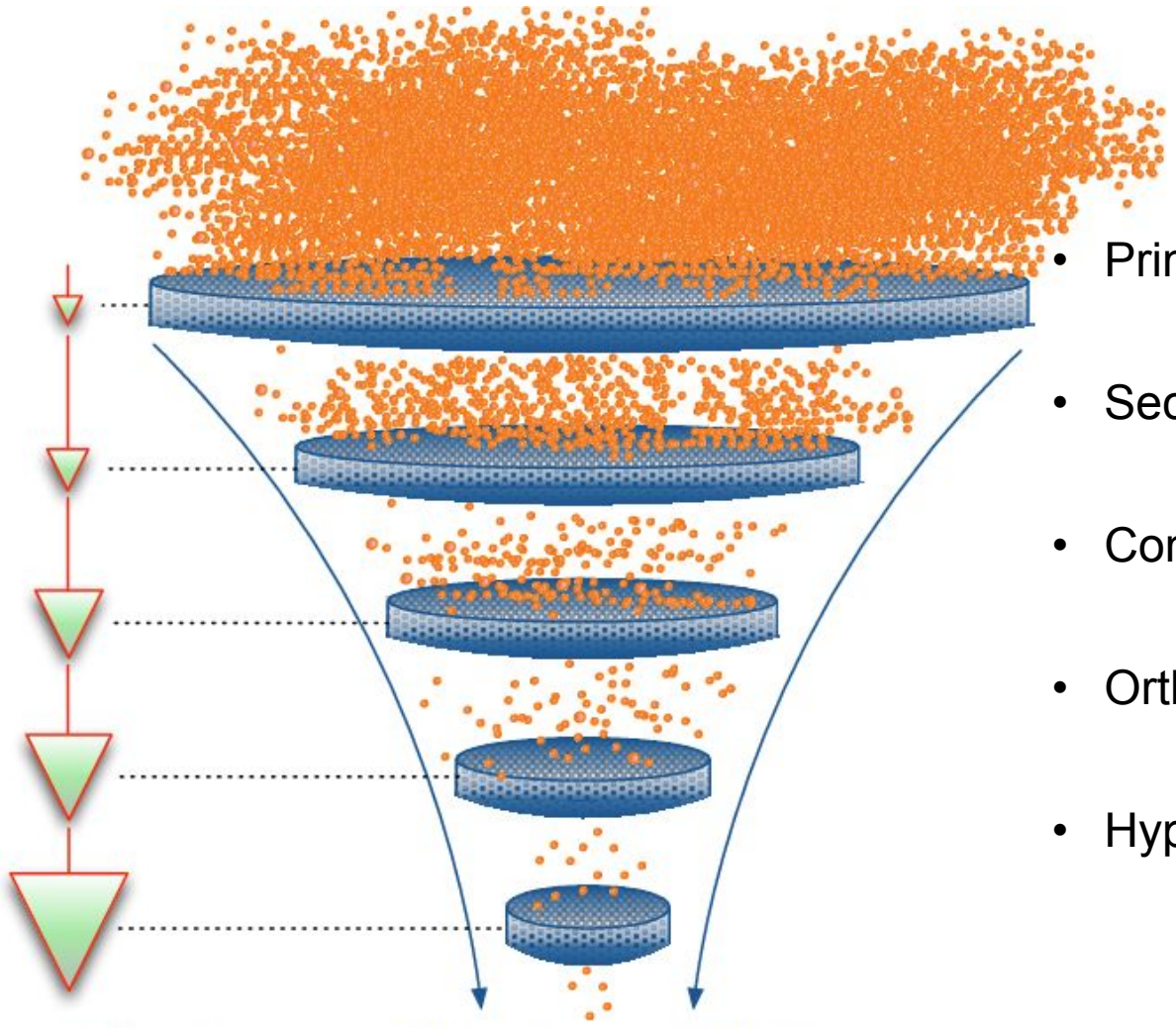
SARS-Cov-2 Mpro structure



Included an HTS – noting some compounds like Ebselen and Disulfuram as hits

Jin, Z., Du, X., Xu, Y. et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature 582, 289–293 (2020). https://doi.org/10.1038/s41586-020-2223-y

HTS-driven screening funnel



Assay development and screen

- Primary assay
- Secondary or Counter assay
- Confirmation assay
- Orthogonal assays
- Hypothesis confirmation (SAR)

Emphasis on **Throughput**

Triage

Costs/compound increases
– but so does confidence

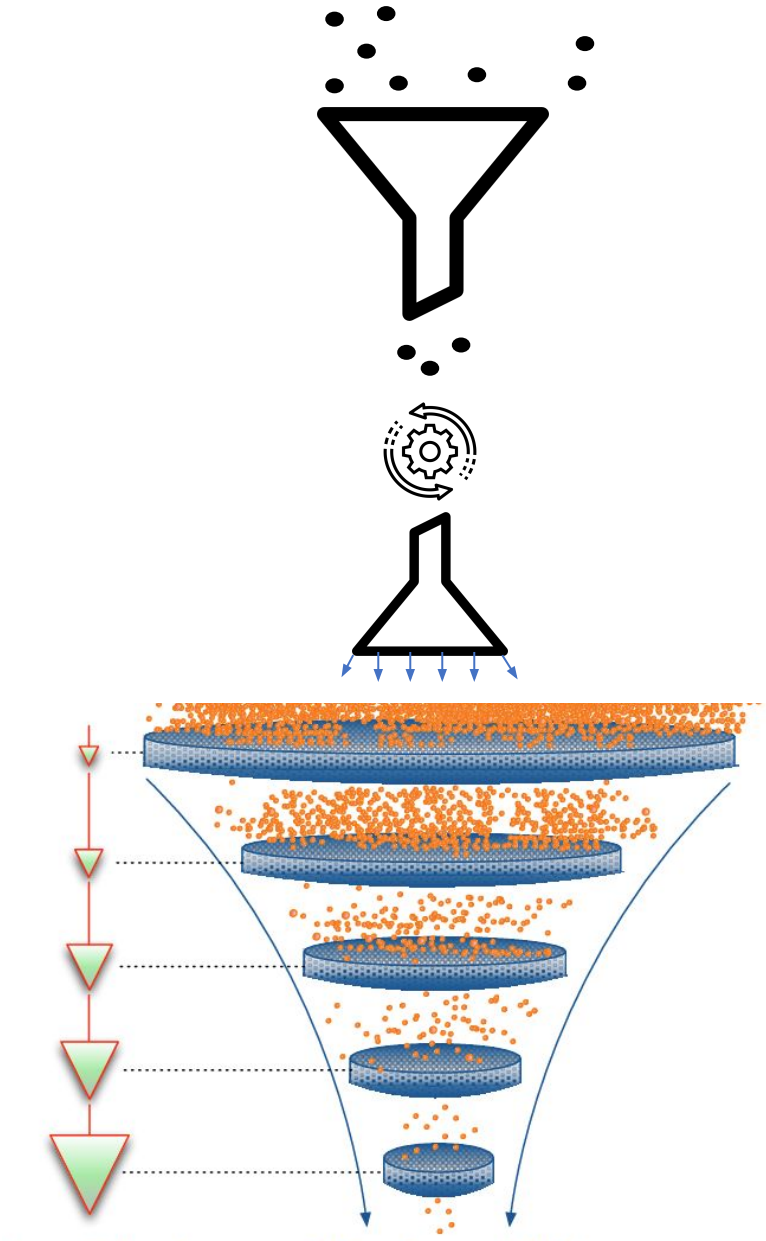
Screening campaigns targeting SARS-Cov-2 Mpro

# Compounds	Publication
Selleck Bioactive Library (~3000)	Cell Res. 2020; 30: 678-692
10,000	Nature. 2020; 582: 289-293
2,400	PLoS One. 2020; 15e0240079
23,000	Angew. Chem. Int. Ed. Engl. 2021; 60: 10423-10429
6,000	PLoS One. 2021; 16e0245962
8,700	ACS Pharmacol. Transl. Sci. 2021; 4: 1096-1110
10,755	ACS Pharmacol. Transl. Sci. 2020; 3: 1008-1016
1,800,000	Protein & Cell, Volume 14, Issue 1, January 2023, Pages 17–27
185,365	Unpublished - Weizmann Institute/Covid-19 Moonshot

Target	Campaign	Compounds	Output
Mpro	Moonshot	1,400	3 Pre-clin candidates
Mpro	ASAP	1,247	DNDi-6510

Ed Griffen

https://www.youtube.com/watch?v=sWzE6qJs6fk&t=989s



High value, high confidence inhibitors

P2 – Target Enablement – Structural Biology + Fragment screening

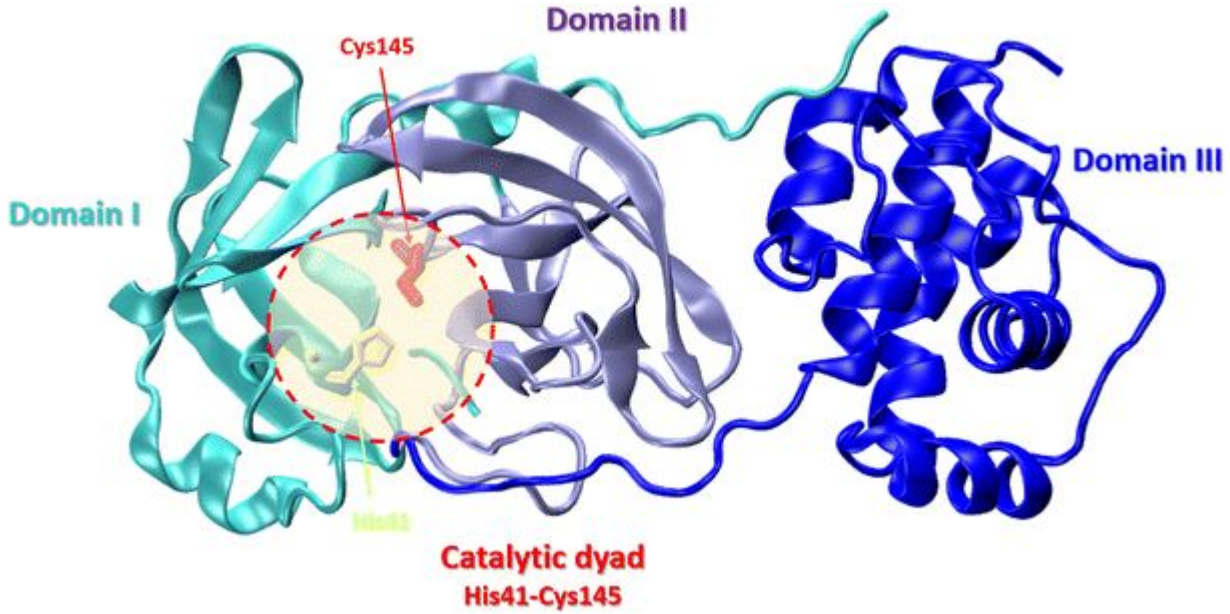
P3 – Hit to Lead
Or
P4 – Covalents
Or
P5 – Lead Optimization

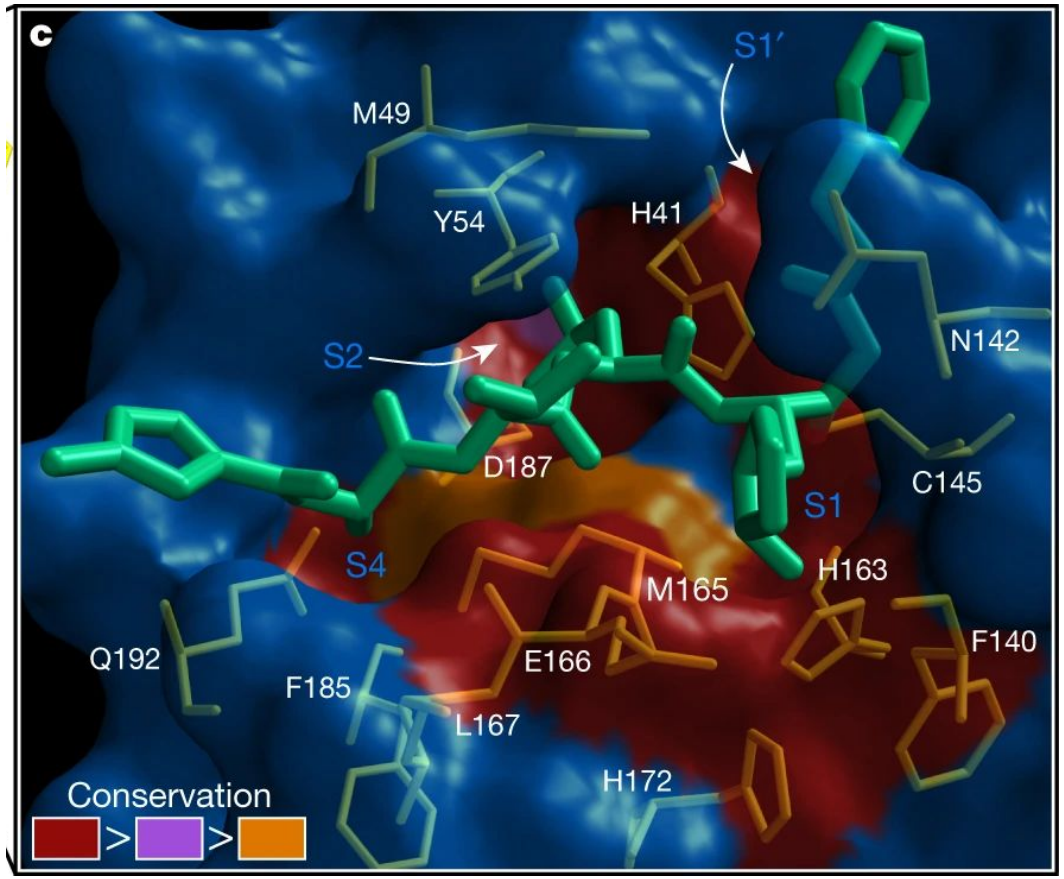
Biochemical Core

- Better compounds than HTS deck
- Hypothesis driven
- Many eyes on data
- Weekly iterations

Structural Biology Core

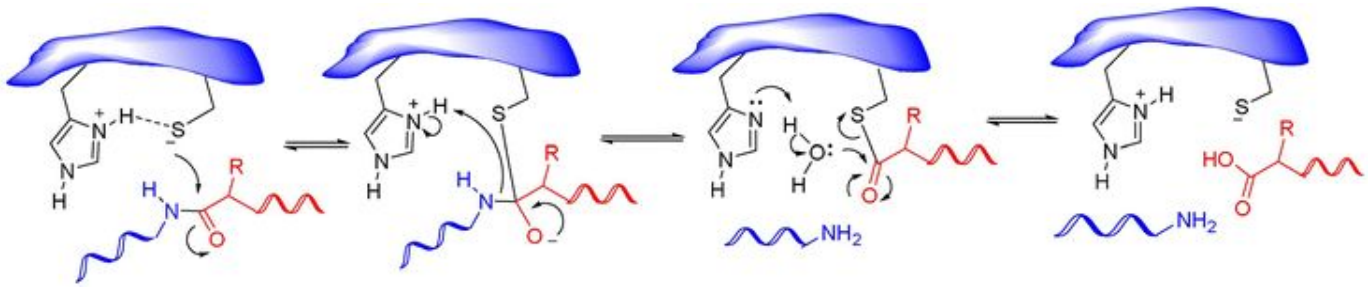
Antiviral Core





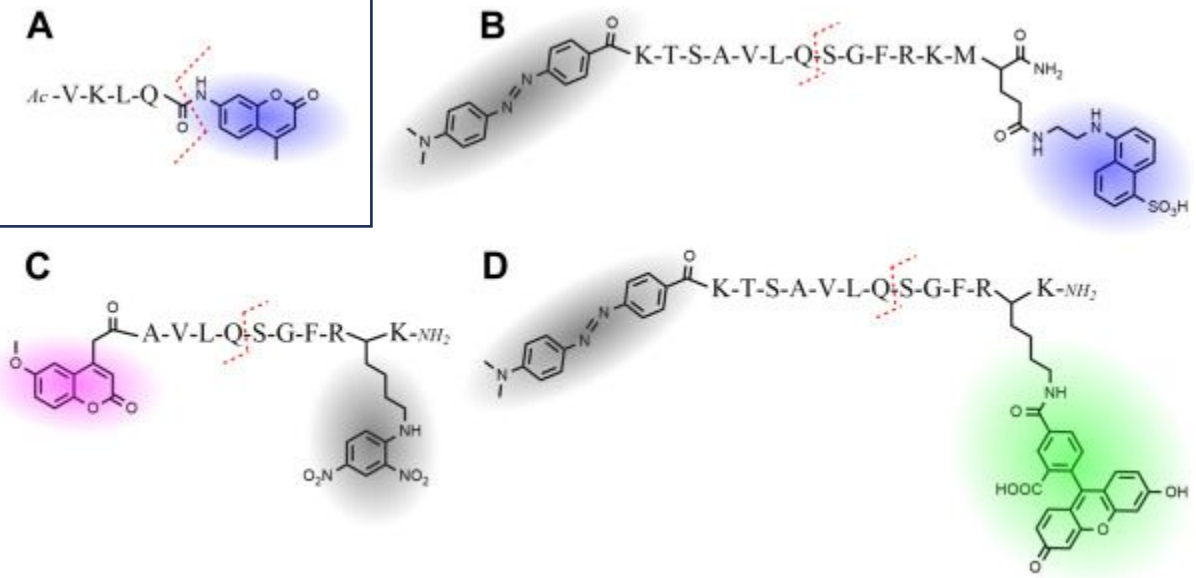
Jin, Z., Du, X., Xu, Y. et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature 582, 289–293 (2020). https://doi.org/10.1038/s41586-020-2223-y

RSC Adv., 2023, 13, 35500-35524



Catalytic dyad of 3C proteases

Fluorogenic



Fluorogenic or FRET based Mpro substrates
Derived from Nsp4-5 site in polyprotein

https://doi.org/10.1016/j.jbc.2022.101739

Mpro substrates

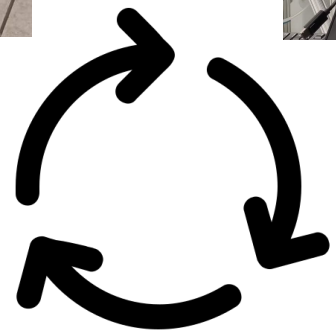
MCA-AVLQ ↓ SGFR-Lys(Dnp)-Lys-NH2	https://doi.org/10.1093/procel/pwac016
2-aminobenzoyl — AVLQ ↓ SGFRKK — tyrosine(3-NO2)R-COOH	https://doi.org/10.1042%2FBCJ20210197
Dabsyl-KTSAVLQ ↓ SGFRKME-EDANS	https://doi.org/10.1021/acsptsci.0c00108
DABCYL-KTSAVLQ↓SGFRKM-EDANS	https://doi.org/10.1021/acsptsci.0c00216
Ac-VKLQ-AFC Ac-Abu-Tle-LQ-AFC Dabcyl-VKLQ-EDANS MCA-AVKQSGFK-DNP-K	https://doi.org/10.1371/journal.pone.0245962
5-FAM-AVLQ ↓ SGFR-Lys(dabcyl)-Lys	Ours…

Final Mpro assay

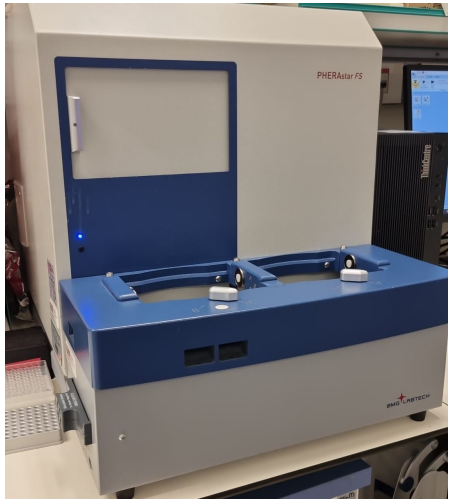
Design > Make > Test (repeat, repeat, repeat….)

5 nM SARS-CoV-2 3CL protease (Diamond Light Source)
375 nM [5-FAM]-AVLQSGFR-[Lys(Dabcyl)-K-amide substrate
20 mM HEPES (pH 7.3)
50 mM NaCl
10% Glycerol
1 mM TCEP
0.01 % Tween-20

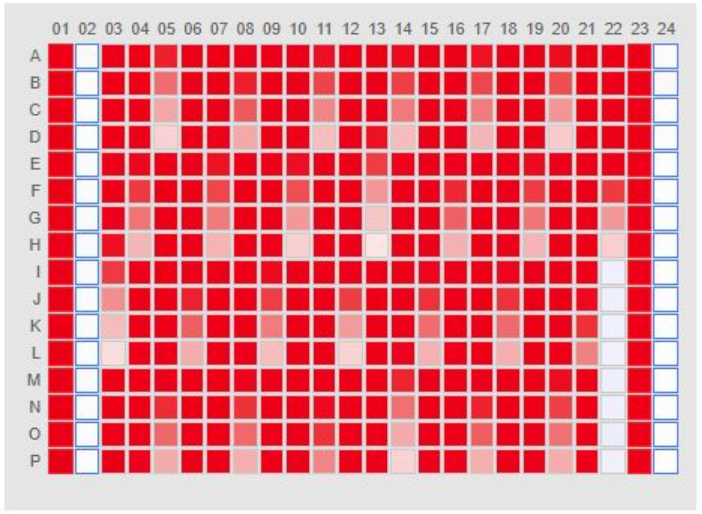
Shipment
Registration
Assay ready plate
Assay
Discuss results



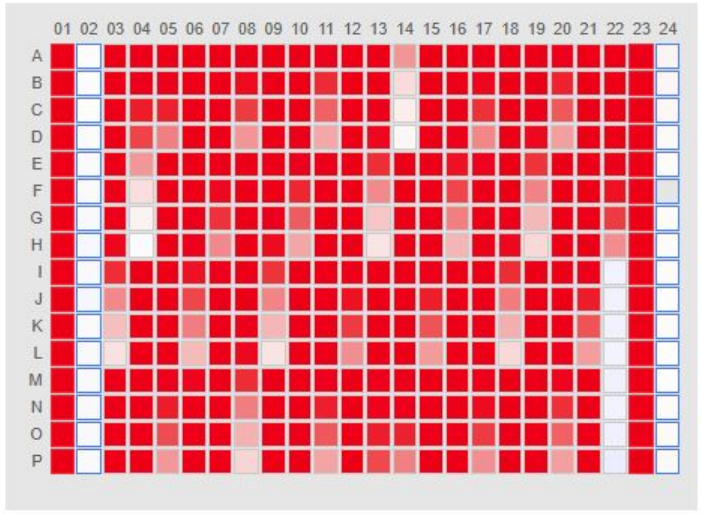
Assay ready plate with compounds
Protease
Incubate 15 minutes
Substrate
Incubate 30 minutes
read signal ex 480 nm/em 520 nm



Typical SARS-Cov-2 Mpro assay run

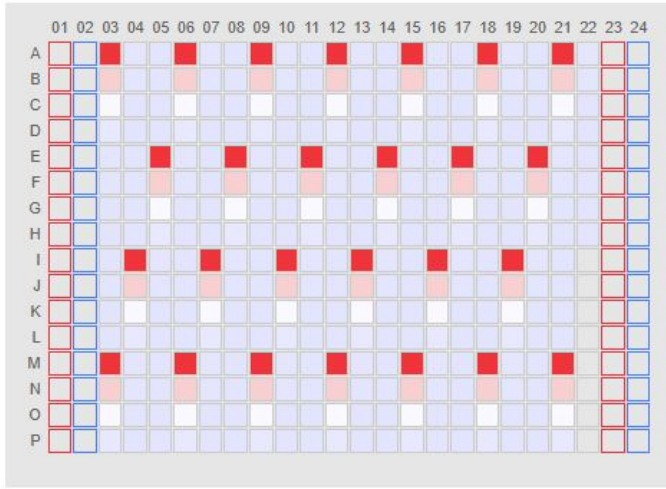


Z'-factor (controls): 0.96 Z-factor: -4.16

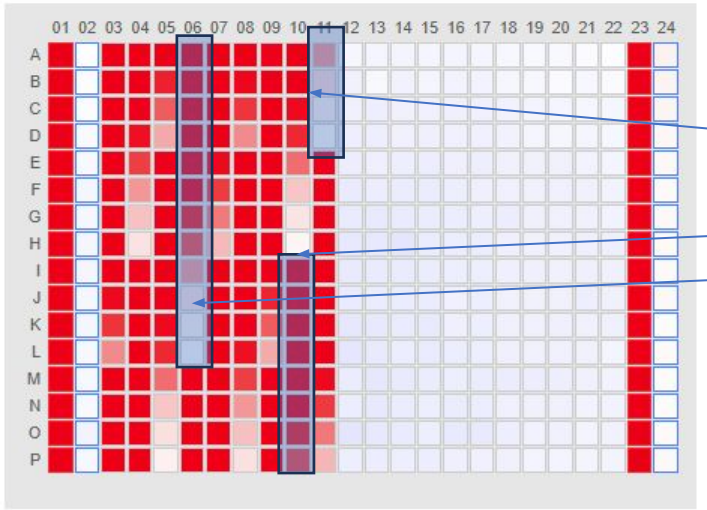


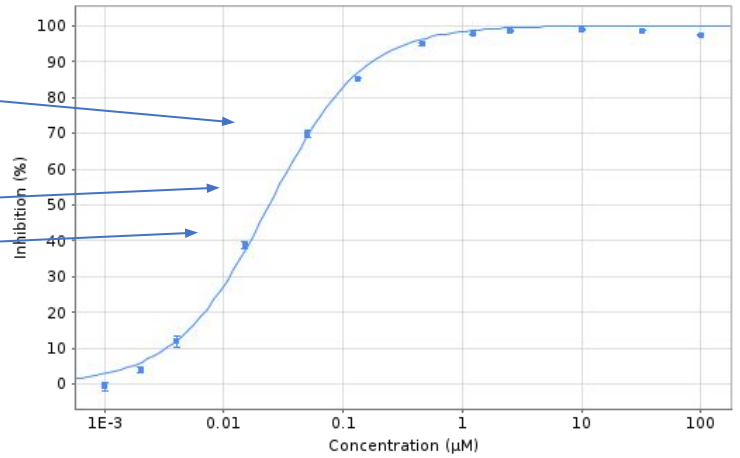
Z'-factor (controls): 0.94 Z-factor: -4.30

Dose-response template

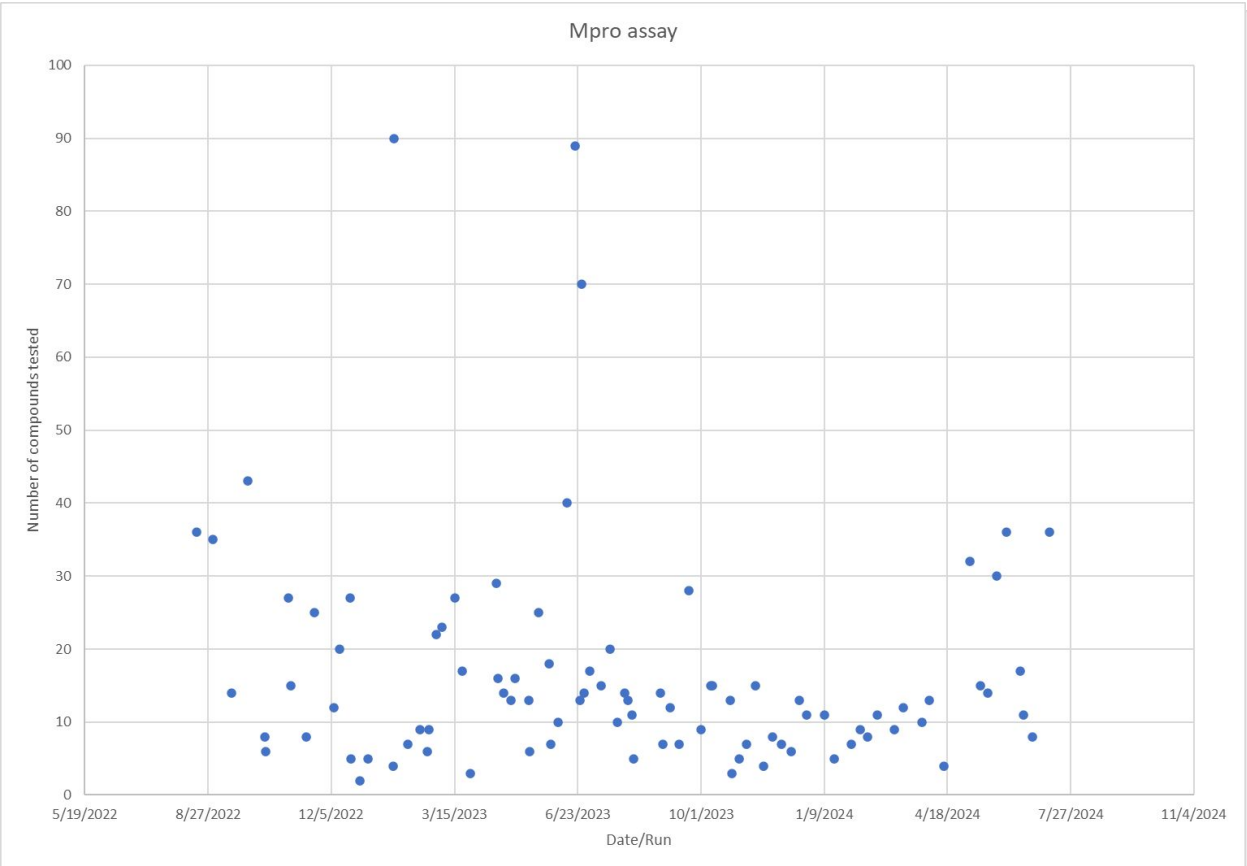


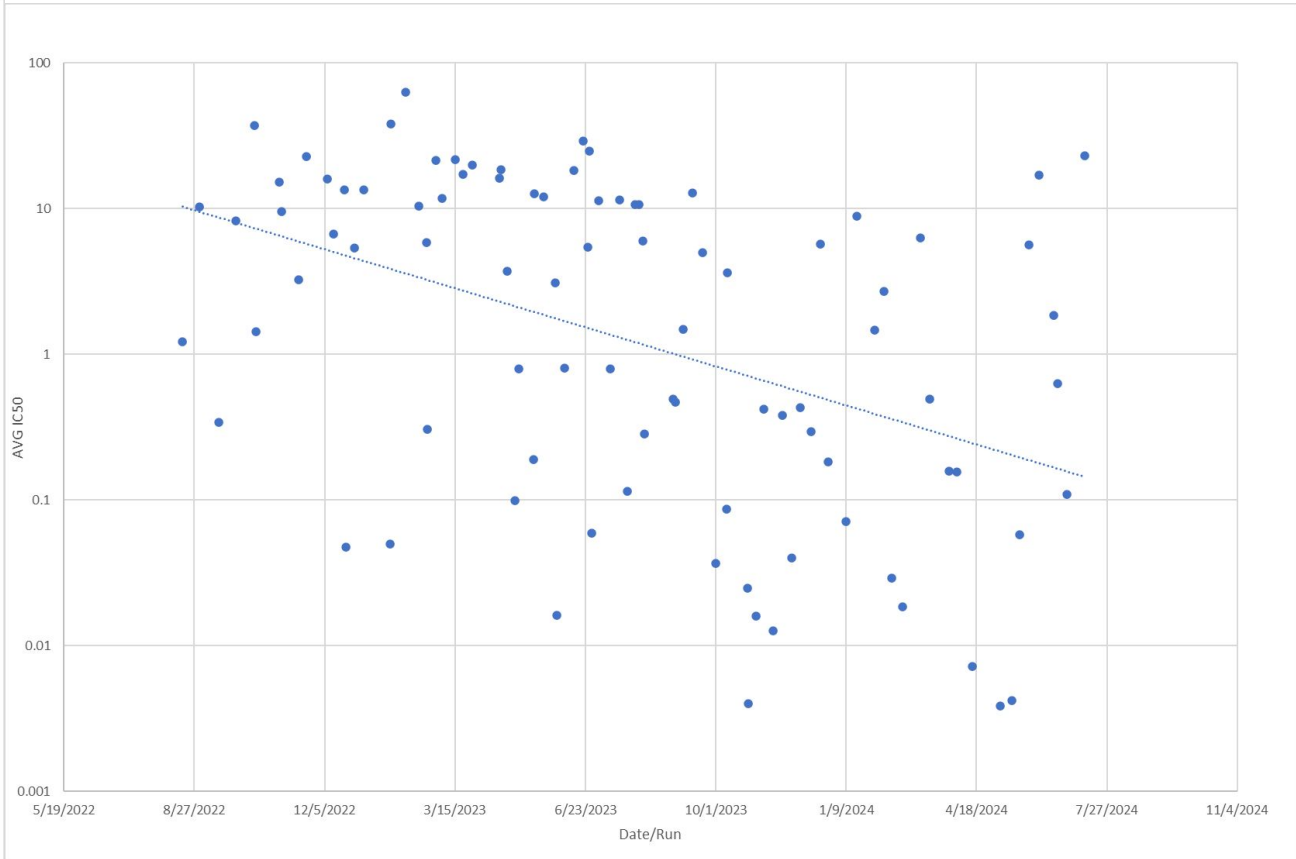
21272-SARS3





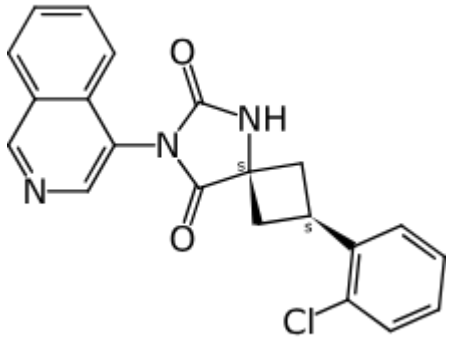
Z'-factor (controls): 0.91 Z-factor: -3.78

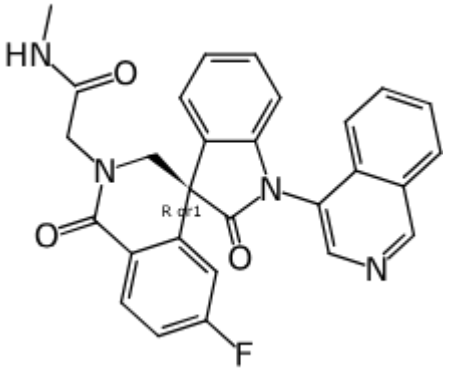

Mpro assay
Number of compounds tested
100
90
80
70
60
50
40
30
20
10
0
5/19/2022
8/27/2022
12/5/2022
3/15/2023
6/23/2023
10/1/2023
1/9/2024
4/18/2024
7/27/2024
11/4/2024
Date/Run


AVG IC50
100
10
1
0.1
0.01
0.001
5/19/2022
8/27/2022
12/5/2022
3/15/2023
6/23/2023
10/1/2023
1/9/2024
4/18/2024
7/27/2024
11/4/2024
Date/Run

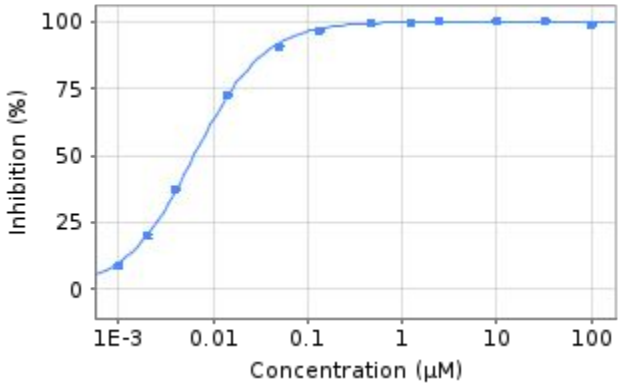
What is going on with MERS Mpro??

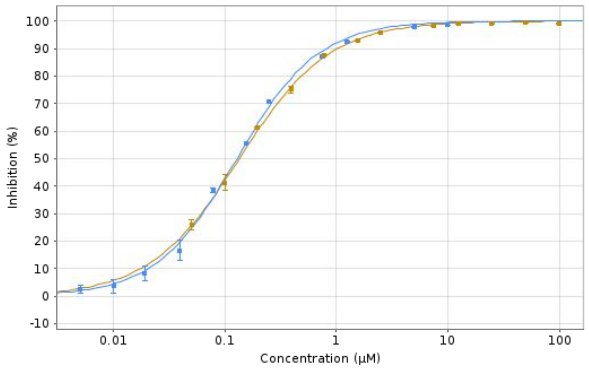
https://doi.org/10.1021/jacs.1c08402
"The Carlsson Compound"



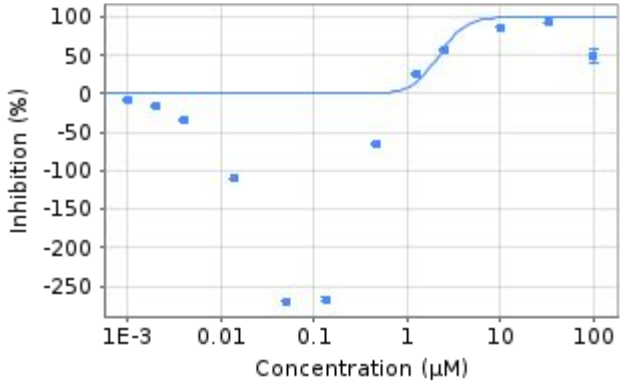


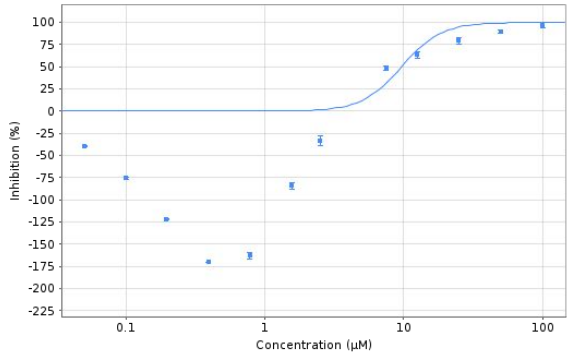
SARS-Cov2 Mpro





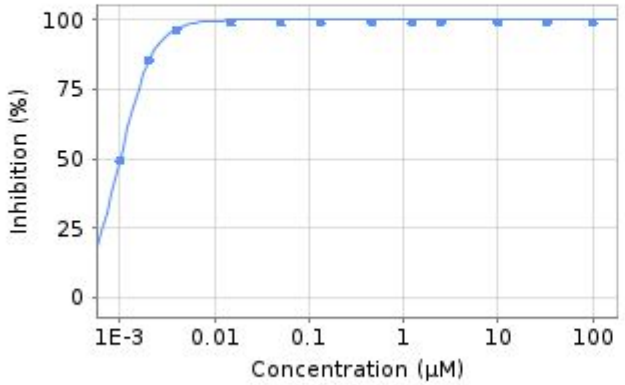
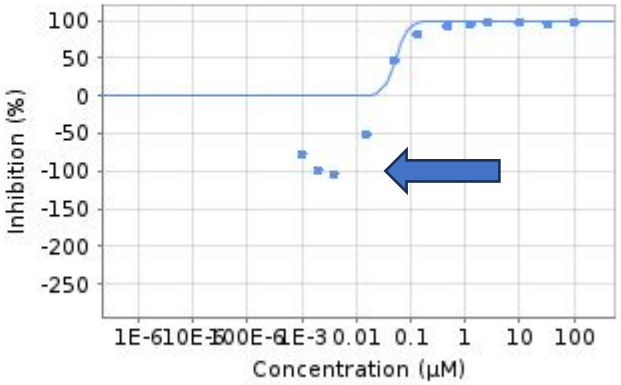
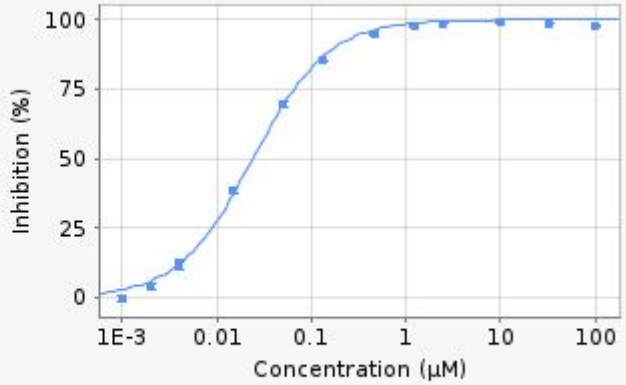
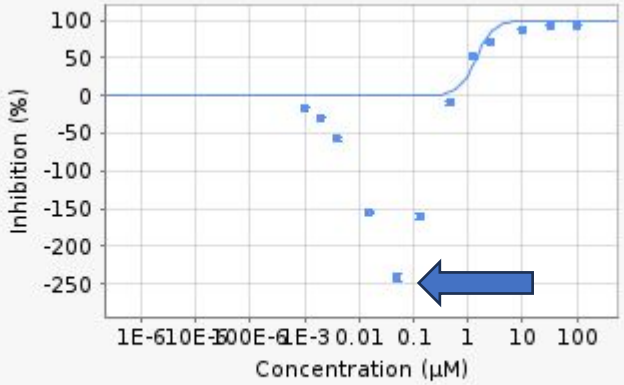
MERS Mpro



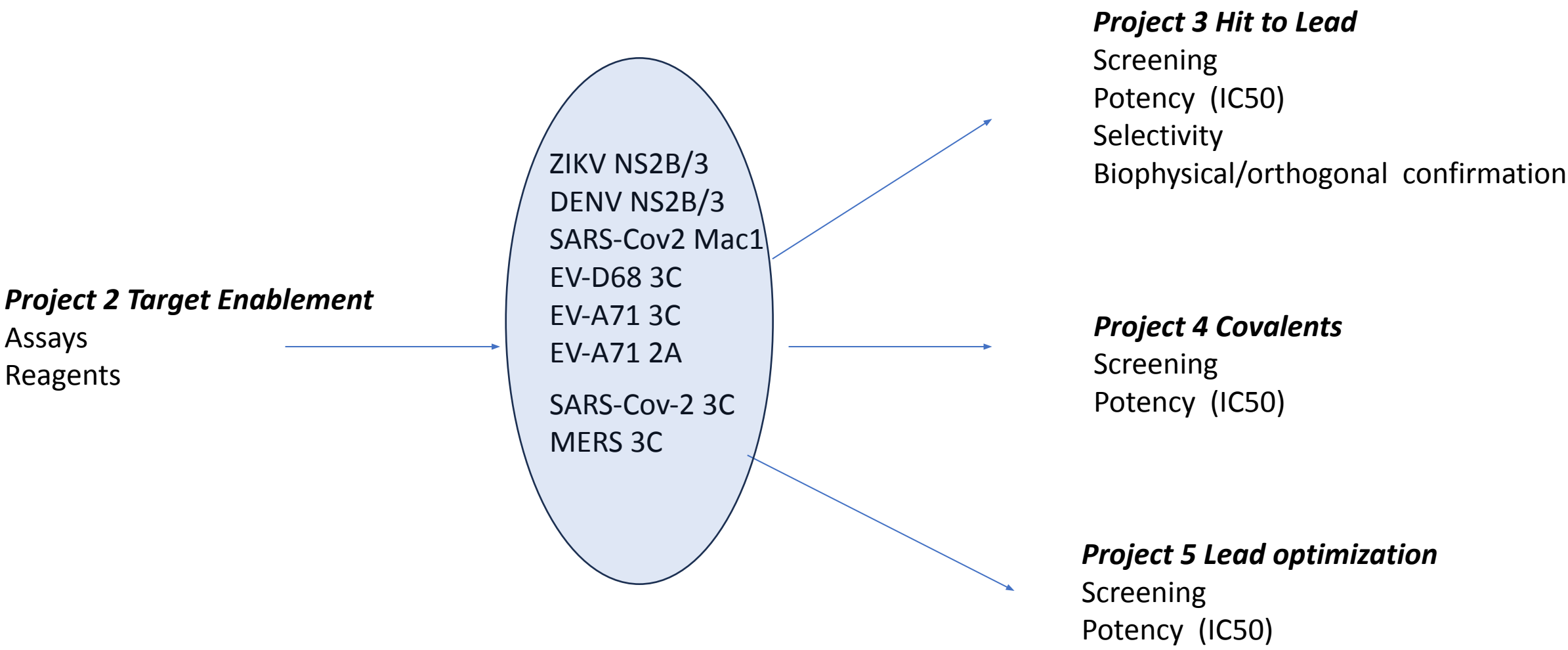


See David Minh's Open-Science Talk
https://www.youtube.com/watch?v=0o8QrKIQKk4

Activation is less prominent with potent compounds

Molecule	SARS-CoV-2-MPro_fluorescence-dose-response_weizmann Plot Concentration - Inhibition	MERS-CoV-MPro_fluorescence-dose-response_weizmann Plot Concentration - Inhibition
ASAP-0027675 ASAP Discovery Vault	 Flag outliers & Override	 Flag outliers & Override
ASAP-0027690 ASAP Discovery Vault	 Flag outliers & Override	 Flag outliers & Override

We assume the activation is compound-induced transitioning from inactive monomer to active dimer



Biochemical core

- Turn working assay in screen-able assays with a priority on reproducibility
- Deliver data (and sometimes compounds) to Project clients

Projects/Assays in Biochemical Core

ASAP
Accessible Antivirals to Prevent Pandemics

Family	Virus	Target	Function	Assays	Stage
Corona	SARS-Cov-2	Mpro	Protease	Fluorogenic, covalent MS	Production
Corona	MERS-Cov	Mpro	Protease	Fluorogenic, covalent MS	Production
Corona	SARS-Cov-2	Nsp3 Mac1	ADPr hydrolase	Binding, Thermal shift, hydrolase	Production
Corona	SARS-Cov-2	N protein	Nucleocapsid	VLP	Assay transfer
Corona	SARS-Cov-2	Nsp13	Helicase	Fluorogenic (ss-DNA capture)	Development finished
Flavi	DENV-2	NS2B/3	Protease	Fluorogenic (Bienta site)	Production
Flavi	DENV-2/ZIKV	NS2B/3	Protease	Fluorogenic, Thermal shift	Production
Flavi	DENV-1/3/4/WNV	NS2B/3	Protease	Fluorogenic	Production
Picorna	EV-A71	3C	Protease	Fluorogenic (Bienta site)	Production
Picorna	EV-D68/A71	3C	Protease	Fluorogenic, Thermal shift, LC-MS	Production
Picorna	EV-D68	3C	Protease	SPR	Production
Picorna	EV-A71	2A	Protease	Fluorogenic	Production

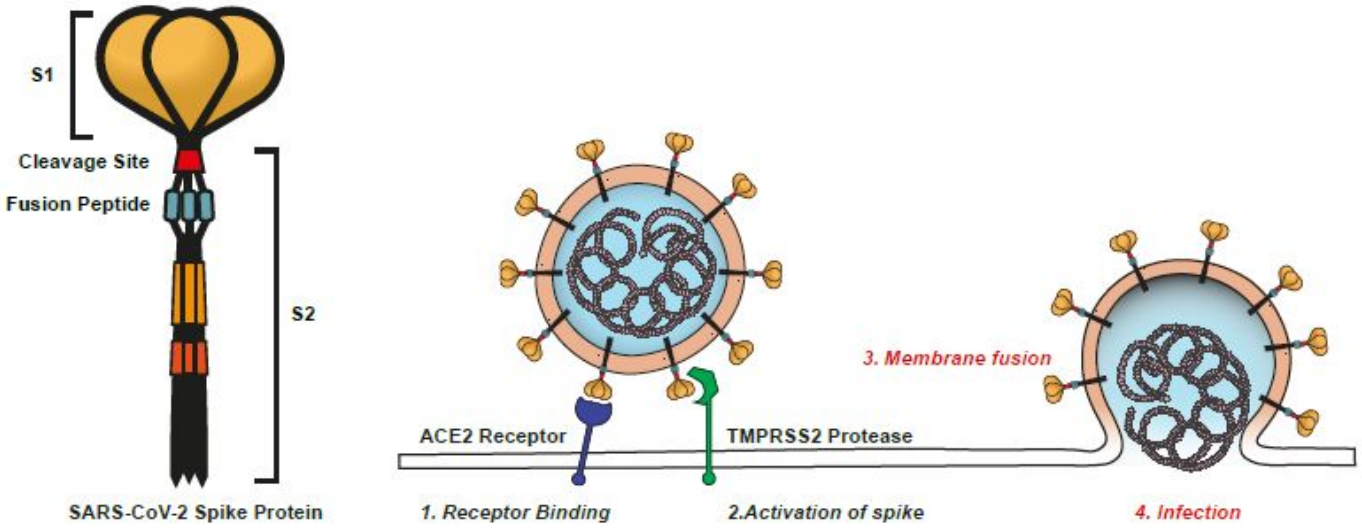
Dr. Noga Kozer
Drug Discovery Unit
The Weizmann Institute of Science

Dr. Khriesto Shurrush
Medicinal Chemistry Unit
The Weizmann Institute of Science

Fusion-inhibitor screening

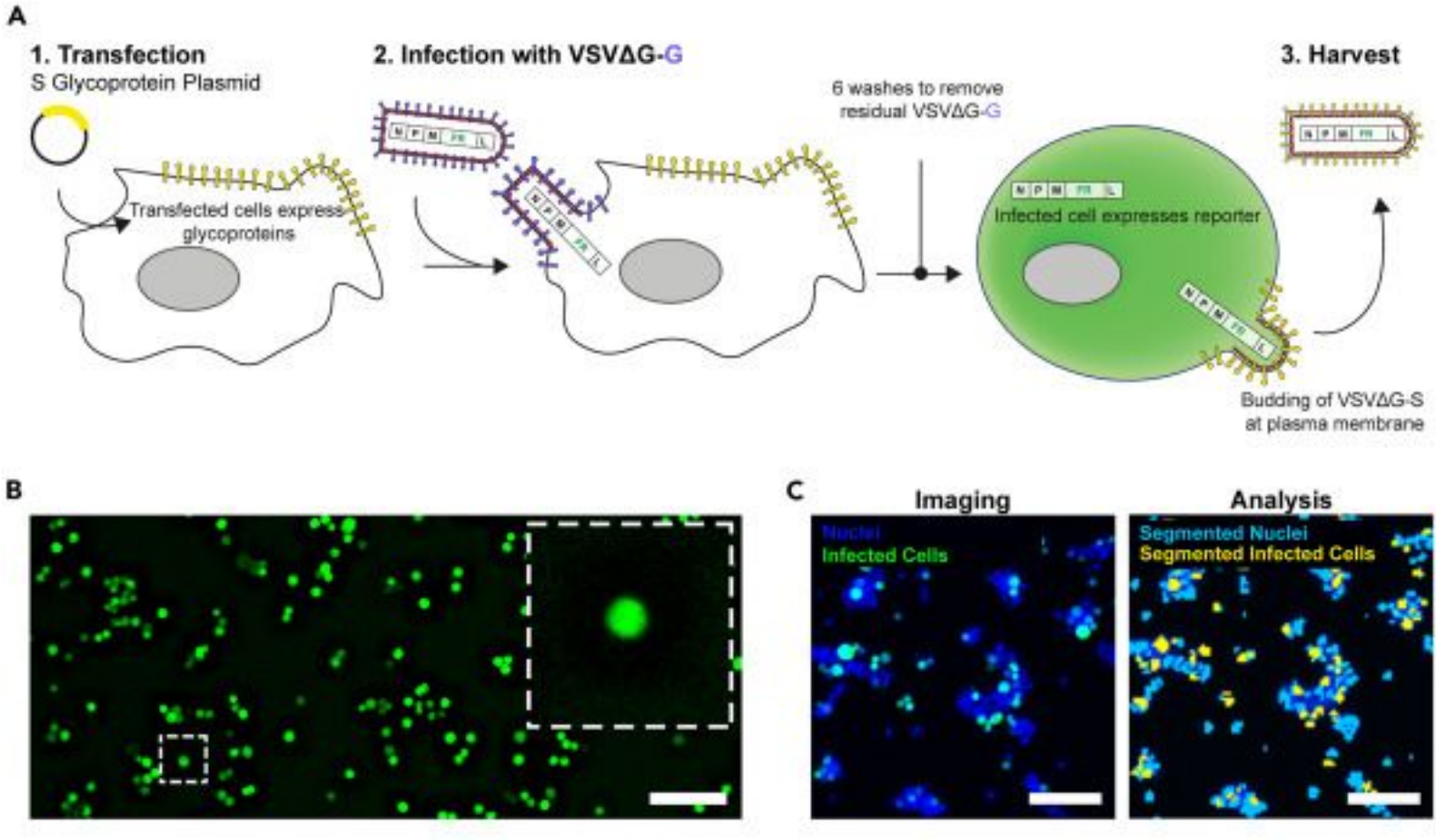
Dr. Ori Avinoam
Department of Biomolecular Science
The Weizmann Institute of Science

Dr. Suman Khan
Department of Biomolecular Science
The Weizmann Institute of Science

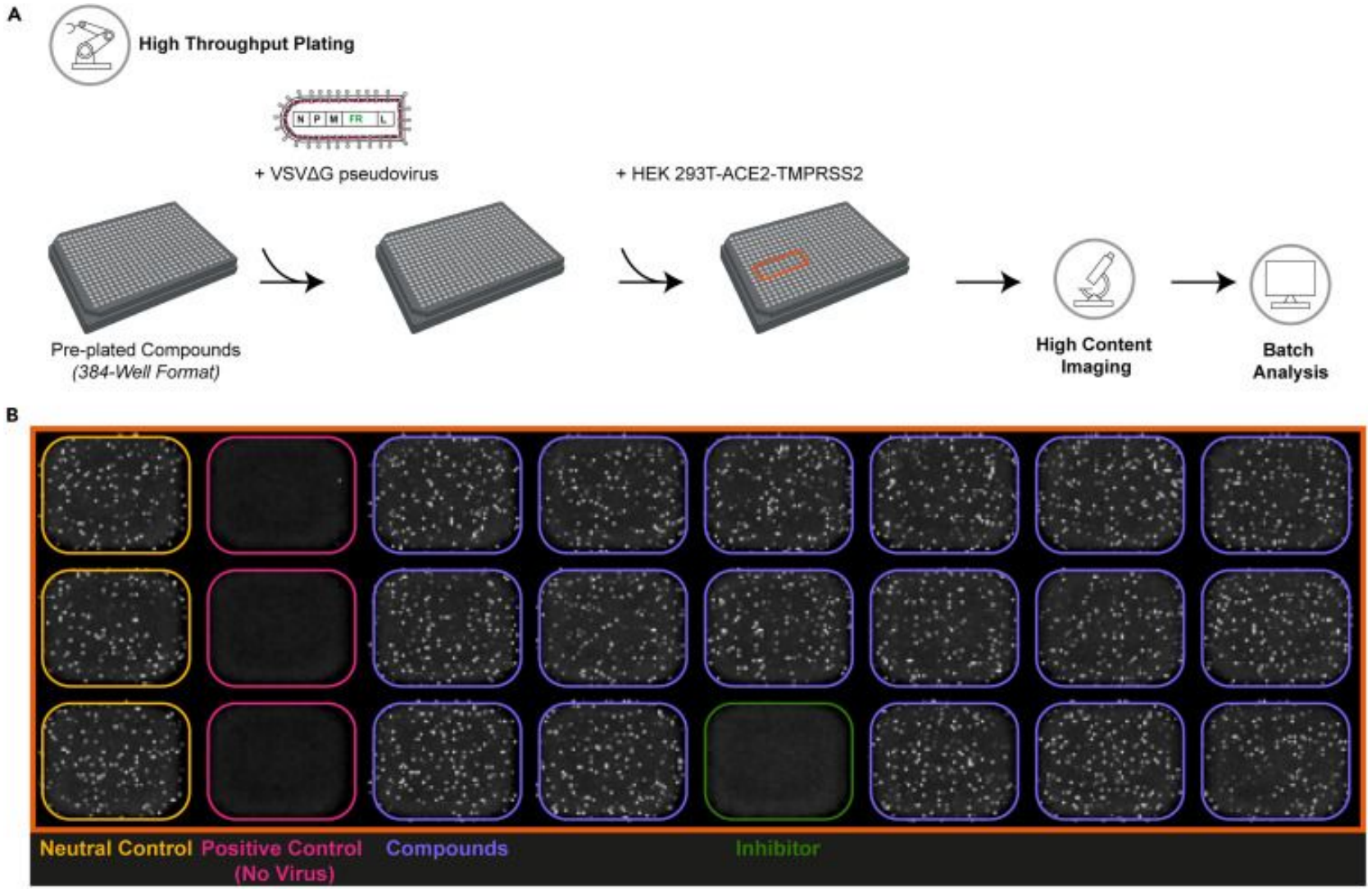


- Target this event with small molecules
- Screen in a safe manner (not with hazardous virus)

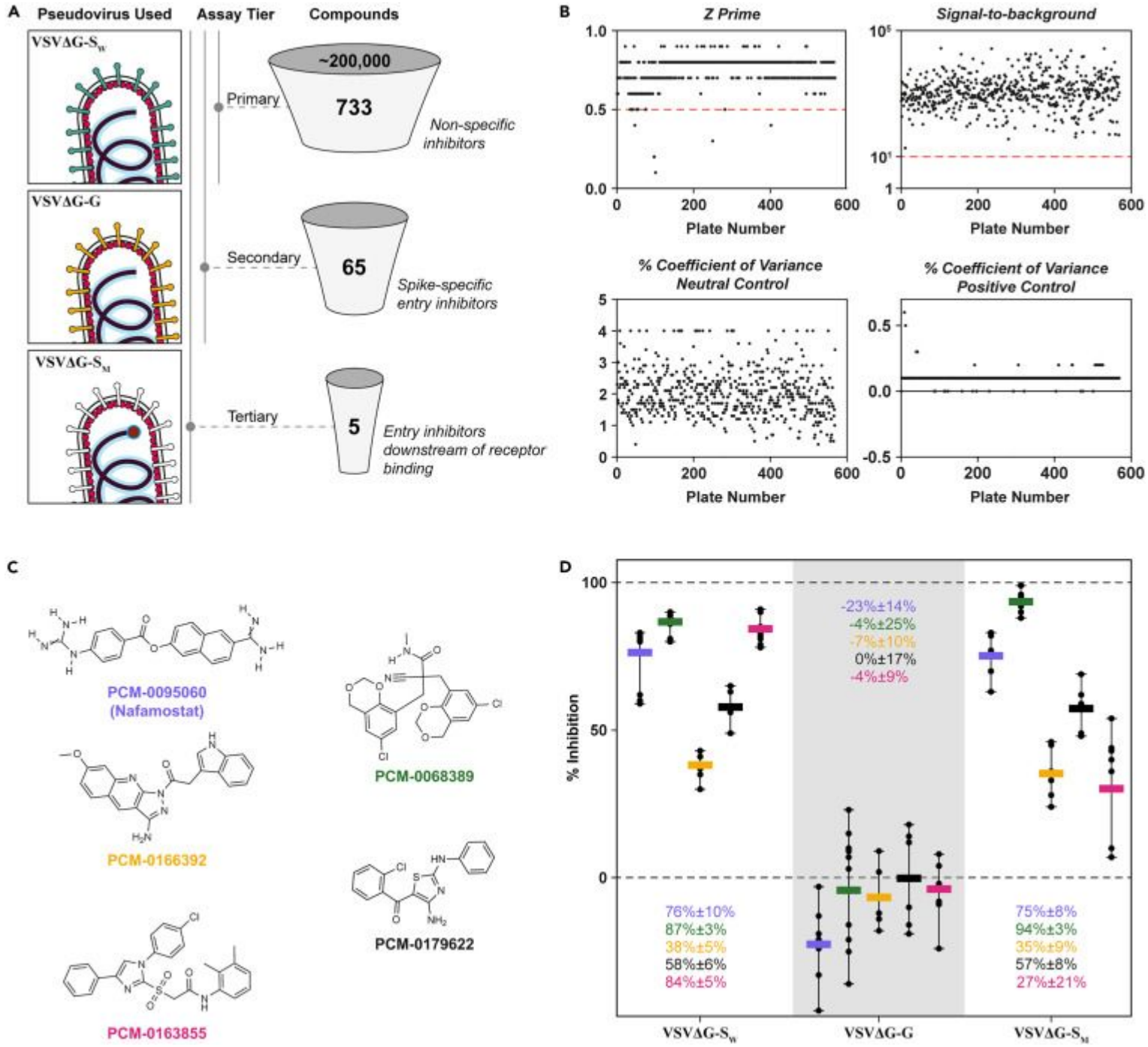
Automated microscopy-based screening assay

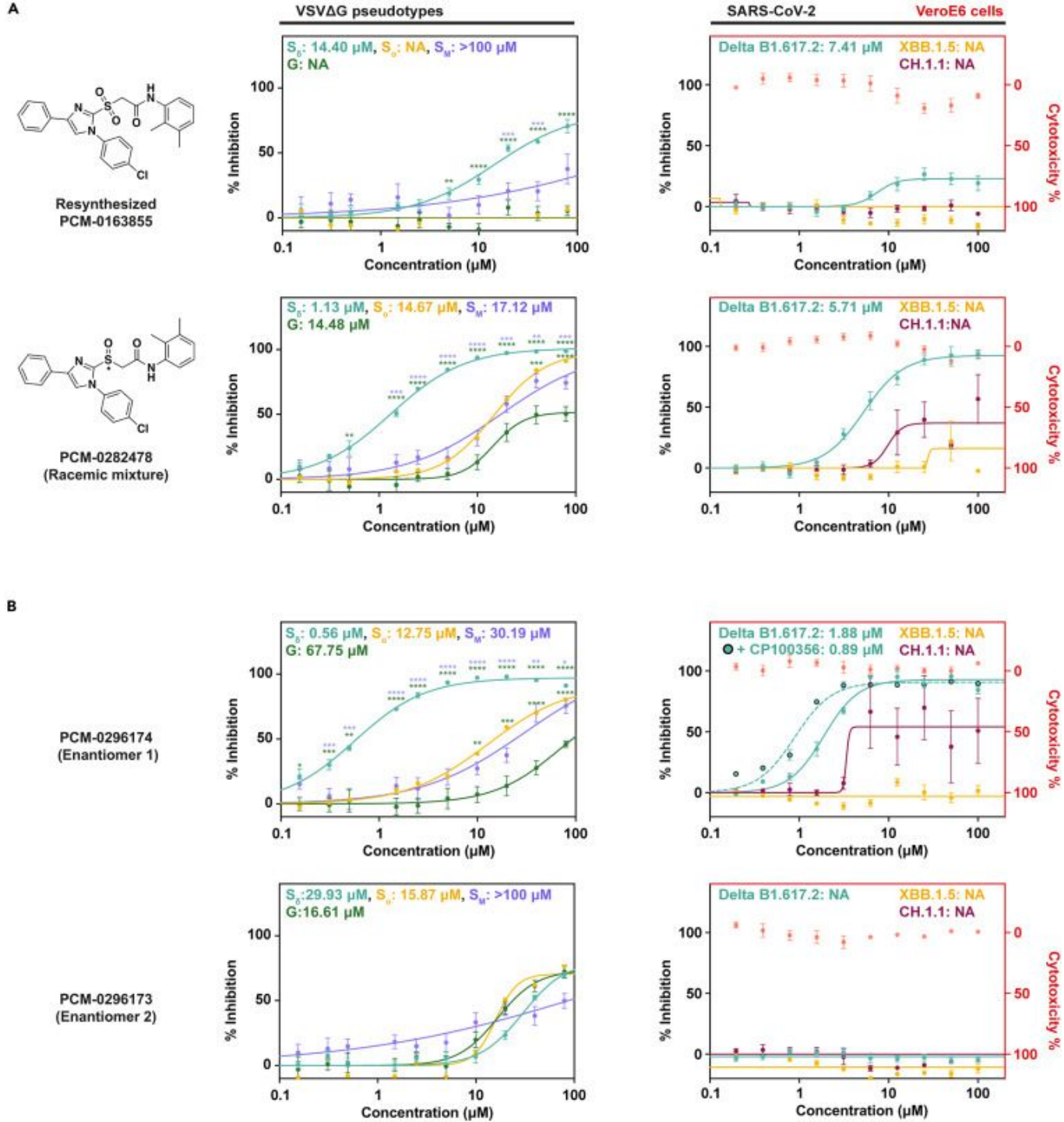


Khan et al, iScience. 2024 May 17;27(6):110019



Khan et al, iScience. 2024 May 17;27(6):110019


A
Pseudovirus Used
Assay Tier
Compounds
VSVΔG-S$_W$
VSVΔG-G
VSVΔG-S$_M$
Primary
~200,000
733
Non-specific inhibitors
Secondary
65
Spike-specific entry inhibitors
Tertiary
5
Entry inhibitors downstream of receptor binding
B
Z Prime
Signal-to-background
% Coefficient of Variance Neutral Control
% Coefficient of Variance Positive Control
Plate Number
C
PCM-0095060 (Nafamostat)
PCM-0068389
PCM-0166392
PCM-0179622
PCM-0163855
D
% Inhibition
-23%±14%
-4%±25%
-7%±10%
0%±17%
-4%±9%
76%±10%
87%±3%
38%±5%
58%±6%
84%±5%
75%±8%
94%±3%
35%±9%
57%±8%
27%±21%
VSVΔG-S$_W$
VSVΔG-G
VSVΔG-S$_M$


A
VSVΔG pseudotypes
SARS-CoV-2
VeroE6 cells
Resynthesized
PCM-0163855
S0: 14.40 μM, SΩ: NA, SM: >100 μM
G: NA
Delta B1.617.2: 7.41 μM
XBB.1.5: NA
CH.1.1: NA
PCM-0282478
(Racemic mixture)
S0: 1.13 μM, SΩ: 14.67 μM, SM: 17.12 μM
G: 14.48 μM
Delta B1.617.2: 5.71 μM
XBB.1.5: NA
CH.1.1:NA
% Inhibition
Cytotoxicity %
Concentration (μM)
B
PCM-0296174
(Enantiomer 1)
S0: 0.56 μM, SΩ: 12.75 μM, SM: 30.19 μM
G: 67.75 μM
Delta B1.617.2: 1.88 μM
+ CP100356: 0.89 μM
XBB.1.5: NA
CH.1.1: NA
PCM-0296173
(Enantiomer 2)
S0:29.93 μM, SΩ: 15.87 μM, SM: >100 μM
G:16.61 μM
Delta B1.617.2: NA
XBB.1.5: NA
CH.1.1: NA

Summary

- Leveraging HTS infrastructure for iterative screening in Moonshot > ASAP consortia enables rapid turnaround and dissemination of data
- Running multiple targets in parallel
- Example of phenotypic screening for fusion inhibitors which translates from pseudovirus to live virus
- Importance of medicinal chemistry linked with screening platform to mitigate risk

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