NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE

Broad-Spectrum Antiviral Peptide Engineering for Future Pandemic Preparedness

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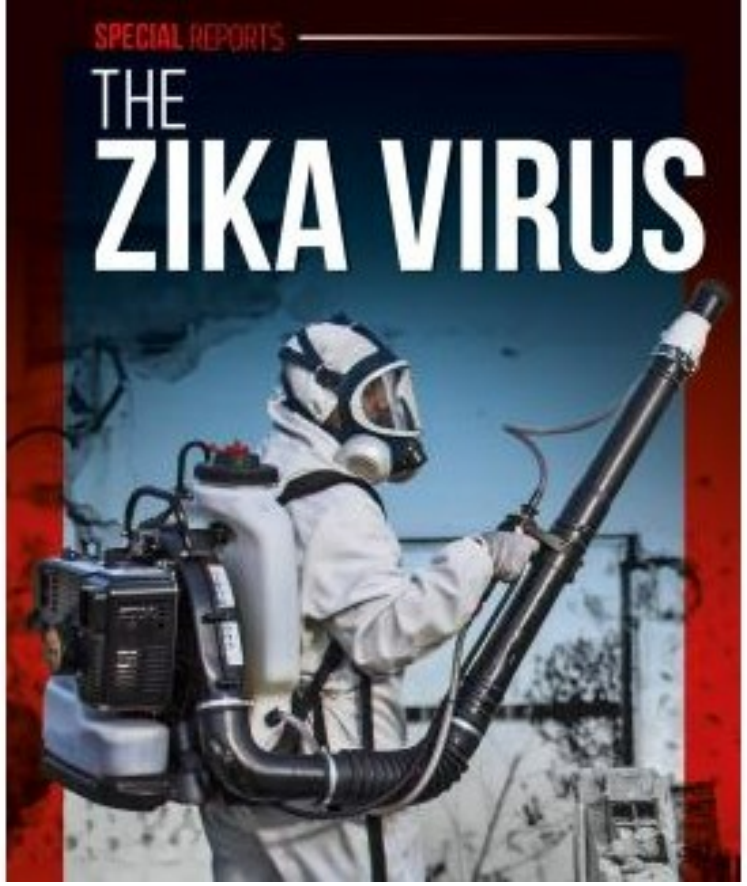
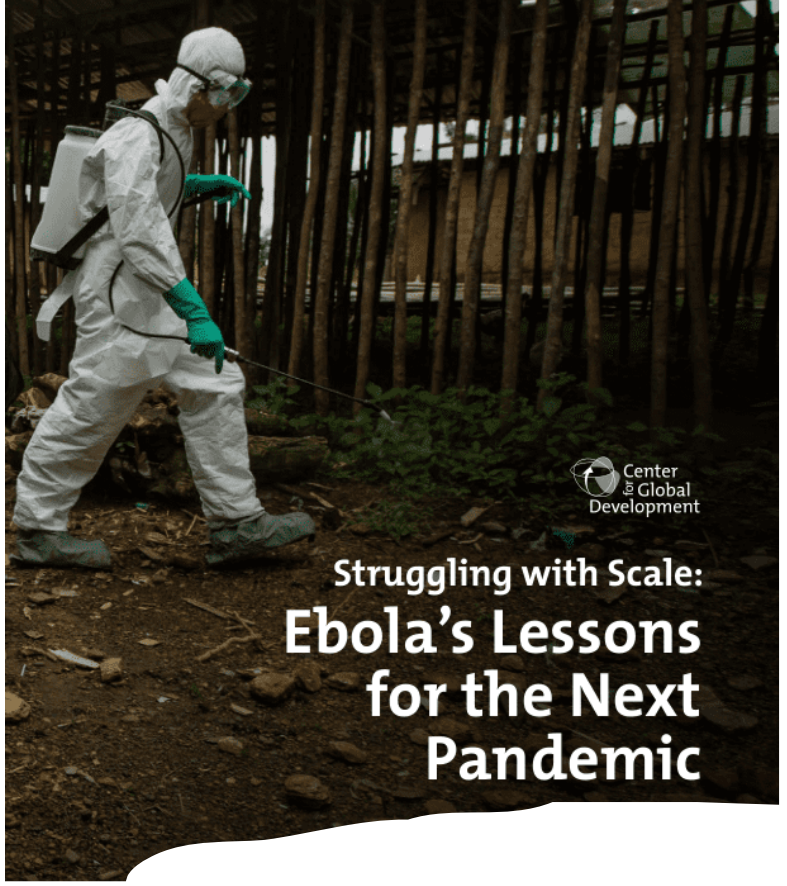
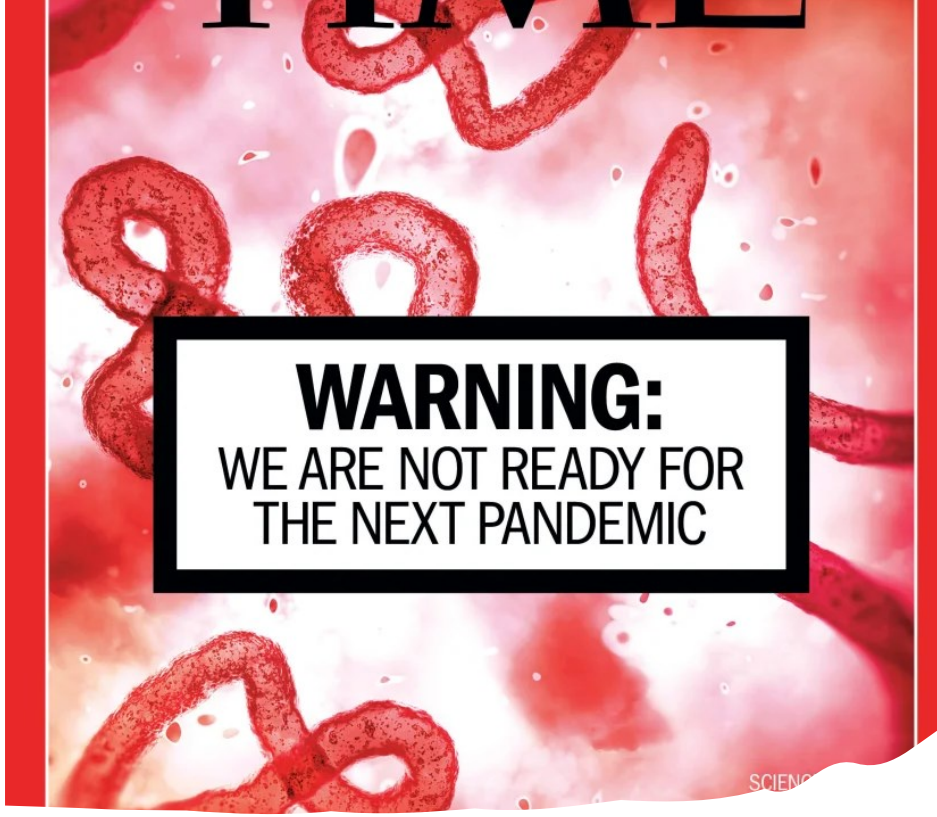
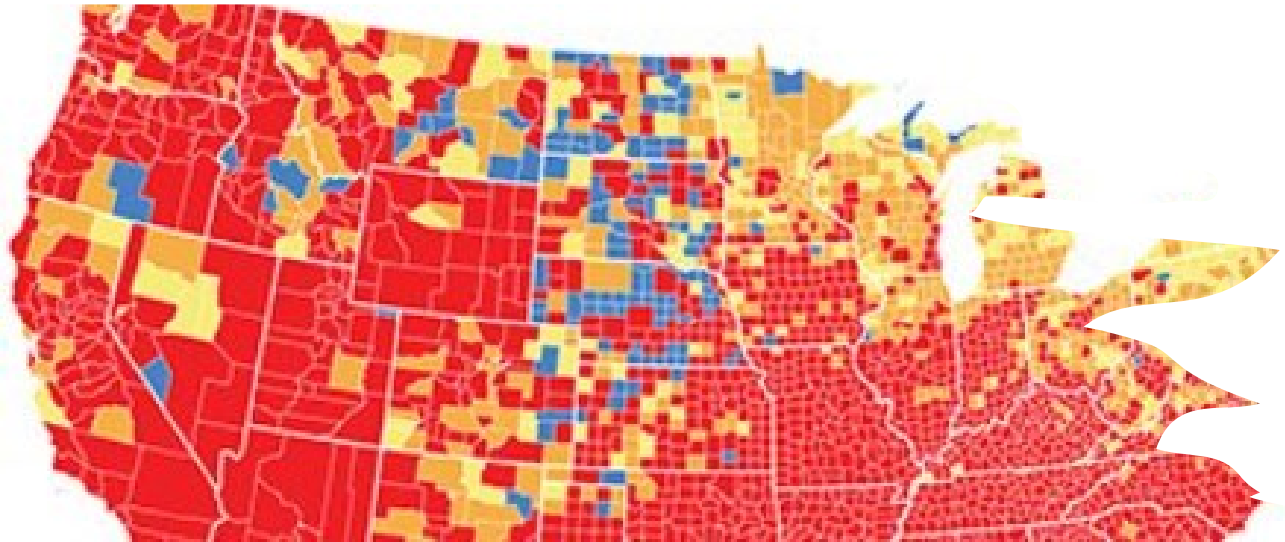
School of Materials Science & Engineering,
Nanyang Technological University

2023년 재외한인공학자 네트워킹 신기술 세미나









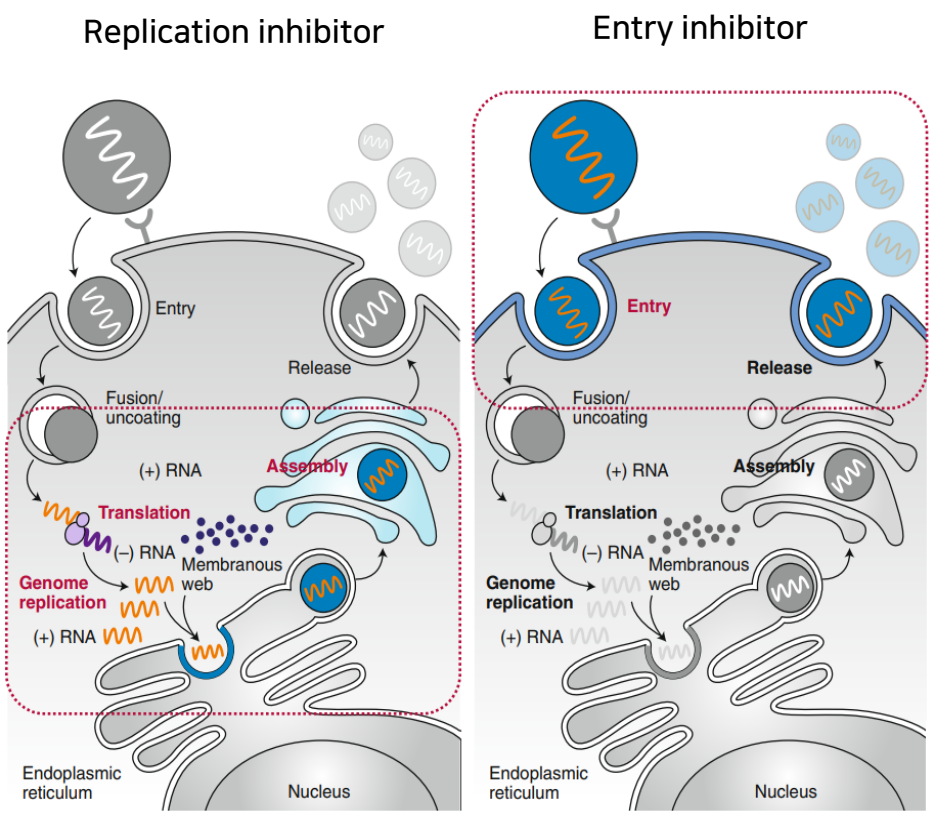
Are we ready for the next virus pandemic?

New Antiviral Strategies Are Urgently Needed

STOCKING THE SHELVES FOR THE NEXT PANDEMIC

Despite previous warnings, drug makers failed to prepare a stockpile of compounds to fight viral pandemics. Can they finally do the right thing? **By Elie Dolgin**

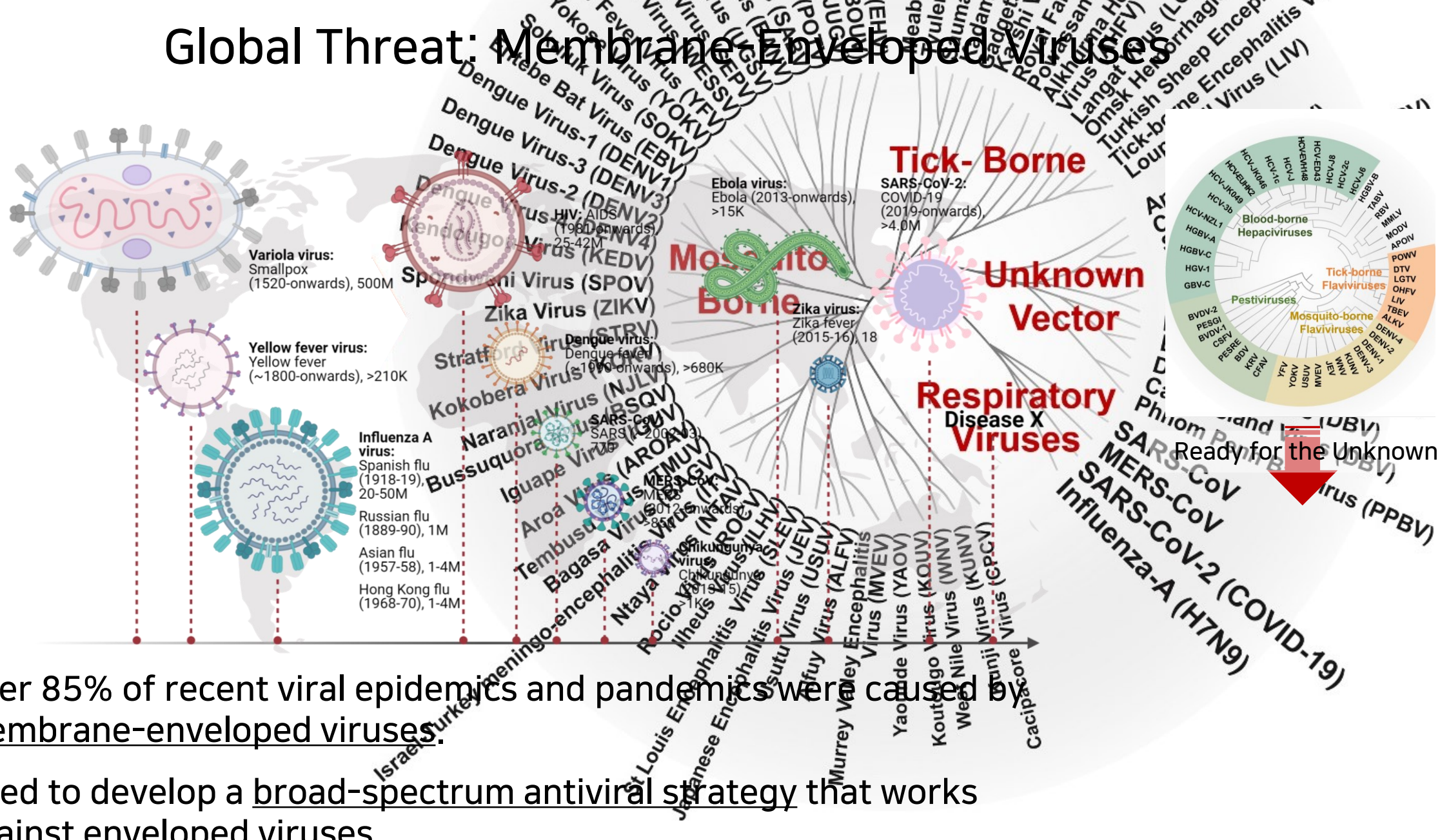
340 | Nature | Vol 592 | 15 April 2021



- Replication inhibitors cannot prevent infection and drug resistance often easily emerges.
- Most entry inhibitors such as antibodies only work against one virus and can also have challenges with resistance development.

Cho et al., *Nat. Mater. (*2020).
Dolgin et al., *Nature* (2021).

Global Threat: Membrane-Enveloped Viruses

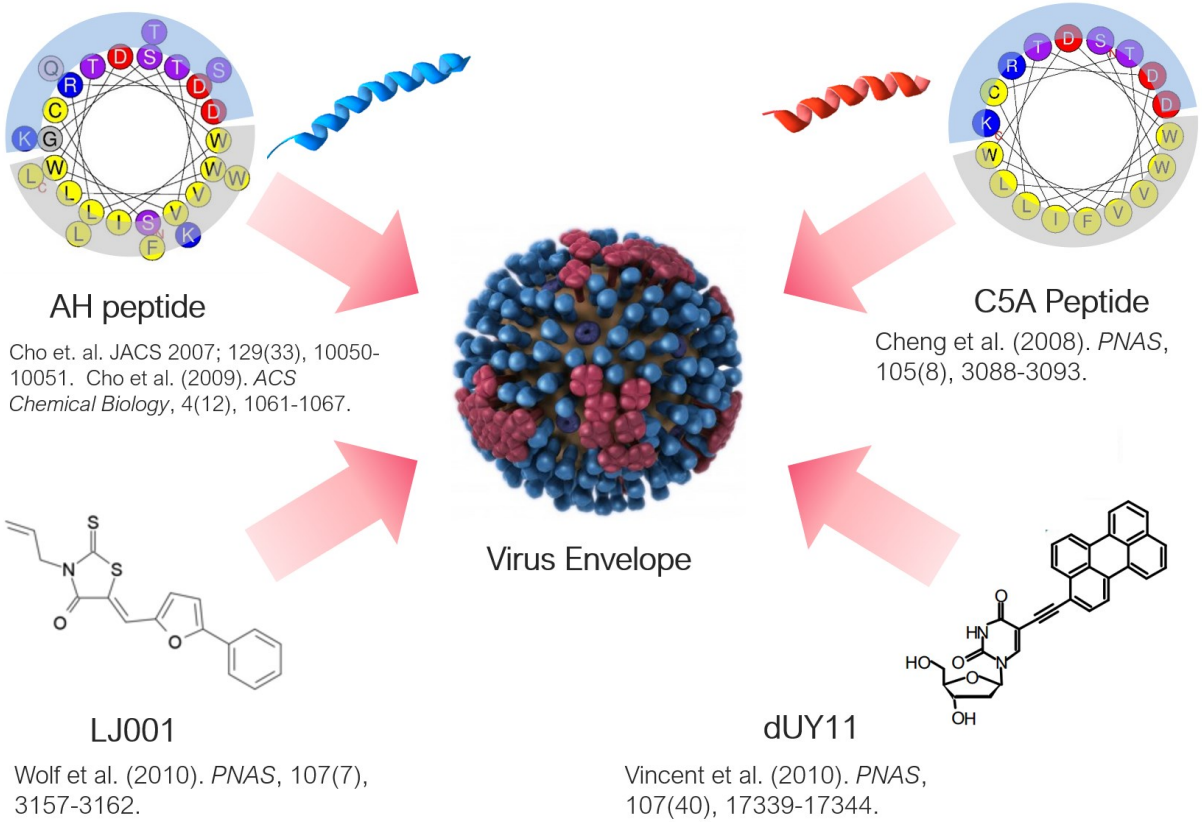


- Over 85% of recent viral epidemics and pandemics were caused by membrane-enveloped viruses.
- Need to develop a broad-spectrum antiviral strategy that works against enveloped viruses.

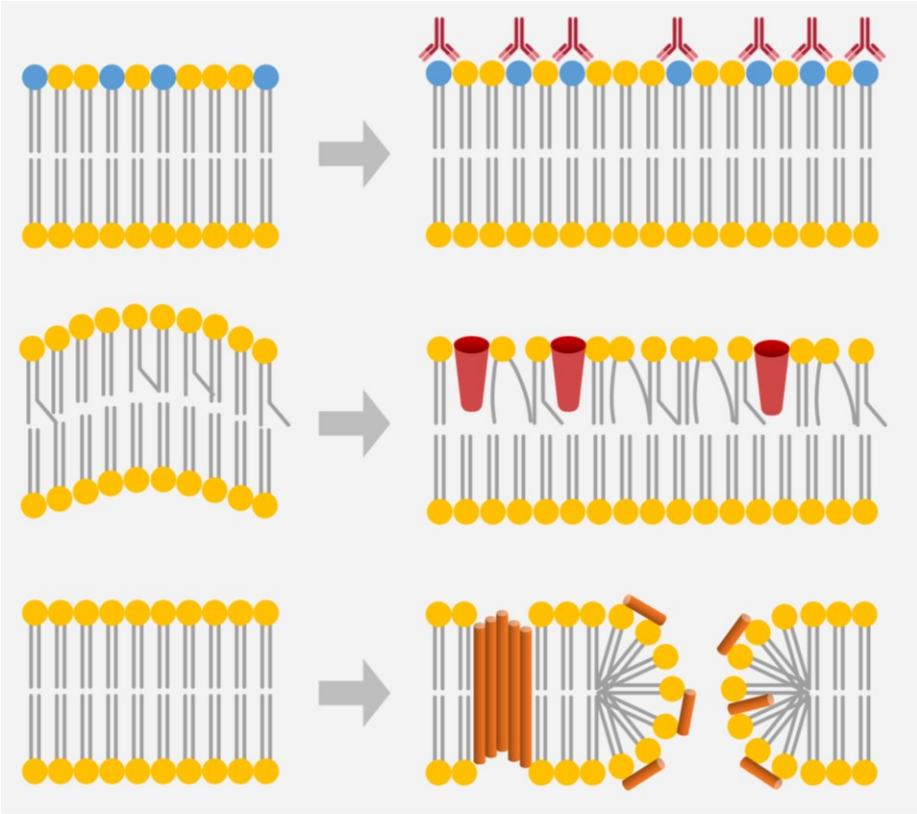
Cho et al., *Nat. Mater. (*2020).
Park and Cho et al., *Acc. Chem. Res.* (2021).

Viral Membrane Disruption Strategies

Molecular Examples



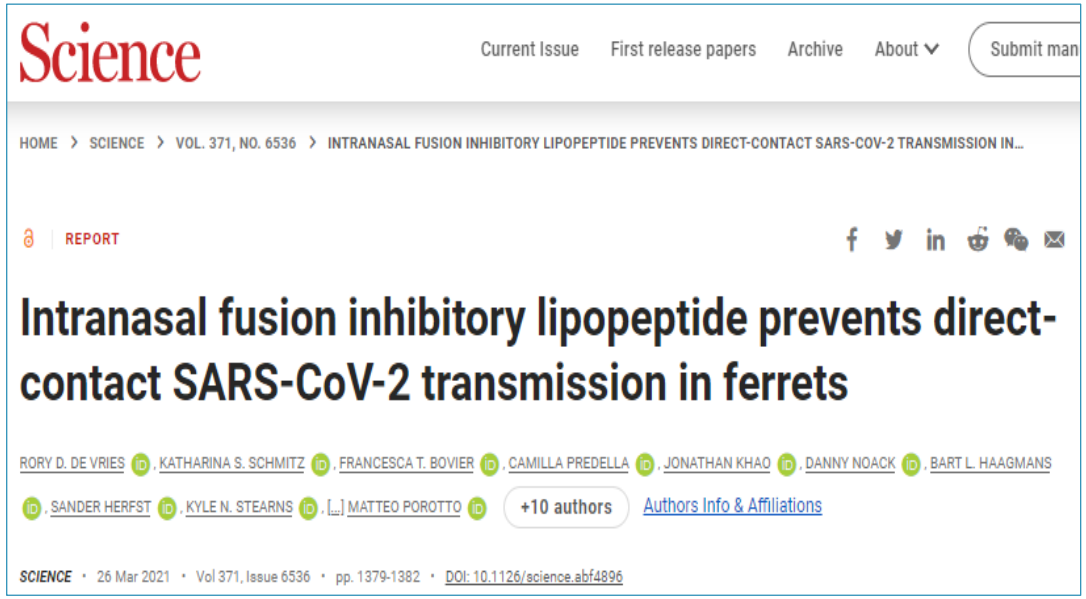
Mechanisms of Membrane Targeting & Disruption



- Viral membrane is a conserved, host cell-derived structural target of enveloped viruses.
- Challenging to disrupt viral membrane in a potent and selective manner that works *in vivo*.

Antiviral Peptides: Next-Generation Technology

Fusion Inhibitors: COVID-19 Pandemic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HOME › SCIENCE › VOL. 371, NO. 6536 › INTRANASAL FUSION INHIBITORY LIPOPEPTIDE PREVENTS DIRECT-CONTACT SARS-COV-2 TRANSMISSION IN...

REPORT

Intranasal fusion inhibitory lipopeptide prevents direct-contact SARS-CoV-2 transmission in ferrets

RORY D. DE VRIES, KATHARINA S. SCHMITZ, FRANCESCA T. BOVIER, CAMILLA PREDELLA, JONATHAN KHAO, DANNY NOACK, BART L. HAAGMANS, SANDER HERFST, KYLE N. STEARNS, [...] MATTEO POROTTO +10 authors Authors Info & Affiliations

SCIENCE · 26 Mar 2021 · Vol 371, Issue 6536 · pp. 1379-1382 · DOI: 10.1126/science.abf4896

Envelope Disruptors: Zika Epidemic


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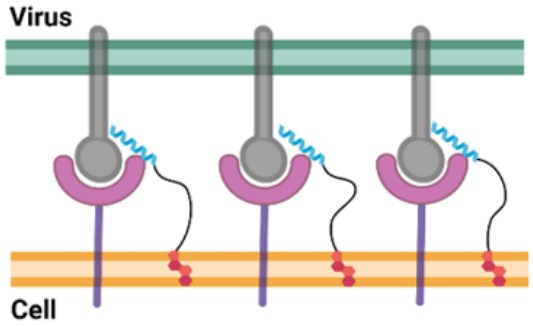
Letter | Published: 22 October 2018

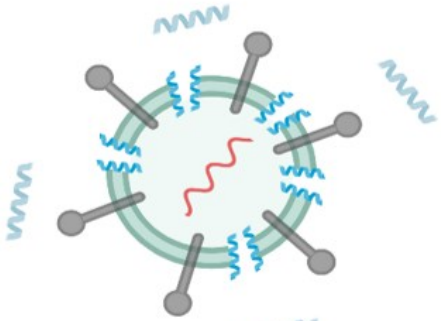
Therapeutic treatment of Zika virus infection using a brain-penetrating antiviral peptide

Joshua A. Jackman, Vivian V. Costa, Soohyun Park, Ana Luiza C. V. Real, Jae Hyeon Park, Pablo L. Cardozo, Abdul Rahim Ferhan, Isabella G. Olmo, Thaiane P. Moreira, Jordana L. Bambirra, Victoria F. Queiroz, Celso M. Queiroz-Junior, Giselle Foureaux, Danielle G. Souza, Fabiola M. Ribeiro, Bo Kyeong Yoon, Evelien Wynendaele, Bart De Spiegeleer, Mauro M. Teixeira & Nam-Joon Cho

Fusion Inhibitors

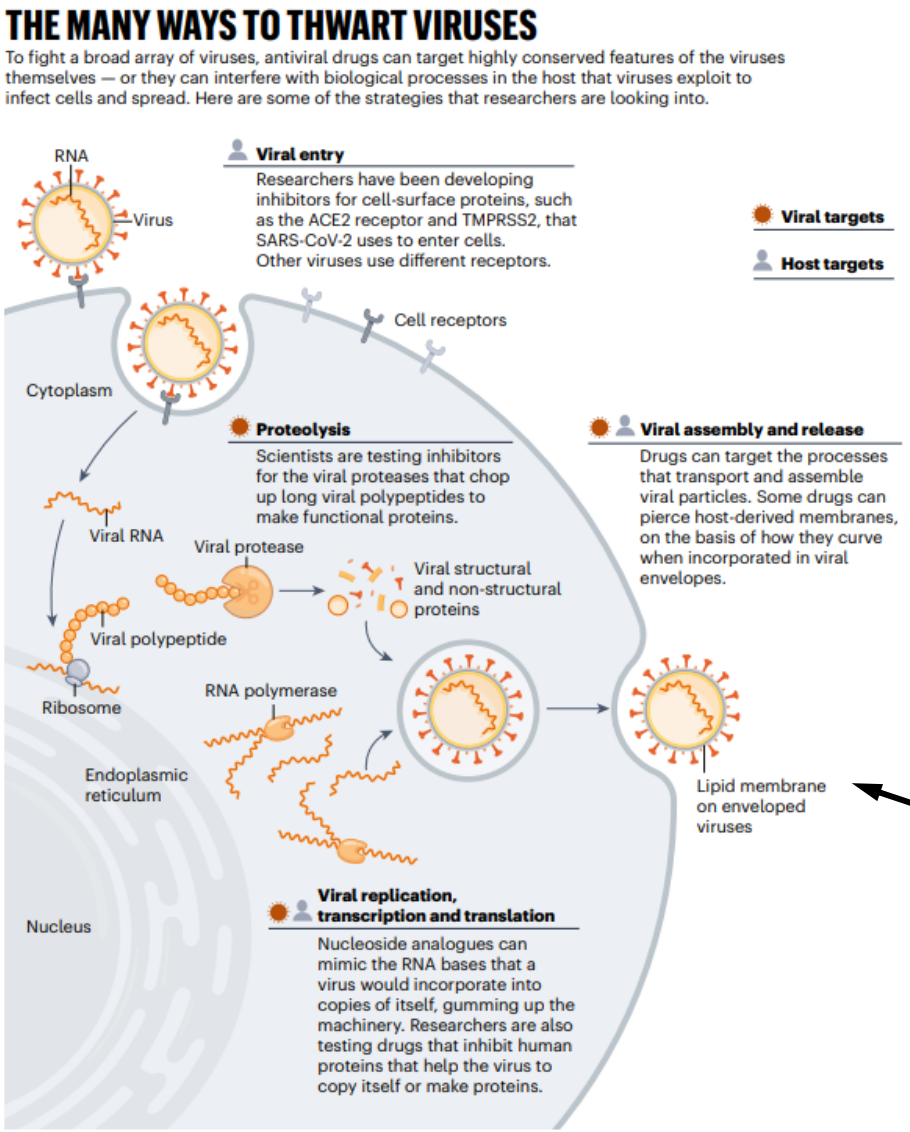


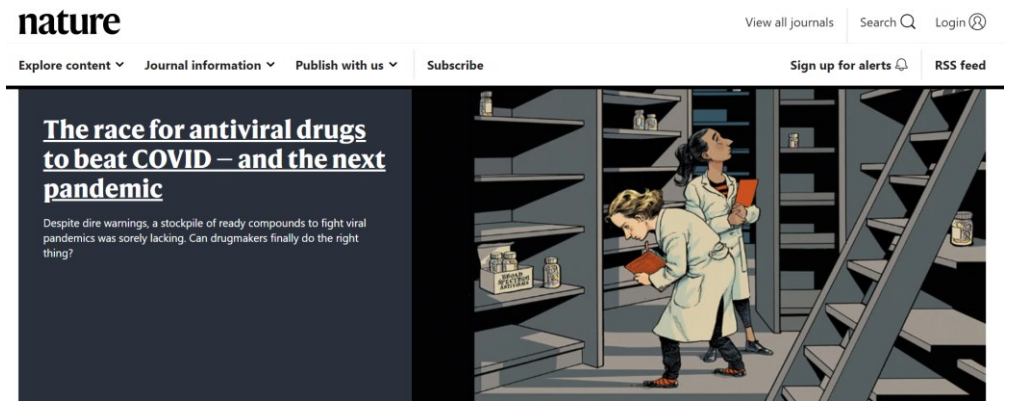
Envelope-Disrupting Inhibitors



BBA-Biomembranes 17, no. 11 (2022): 971-977.
Science 371, no. 6536 (2021): 1379-1382.
Nature Materials 17, no. 11 (2018): 971-977.

Envelope-Disruptors to Stop Future Pandemics





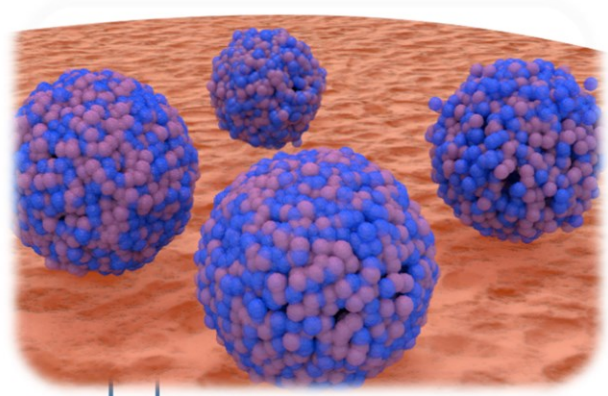
– Nam-Joon Cho, a materials scientist at Nanyang Technological University in Singapore, and Joshua Jackman, a chemical engineer at Sungkyunkwan University in Seoul. They have developed small peptide drugs that poke holes in the lipid wrappings found around enveloped viruses[7]. These lipids come from the membrane surface of human cells. But the peptides penetrate only lipids that encase viruses, not cells, because of differences in the size of the membrane structure and how much it bends (see 'The many ways to thwart viruses').

Cho describes the lipid coating as the "common denominator" of all enveloped viruses – a group that includes flaviviruses, alphaviruses, coronaviruses, filoviruses, retroviruses and

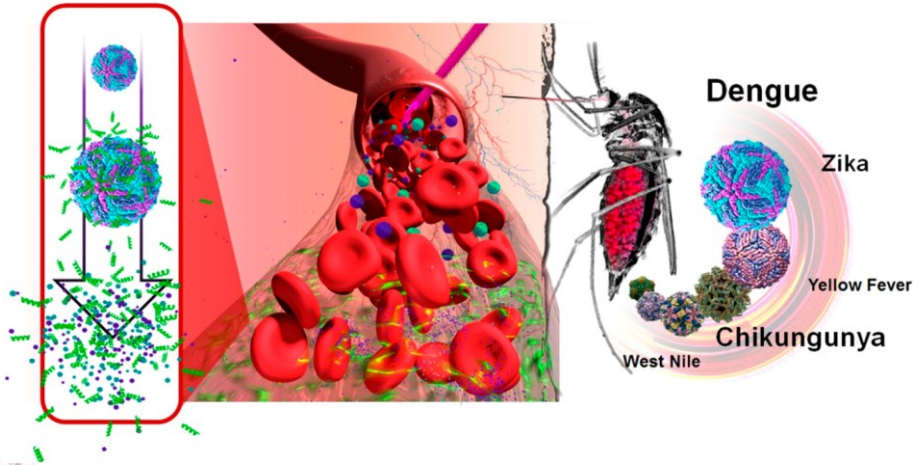
Next-Generation Antiviral Strategy

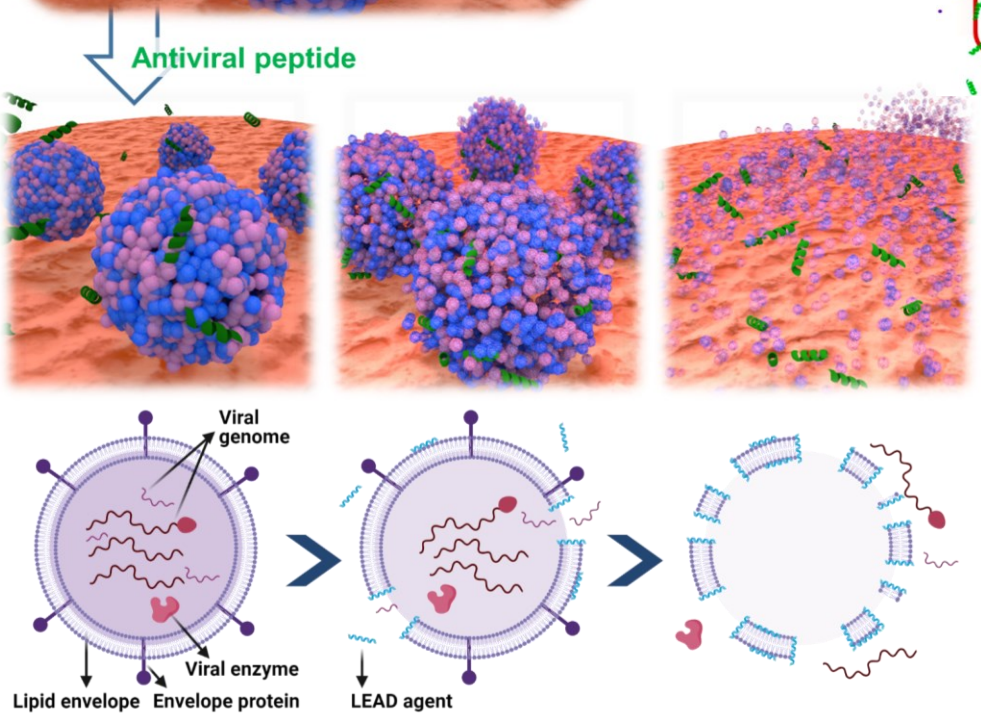
Nature 592, no. 7854 (2021): 340-343.

Our LEAD Approach



Lipid
Envelope
Antiviral
Disruption





Biophysical + Computational
+ Virological Approaches

Investigate the design & function
of membrane-active peptides

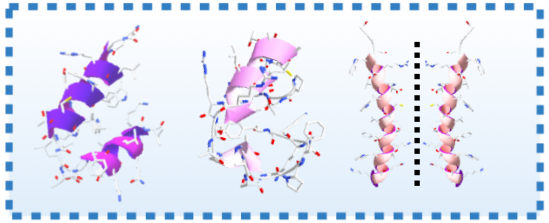
Next-generation antiviral peptides

Cho et al., *Nat. Mater. (*2020).

Antiviral Peptide Engineering Strategy

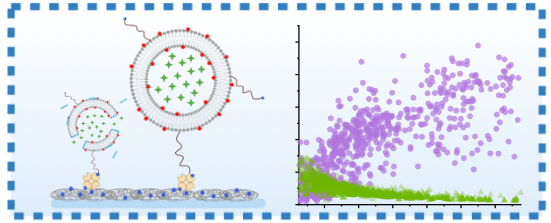
Integrated Experimental and Theoretical Approaches

Peptide Design



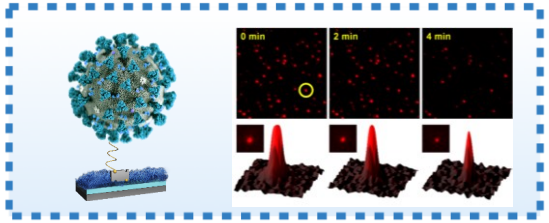
- Sequence optimization
- Secondary structure prediction
- Interfacial hydrophobicity

Biophysical Evaluation

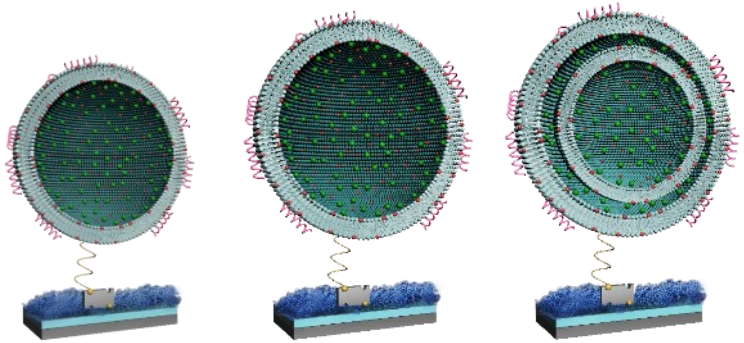


- Peptide concentration
- Solution conditions
- Membrane composition/curvature

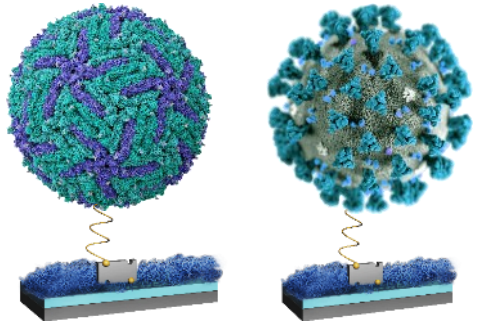
Virus Particle Tracking



- Real-time interaction tracking
- Model & authentic virus particles
- Virus conformational effects

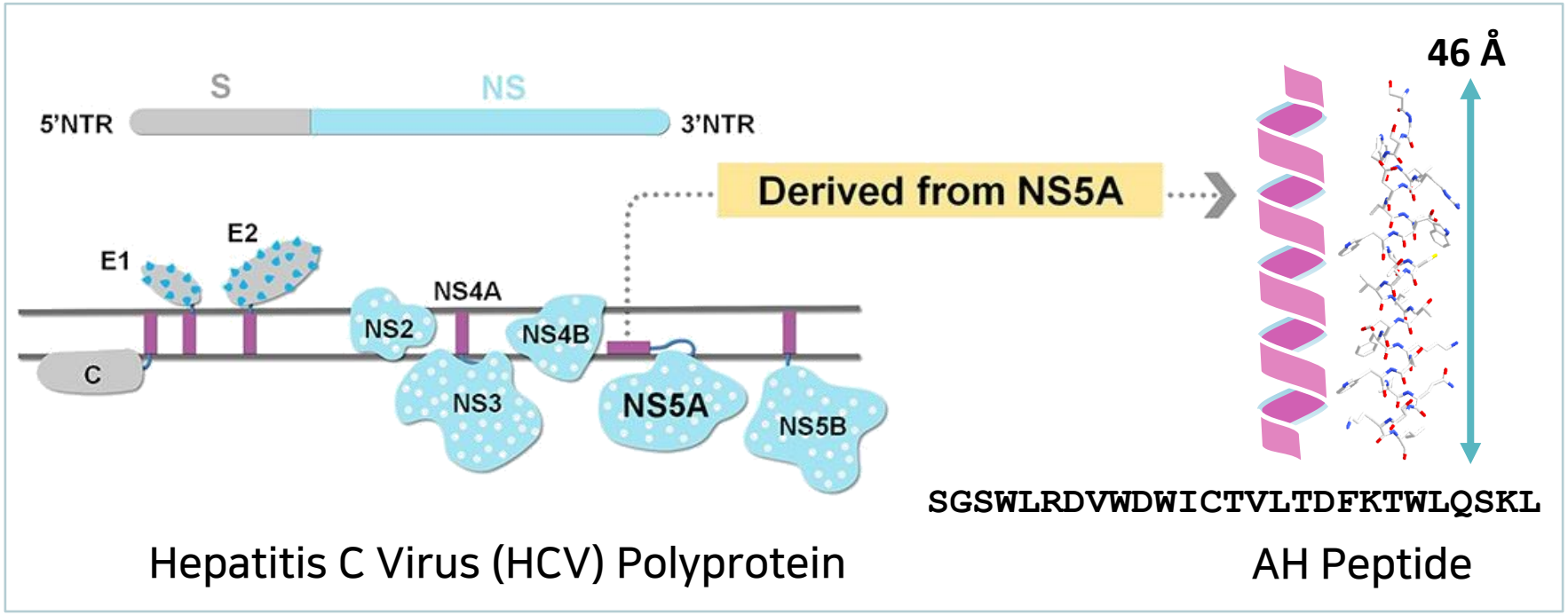


Virus-mimicking liposomes



Enveloped virus particles

AH Peptide: First LEAD Candidate



- AH peptide is derived from the N-terminus of HCV NS5A protein and involved in viral membrane association.
- Serendipitously discovered that AH peptide can <u>rupture small liposomes and enveloped viruses</u> (<160 nm diameter).

Cho et *al. JACS* 2007; Cho et *al. Analytical Chemistry* 2007; Cho et al., *ACS Chemical Biology* (2009).

AH vs NH Peptides

AH Peptide

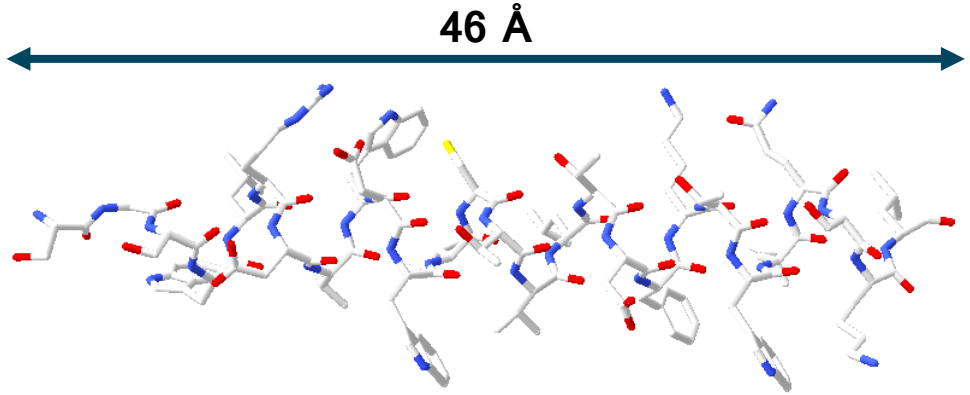
SGSWLRDVWDWICTVLTDFKTWLQSKL

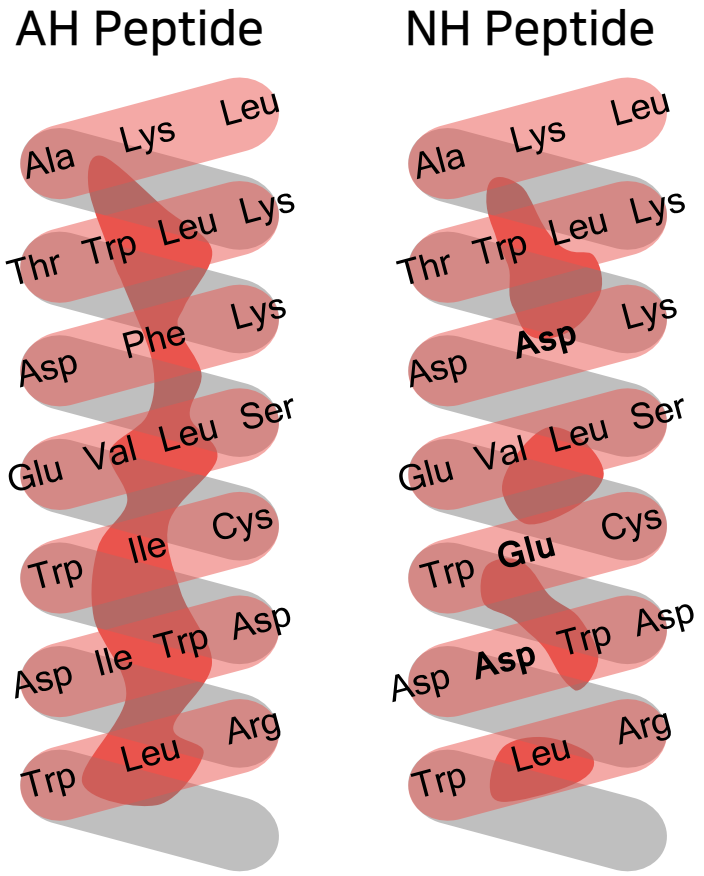
Theoretical Mass (M+H+): 3282.3

NH Peptide

SGSWLRDDWDWECTVLTDDKTWLQSKL

Theoretical Mass (M+H+): 3282.5

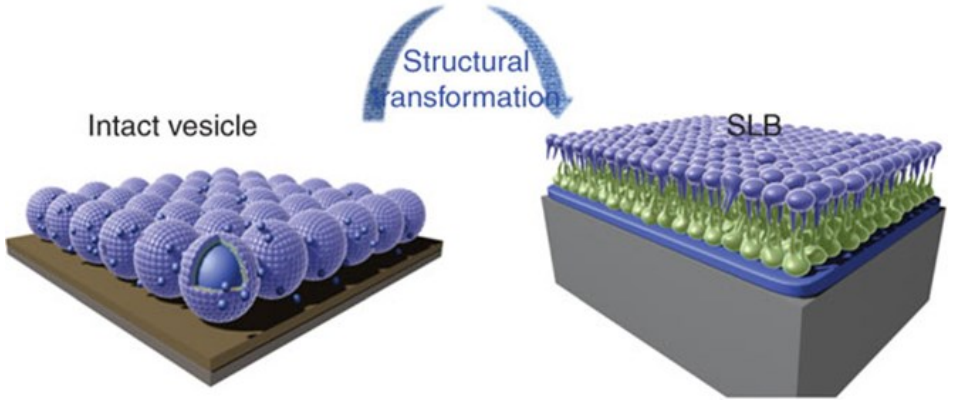


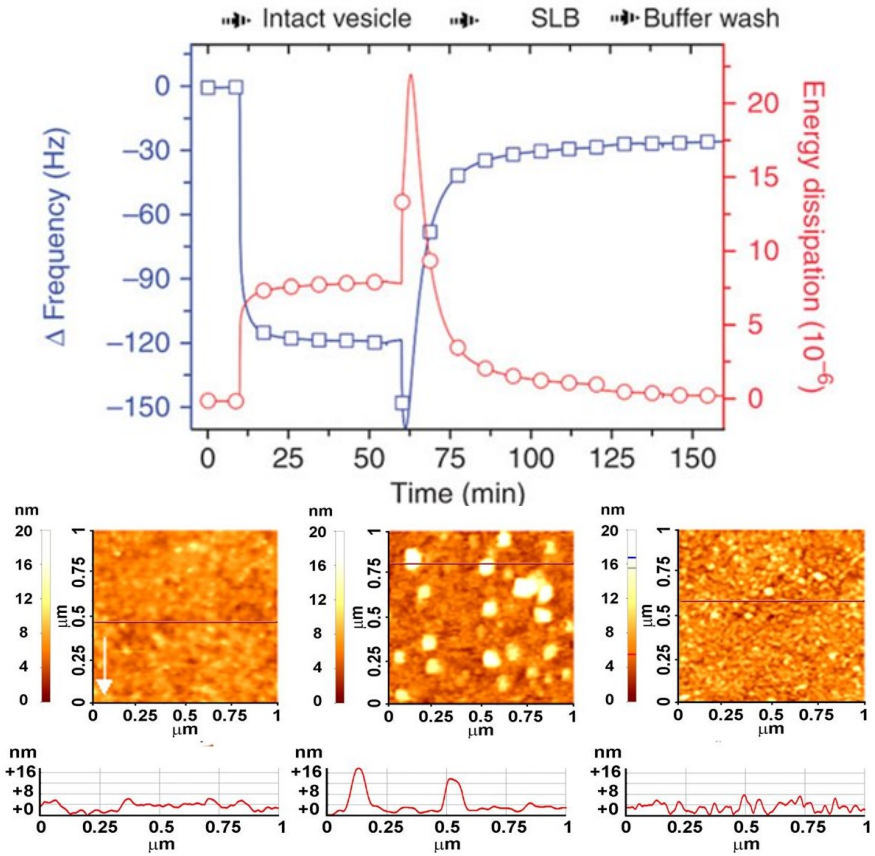


AH peptide preserves α-helical character, and NH peptide does not.

Discovery of a Vesicle-Rupturing Peptide

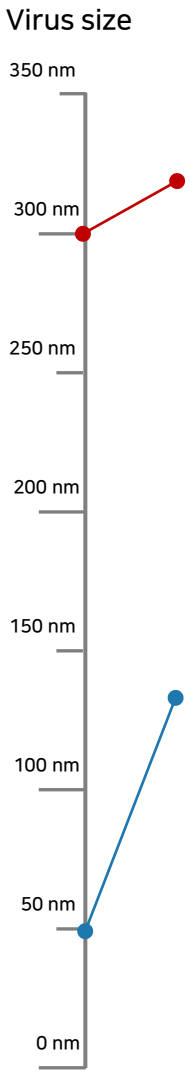
- Structural Transformation
  - AH peptide promotes vesicle rupture.
- Bilayer Formation
  - Values correspond to a planar bilayer.
- Multiple Substrates
  - Gold, TiO_2, Al_2O_3.



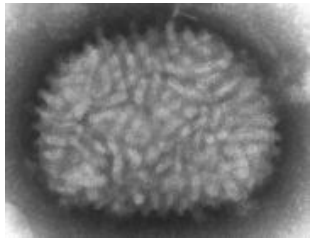


Cho et *al. JACS* 2007; Cho et *al. Analytical Chemistry* 2007; Cho et *al. Nature Protocols* 2010; Jackman et *al. J Phys Chem B* 2013

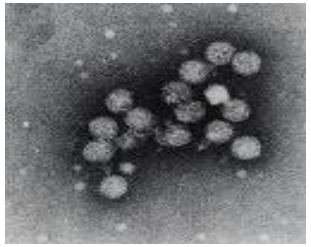
Effects of AH Peptide on Virus Infectivity



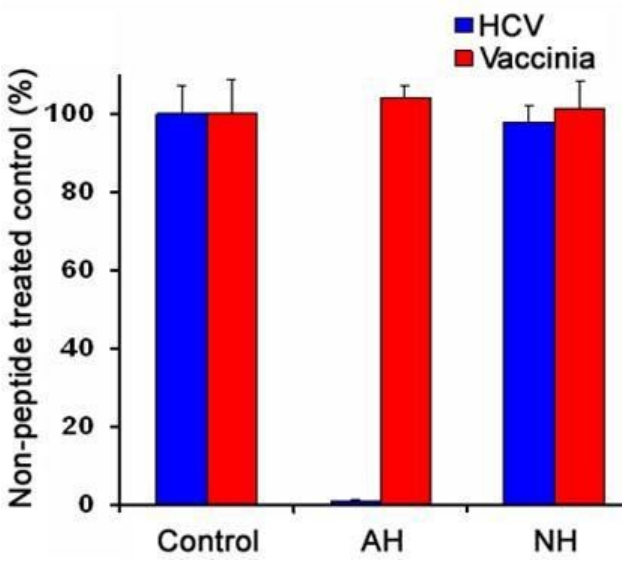
Vaccinia virus (VACV or VV)
large, complex, enveloped virus
~ 360 × 270 × 250 nm

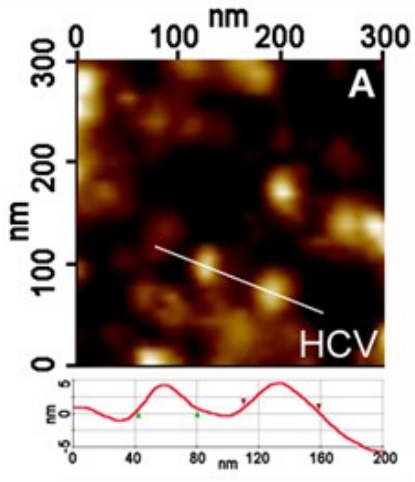


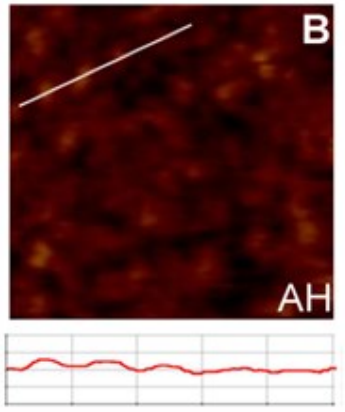
Hepatitis C virus (HCV)
small (50 nm in size),
enveloped, single strand RNA virus



AH peptide prevented infection in HCV while neither AH/NH peptide prevented vaccinia infection.

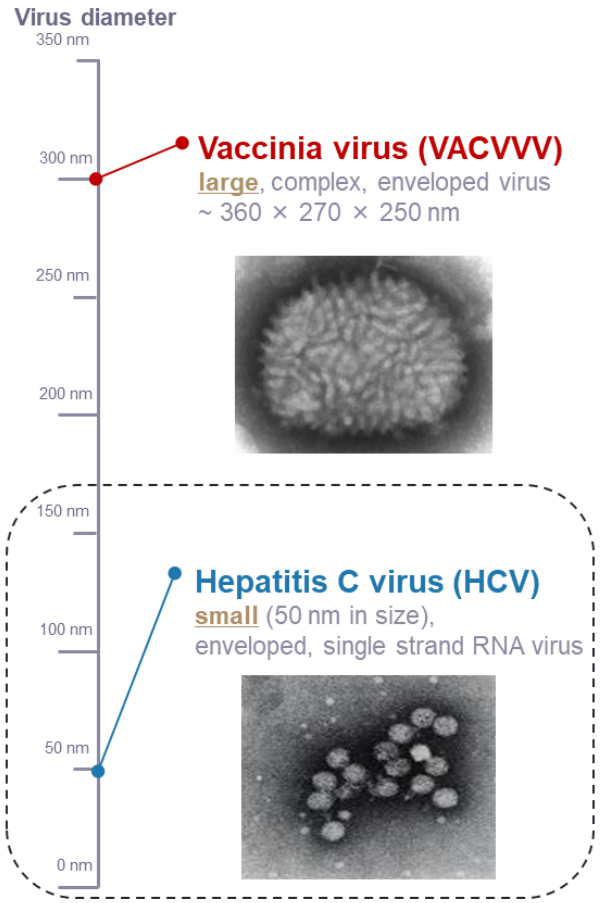


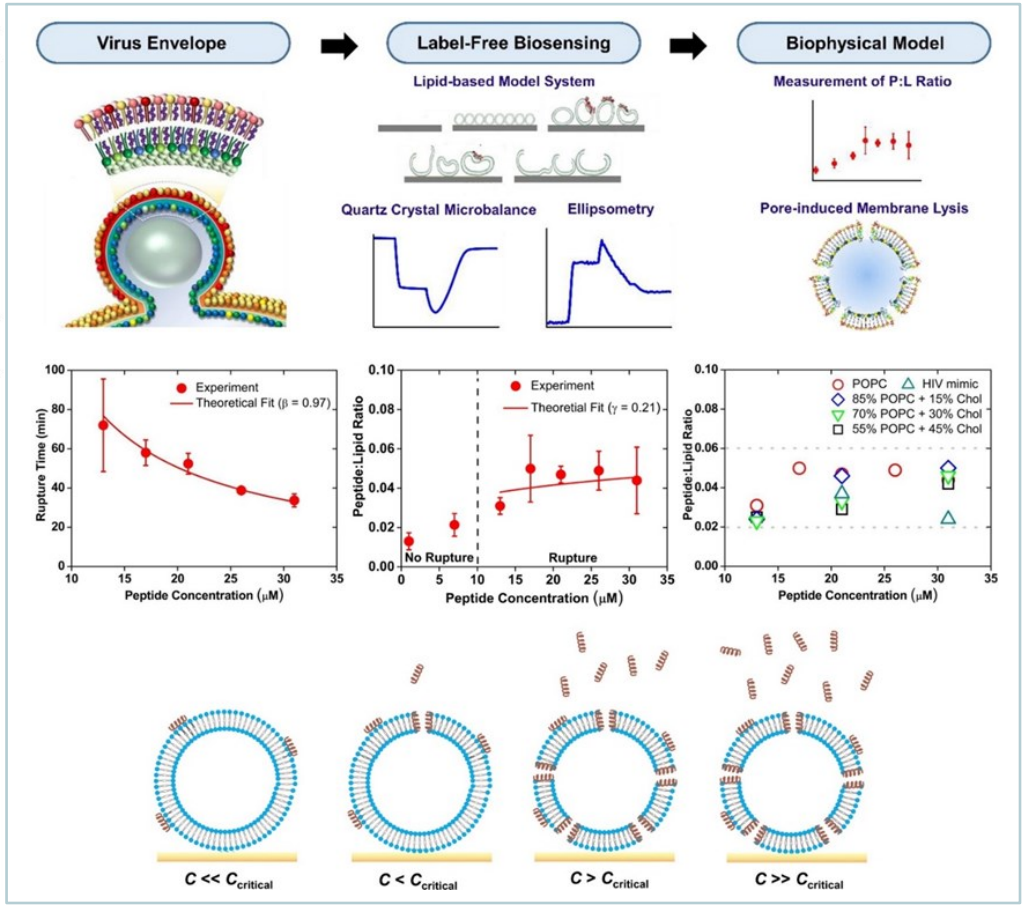




Membrane-Curvature-Dependent Antiviral Activity

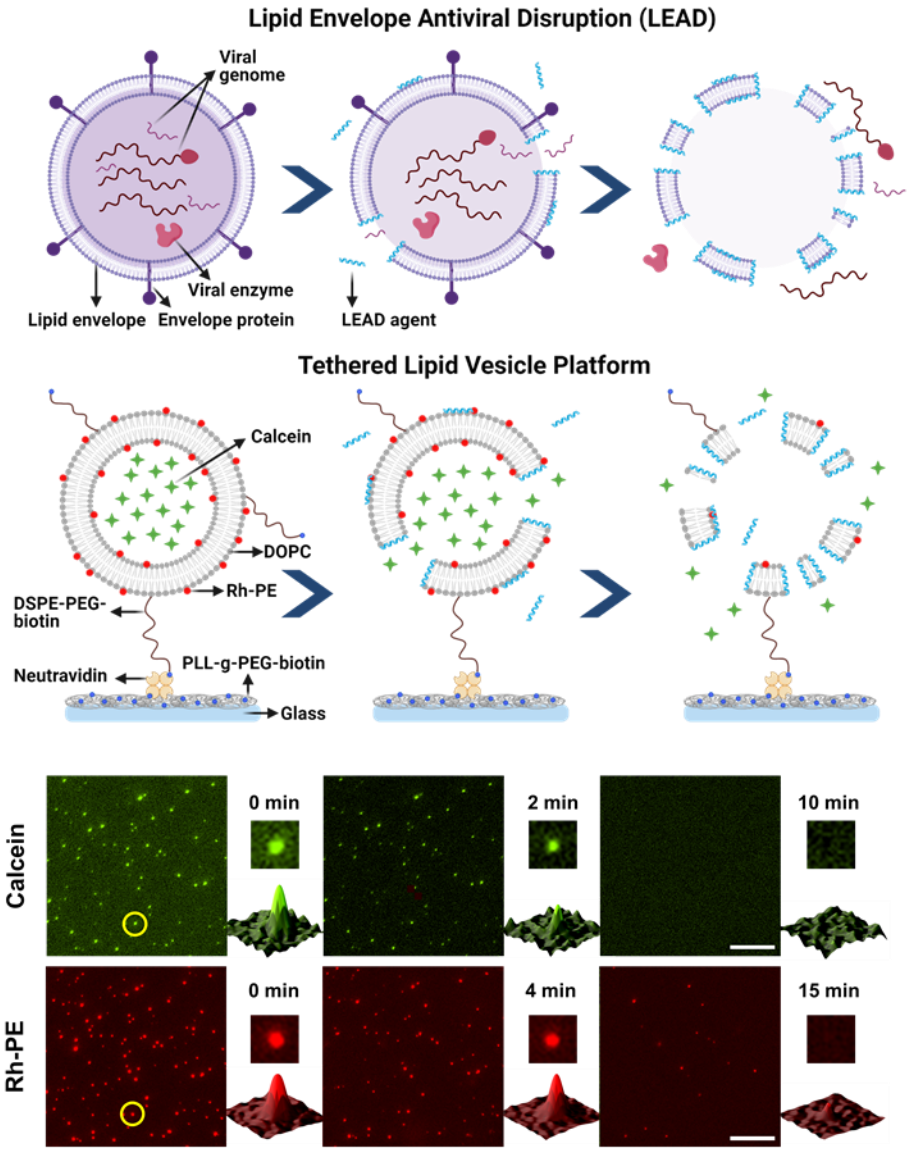
AH peptide forms pores in curved lipid membranes and triggers membrane lysis.

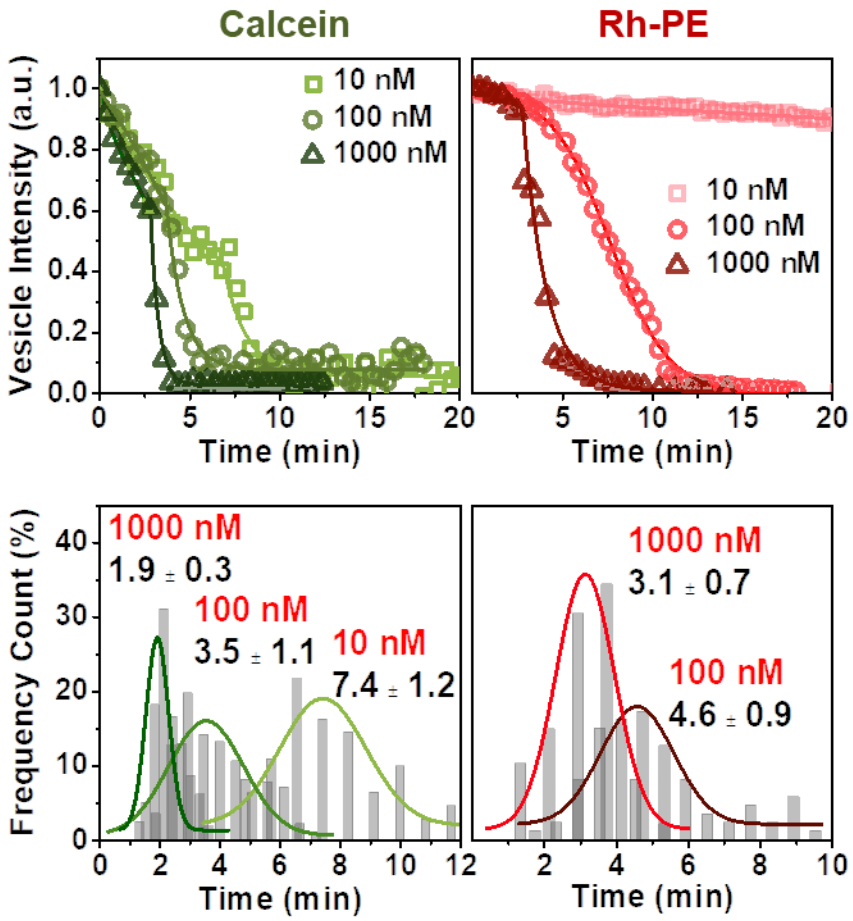




Cho et al., *Nat. Mater. (*2020).

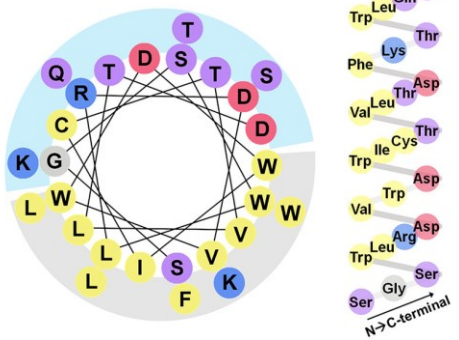
Tethered Lipid Vesicle: Model Viral Particle



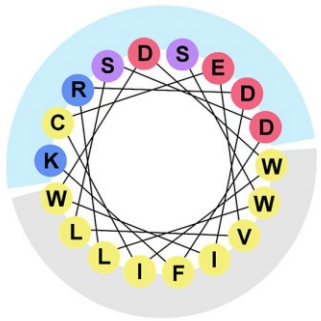


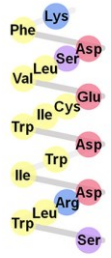
AH peptide has distinct selectivity principles compared to structural analogues.

AH Peptide

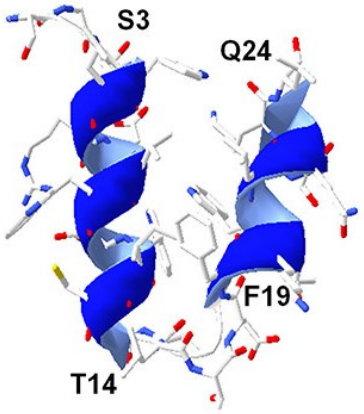


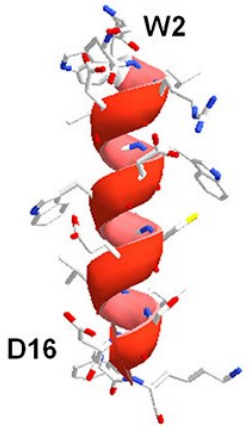
C5A Peptide

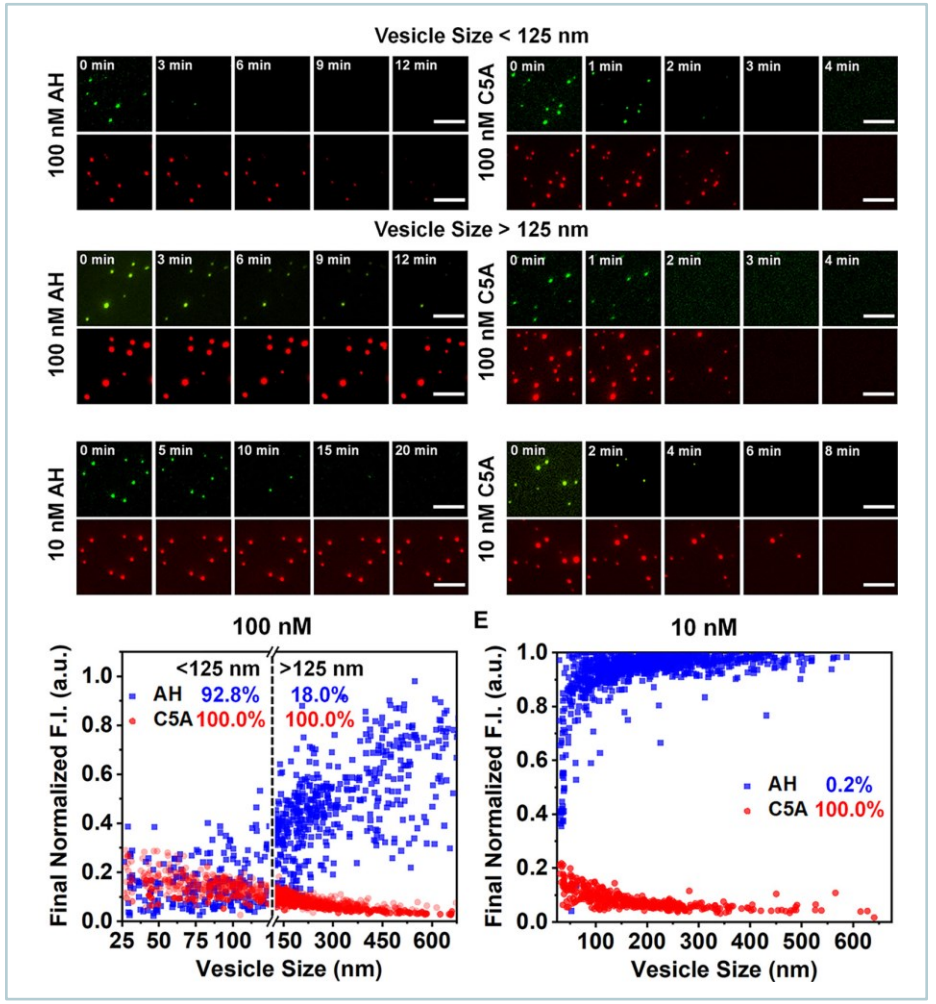




***De Novo* Folding Simulation**



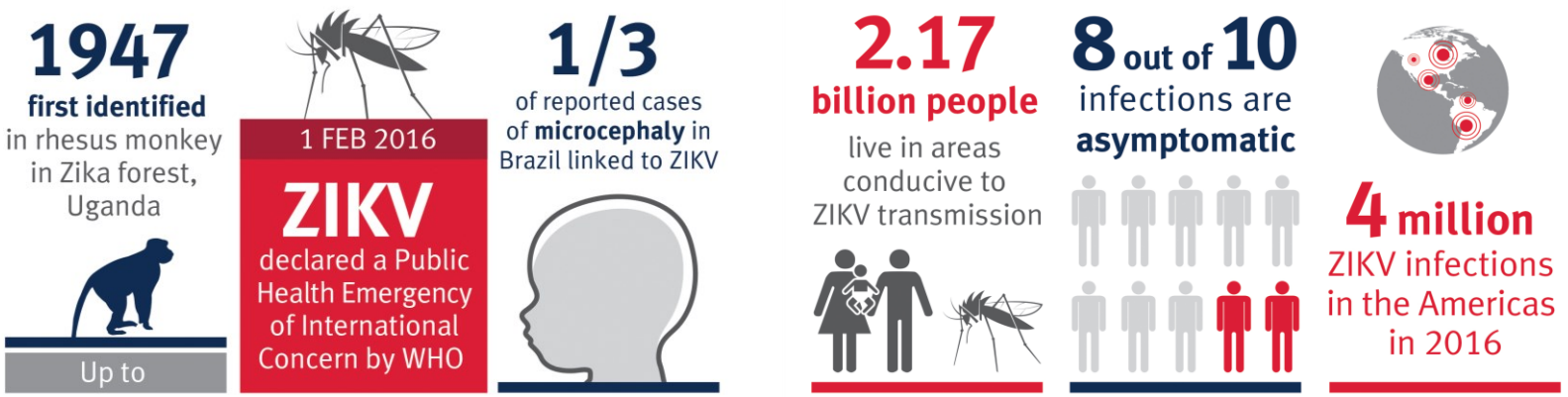


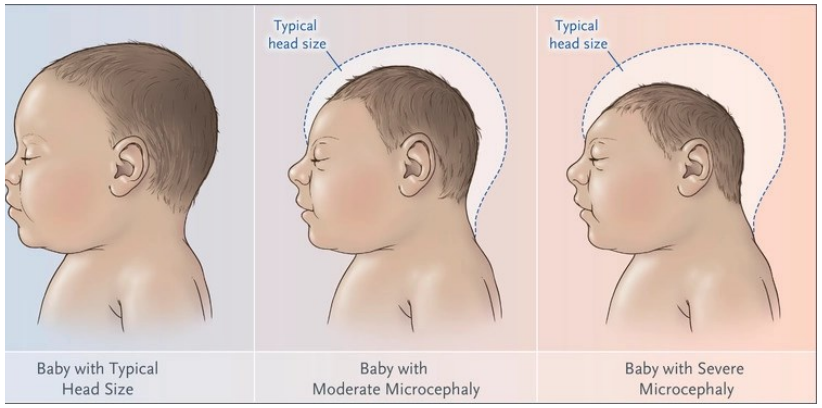


Langmuir 35, no. 30 (2019): 9934-9943.
Langmuir (**2021**) *37* (45), 13390-13398

Therapeutic Treatment of Zika Virus Infection Using a Brain-Penetrating Antiviral Peptide

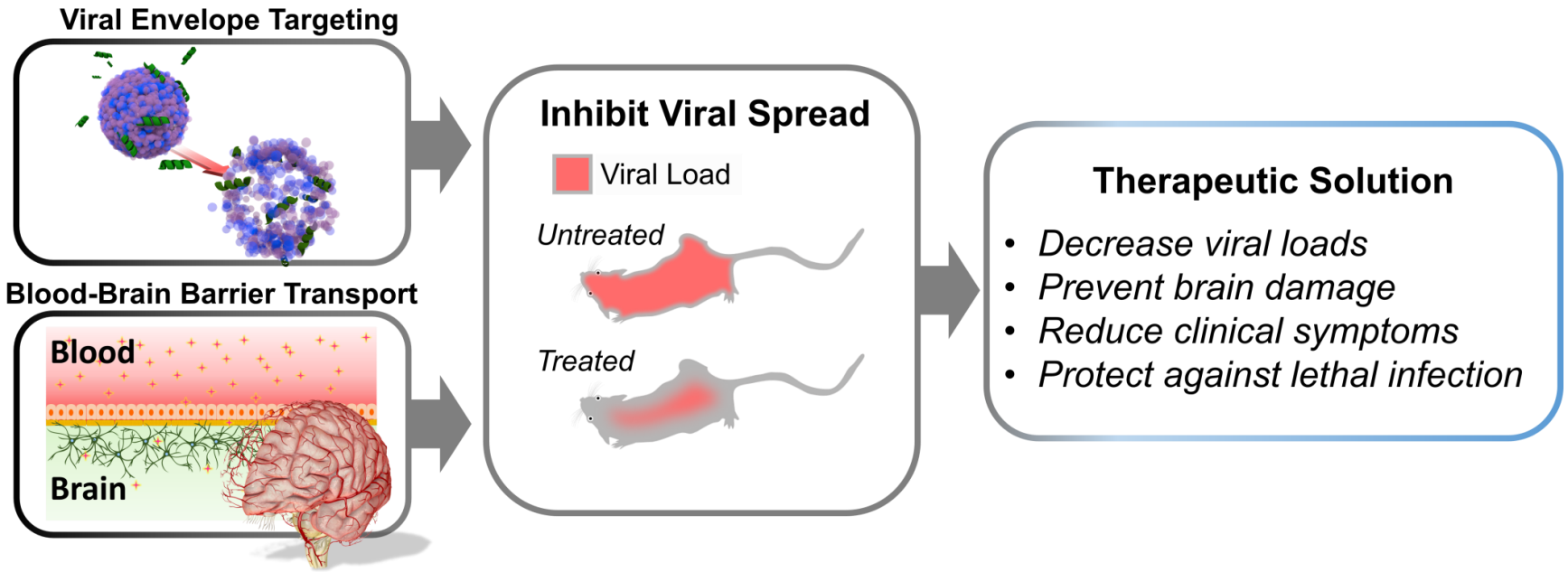
Zika Virus Epidemic: Neurodegenerative Disease

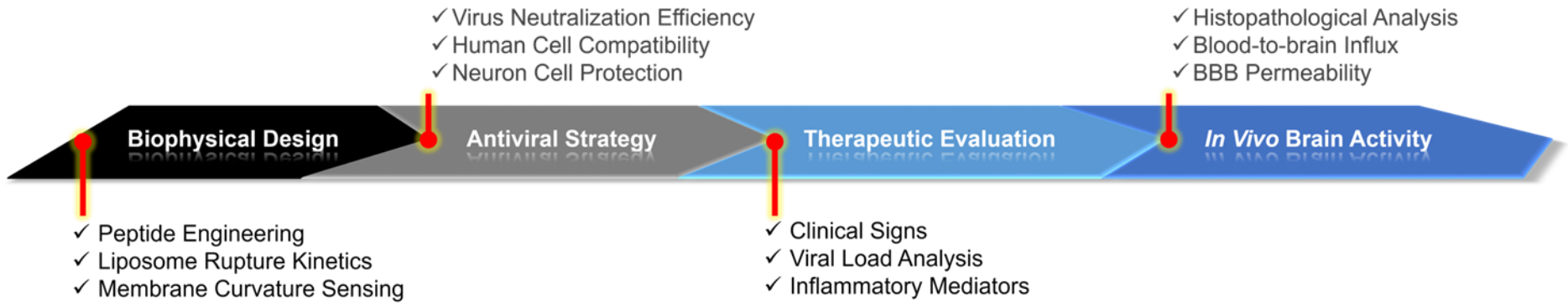




New England Journal of Medicine 374, no. 16 (2016): 1552-1563.

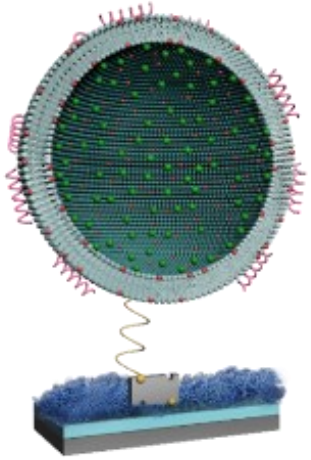
LEAD Concept





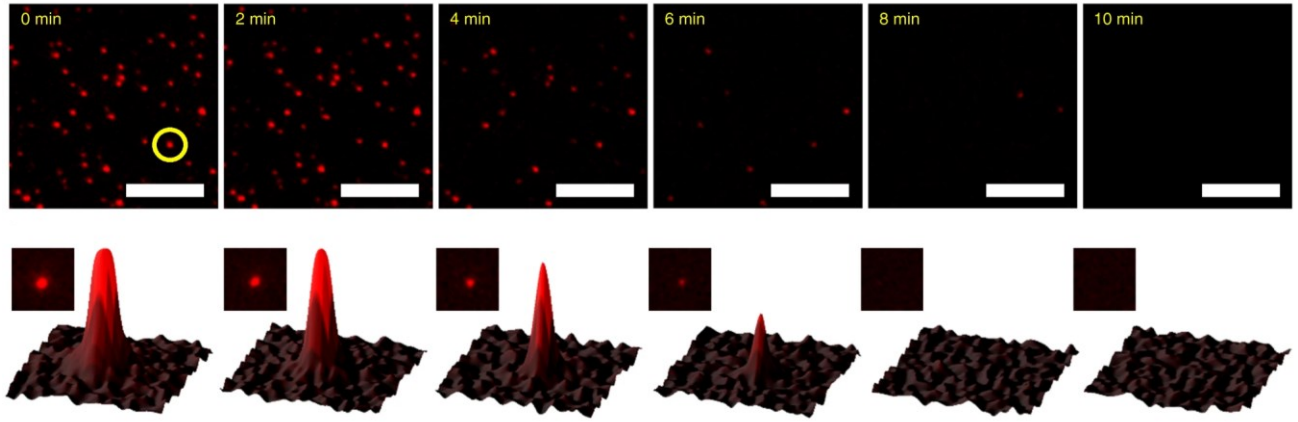
Jackman et al., *Nat. Mater. (*2018).

Stereochemical Engineering of AH-D Peptide

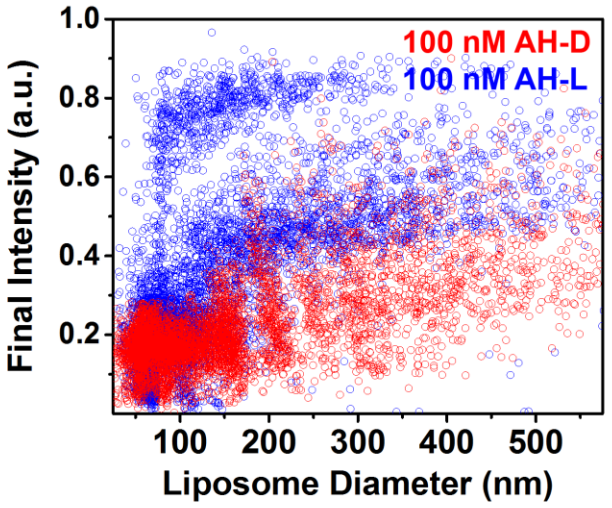


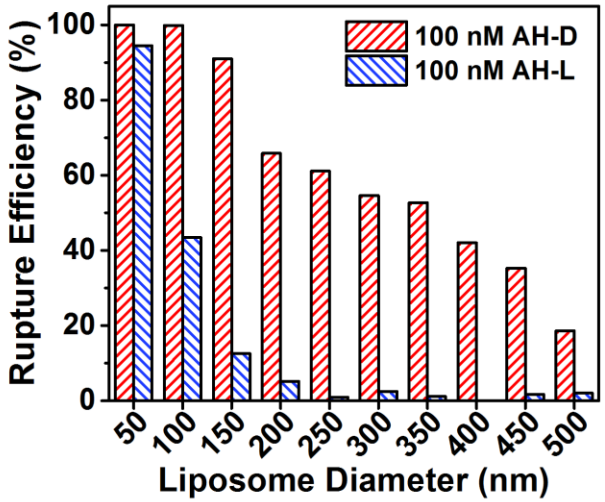
Tethered Vesicle Platform

Highly Parallel Imaging of Peptide-Induced Liposome Disruption



AH-D Peptide Has Refined Membrane-Curvature Selectivity

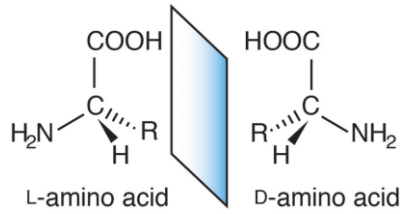




Peptide Engineering

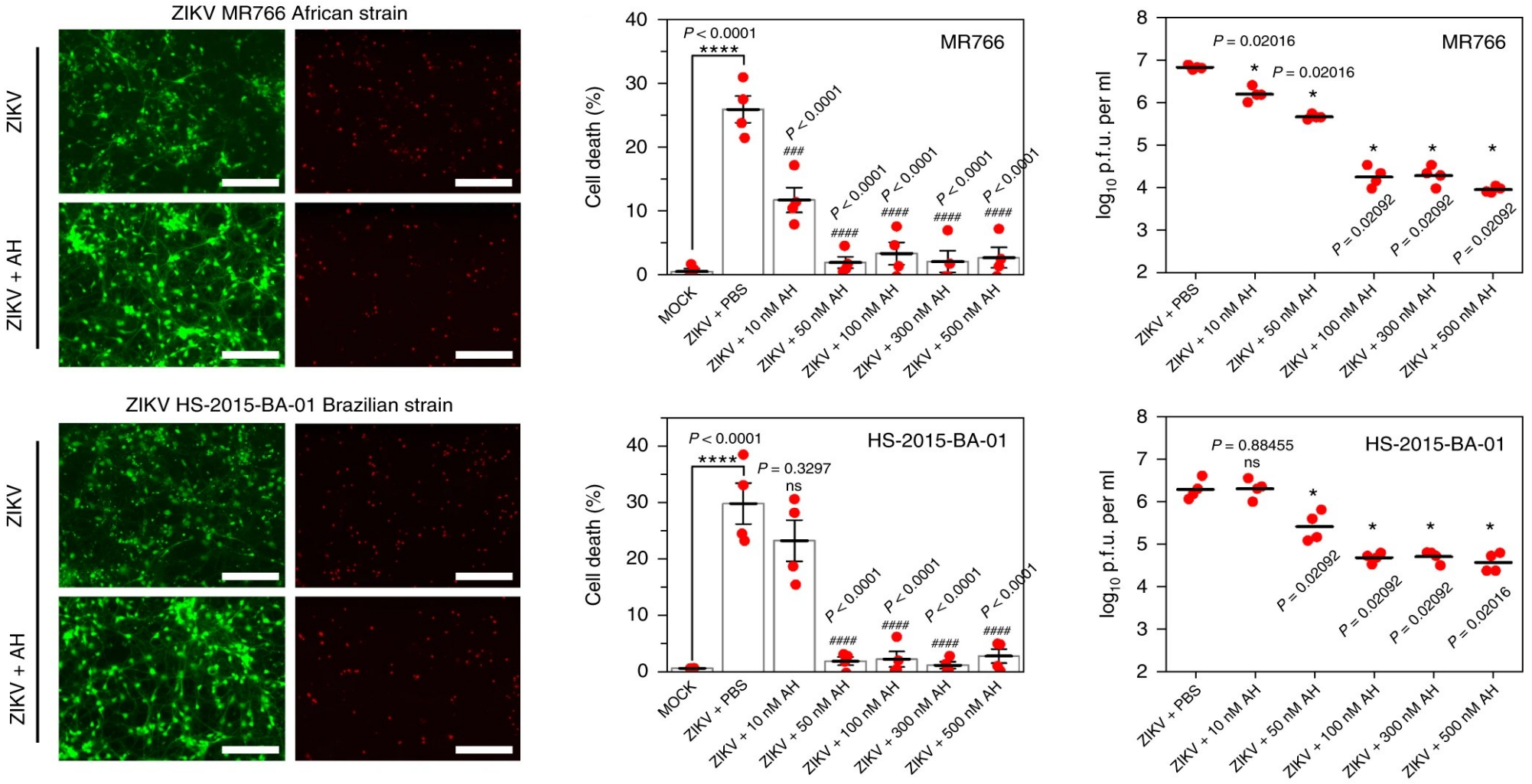
Achiral lipid membrane targeting

AH-L → AH-D



Jackman et al., *Nat. Mater.* (2018).

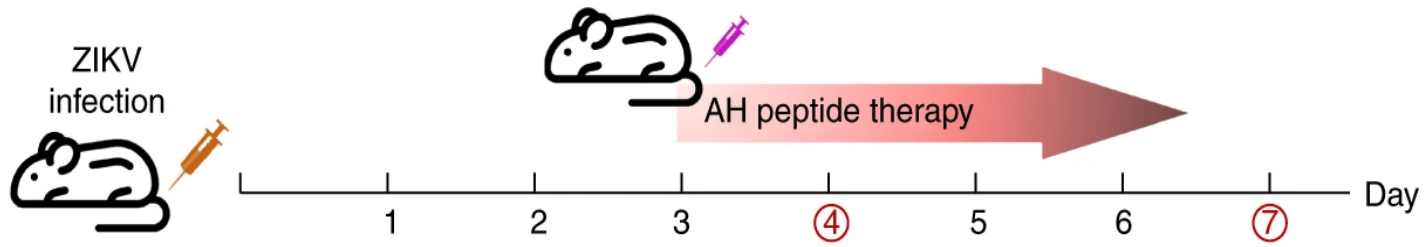
In Vitro Antiviral Characterization of AH-D Peptide

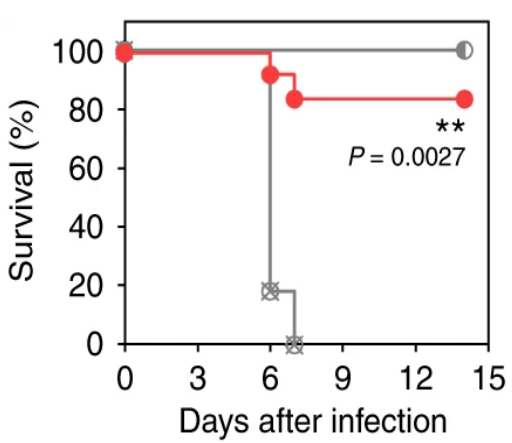


Nanomolar antiviral activity and >300-fold selectivity index

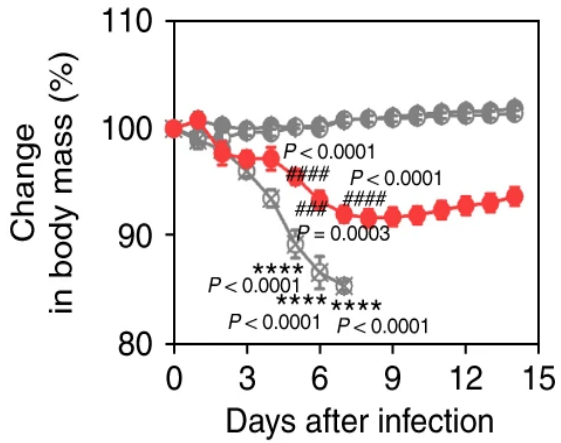
Jackman et al., *Nat. Mater. (*2018).

Therapeutic Testing in Lethal Mouse Model

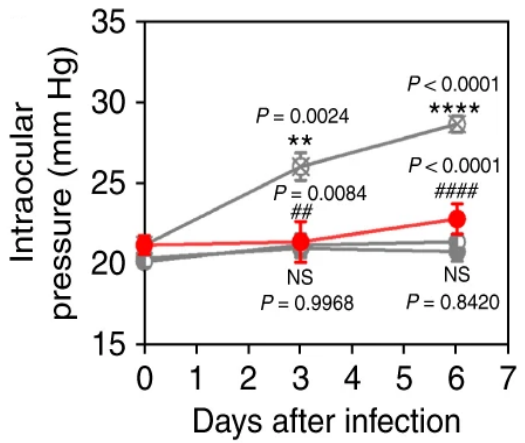




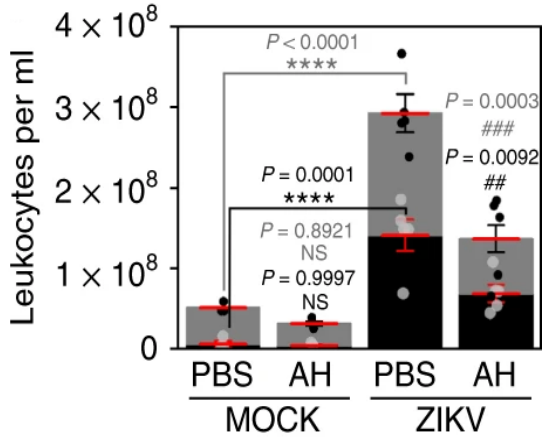
>80% mortality reduction



Decreased weight loss



Reduced eye pressure increase



Reduced white blood cell increase

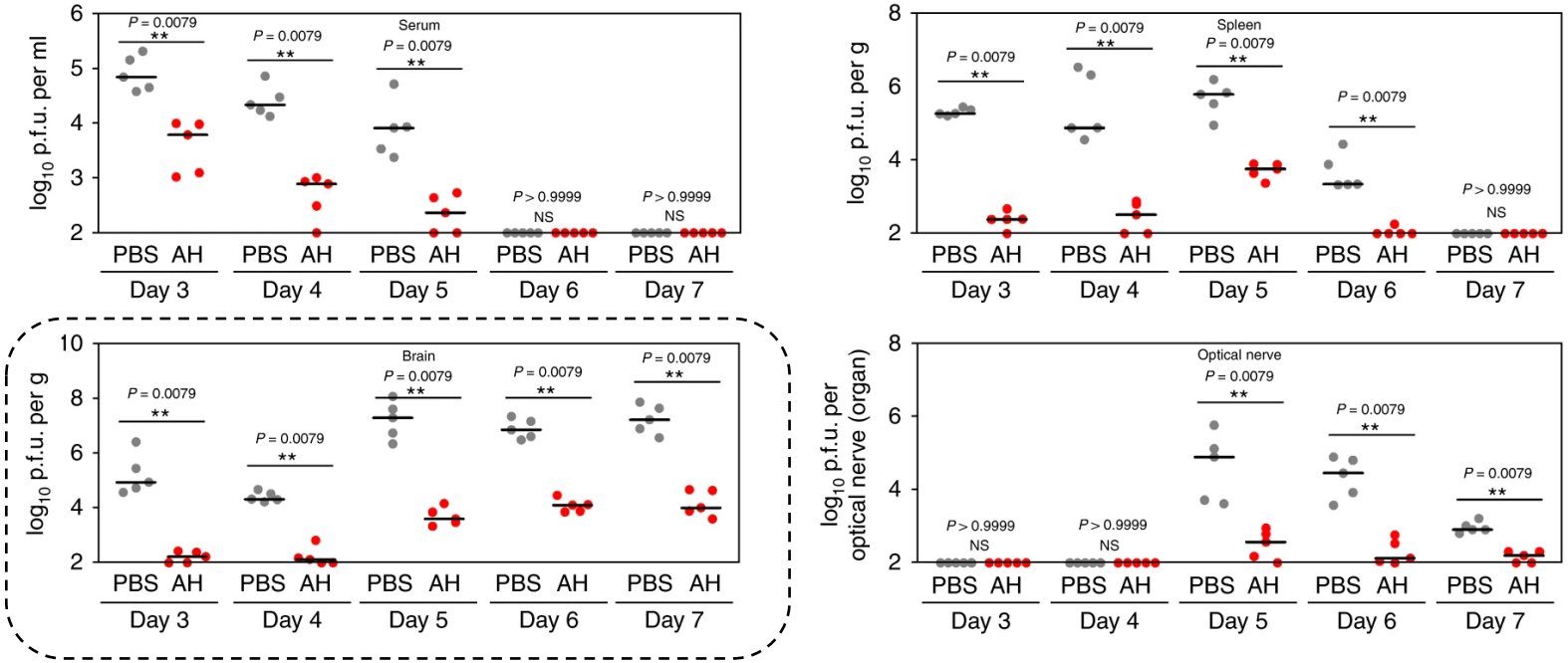
AH-D peptide therapy shows excellent clinical benefits.

Jackman et al., *Nat. Mater. (*2018).

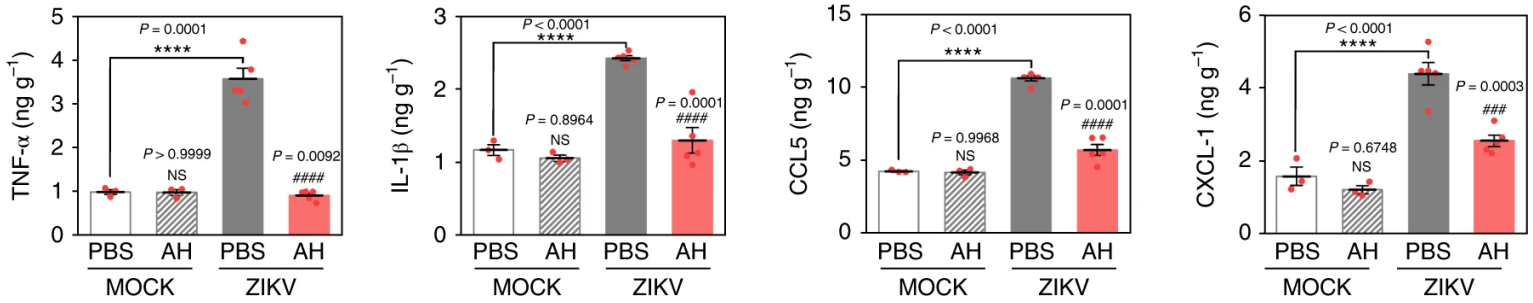
Antiviral and Anti-Inflammatory Treatment Effects

Decreased viral loads in serum, spleen, brain, & optical nerve

Brain



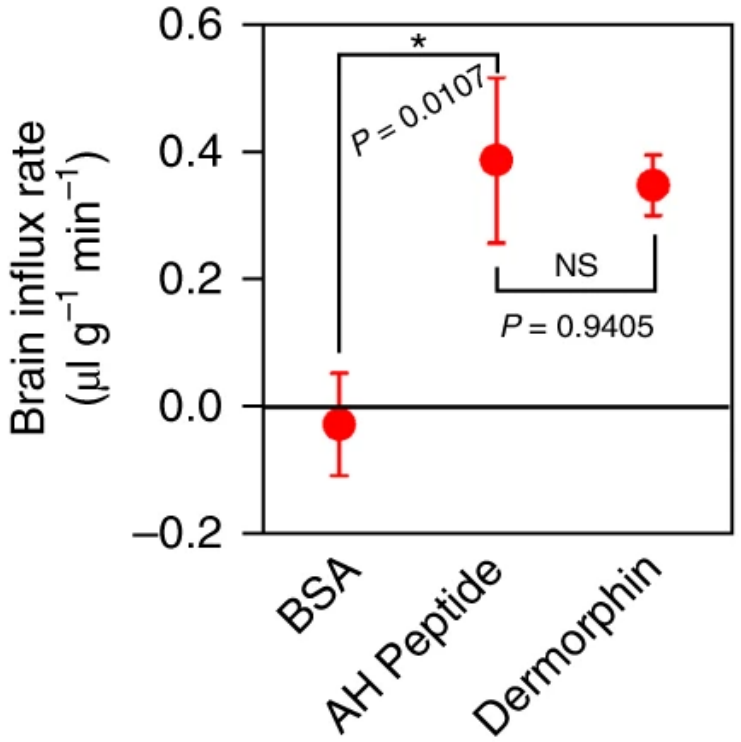
Decreased cytokine and chemokine levels in the brain

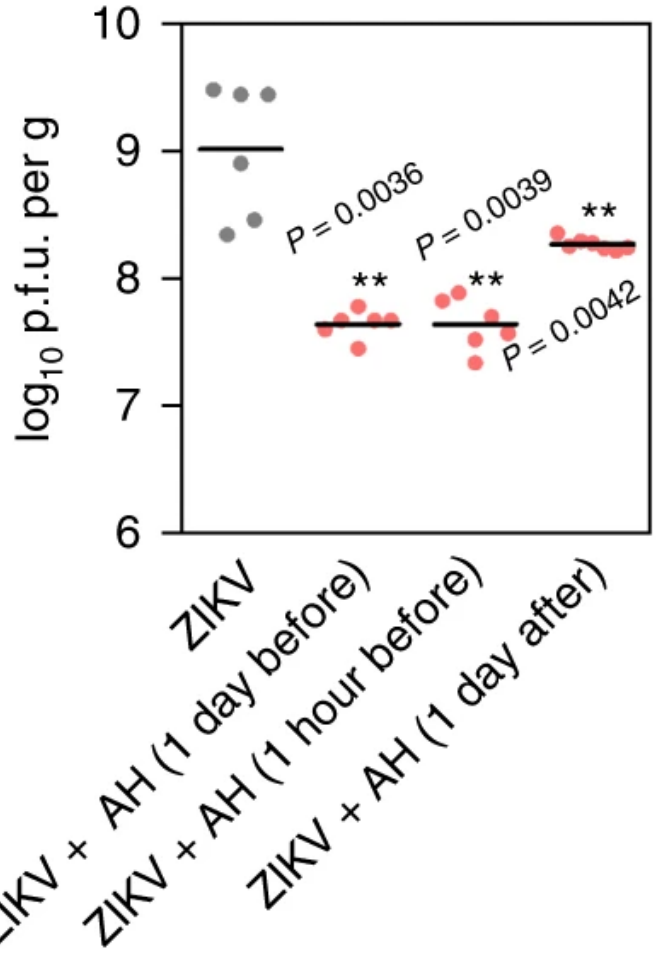


Jackman et al., *Nat. Mater.* (2018).

AH-D Peptide Inhibits Zika Virus in the Brain

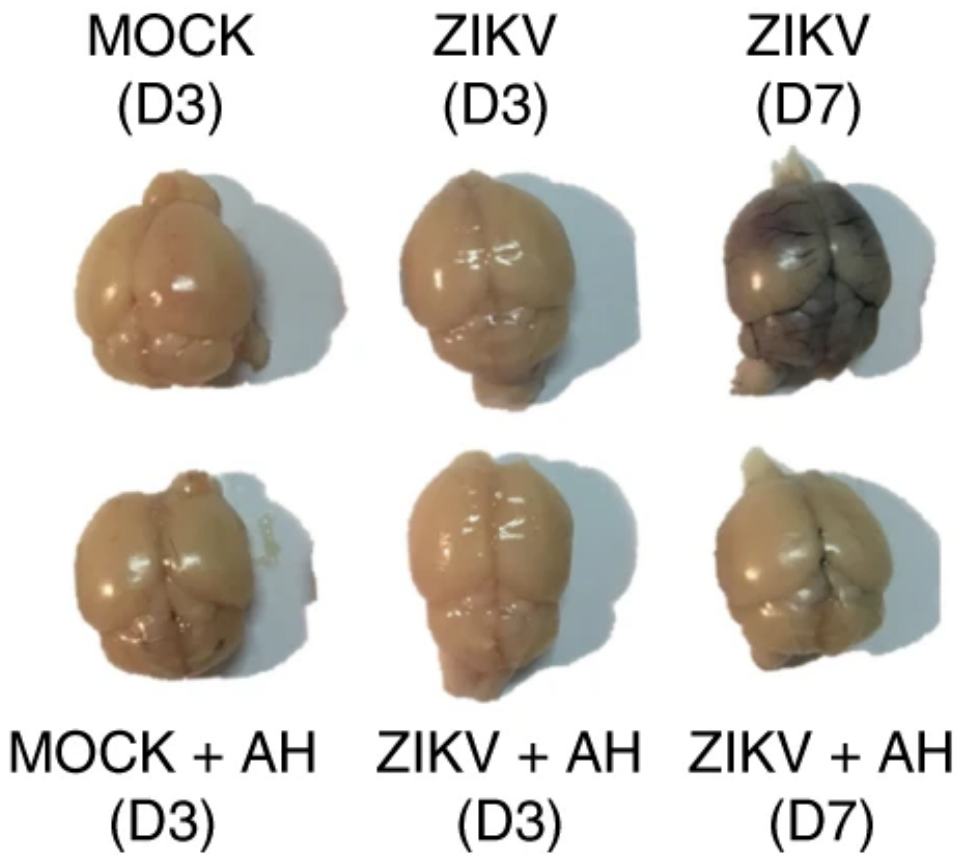
AH-D peptide cross blood-brain barrier and is active in brain.

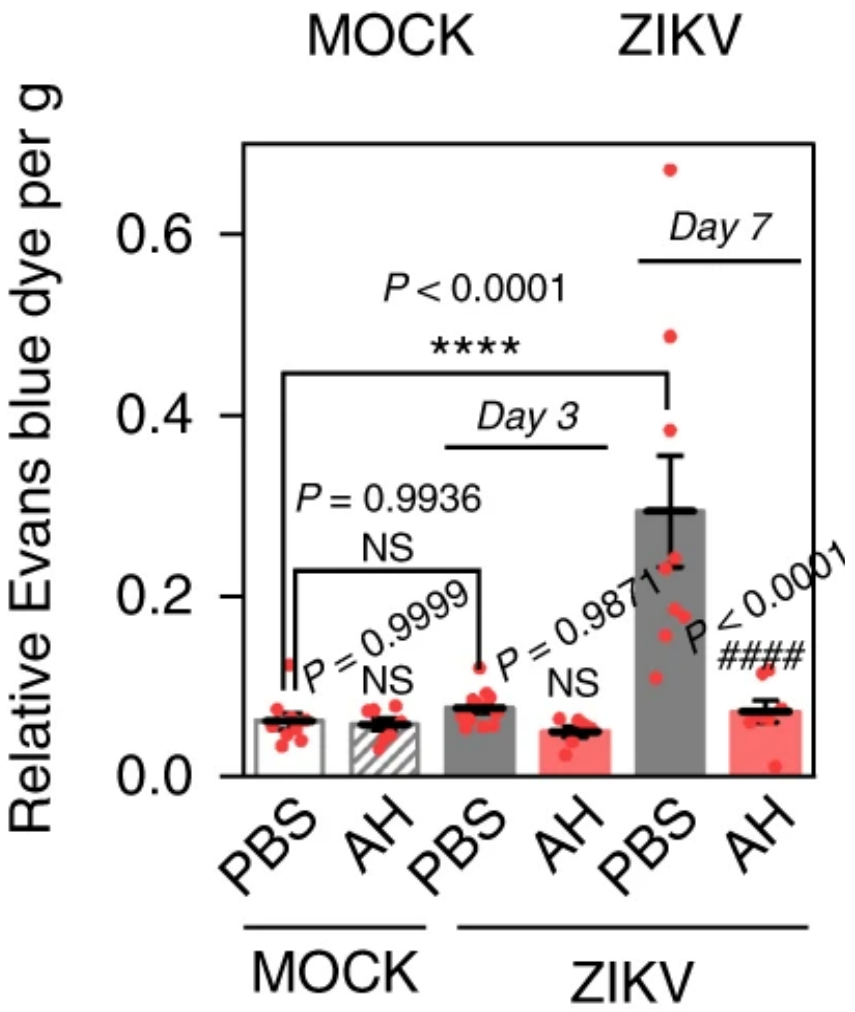




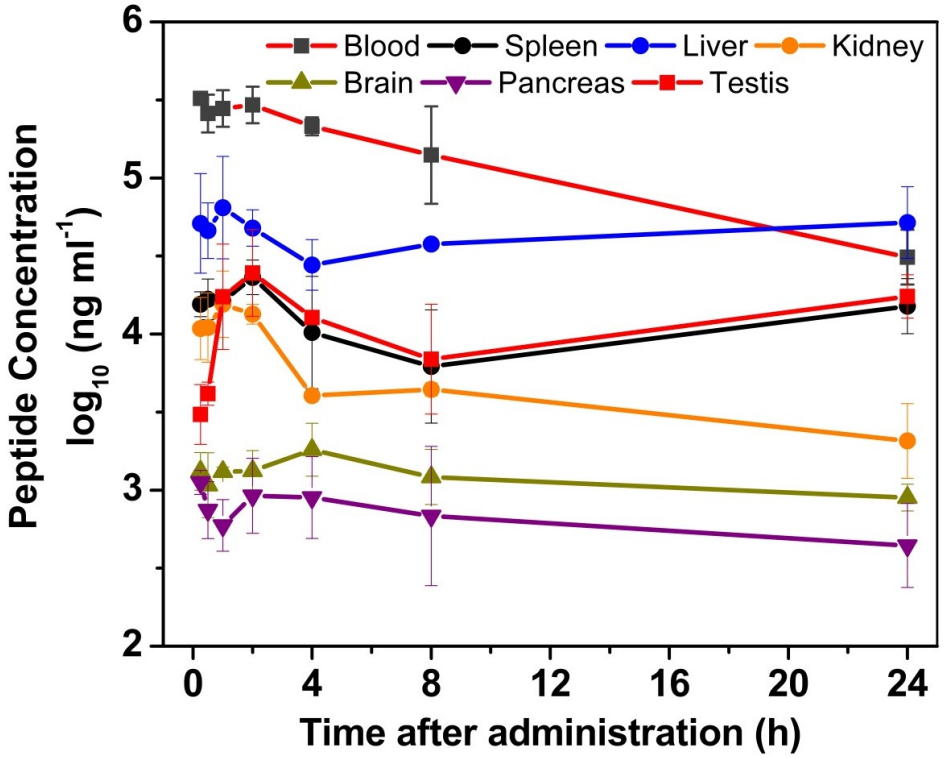
Jackman et al., *Nat. Mater. (*2018).

AH-D peptide can cross intact BBB and maintain BBB integrity.





PK/PD Evaluation

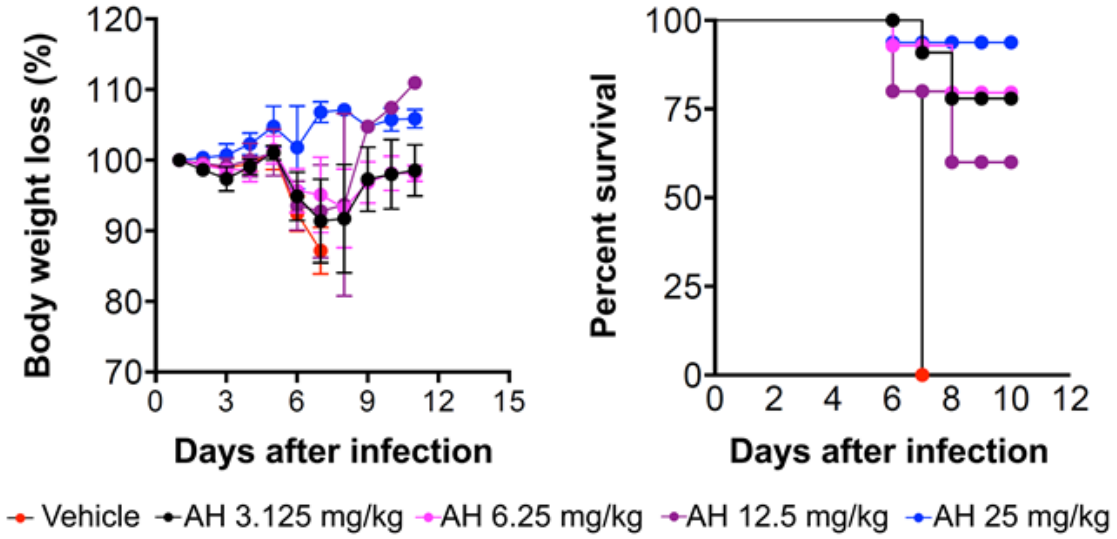


- 25 mg/kg i.v. bolus administration → sampling over 24 hrs
- High peptide concentrations in blood and relevant tissues.
- Brain concentration is around 400-600 nM.

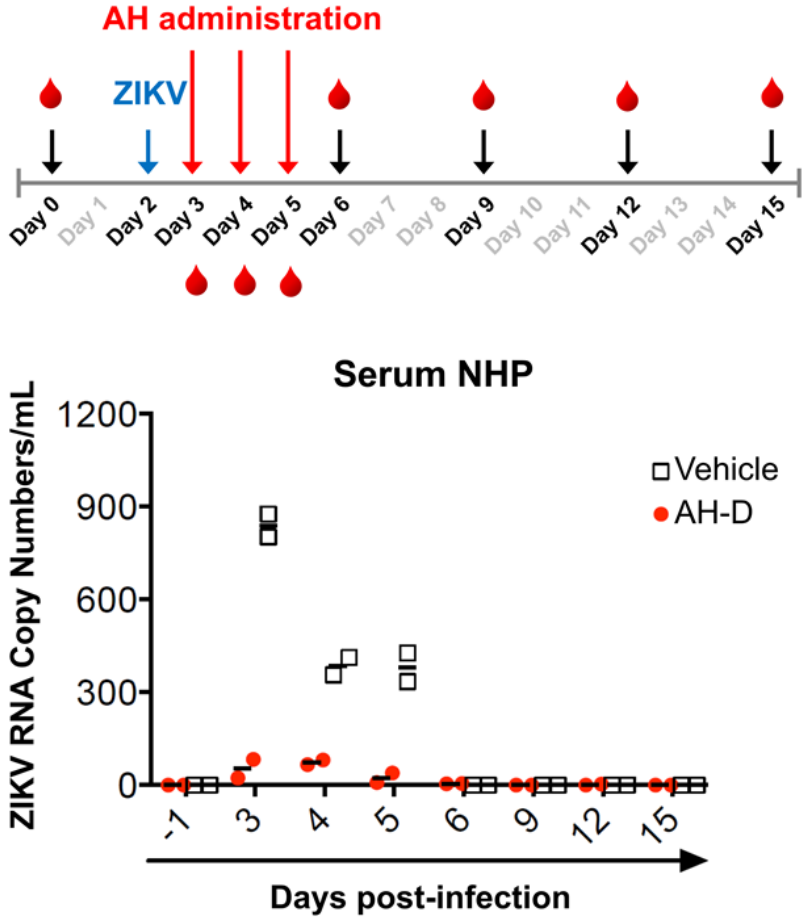
Jackman et al., *Nat. Mater. (*2018).

Antiviral *In Vivo* Tests for Zika & Dengue

Antiviral *in vivo* testing of AH peptide to inhibit dengue virus infection in <u>mice</u>



Antiviral *in vivo* testing of AH peptide to inhibit Zika virus infection in <u>marmoset</u>



Broad-Spectrum Activity of AH Peptide

Virus	EC_{50} (μM)
SARS-CoV-2 virus	0.030
Zika (FSS13025)	0.960
Zika (PRVABC-59)	1.240
Zika (MR766)	1.040
Dengue-1 (PRS41393)	1.040
Dengue-2 (New Guinea C)	1.150
Dengue-3 (H87)	1.200
Dengue-4 (H241)	1.140
Yellow Fever (17D)	0.098
Japanese Encephalitis (SA 14-14-2)	0.150
Powassan Virus (BL)	5.170
Chikungunya (181/25)	2.520
Ebola (Zaire)	0.930
Marburg (Angola)	0.640
Rift Valley Fever Virus (MP12)	0.260
Human cytomegalovirus (AD169)	<4
Vaccinia Virus (NYCBH)	>12
Polio Virus (Malhoney)	>30

Works against a wide range of viruses that are of importance to clinical medicine and biodefense.

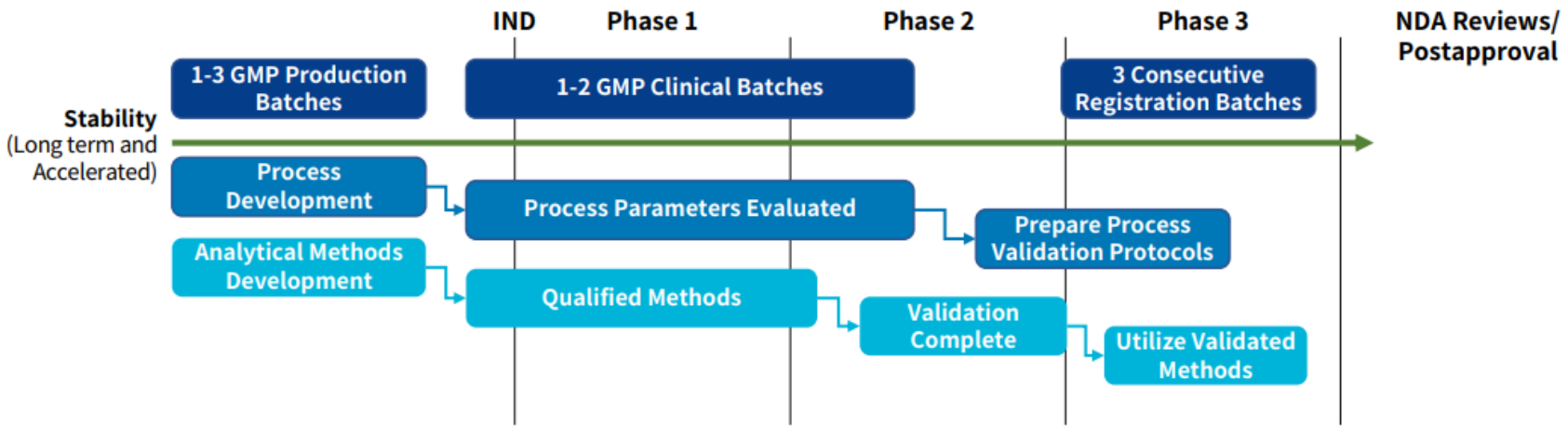
Tested in collaboration with

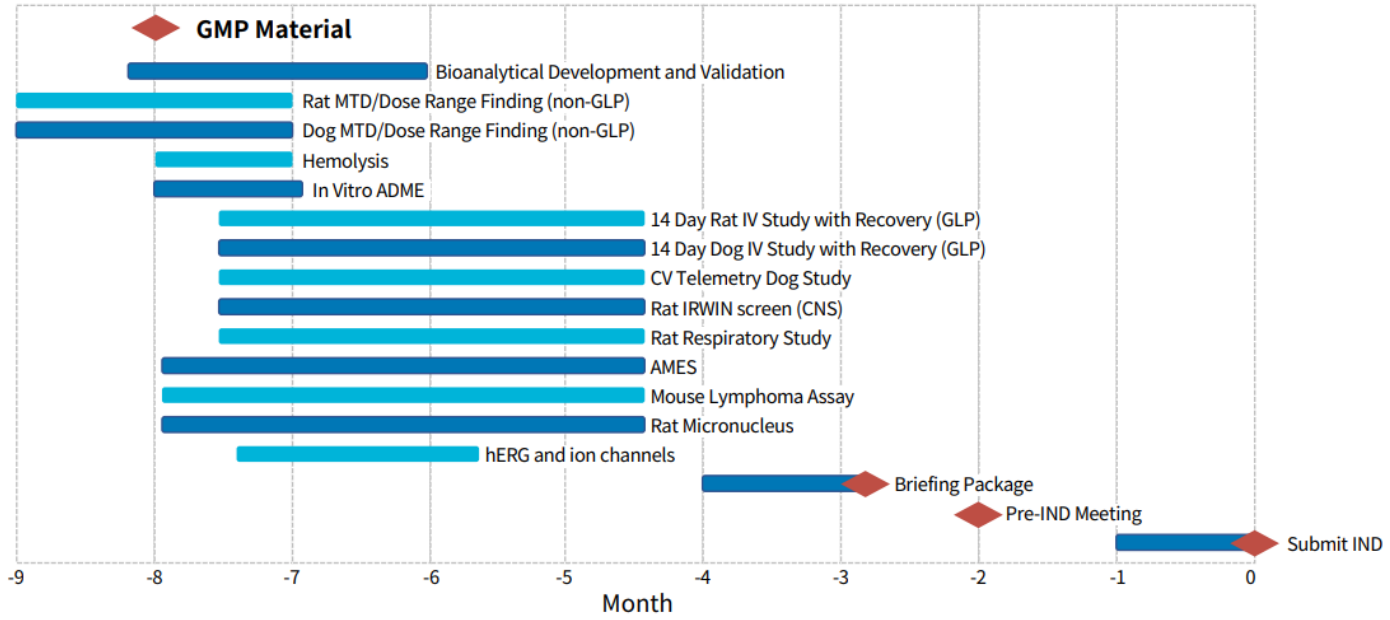
USAMRIID
United States Army Medical Research Institute of Infectious Diseases

Public Health England

NIH National Institute of Allergy and Infectious Diseases

Nonclinical Program to Initiate IND





Deep Learning from Nature: AH Peptide Engineering

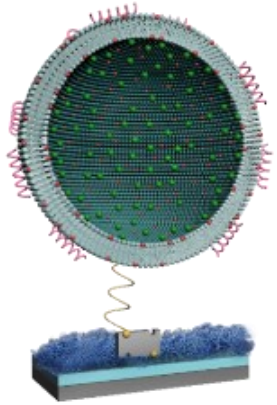
Blue Letter: Conservative mutation
Red Letter: Non-conservative mutation

Conserved residues: Acidic, Basic, Hydrophobic, Thiol

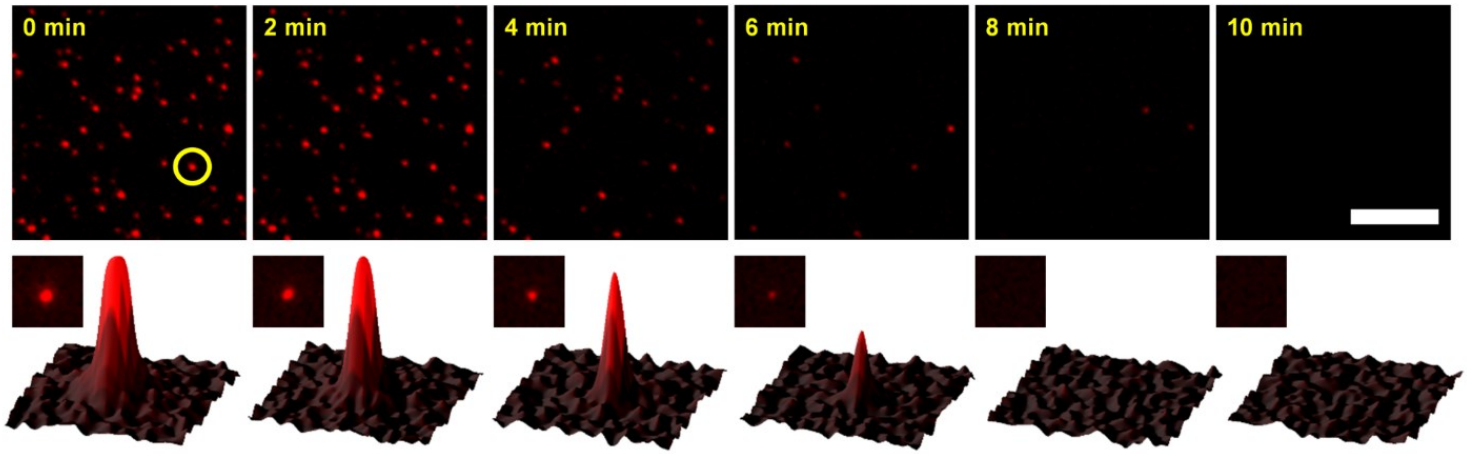
	Amino Acid Sequence (NH_2 5 10 15 20 25 COOH)	pI	pH 7
TMIG1	S G S W L R D I W D W I C E V L S D F K T W L K A K L	6.3	-0.1
1b (AH)	S G S W L R D V W D W I C T V L T D F K T W L Q S K L	6.1	-0.1
TMIG2	S G S W L R D V W D W V C T I L T D F K N W L T S K L	6.1	-0.1
TMIG3	S G S W L R D I W E W V C S I L T D F K N W L S A K L	6.2	-0.1
TMIG4	S D D W L R I I W D W V C S V V S D F K A W L S A K I	4.2	-1.1
TMIG5	S G D W L R I I W D W V C S V V S D F K T W L S A K I	6.1	-0.1
TMIG6	S D D W L R T I W D W V C S V L A D F K A W L S A K I	4.2	-1.1
TMIG7	G D D W L H D I W D W V C I V L S D F K T W L S A K I	3.9	-3.0
TMIG8	D G N W L Y D I W N W V C T V L A D F K L W L G A K I	4.0	-1.1
TMIG9	A E S W L W E V W D W V L H V L S D F K T C L K A K F	5.3	-1.0
TMIG10	G S T W L R D I W D W V C T V L S D F R V W L K S K L	8.8	0.9

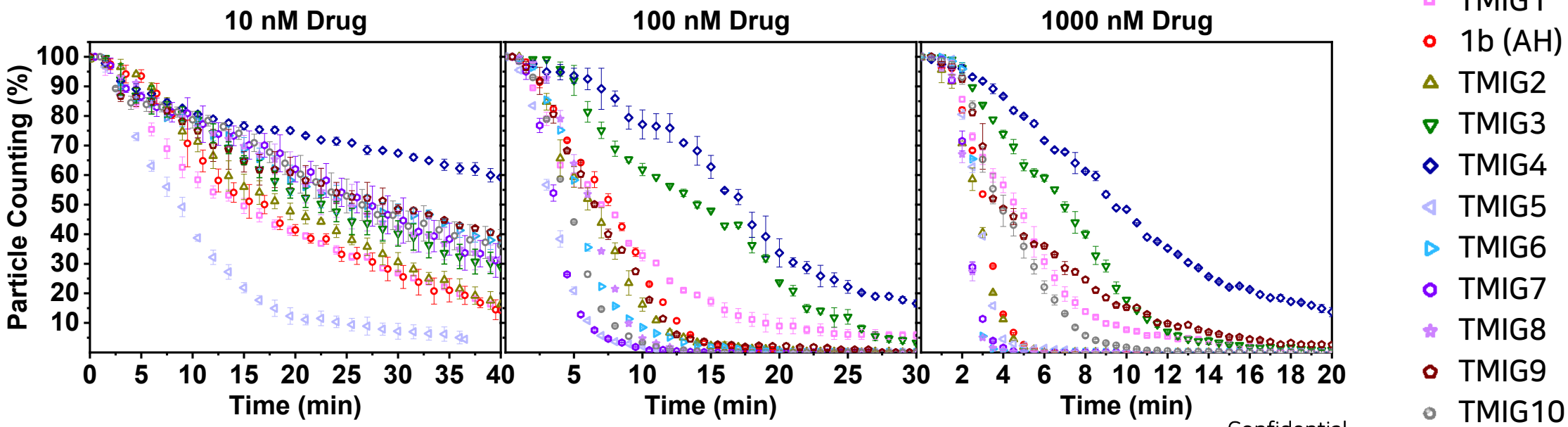
Note: All sequences start with amino group (N-terminus) and end with carboxyl group (C-terminus). In case of C-terminal amidation, isoelectric point (pI) is ~2.4 higher than carboxylation.

Single Tethered Vesicle Platform

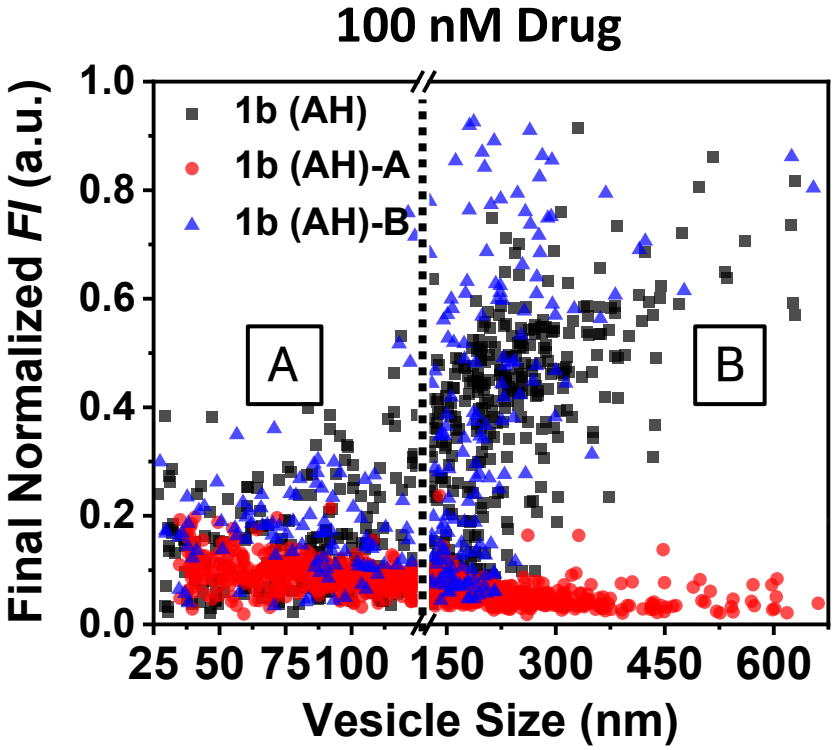


Synthetic lipid envelope particles (controllable size)

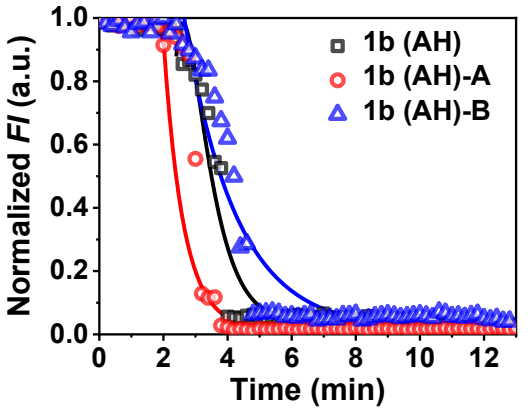




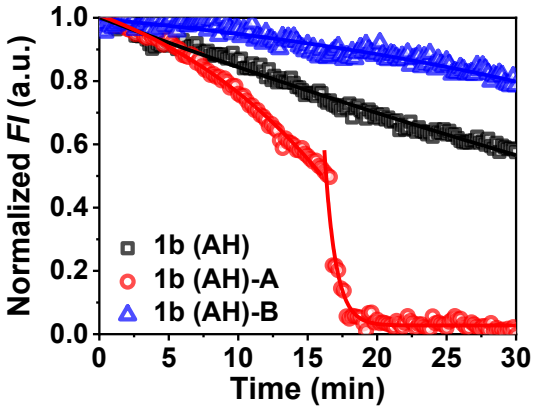
AH-D Functional Terminals: Single Vesicles



A < 125 nm vesicles

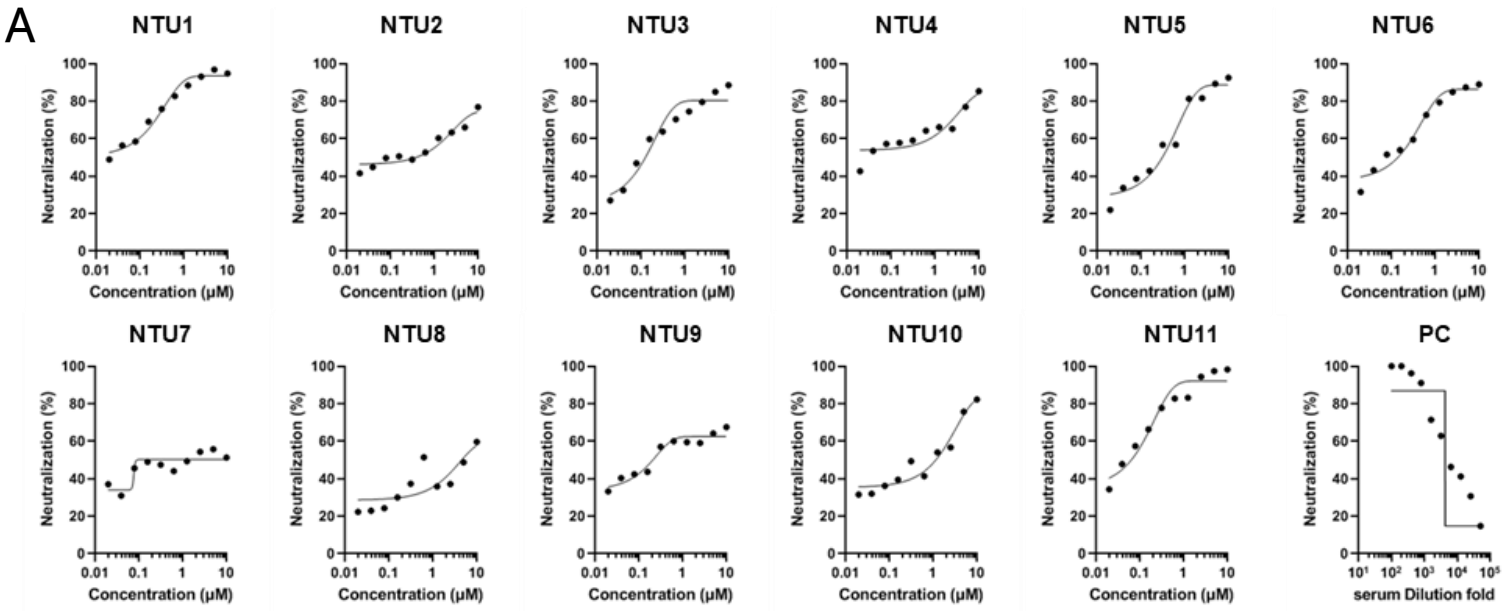


B > 125 nm vesicles



Screening Data: Engineered Peptides

A



Peptide	NTU1	NTU2	NTU3	NTU4	NTU5	NTU6	NTU7	NTU8	NTU9	NTU10	NTU11
IC_{50} (μM)	0.02	0.23	0.1	0.03	0.23	0.09	0.81	4.81	0.2	0.88	0.05

New potent viral inhibitors in the low nanomolar range (~30 nM).

Antiviral testing of engineered peptides to inhibit SARS-CoV-2 virus

Economic & Societal Impact

THE STRAITS TIMES

Singapore economy will take longer to recover from Covid-19 crisis than past recessions: MAS

A Collapse That Wiped Out 5 Years of Growth, With No Bounce in Sight

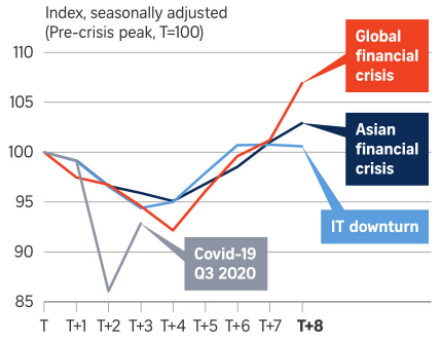
The New York Times

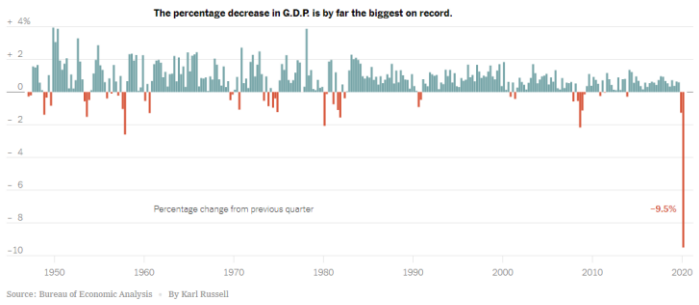
The Flu Vanished During Covid. What Will Its Return Look Like?

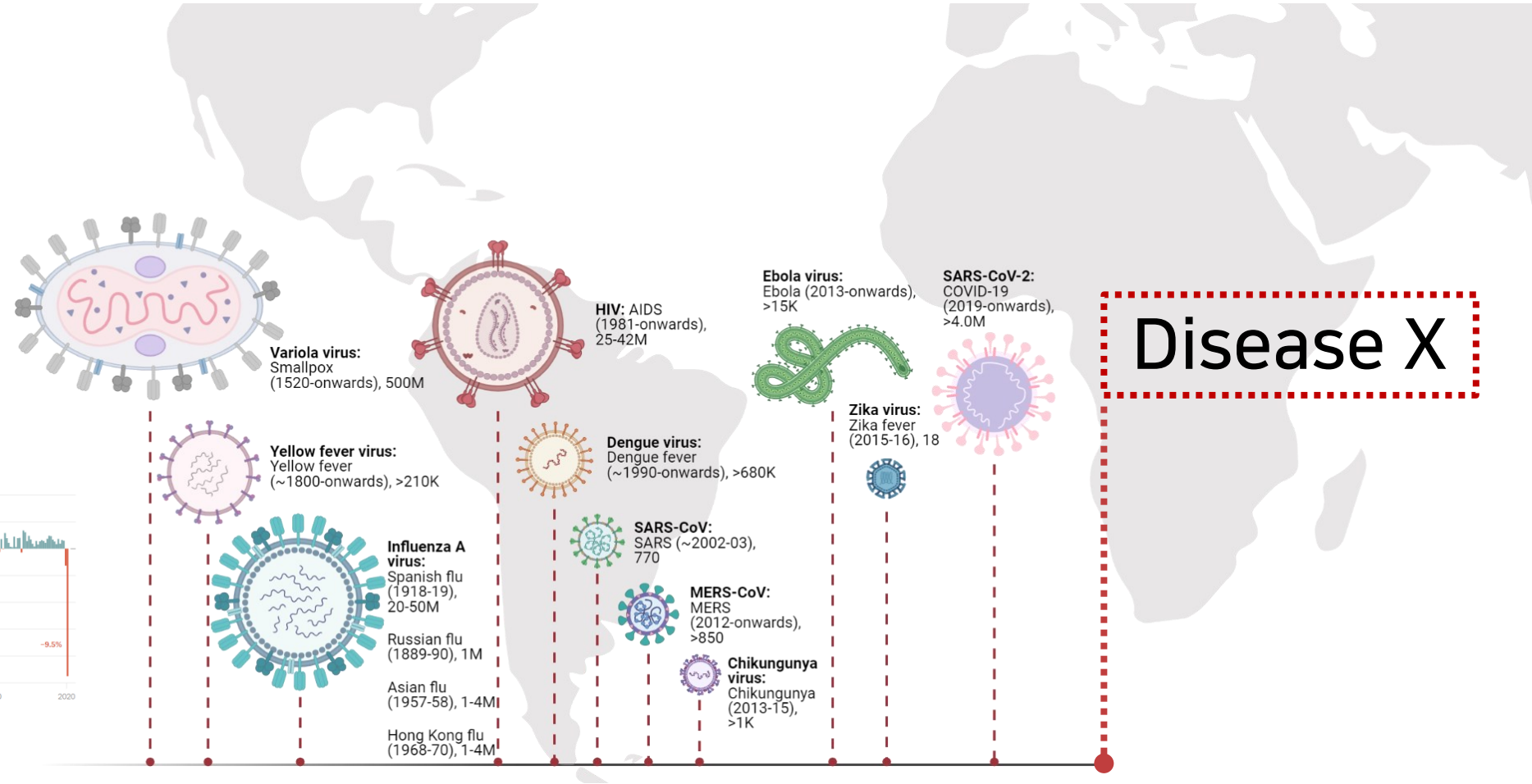
To me these seem like a glimpse into the future where we are going to be in an arms race with this virus – Michael Worobey BBC

Current downturn deeper than previous recessions

SINGAPORE'S GDP PROFILE ACROSS DOWNTURNS (T=PEAK QUARTER)







Concluding Remarks

- Peptide therapy inhibits ZIKV infection in mice through a combination of systemic control and inhibitory activity in organs, including the brain.

- Might also address other ZIKV-related medical complications such as viral persistence in tissues, maternal-fetal and sexual transmission, and eye infections.

- Motivates new antiviral strategies for treating mosquito-borne virus infections and possibly other classes of neurodegenerative diseases with possible viral etiologies.

Collaborator Acknowledgement

Academic Collaboration

Profs. Jeffrey S. Glenn and Curtis W. Frank, Stanford University
Profs. Ram Sasisekharan and Jianzhu Chen, Massachusetts Institute of Technology
Profs. Dr. Mauro Teixeira and Vivian Vasconcelos Costa, Federal University of Minas Gerais
Profs. Brian Gowen and Donald Smee, Utah State University
Prof. Brent Korba, Georgetown University
Profs. Mark Denison and Jim Campbell, Vanderbilt University
Prof. Thiravat Hemachudha, Chulalongkorn University/WHO
Prof. Bart De Spiegeleer, Ghent University
Prof. Johan Neyts, Catholic University of Leuven
Drs. Hannes Martin Hentze and Vijay Saradhi, A*Star

Government Collaboration

Dr. John Dye, US Army Medical Research Institute for Infectious Diseases
Dr. Cristina Cassetti and team, National Institute of Health (NIAID)
Dr. Roger Hewson and team, National Infection Service, Public Health England
Dr. Thomas Fuerst, University of Maryland Institute for Bioscience & Biotechnology Research

Industry Partners and Collaboration

Dr. Javad Aman, Integrated BioTherapeutics
Dr. Justin Richner, IIT Research Institute
Dr. Fusataka Koide, Southern Research
Dr. Robert Buckheit, ImQuest
Charles River Laboratories
Bachem & Polypeptide

USAMRIID United States Army Medical Research Institute of Infectious Diseases
Public Health England
UNIVERSITY OF MARYLAND | NIST INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH
National Institute of Allergy and Infectious Diseases

AViDD U19

- Center title: **Development of outpatient antiviral cocktails against SARS-CoV-2 and other potential pandemic RNA viruses (RFA-AI-21-050)**

- Project title: **Targeting viral envelopes with antiviral peptides and peptoids and degraders, and surface proteins with small molecules**
  - Award period: 05/01/2022 – 04/30/2027 (5 years)
  - Amount: 1,547,000 USD (overhead 8%)

- Project title: **Oral small molecule inhibitors of NSP4-mediated membrane-associated RNA replication of SARS-CoV-2 and other RNA viruses**
  - Award period: 05/01/2022 – 04/30/2027 (5 years)
  - Amount: 1,032,000 USD (overhead 8%)

NIAID 2022 DMID Omnibus Broad Agency Announcement (BAA)

- Project title: **Advancing an oral NSP4 inhibitor DAA to the clinic for outpatient SARS-CoV-2 infections**
  - Award period: 11/01/2022 – 10/31/2027 (5 years)
  - Amount: 1,346,875 USD (overhead 8%)

Acknowledgement

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