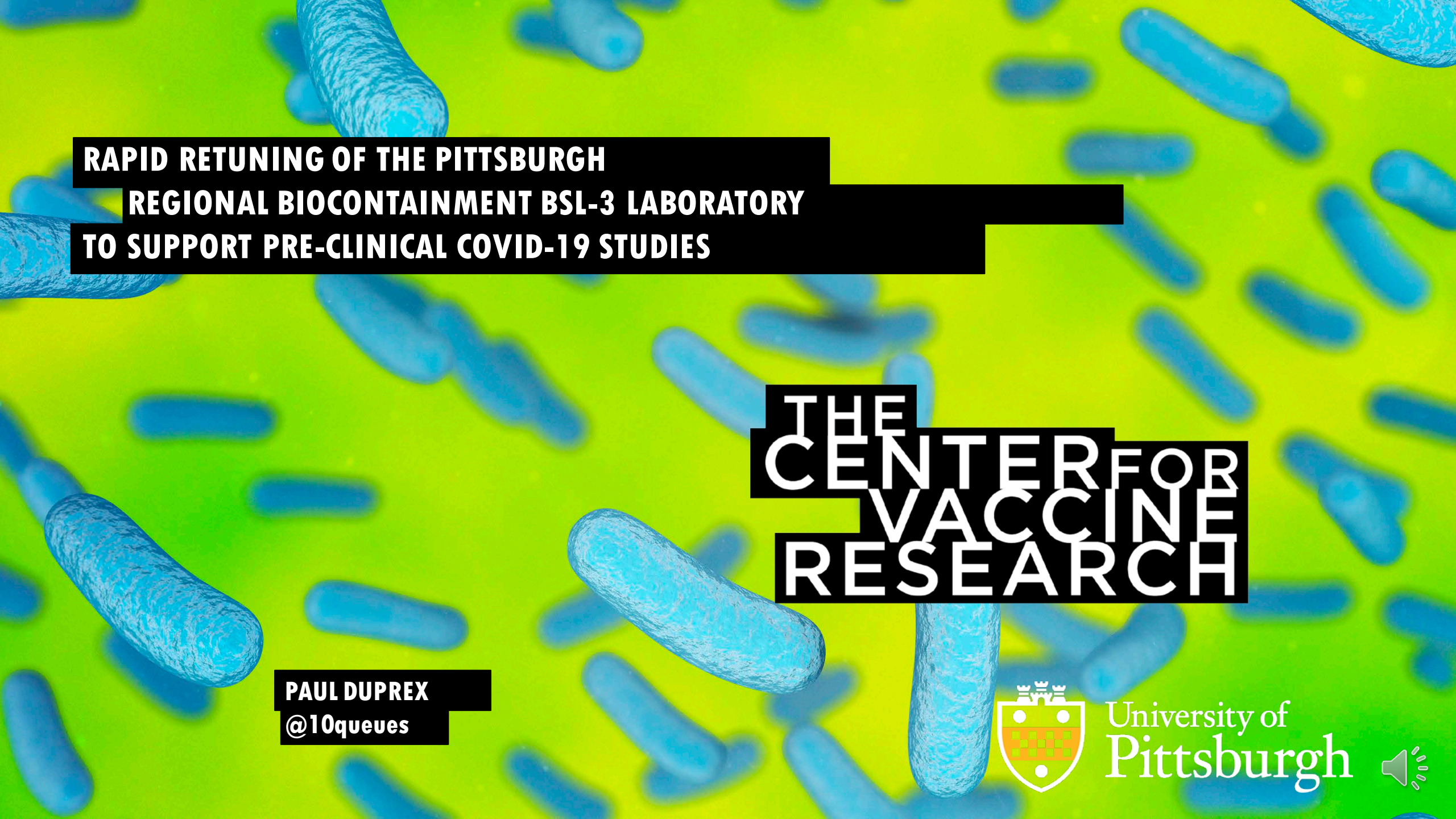
RAPID RETUNING OF THE PITTSBURGH REGIONAL BIOCONTAINMENT BSL-3 LABORATORY TO SUPPORT PRE-CLINICAL COVID-19 STUDIES

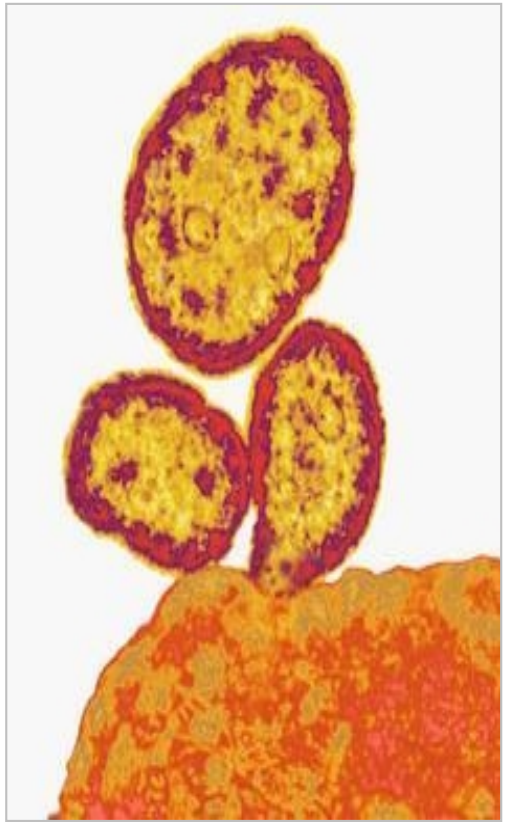


PAUL DUPREX
@10queues

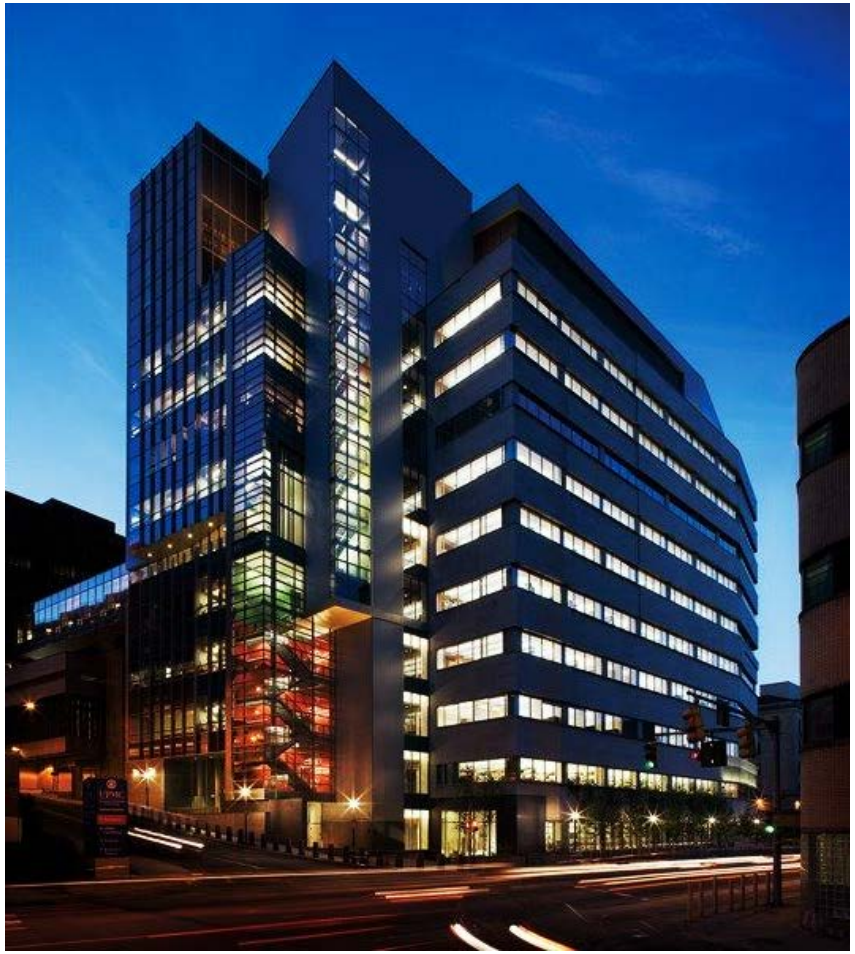
University of Pittsburgh

outline

- *… introducing The Center for Vaccine Research*
  - foundations
  - facilities
  - focus
  - faculty
- *… responding to SARS-CoV-2 emergence*
  - *in vitro studies*
  - *in vivo studies*
- *… creativity drives a diversity of interventions*
  - vaccines
  - antibodies and nanobodies
  - variants



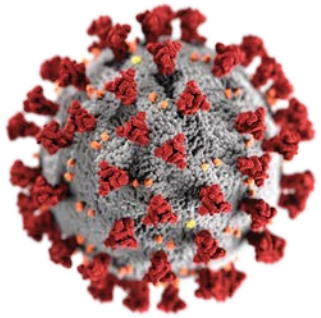
The Center for Vaccine Research



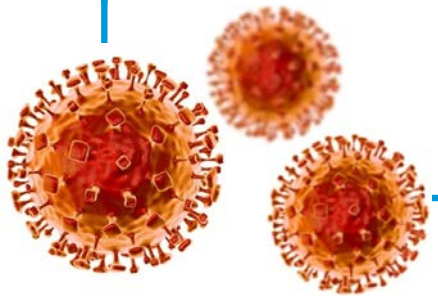
The Center for Vaccine Research

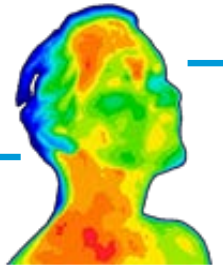
the foundations of CVR …

creative approaches to mitigate infectious disease

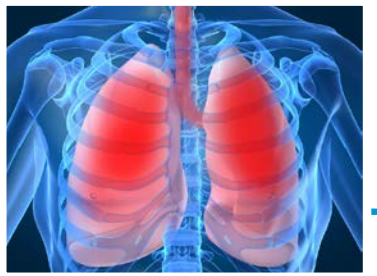


EMERGING VIRUSES





DISEASE



INTERVENTIONS

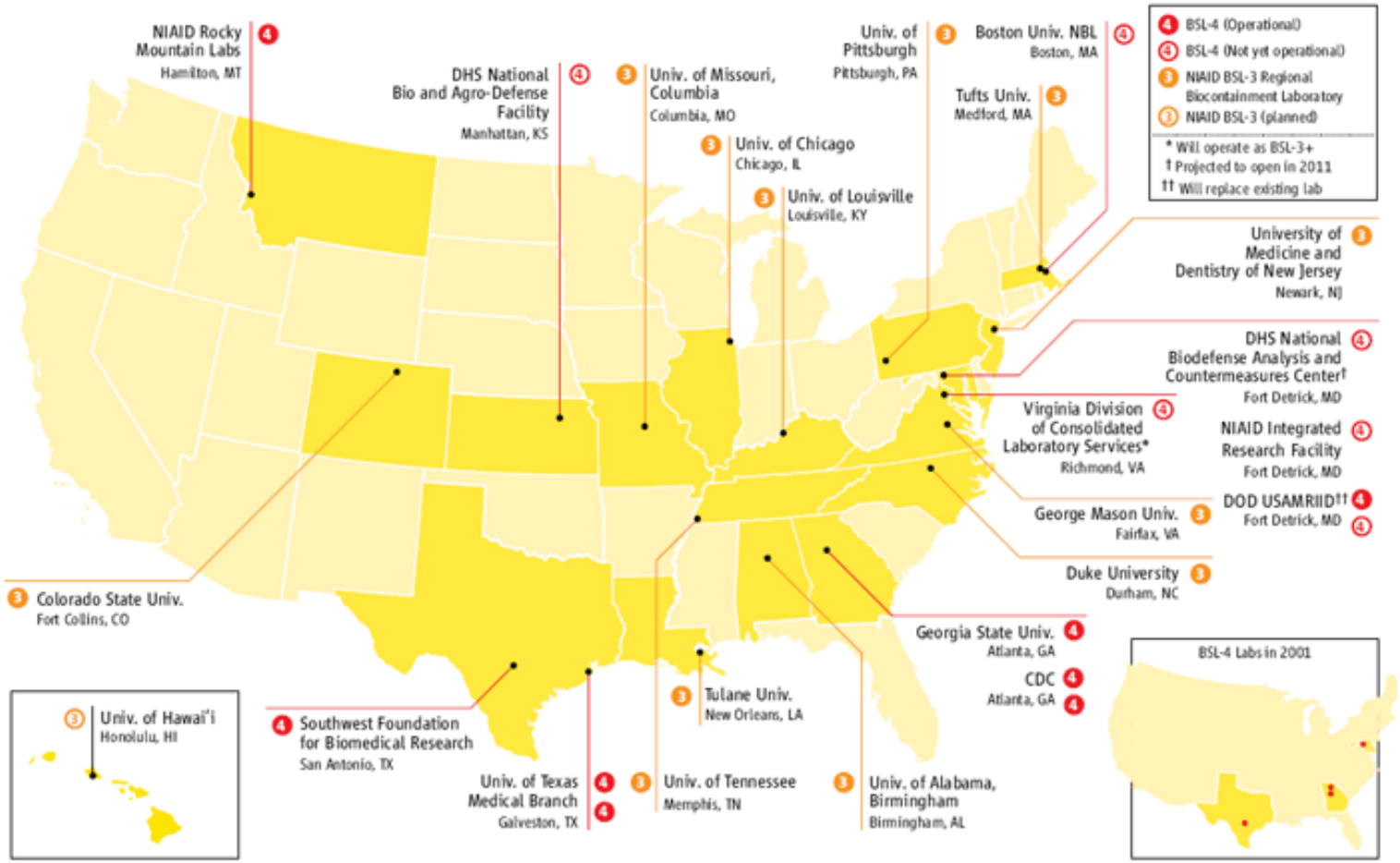
Office of Biodefense Research Resources and Translational Research

National Biocontainment Laboratories (NBL) and Regional Biocontainment Laboratories (RBL)

National Institute of Allergy and Infectious Diseases

Homeland Security

Department of Defense



Regional Biocontainment Laboratory (RBL) at Pittsburgh

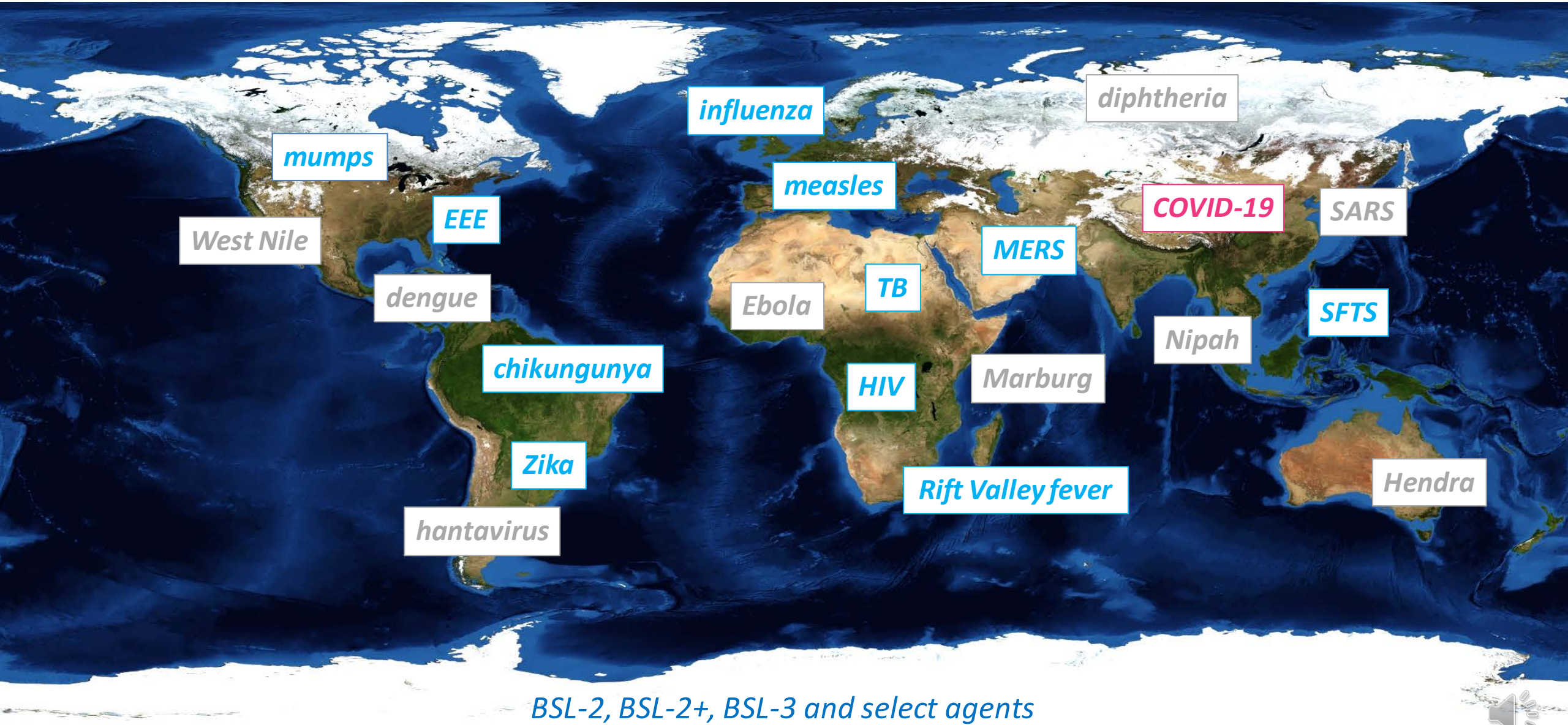
from cells … to tissues … to animal models of disease

understanding respiratory infection and testing interventions

from cells … to tissues … to animal models of disease

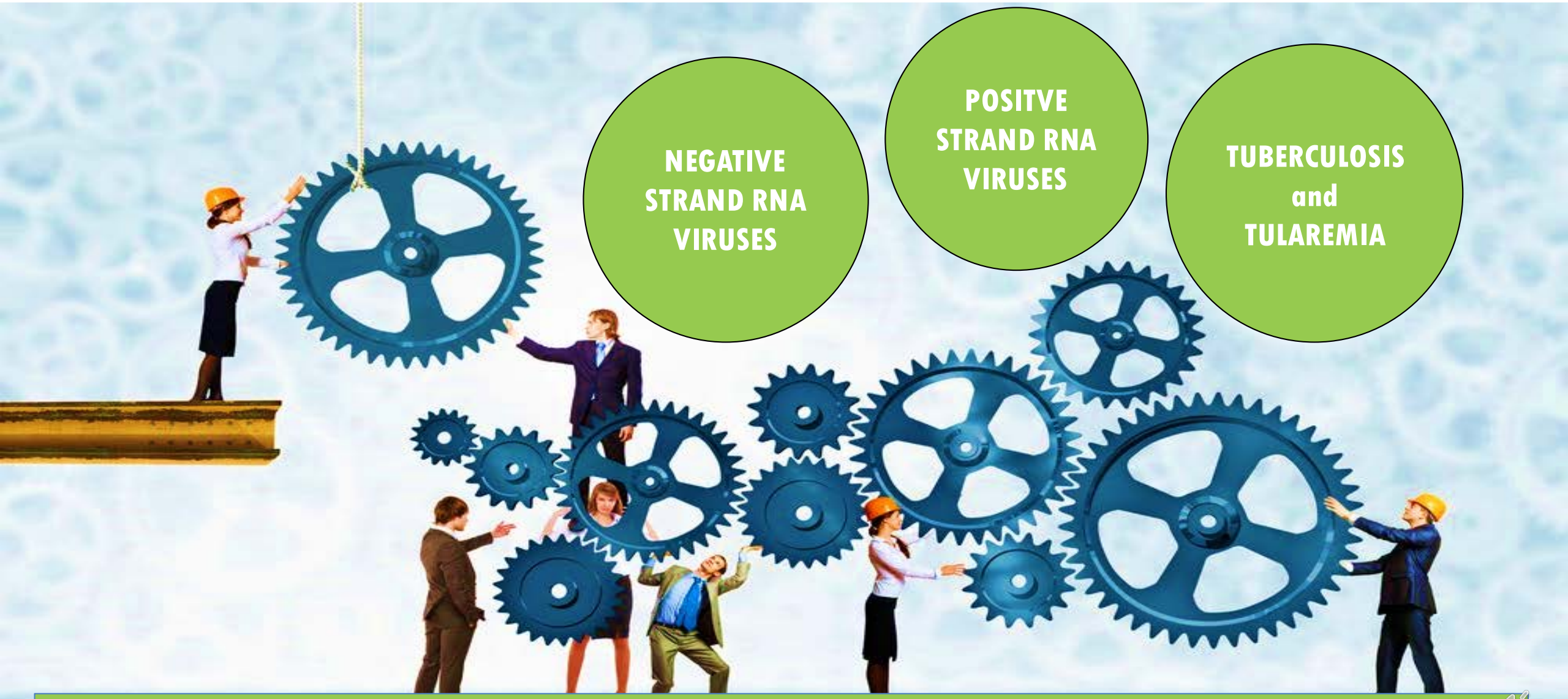
state-of-the-art capabilities for inhalation of pathogenic agents and imaging infections temporally

emerging and re-emerging infectious diseases



BSL-2, BSL-2+, BSL-3 and select agents

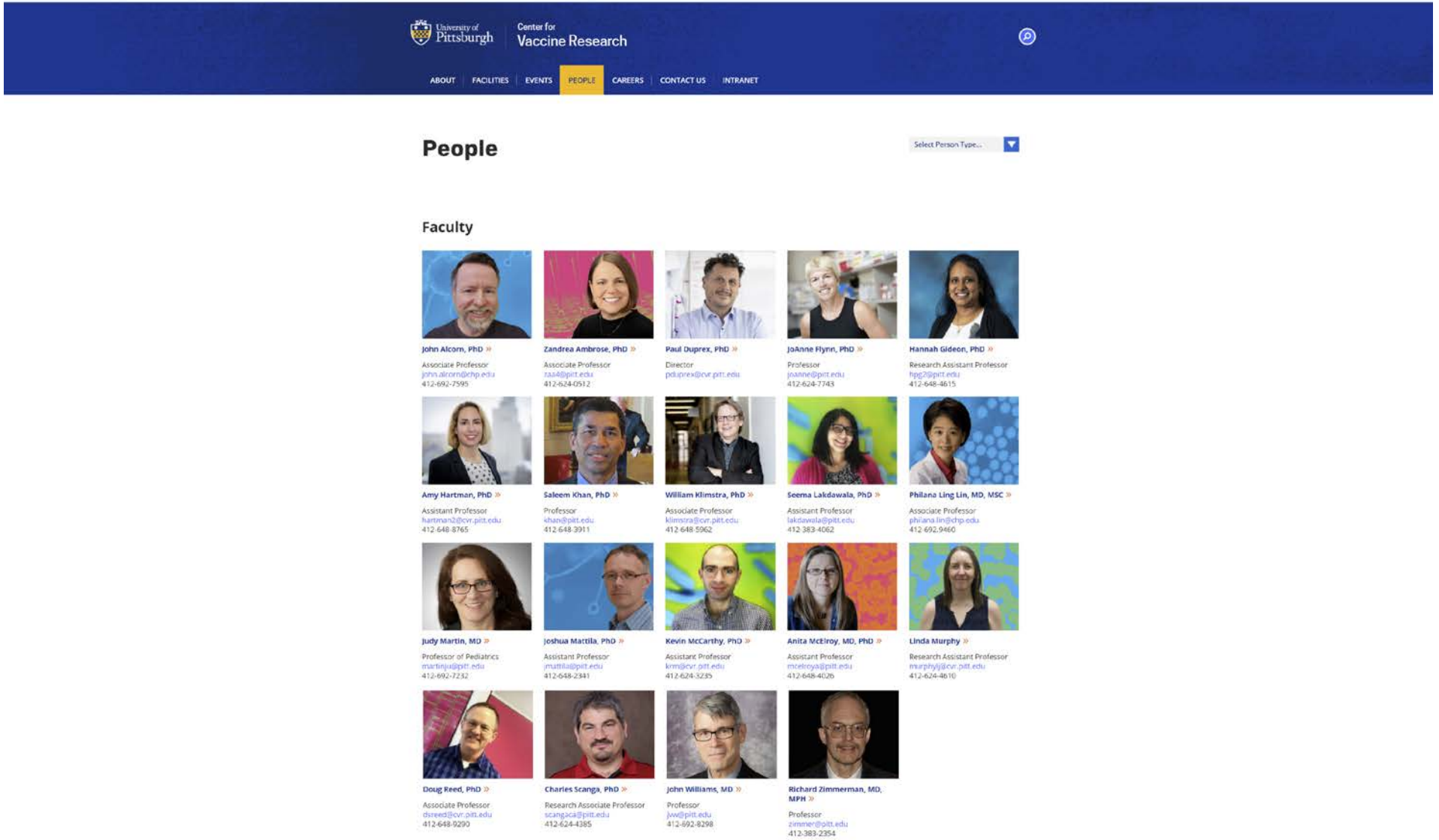
a hub for host-pathogen research and pre-clinical intervention development



ANIMAL MODELS OF DISEASE, MULTIMODAL BIOIMAGING, BIOCONTAINMENT

a hub for host-pathogen research and pre-clinical intervention development

Center for Vaccine Research



NIAID vaccine and treatment evaluation units (VTEUs)

established in 1962 and conducted hundreds of clinical trials, many of which have contributed to vaccine licensure.

state-of-the-art capabilities for inhalation of pathogenic agents and modeling physiological response to disease

Pittsburgh Vaccine Trials Unit

FROM NIH VACCINE TESTING AND EVALUATION UNIT TO …

PITTSBURGH VACCINE TRIALS UNIT

University of Pittsburgh








outline

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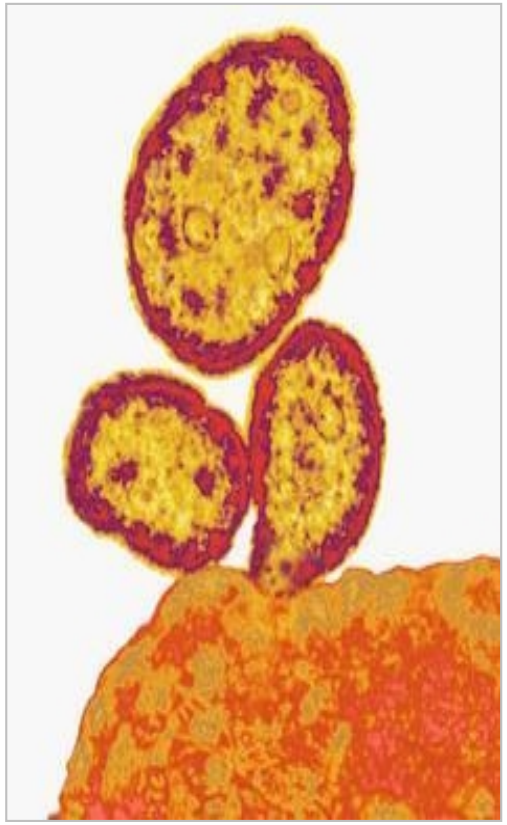
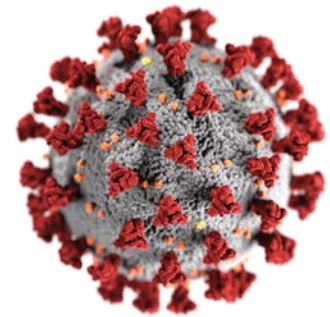


outline

creative approaches to mitigate infectious disease



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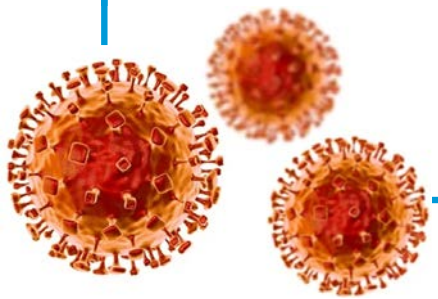

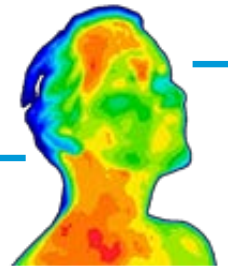

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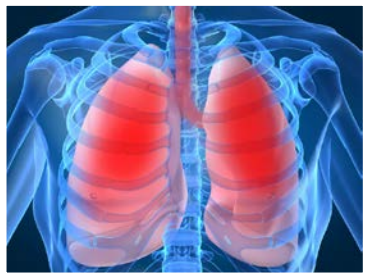
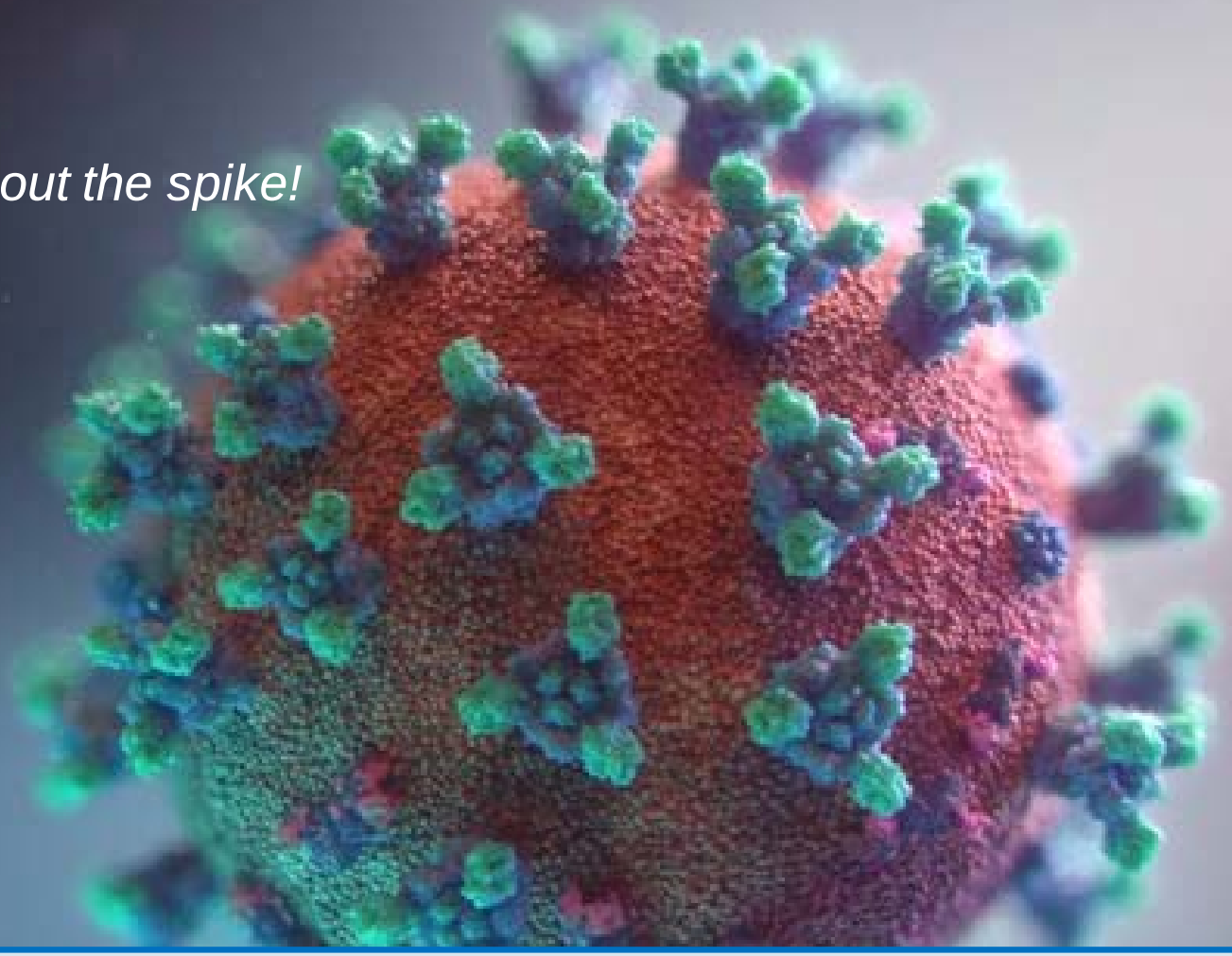
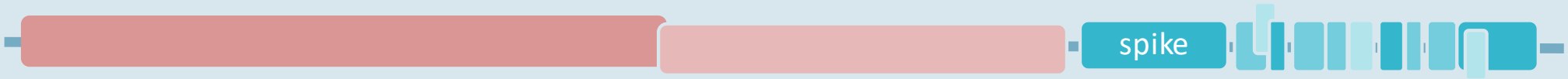


it’s all about the spike!

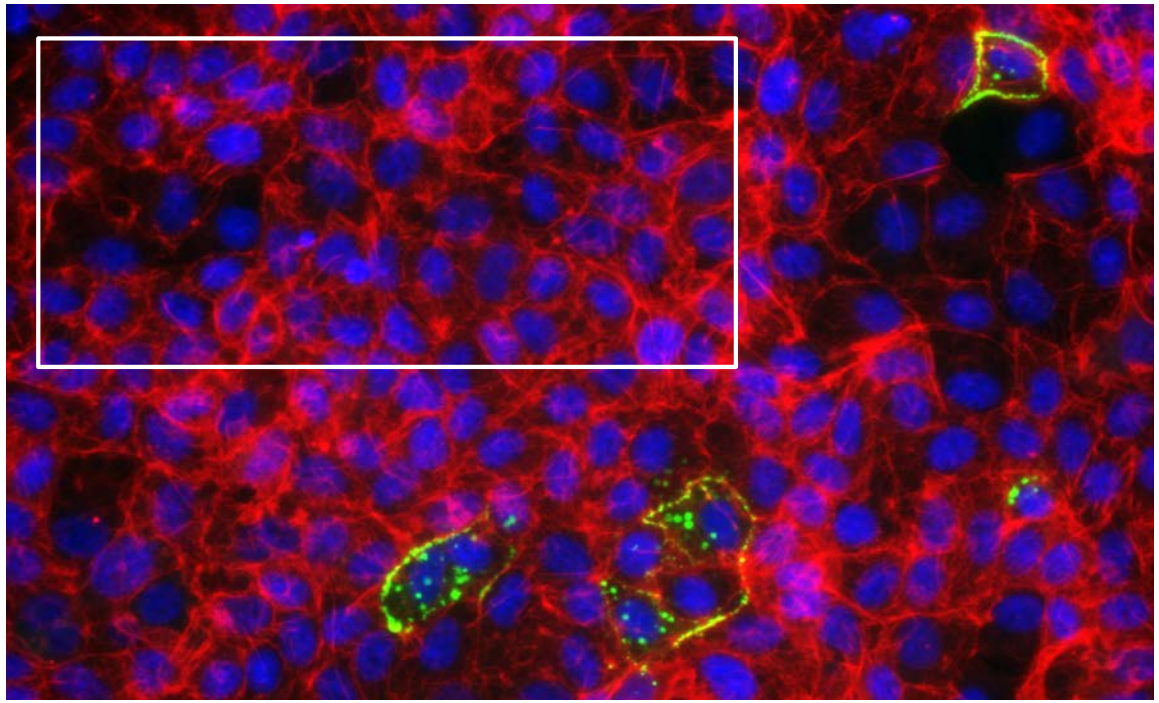


the genome of SARS-CoV-2



SARS-CoV-2

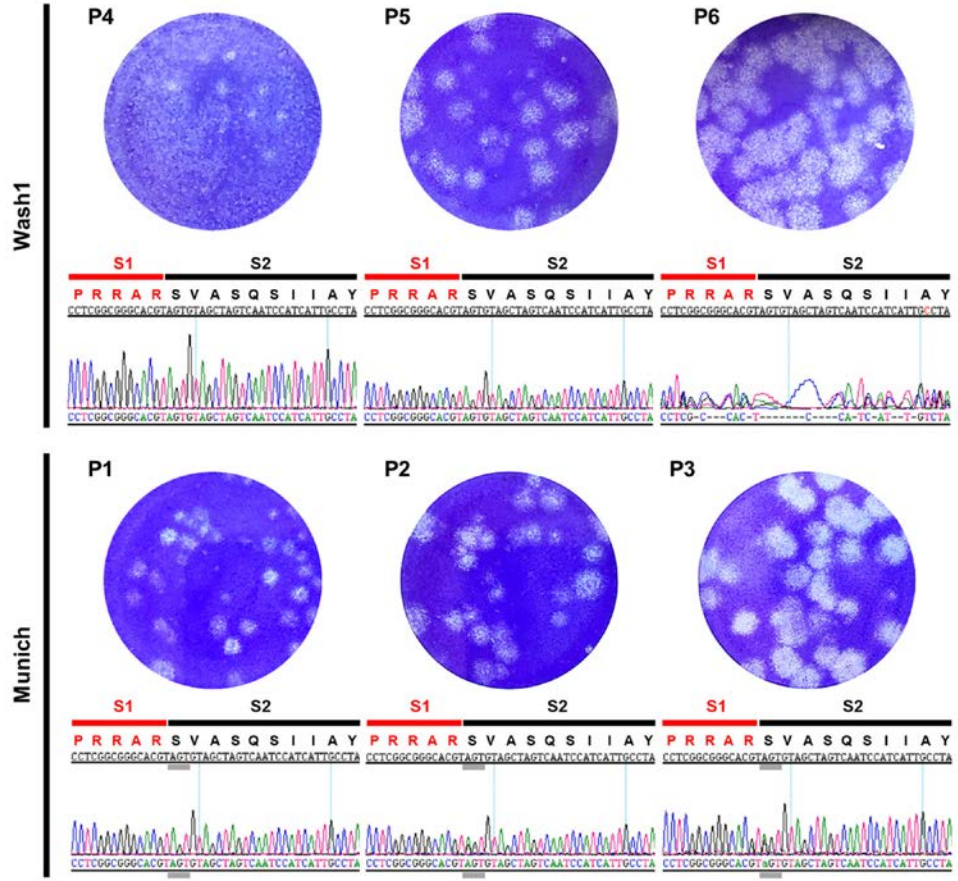
culturing coronavirus … from cells … to tissues … to animal models of disease

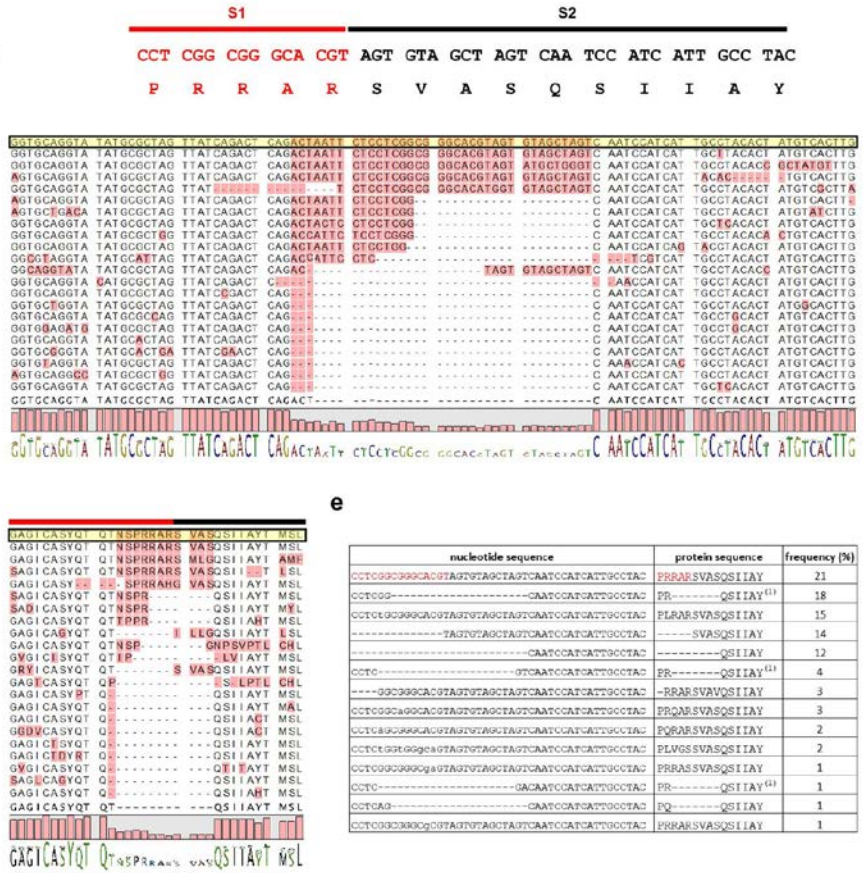


Klimstra et al., 2020 JGV

SARS-CoV-2

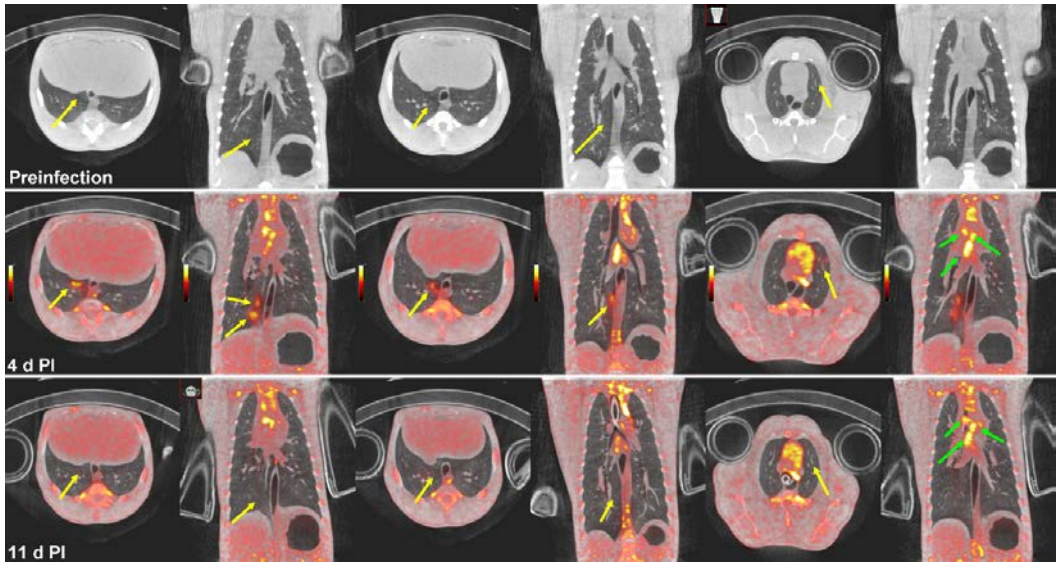
an evolving pathogen: the furin cleavage site

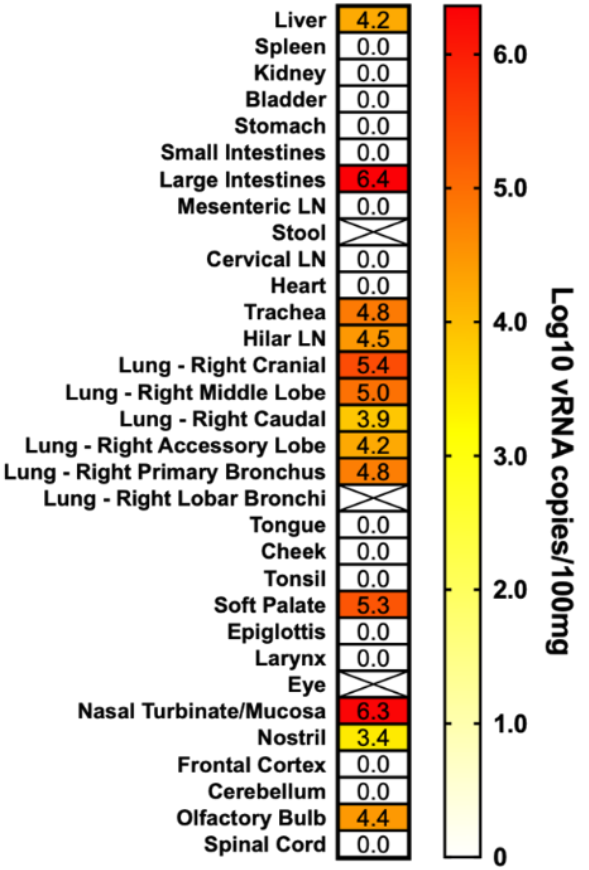




Klimstra et al., 2020 JGV

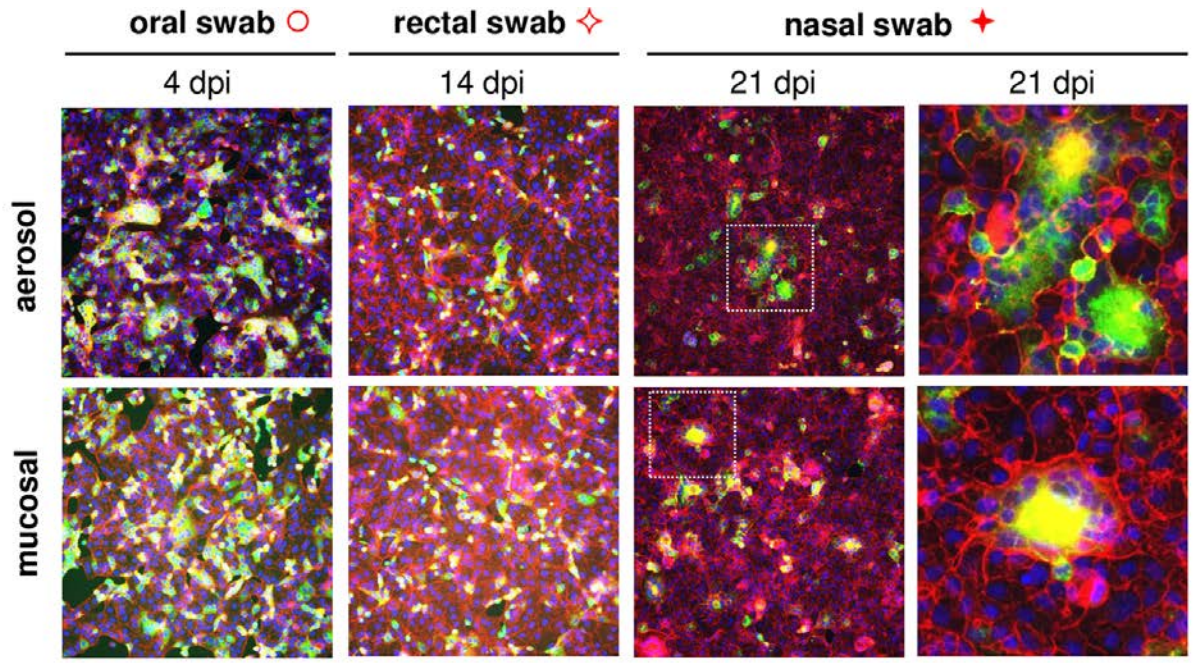
pre-clinical capabilities in CVR





Hartman et al., 2020 PLoS Pathogens

virus isolation from swabs



	2 dpi				4 dpi				7 dpi				11 dpi				14 dpi				21 dpi				28 dpi				35 dpi			
#	O	N	C	R	O	N	C	R	O	N	C	R	O	N	C	R	O	N	C	R	O	N	C	R	O	N	C	R	O	N	C	R
A1	>50	11			3	2			3																							
A2	>50	>50		2	12	>50			2											14												
A3	>50	>50	2	5																												
A4	>50	>50			○11	16			20											✧4	12	✦5										
M1	22	>50	6	2	2			2	11												1	✦2										
M2	28	>50		2	○8	25				19										✧2												

	negative
	positive
	not done

O = oral
N = nasal
C = conjunctival
R = rectal

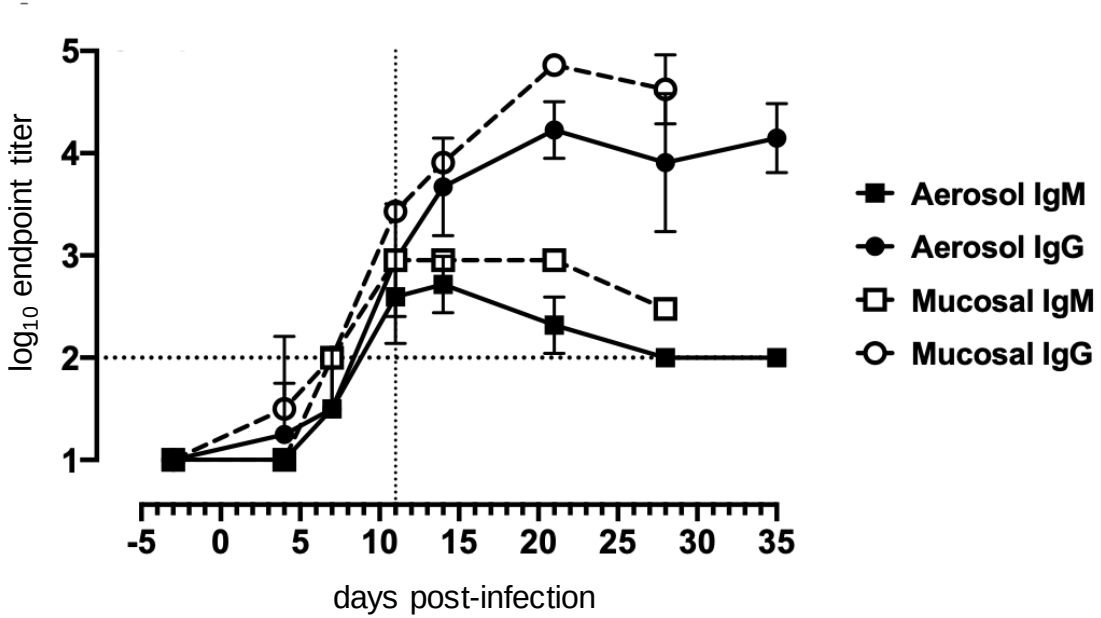
#=plaques per well of isolation

Hartman et al., 2020 PLoS Pathogens

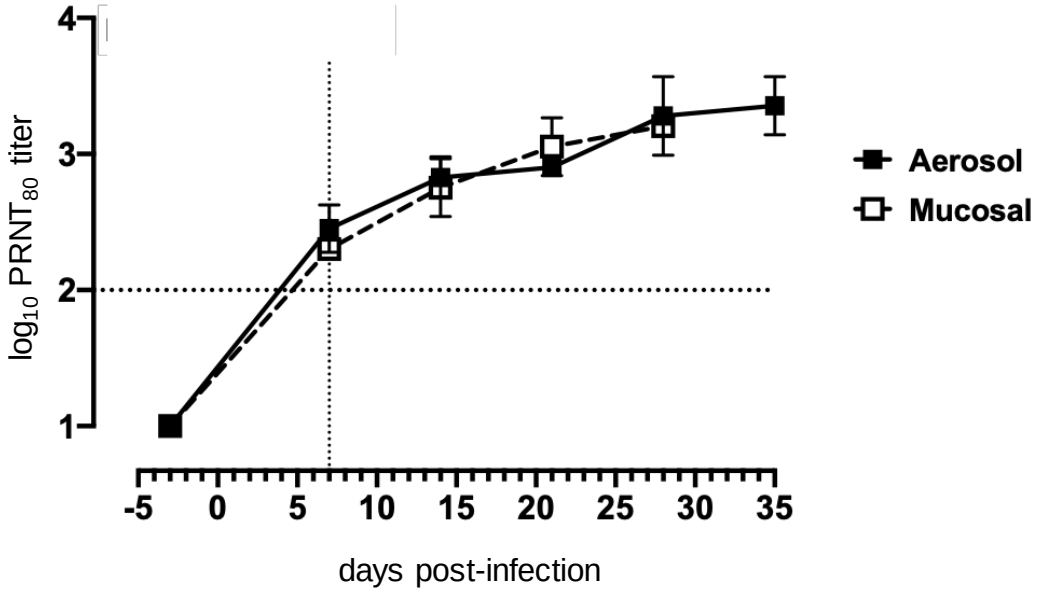
immunological analyses

simultaneous seroconversion for IgM and IgG

serology



neutralization



Hartman et al., 2020 PLoS Pathogens

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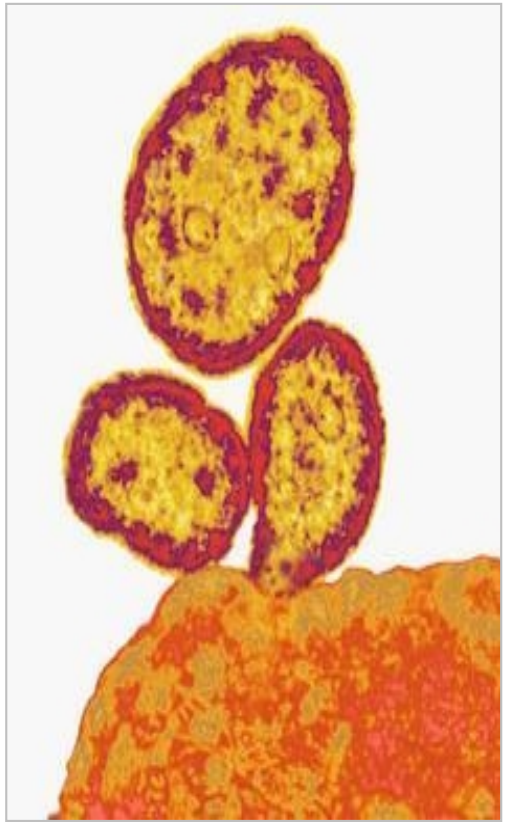
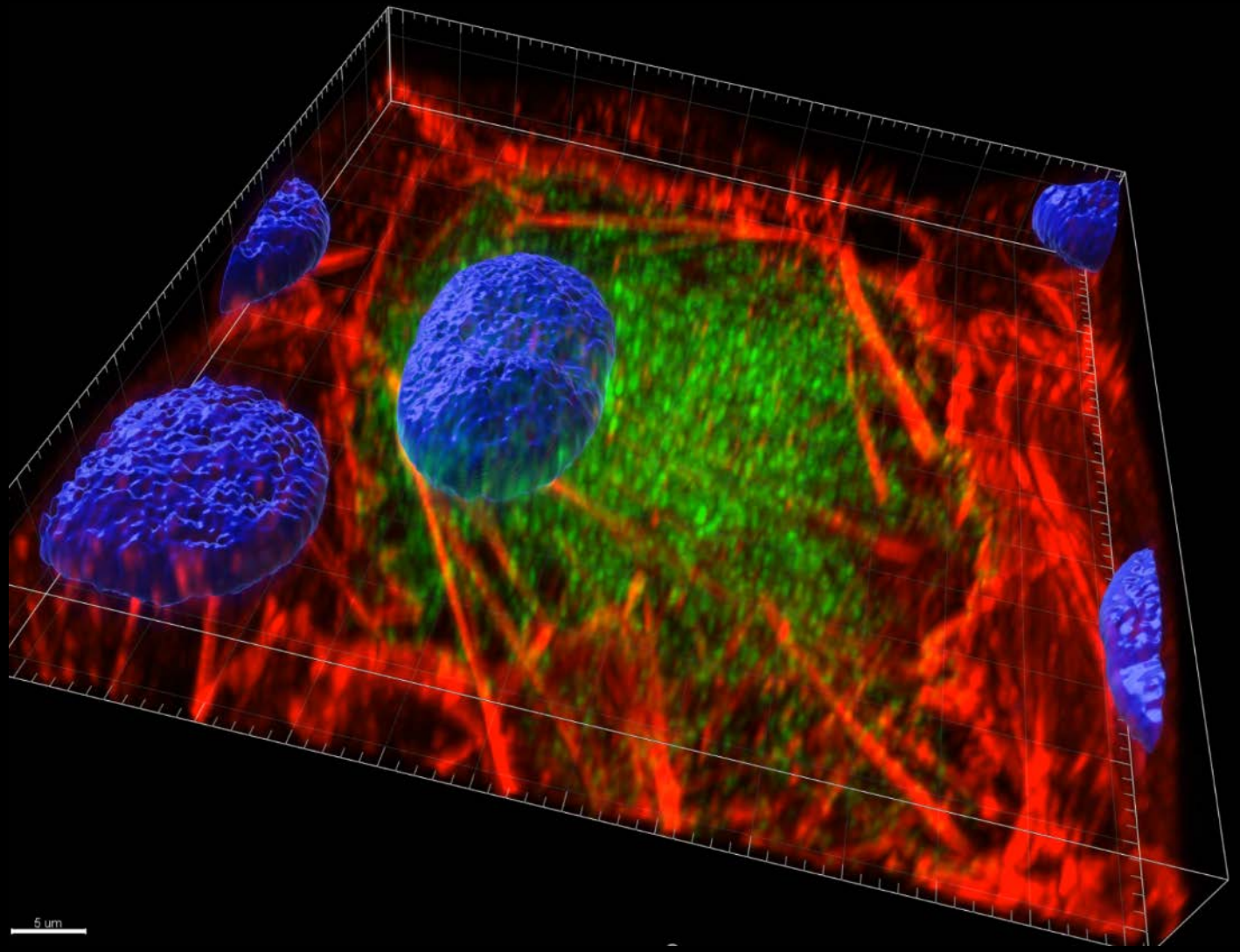


outline

creative approaches to mitigate infectious disease

measles vaccine on the inside … spike on the outside outside …



POONAWALLA GROUP
SERUM INSTITUTE OF INDIA

THE CENTER FOR VACCINE RESEARCH

transchromosomic cow antibodies

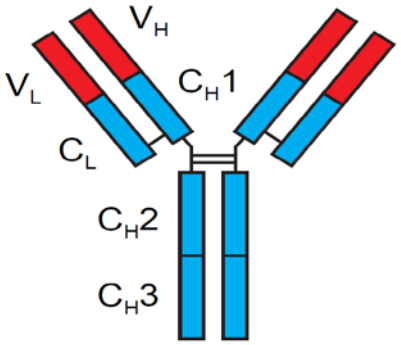
capitalizes on CVR GLP-like Quality Systems (QS) readiness

SAb BIOTHERAPEUTICS

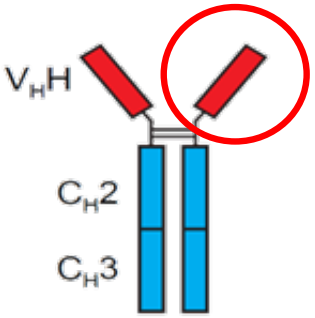
V_HH heavy-chain only antibodies

- minimal V_HH domain for antigen binding
- highly soluble and thermostable
- high sequence similarity with human IgG
- better tissue penetration
- inhalable delivery
- robust and affordable manufacturing

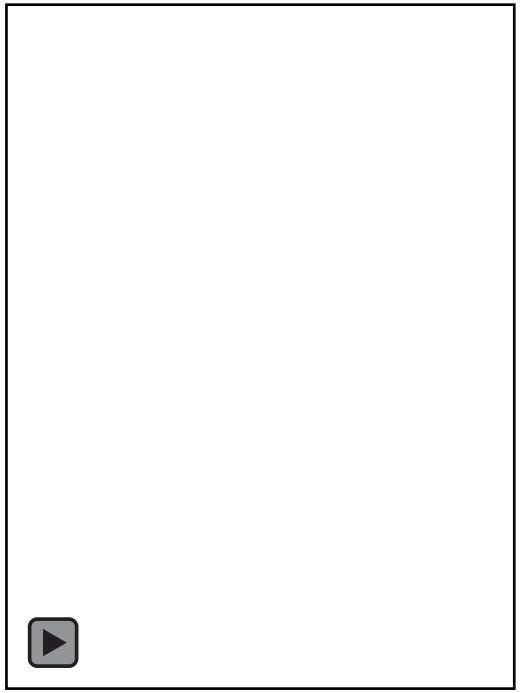
nanobodies



standard IgG



camelid V_HH IgG



nanobody ~15 kDa

V_HH heavy-chain only antibodies

Science

REPORTS

Cite as: Y. Xiang *et al.*, *Science* 10.1126/science.abe4747 (2020).

Versatile and multivalent nanobodies efficiently neutralize SARS-CoV-2

Yufei Xiang[1], Sham Nambulli[2,3]*, Zhengyun Xiao[1]*, Heng Liu[4]*, Zhe Sang[1,5], W. Paul Duprex[2,3], Dina Schneidman-Duhovny[6]†, Cheng Zhang[4]†, Yi Shi[1,5]†

[1]Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA, USA. [2]Center for Vaccine Research, University of Pittsburgh, Pittsburgh, PA, USA. [3]Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA, USA. [4]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA. [5]Pitt/CMU Program for Computational Biology, Pittsburgh, PA, USA. [6]School of Computer Science and Engineering, Institute of Life Sciences, The Hebrew University of Jerusalem, Israel.

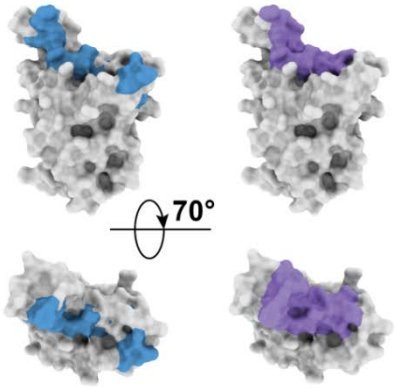
*These authors contributed equally to this work.

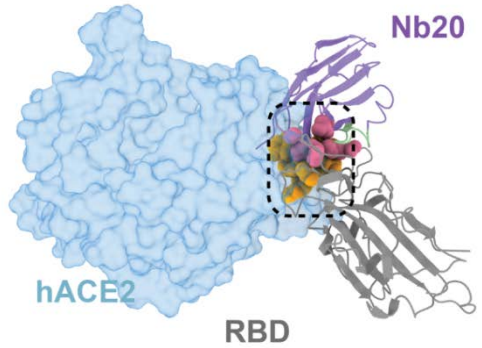
†Corresponding author. Email: dina.schneidman@mail.huji.ac.il (D.S.-D.); chengzh@pitt.edu (C.Z.); yi.shi@pitt.edu (Y.S.)

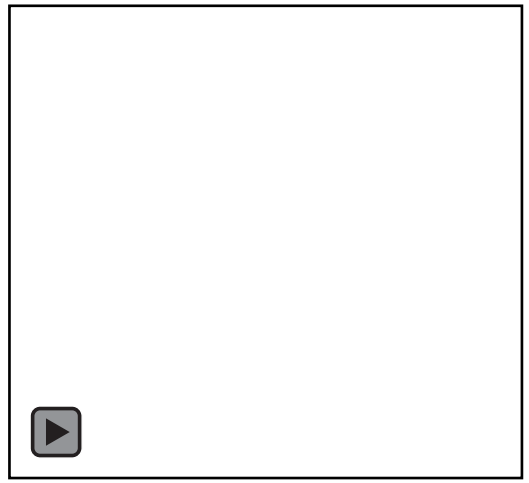
Cost-effective, efficacious therapeutics are urgently needed against the COVID-19 pandemic. Here, we used camelid immunization and proteomics to identify a large repertoire of highly potent neutralizing nanobodies (Nbs) to the SARS-CoV-2 spike (S) protein receptor-binding domain (RBD). We discovered Nbs with picomolar to femtomolar affinities that inhibit viral infection at sub-ng/ml concentration and determined a structure of one of the most potent in complex with RBD. Structural proteomics and integrative modeling revealed multiple distinct and non-overlapping epitopes and indicated an array of potential neutralization mechanisms. We constructed multivalent Nb constructs that achieved ultrahigh neutralization potency (IC50s as low as 0.058 ng/ml) and may prevent mutational escape. These thermostable Nbs can be rapidly produced in bulk from microbes and resist lyophilization, and aerosolization.

discovery of thousands of highly potent, multi-epitope neutralizing nanobodies

number of identifications	unique nanobody clones	CDR3 family "clonotypes"
total	16,555	617
high-affinity	*8,208*	*346*







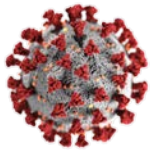
the most efficient nanobodies neutralize SARS-CoV-2 at 0.022 nM

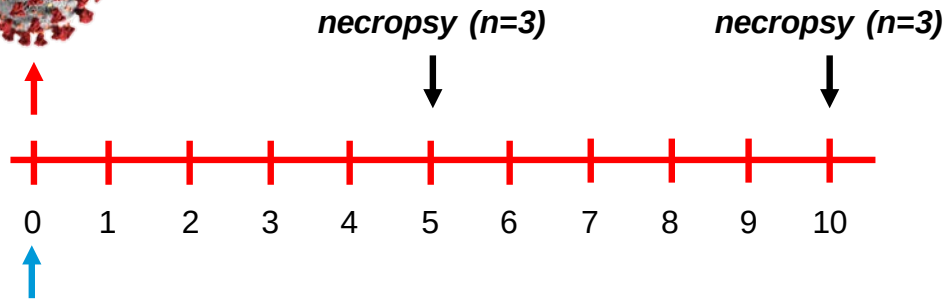
Xiang et al., 2020 Science

inhalable nanobody (PiN-21) treats SARS-CoV-2 infection

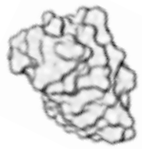
Syrian hamster model: intranasal co-administration

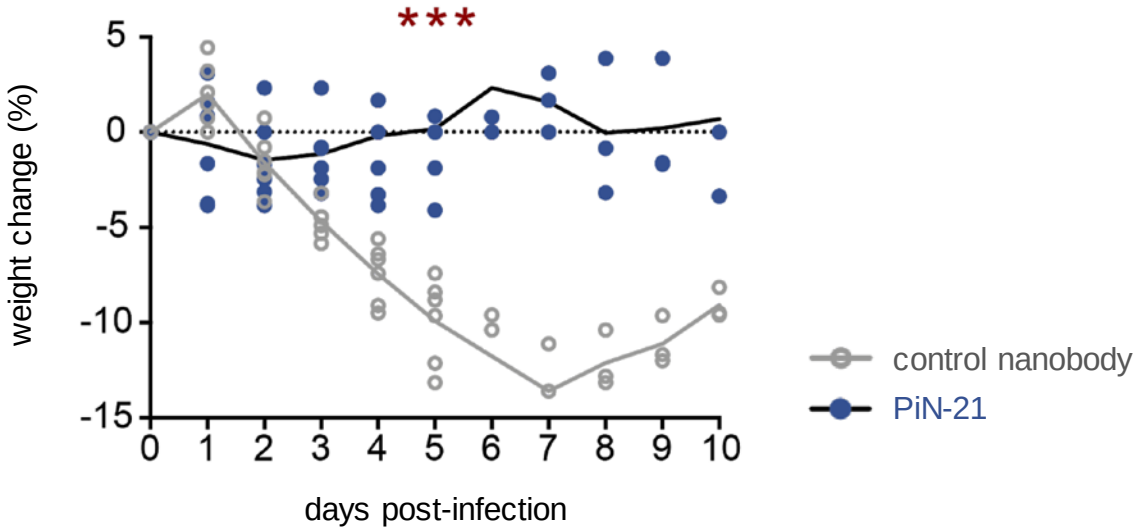
9 x 10^4 p.f.u. virus (IN)





100 µg nanobody (IN)





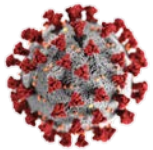
protection from disease at ultra-low doses

Nambulli et al., BioRxiv

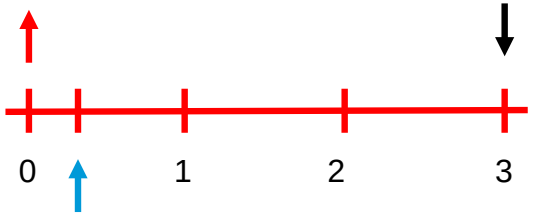
inhalable nanobody (PiN-21) treats SARS-CoV-2 infection

Syrian hamster model: aerosol delivery

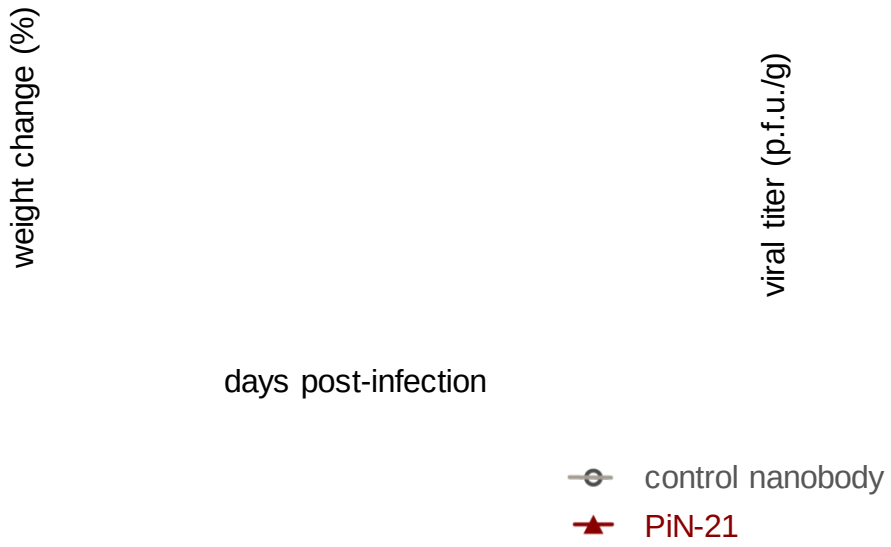
3 x 10^4 p.f.u. virus (IN)

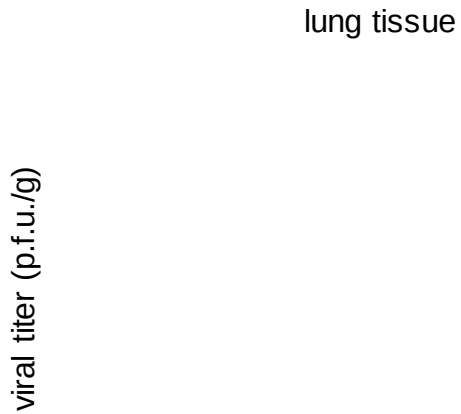


necropsy (n=6)



aerosolization (6 h.p.i.





protection from disease at ultra-low doses

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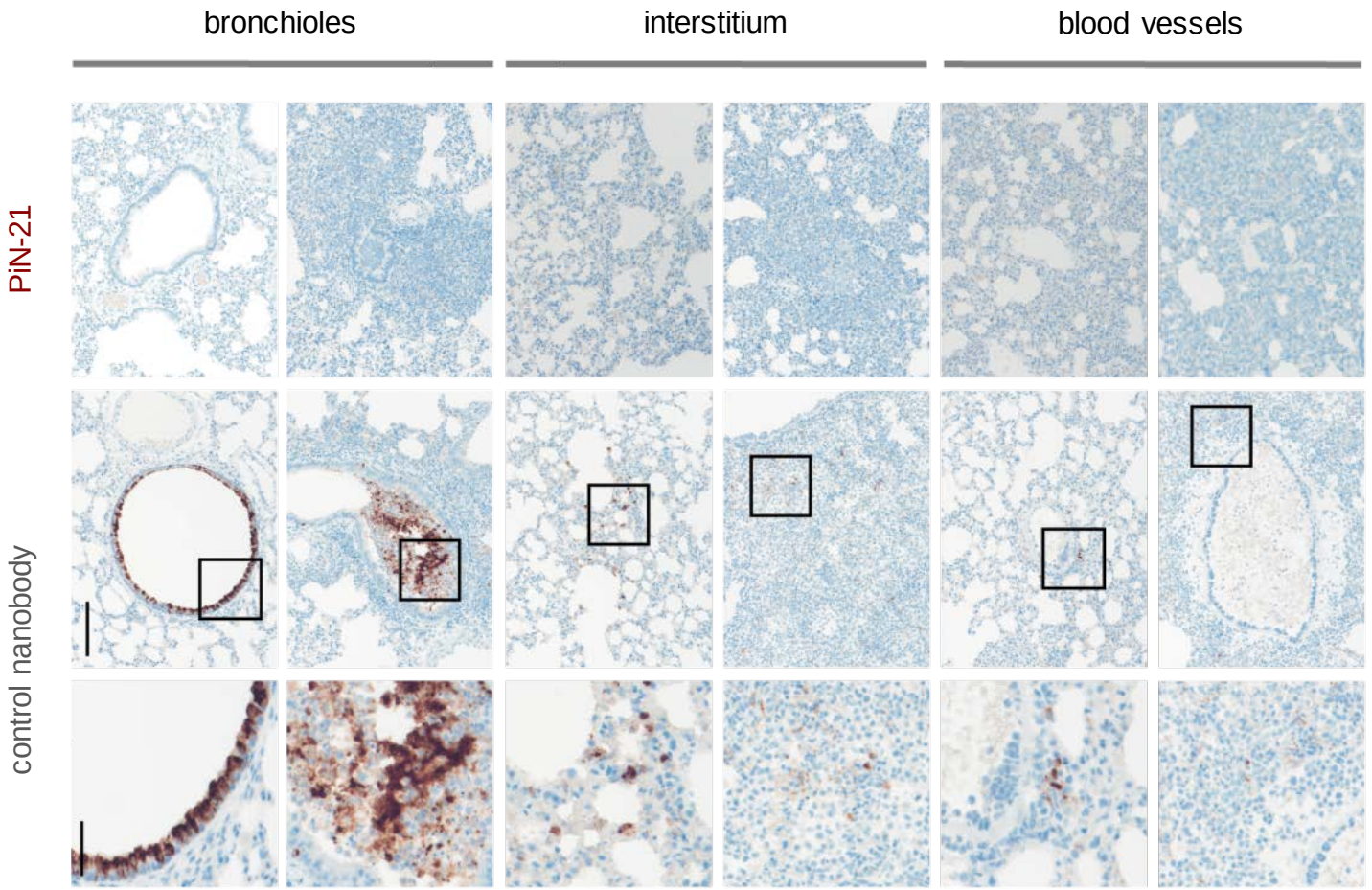
inhalable nanobody (PiN-21) treats SARS-CoV-2 infection

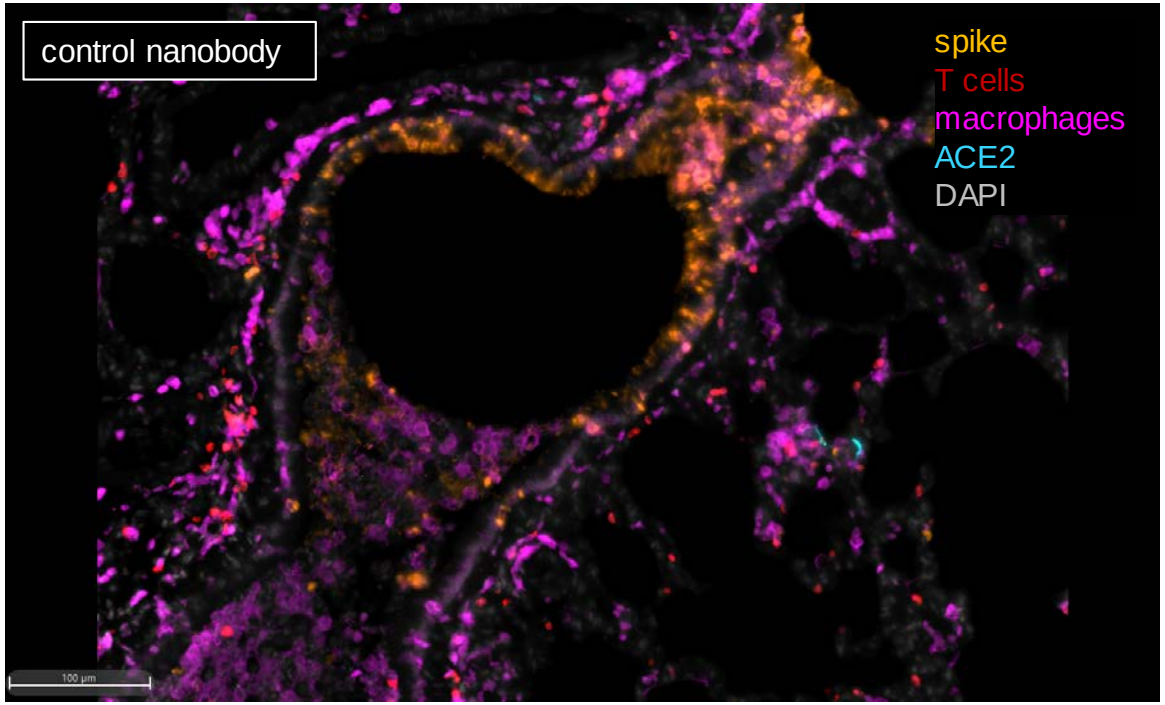
pathological assessment (3 d.p.i.)

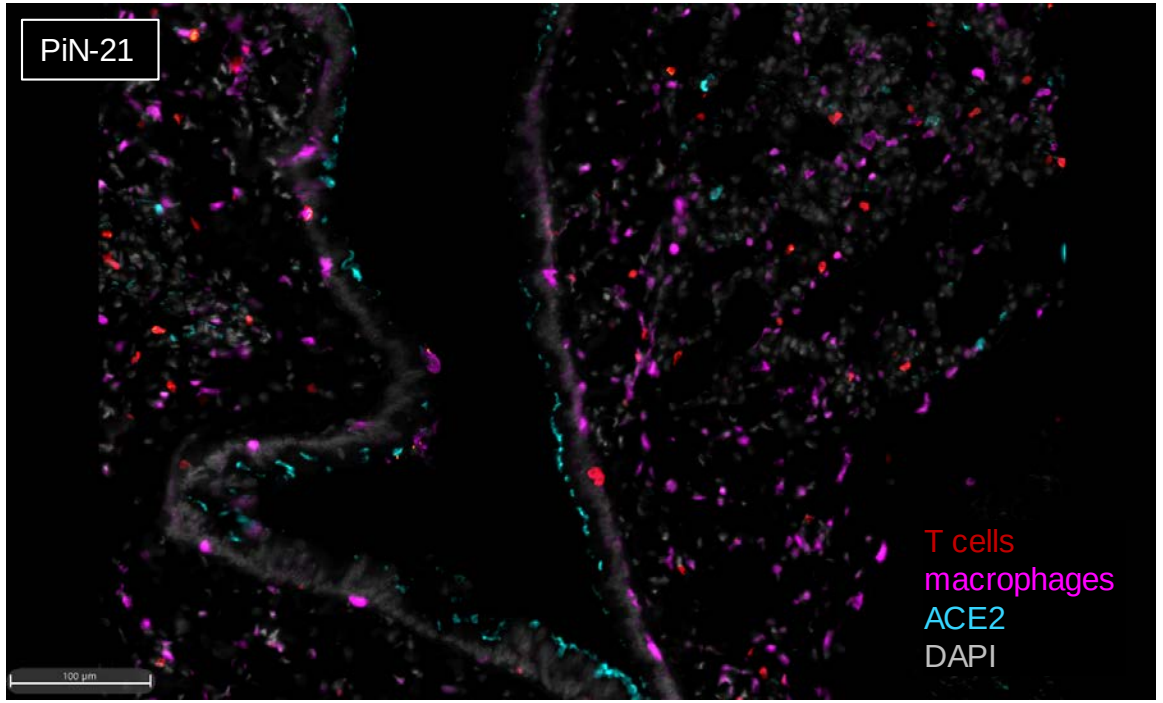


Nambulli et al., BioRxiv

inhalable nanobody (PiN-21) treats SARS-CoV-2 infection

bronchointerstitial compartment

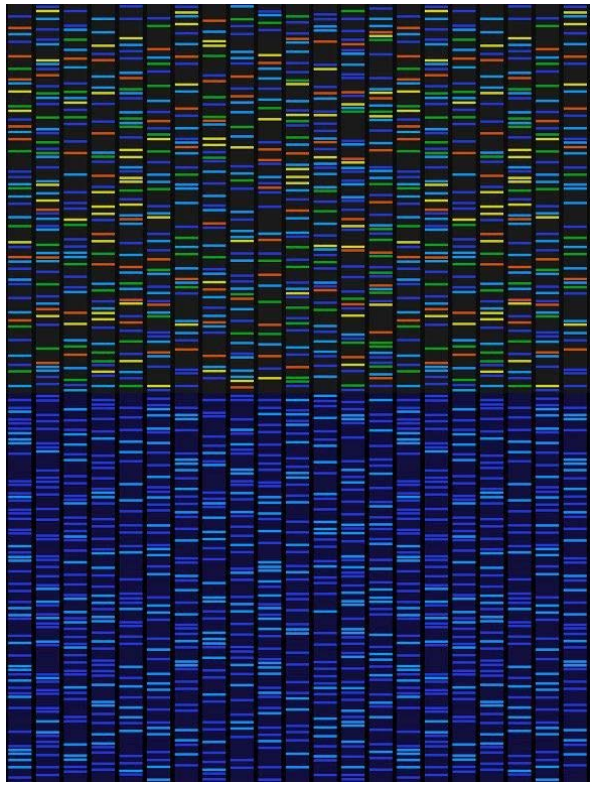


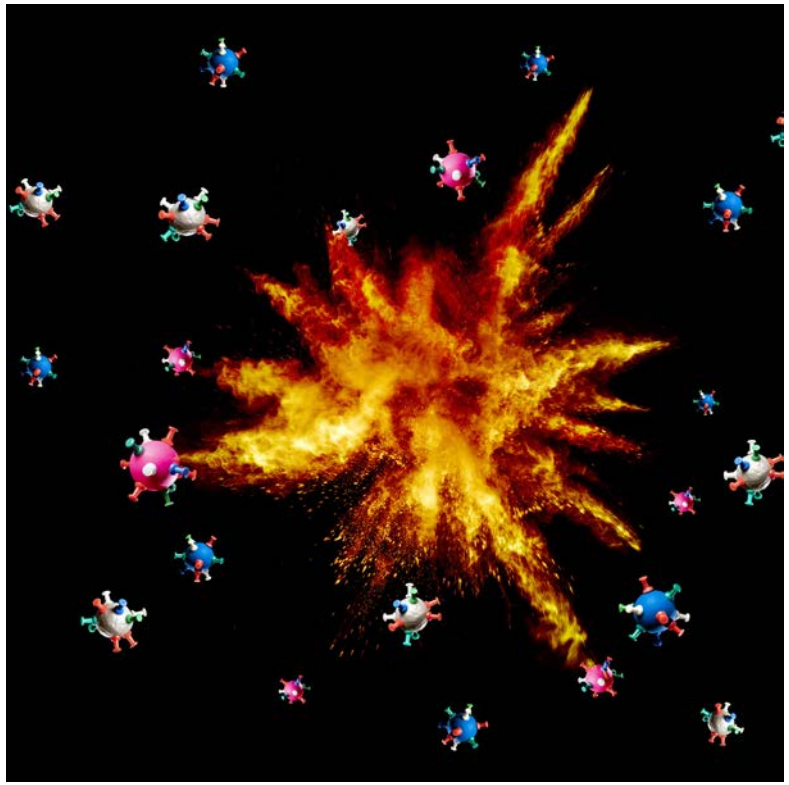


Nambulli et al., BioRxiv

however … the virus is a constantly moving target

SARS-CoV-2 variants of interest and concern: a game of cat and mouse

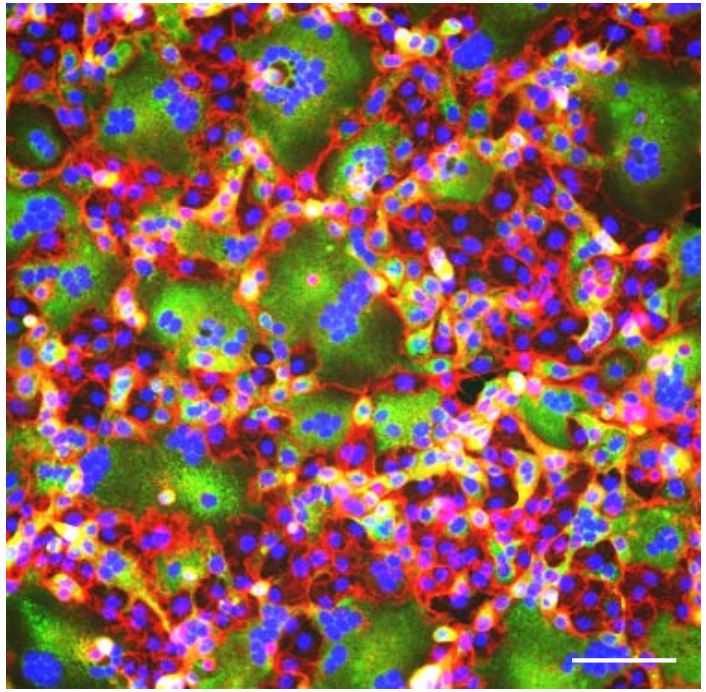


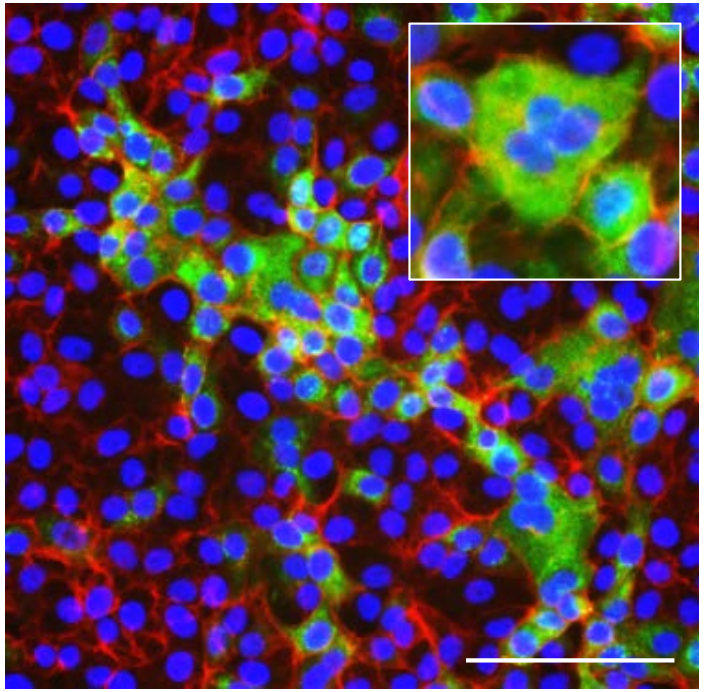


next generation COVID-19 interventions

it all starts with the virus

long term chronic SARS-CoV-2 infection in an immunosuppressed patient





SARS-CoV-2 isolated from day 72 respiratory sample

Hensley et al., 2021 CID

SARS-CoV-2 variants arise during persistent infection of immunosuppressed

recurrent deletion regions (RDRs) in the spike protein

Science

REPORTS

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10.1126/science.abf6950 (2021).

Recurrent deletions in the SARS-CoV-2 spike glycoprotein drive antibody escape

Kevin R. McCarthy[1,2,3]*, Linda J. Rennick[1,2], Sham Nambulli[1,2], Lindsey R. Robinson-McCarthy[4], William G. Bain[5,6,7], Ghady Haidar[8,9], W. Paul Duprex[1,2]*

[1]Center for Vaccine Research, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. [2]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. [3]Laboratory of Molecular Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA. [4]Department of Genetics, Harvard Medical School, Boston, MA, USA. [5]Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Internal Medicine, UPMC, Pittsburgh, PA, USA. [6]Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. [7]VA Pittsburgh Healthcare System, Pittsburgh, PA, USA. [8]Division of Infectious Disease, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. [9]Division of Infectious Disease, Department of Internal Medicine, UPMC, Pittsburgh, PA, USA.

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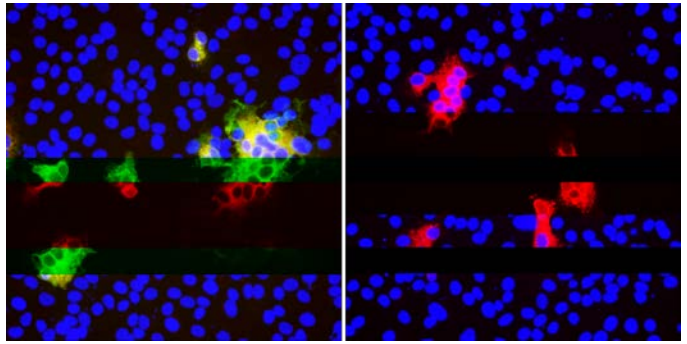
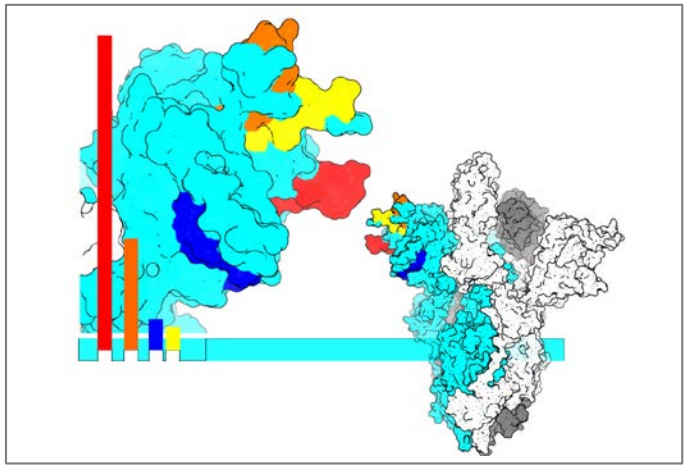
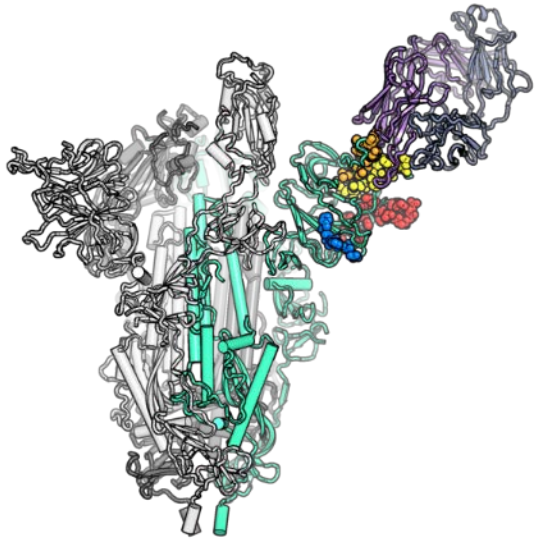
Zoonotic pandemics, like that caused by SARS-CoV-2, can follow the spillover of animal viruses into highly susceptible human populations. Their descendants have adapted to the human host and evolved to evade immune pressure. Coronaviruses acquire substitutions more slowly than other RNA viruses, due to a proofreading polymerase. In the spike glycoprotein, we find recurrent deletions overcome this slow substitution rate. Deletion variants arise in diverse genetic and geographic backgrounds, transmit efficiently, and are present in novel lineages, including those of current global concern. They frequently occupy recurrent deletion regions (RDRs), which map to defined antibody epitopes. Deletions in RDRs confer resistance to neutralizing antibodies. By altering stretches of amino acids, deletions appear to accelerate SARS-CoV-2 antigenic evolution and may, more generally, drive adaptive evolution.

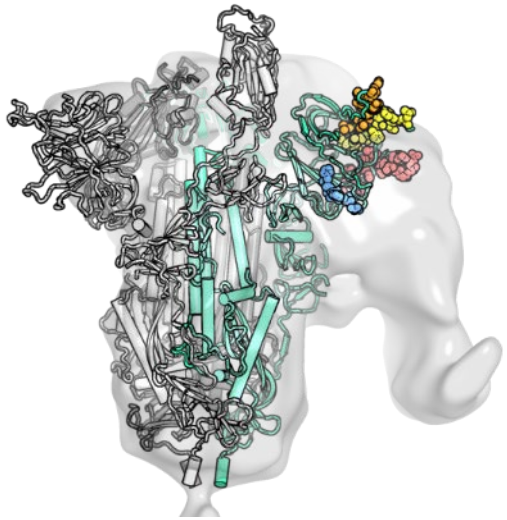


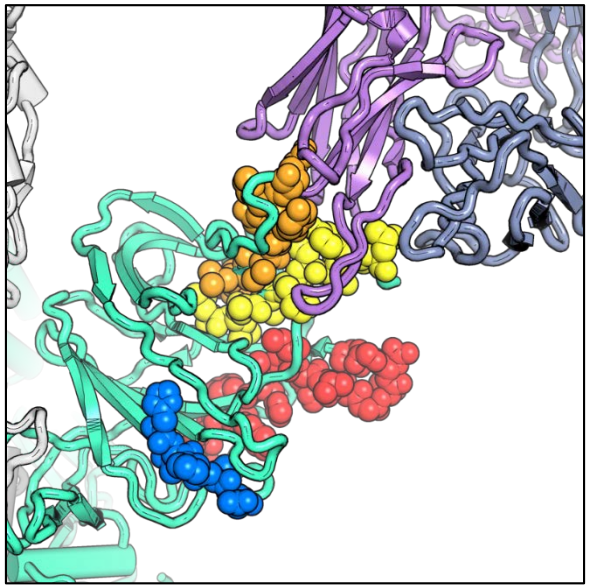


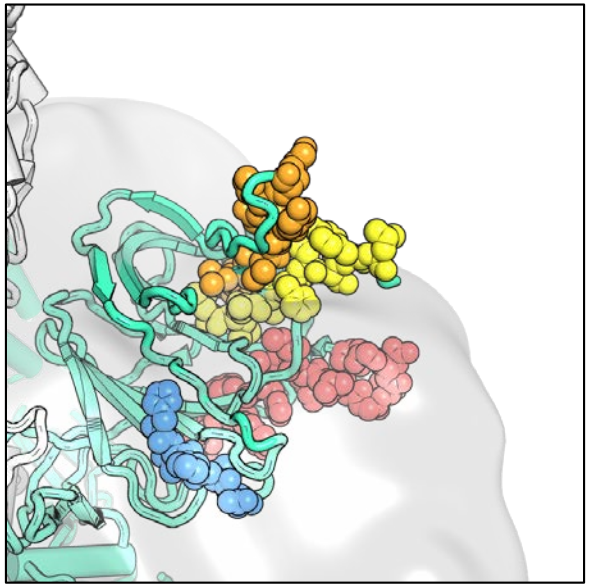
RDRs map to discrete surfaces within antibody epitopes

4A8









RDR1

RDR2

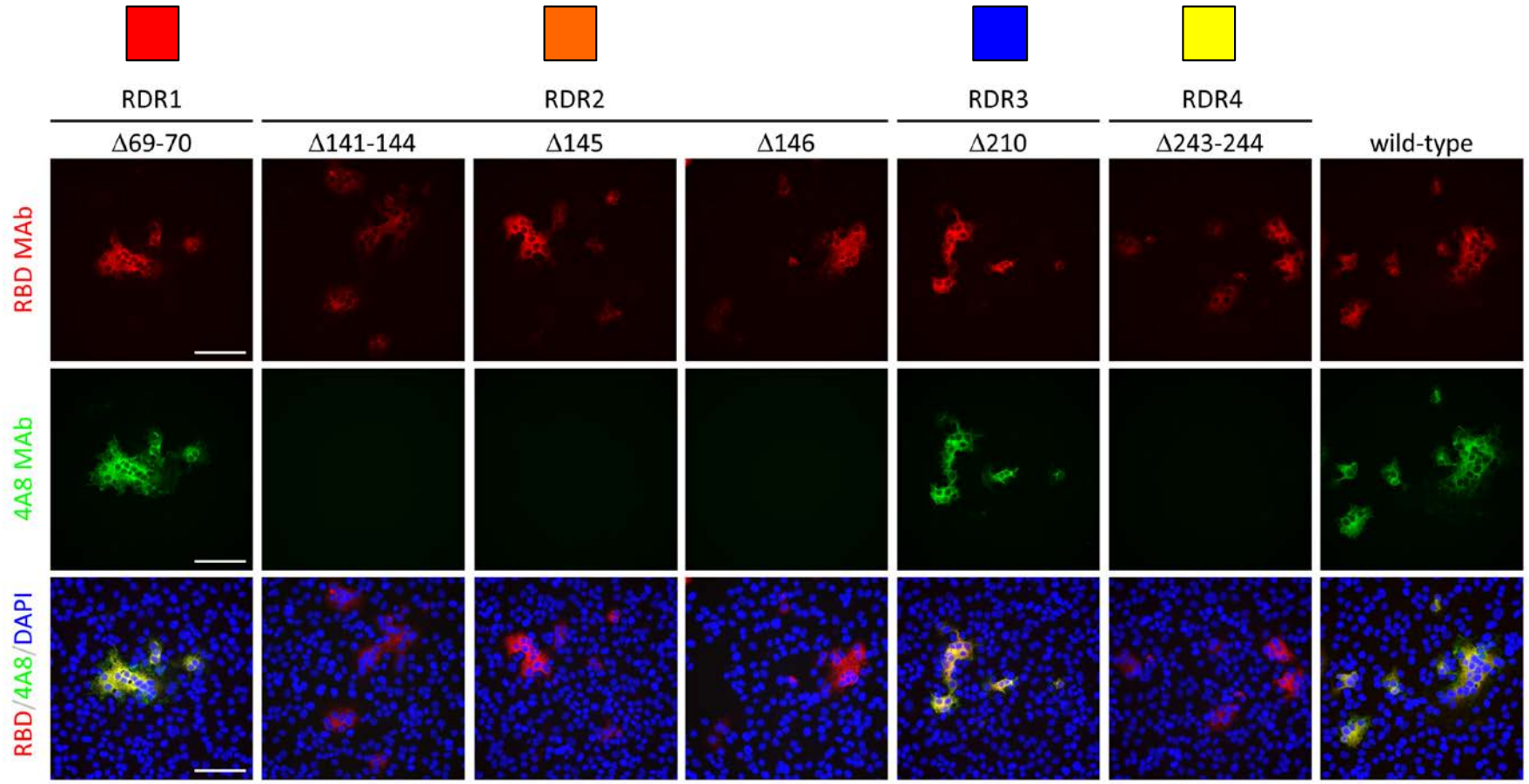
RDR3

RDR4

McCarthy et al., 2021 Science

deletions in RDR1 and 4 disrupt 4A8 binding

indirect immunofluorescence



RDR1
RDR2
RDR3
RDR4

McCarthy et al., 2021 Science

deletions in RDR1 and 4 completely abolish 4A8 neutralization activity

plaque reduction neutralization test 80 percent ($PRNT_{80}$)

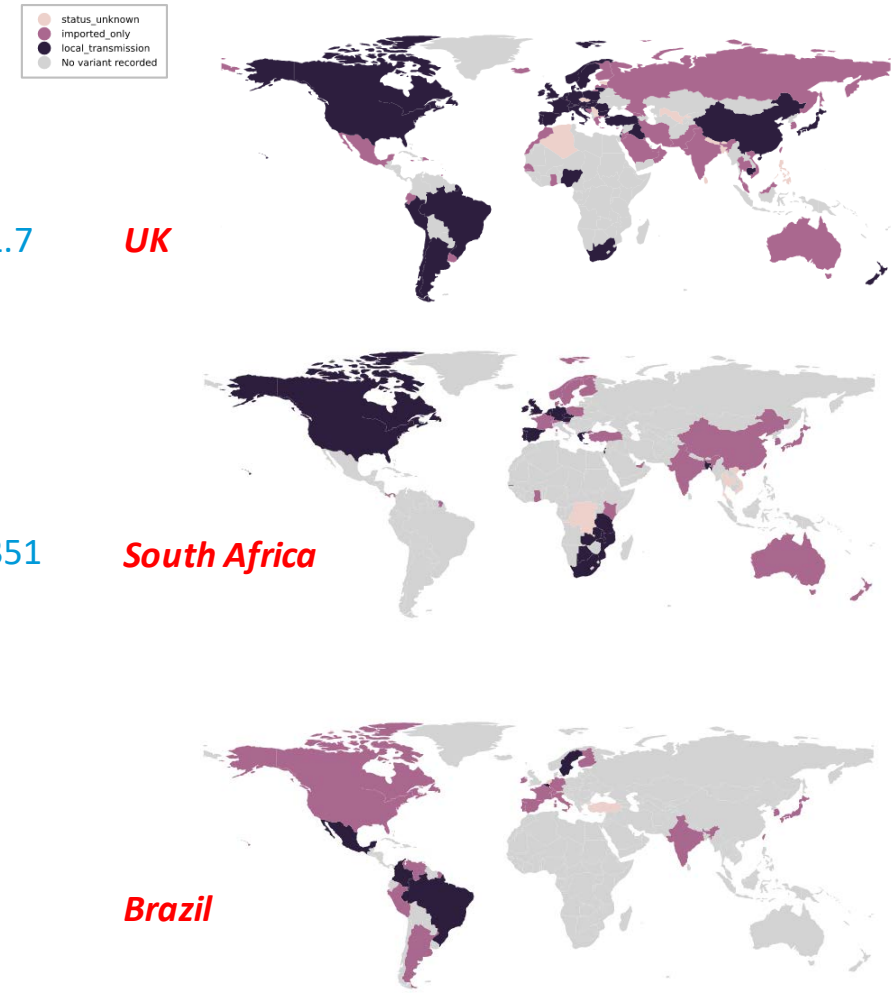
antibody	virus	antibody concentration (µg/ml)									
		25	12.5	6.25	3.125	1.56	0.78	0.39	0.2	0.1	0.05
4A8 (α-S)	*PLTI1*	-	-	-	-	-	-	-	-	-	-
	Munich: P3	+	+	+	+	+	+	-	-	-	-
2214 (α-HA)	*PLTI1*	-	-	-	-	-	-	*ND*	*ND*	*ND*	*ND*
	Munich: P3	-	-	-	-	-	-	*ND*	*ND*	*ND*	*ND*

	virus	serum dilution					
		1:100	1:200	1:400	1:800	1:1600	1:3200
human convalescent serum	*PLTI1*	+	+	+	+	+	-
	Munich: P3	+	+	+	+	+	-

these virus variants are monoclonal antibody escape mutants

McCarthy et al., 2021 Science

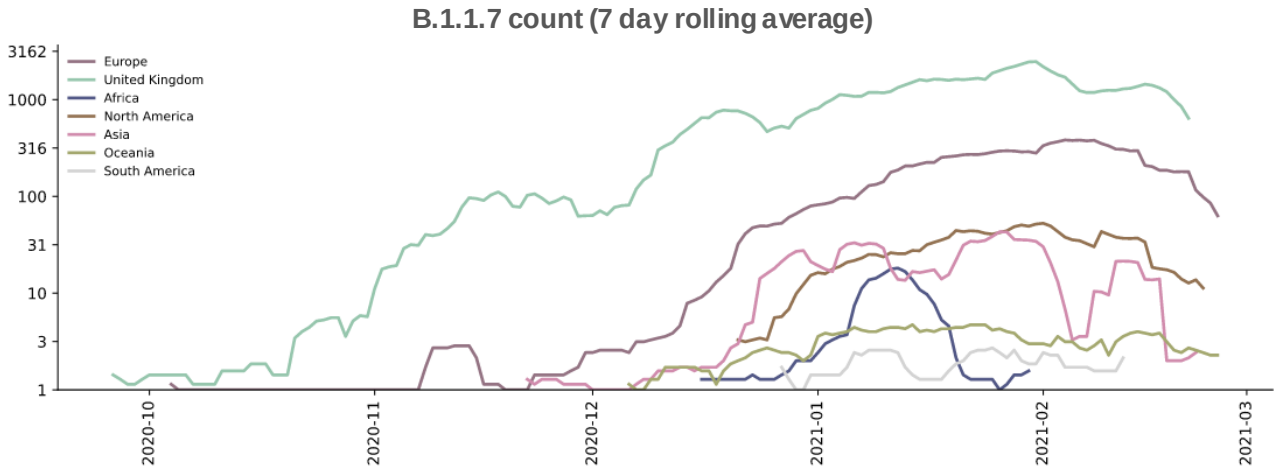
multi-epitope, multivalent constructs to suppress virus mutational escape

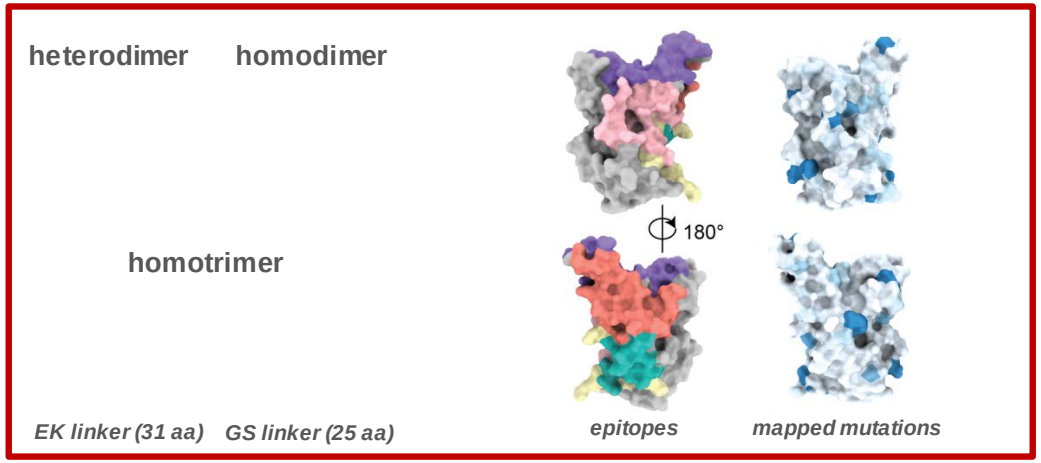


B.1.1.7 ***UK***

B.1.351 ***South Africa***

P.1 ***Brazil***

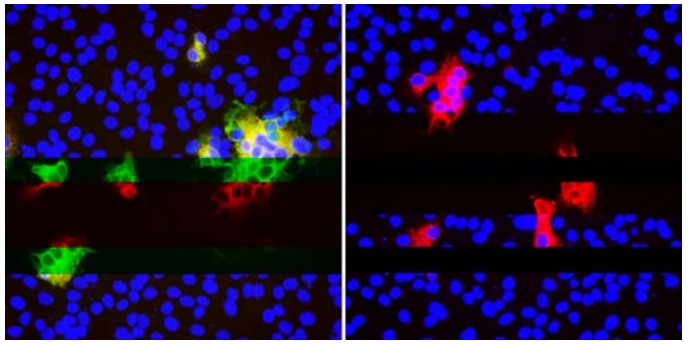




summary

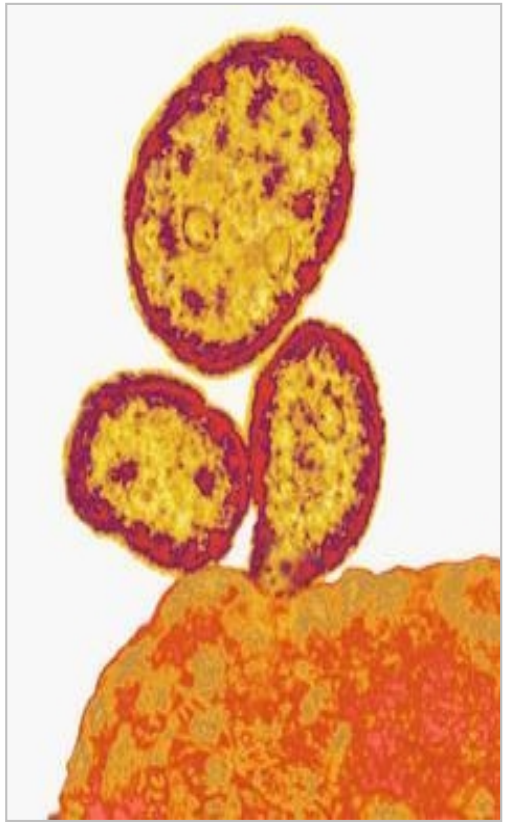
the utility of nanobodies as a COVID-19 intervention

- exquisite potency *in vitro* (up to 0.058 ng/ml) and *in vivo* (< 0.6 mg/kg)
- highly solubility and stability for drug storage and administration
- albumin fusion to nanobody extends *in vivo* half-life
- inhaled or intranasal delivery significantly impacts disease
- low cost of goods
- bind to five separate neutralization epitopes permitting heterodimeric constructs to be generated to address escape due to viral variants



summary

- *… introducing The Center for Vaccine Research*
  - foundations
  - facilities
  - focus
  - faculty
- *… responding to SARS-CoV-2 emergence*
  - *in vitro*
  - *in vivo*
- *… creativity drives interventions*
  - vaccines
  - antibodies and nanobodies
  - variants



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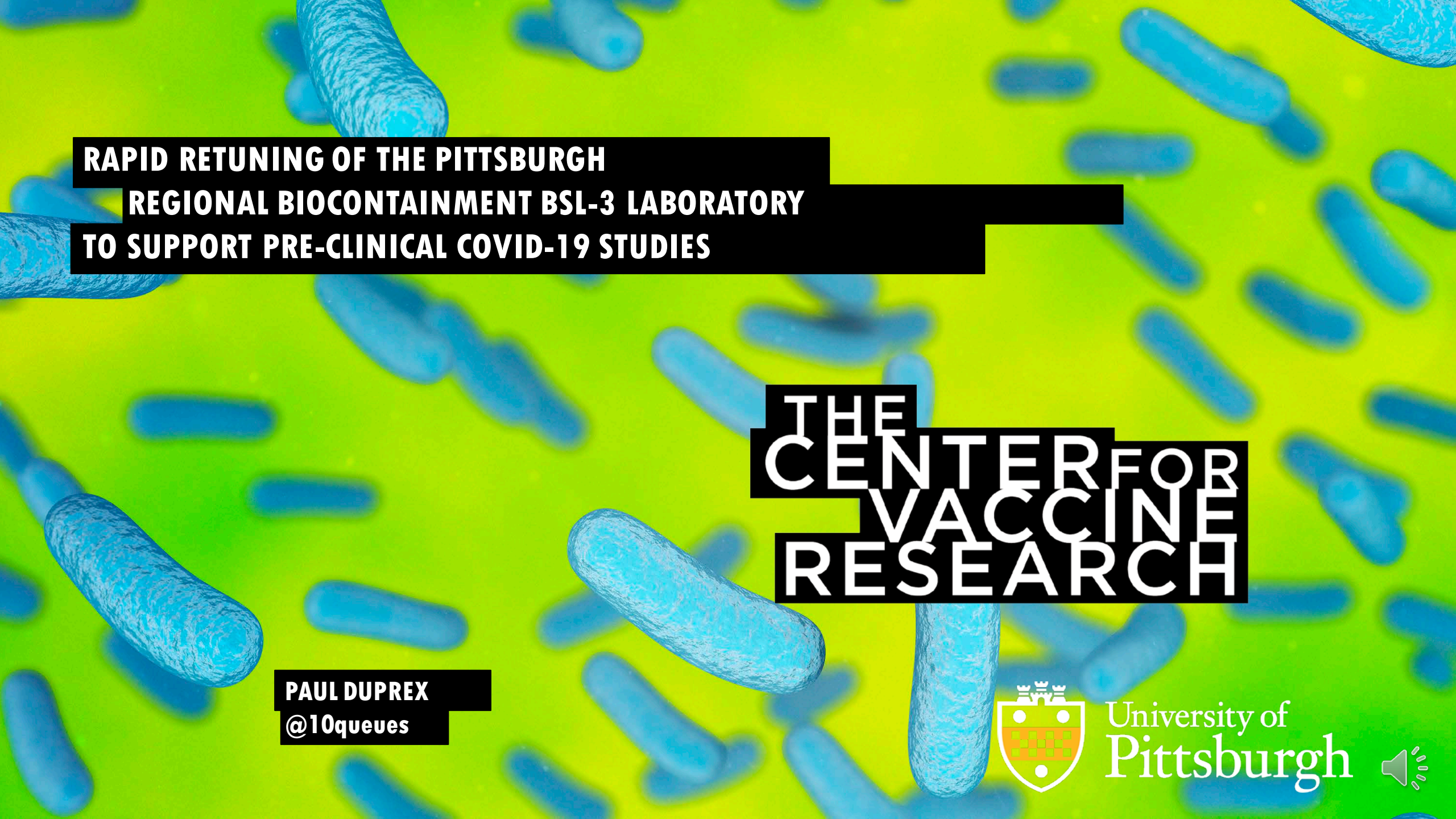
THE HEBREW UNIVERSITY OF JERUSALEM

Dina Schneidman-Duhovny

RAPID RETUNING OF THE PITTSBURGH REGIONAL BIOCONTAINMENT BSL-3 LABORATORY TO SUPPORT PRE-CLINICAL COVID-19 STUDIES



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