Blood-Brain Barrier: Friend and Foe

Understand defense mechanisms and transport across the BBB and move towards targeted, noninvasive study and repair of the brain

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Investigator, Howard Hughes Medical Institute

Caltech

Blood vessels in human brain

Conflict of Interest

Member, Scientific Advisory Boards

Johnson & Johnson Innovative Medicine
Amgen Emerging Technologies

Co-founder

Capsida Biotherapeutics
Receptive Bio

Both natural and diseased behaviors involve distributed, molecularly diverse circuits throughout the brain and spinal cord

To understand and restore brain (and related) function we need ***brain-wide access*** to ***deliver research tools*** and therapies

Alzheimer's
114 M

Epilepsy
70 M

Parkinson's
10 M

Stroke
17 M

Chronic Pain
280 M

Schizophrenia
39 M

Depression
350 M

Impaired Vision
4 M

Attention Disorders
6.4 M

PTSD
8 M

Spinal Cord Injury
12 K

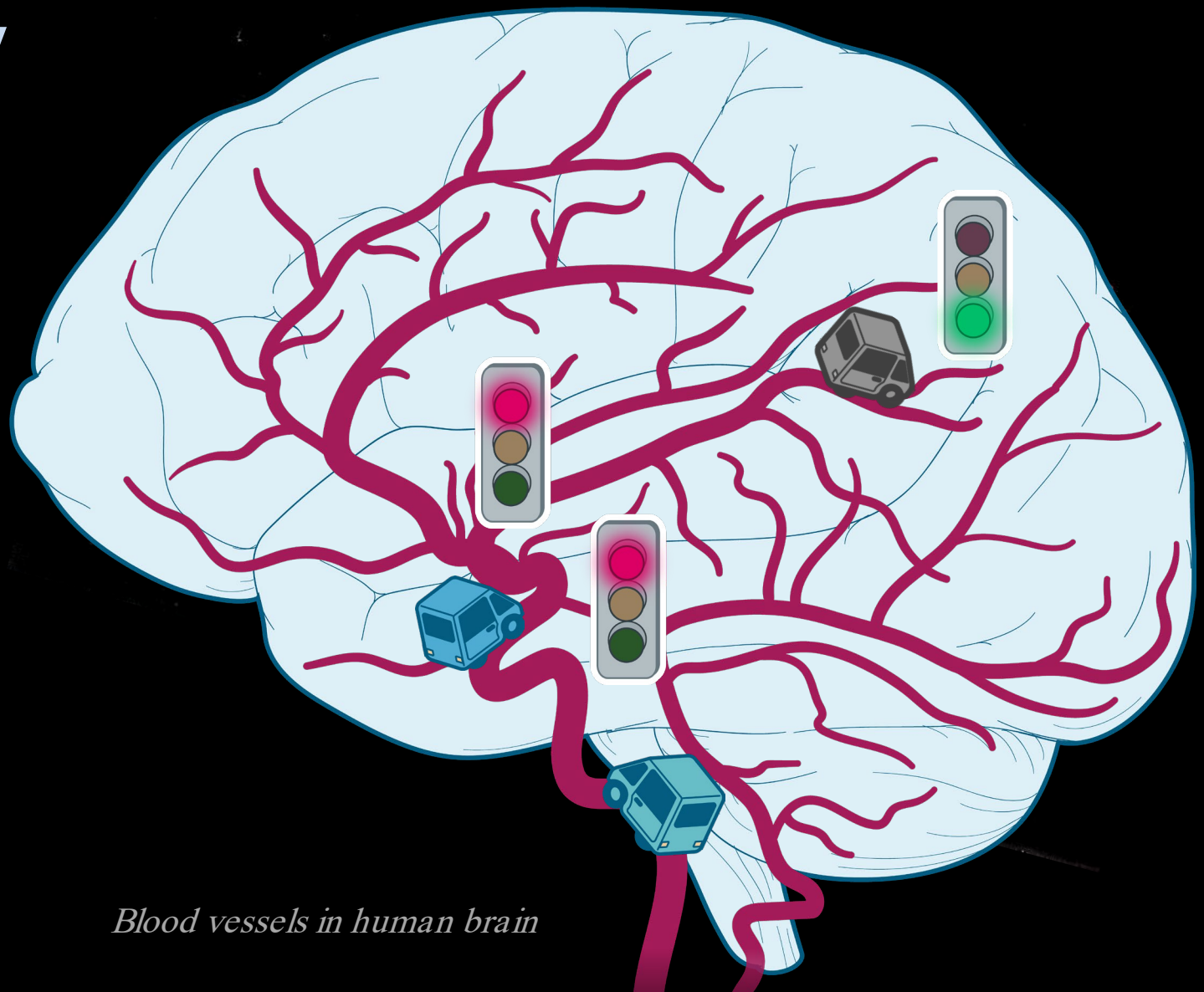
ALS
> 200K

1 billion people worldwide affected by brain disease

+ brain metastasis:
> 200k people diagnosed each year

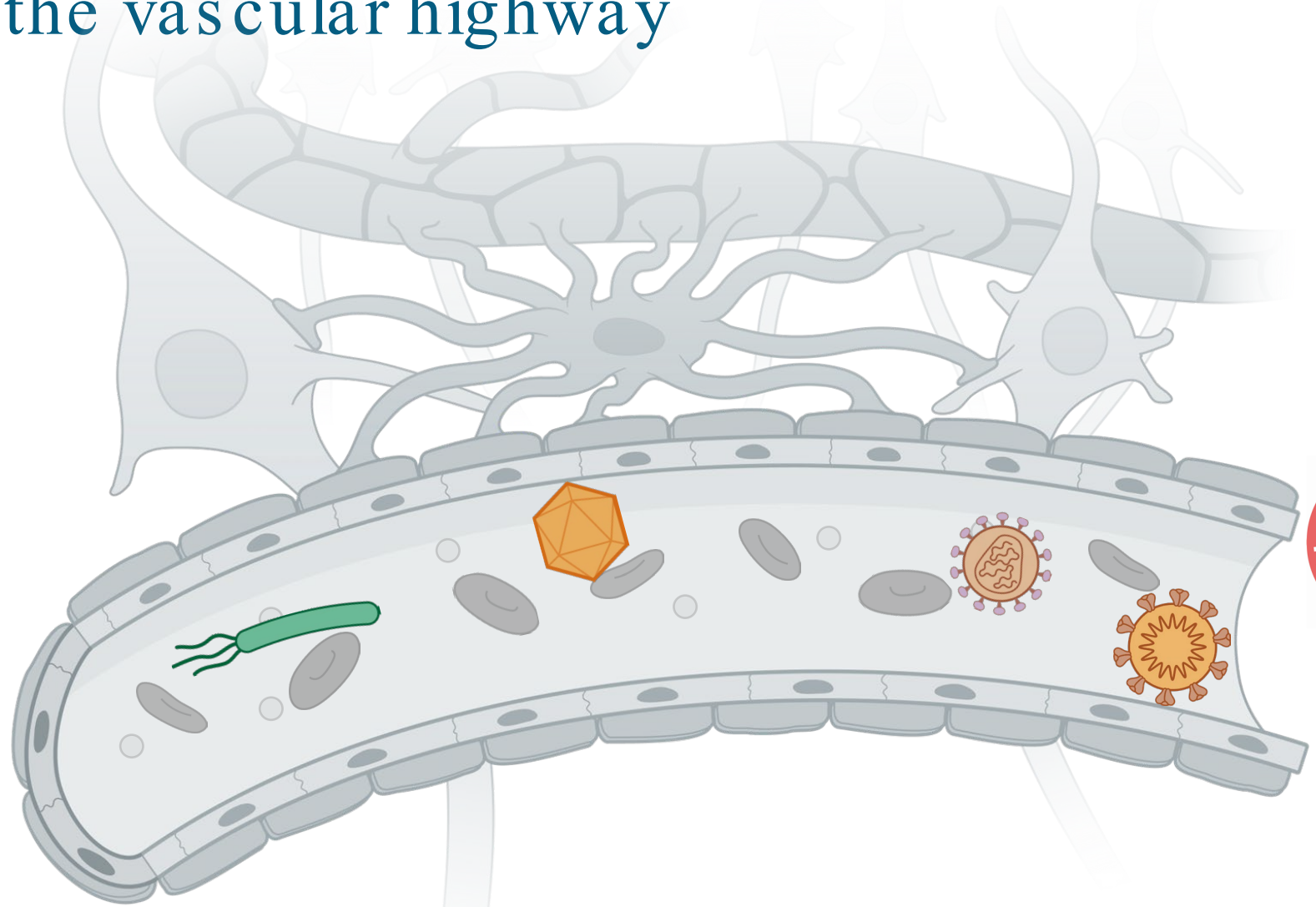
The brain vasculature: 400 miles highway for therapeutic delivery

each of our brain cells
is within 20 µm
of a vascular supply



Blood vessels in human brain

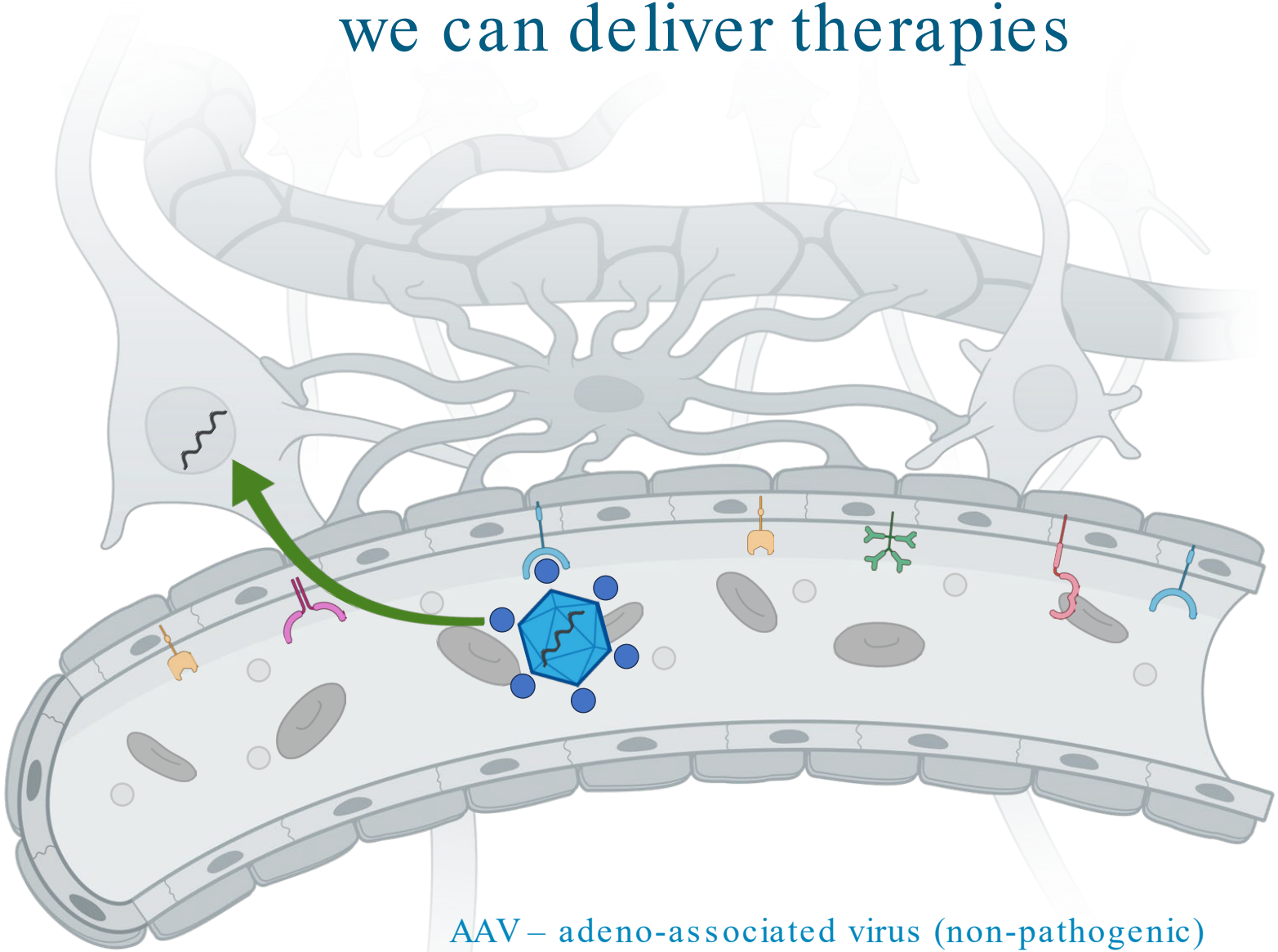
The Blood-Brain-Barrier (BBB) stringently regulates arrivals from the vascular highway



selectively allowing nutrients and immune defenses

but also blocking pathogens: most viruses &bacteria cannot gain entry

By recruiting molecular gatekeepers of the BBB, we can deliver therapies



e.g., 2019 FDA approval of systemic AAV9 for SMA (spinal muscular atrophy), in young patients (more permeant BBB)

Emily, as active 3 years old

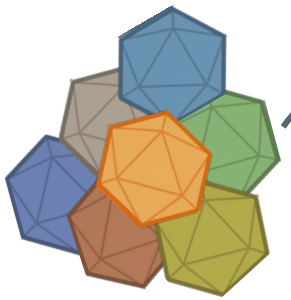
phoenixchildrensfoundation.org

AAV – adeno-associated virus (non-pathogenic)

Intravascular AAV9 mainly targets neonatal-neurons but adult-astrocytes in CNS

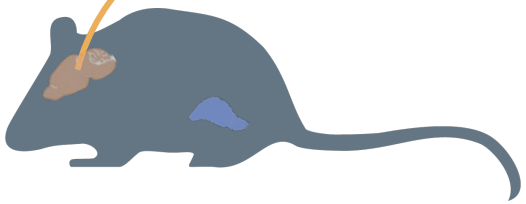
A bioengineering solution: directed evolution of BBB-crossing vehicles

generate library of diversified AAV capsids



20^7 (1.28 billion) amino acid variants

inject library into bloodstream



Apply positive selection pressure (e.g. in Cre+ mice by the CREATE method)

Brain +

selectively recover successful variants from organs/cells of interest

recovered viral variants

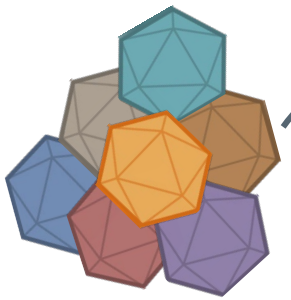
(e.g. PHP.B/eB capsids)

Deverman BE..Gradinaru V (2016) Cre-dependent capsid selection yields AAVs for global gene transfer to the adult brain

Ravindra Kumar S..Gradinaru V (2020) Multiplexed Cre-dependent selection yields systemic AAVs for targeting distinct brain cell types

A bioengineering solution: directed evolution of BBB-crossing vehicles

generate library of diversified AAV capsids



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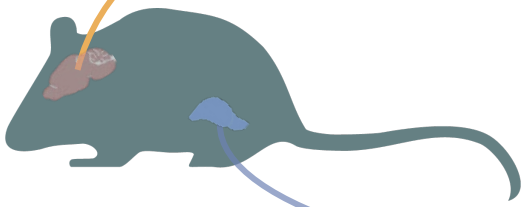


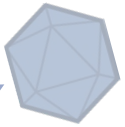
Apply selection pressure: positive and negative (e.g. in Cre+ mice by the CREATE method)

Brain +

selectively recover successful variants from organs/cells of interest

recovered viral variants

Liver -



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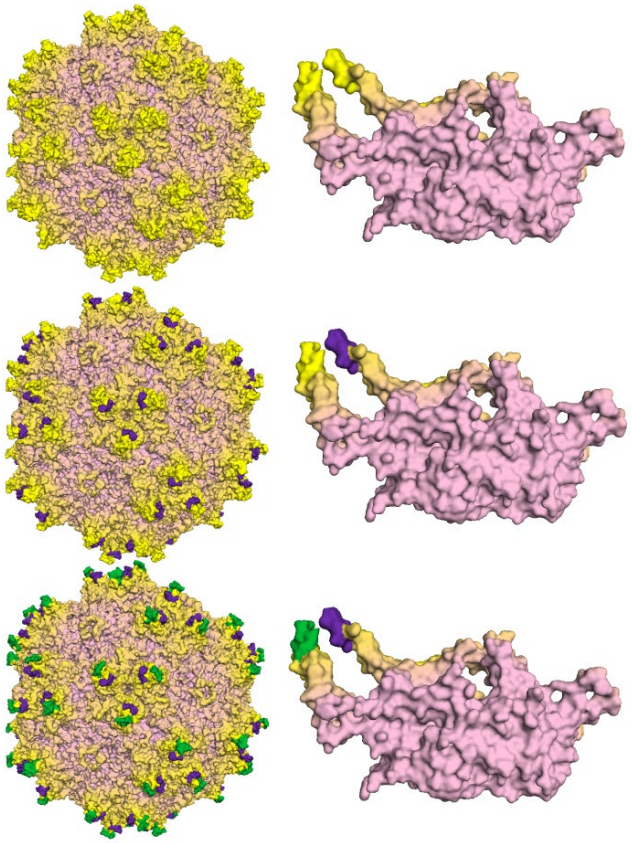
Iterative engineering for widespread and noninvasive Delivery to the Brain with Liver de-targeting in mice

Engineered region

full capsid | monomer

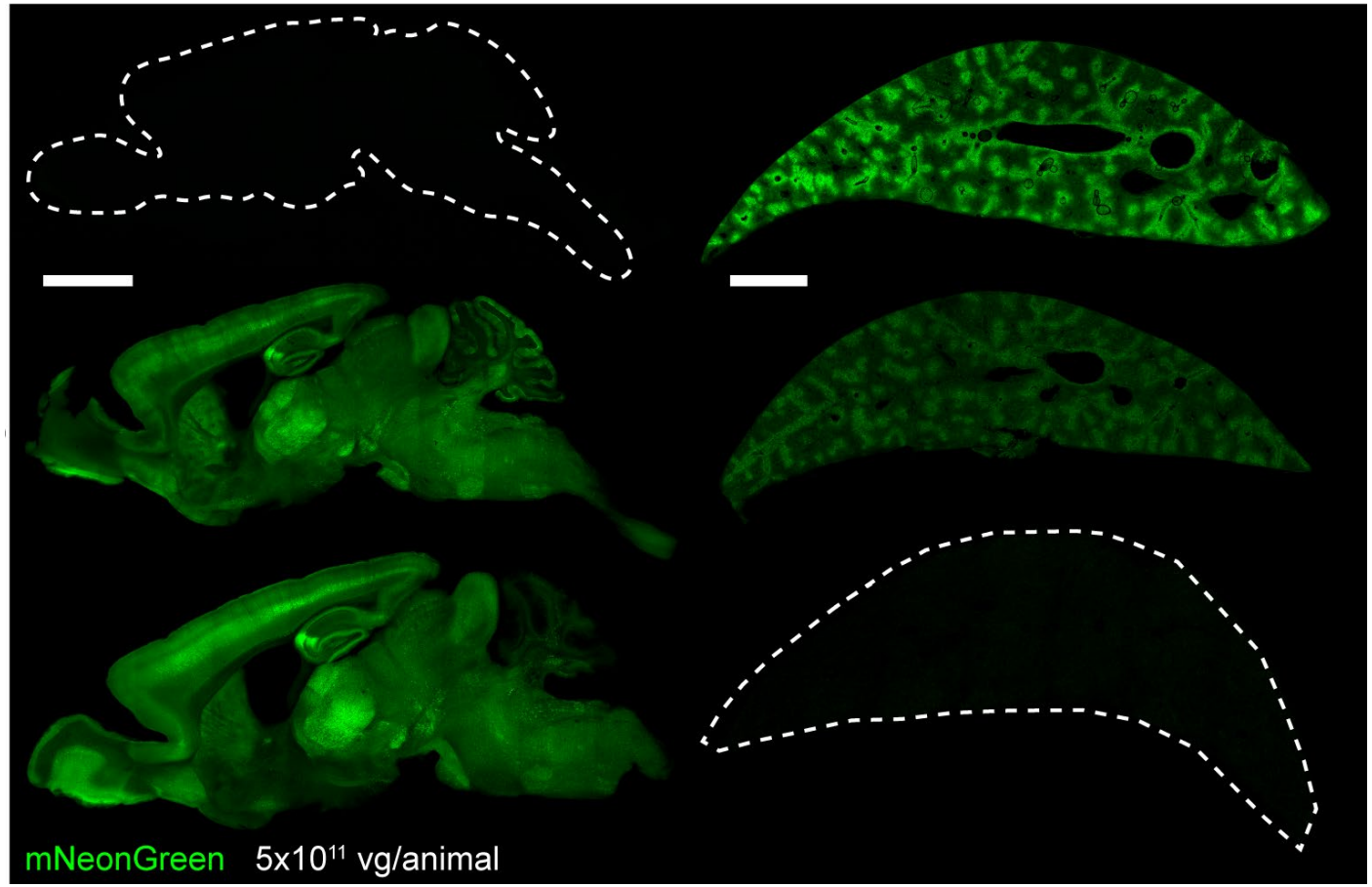


AAV9 (parent)

AAV-PHP.eB (2nd generation)

AAV.CAP-B10 (3rd generation)

Outcome after systemic administration



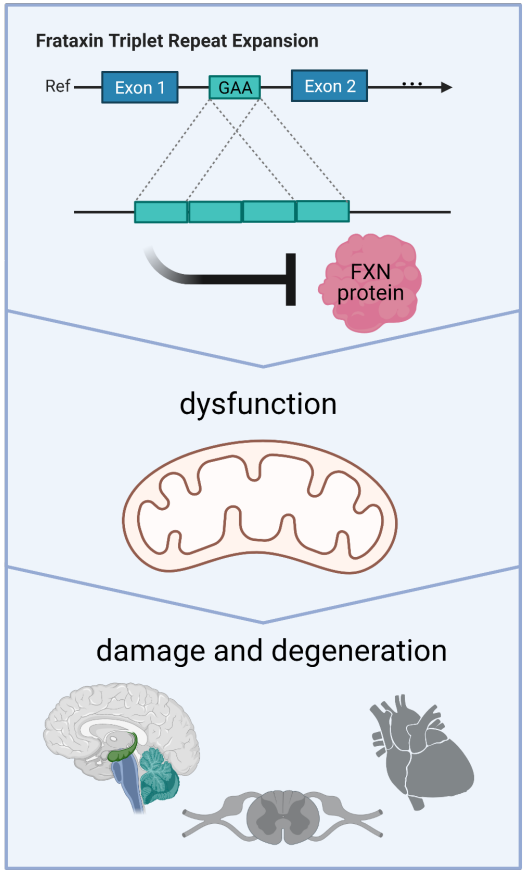
Chan K.Y…Gradinaru V (2017) Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems
Goertsen D, Flytzanis NC, Goeden N..Gradinaru V (2021) AAV capsid variants with brain-wide transgene expression and decreased liver targeting after intravenous delivery in mouse and marmoset

Applications in neurodegeneration: Friedreich's Ataxia (FA)

rare neurodegenerative disease, good candidate for AAV gene therapy

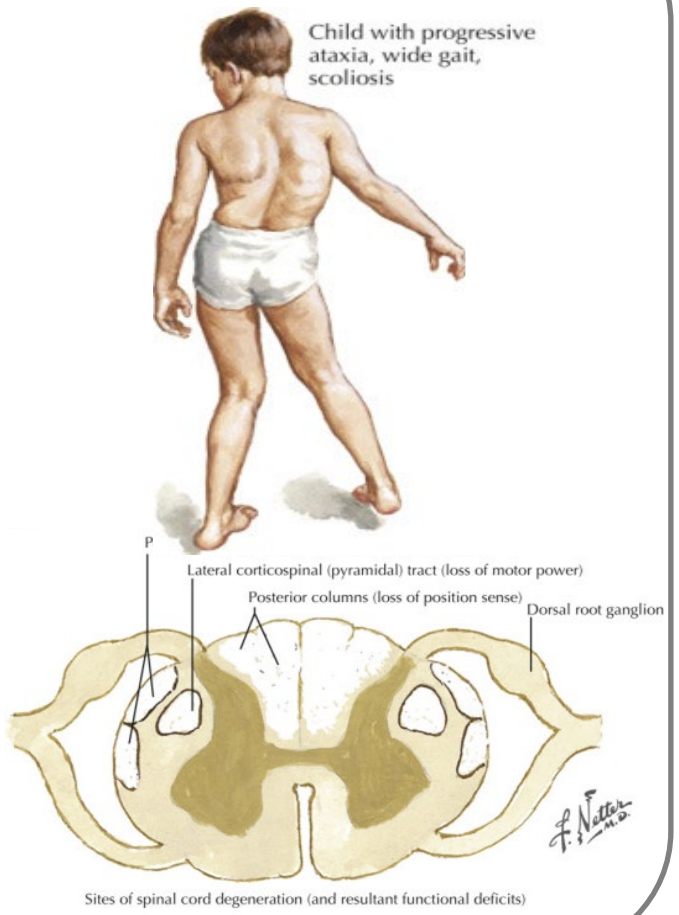
Acacia Mayfield
MD/PhD student

Pathophysiology



Created with BioRender.com

Clinical Manifestation

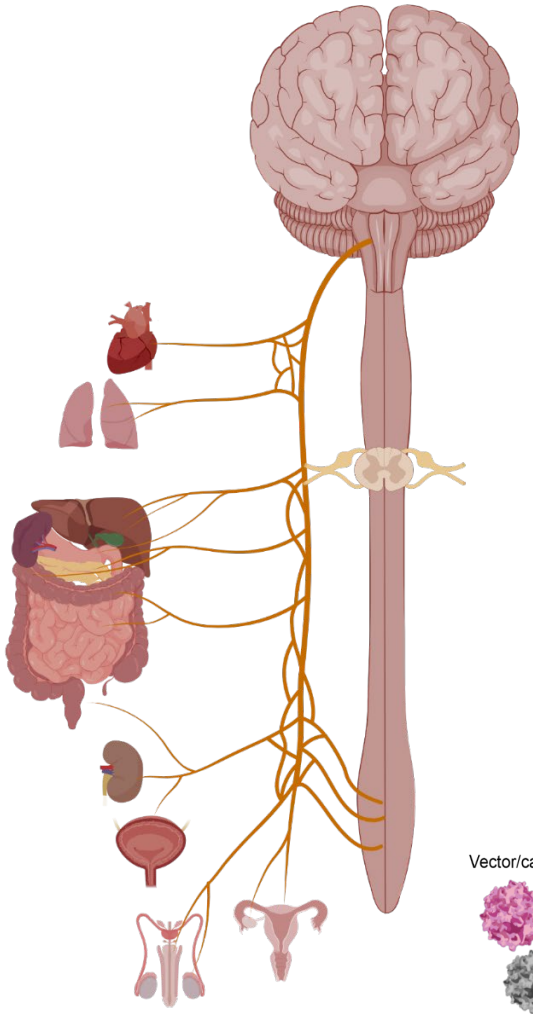


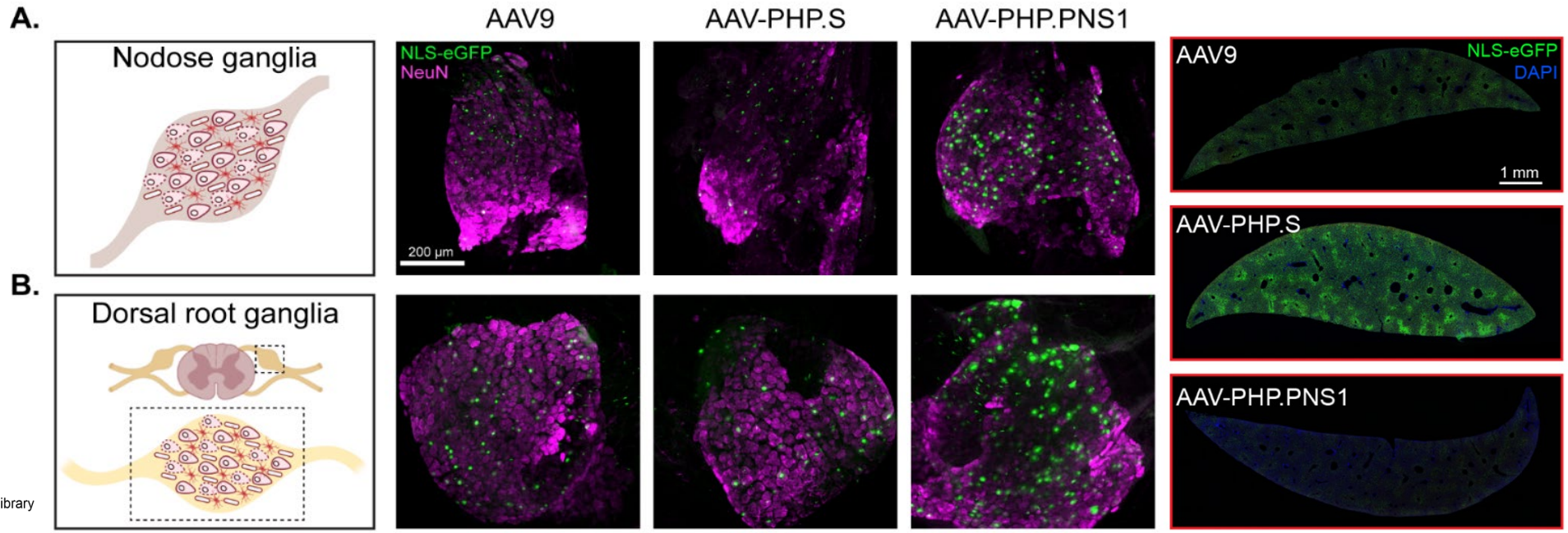
Netter's Neurology 2E

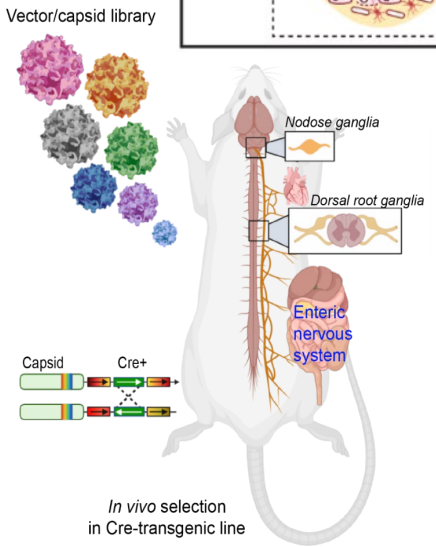
Criteria Encouraging Development of AAV Gene Therapy for FA

- Monogenic Disorder
- Caused by loss of expression
- Promising existing literature on beneficial effects of gene replacement
- ***Transgene Coding Sequence < AAV Packaging Limit***

Beyond CNS, Better PNS Variants (brain to gut, pain, etc.)



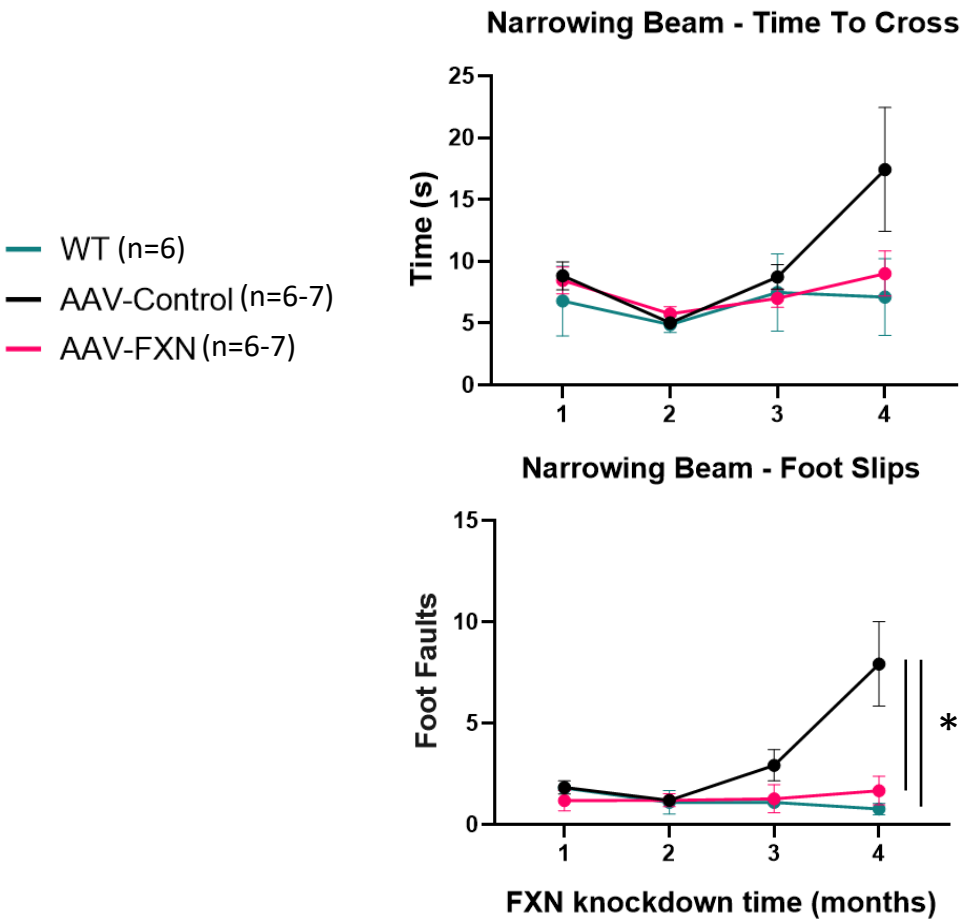


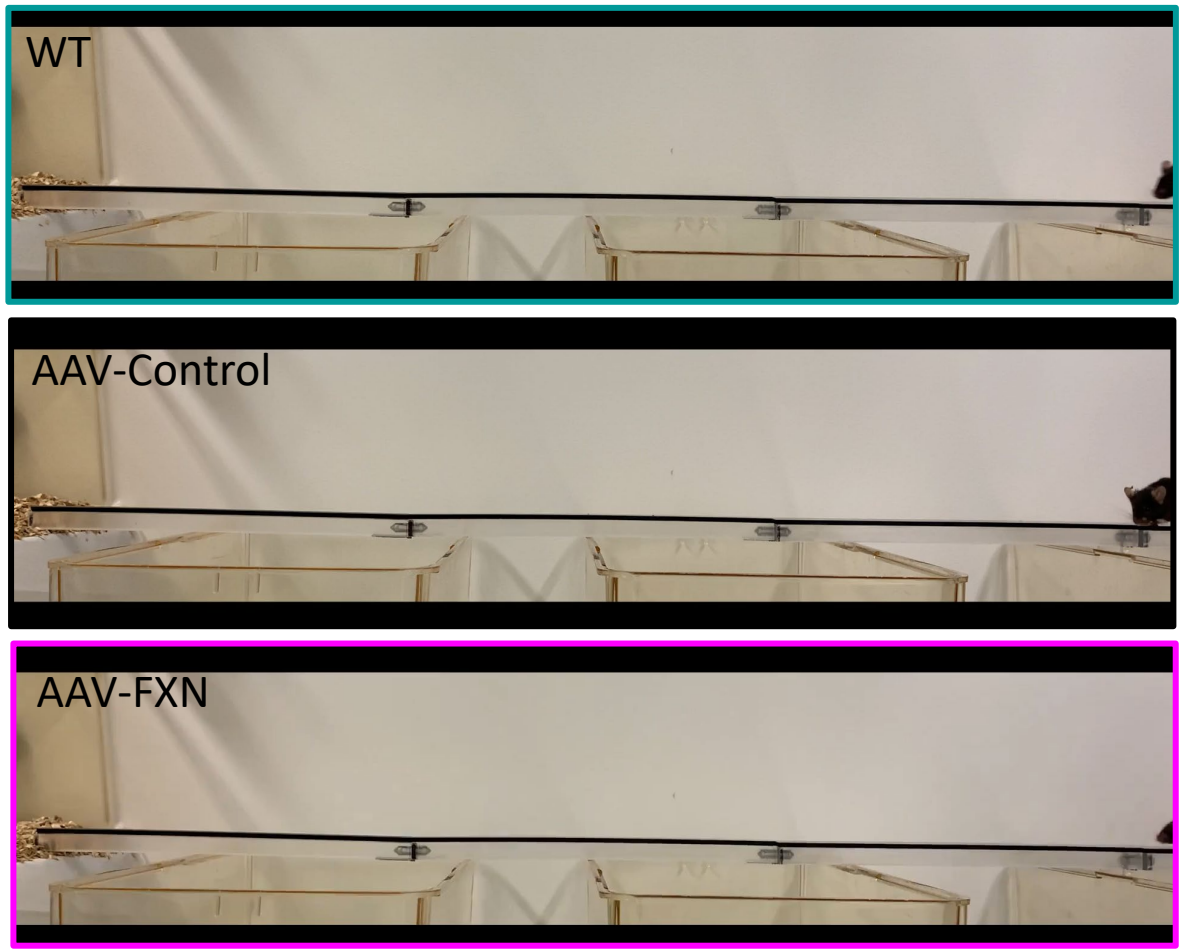


Xinhong Chen & Priya Kumar et al, Neuron 2022

AAV-FXN treated rodents perform near WT in motor coordination tests, AAV-Control rodents deteriorate

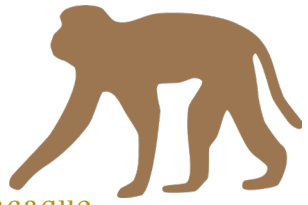
Acacia Mayfield
MD/PhD student





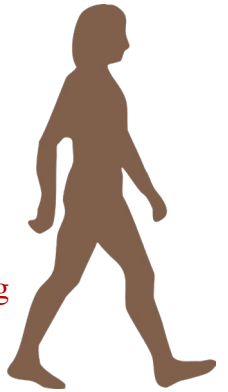
Targeted Gene Therapy with Engineered Systemic AAVs for the Central and Peripheral Nervous Systems Prevents Motor Coordination Phenotypes in a Mouse Model of Friedreich's Ataxia

The BBB varies across species and strains



Rhesus macaque
adult weight ~6 kg
brain weight ~95 g

Old World monkeys
~25 Mya



human
adult weight ~70 kg
brain weight ~1350 g

hominids

marmoset
adult weight ~0.24 kg
brain weight ~8 g

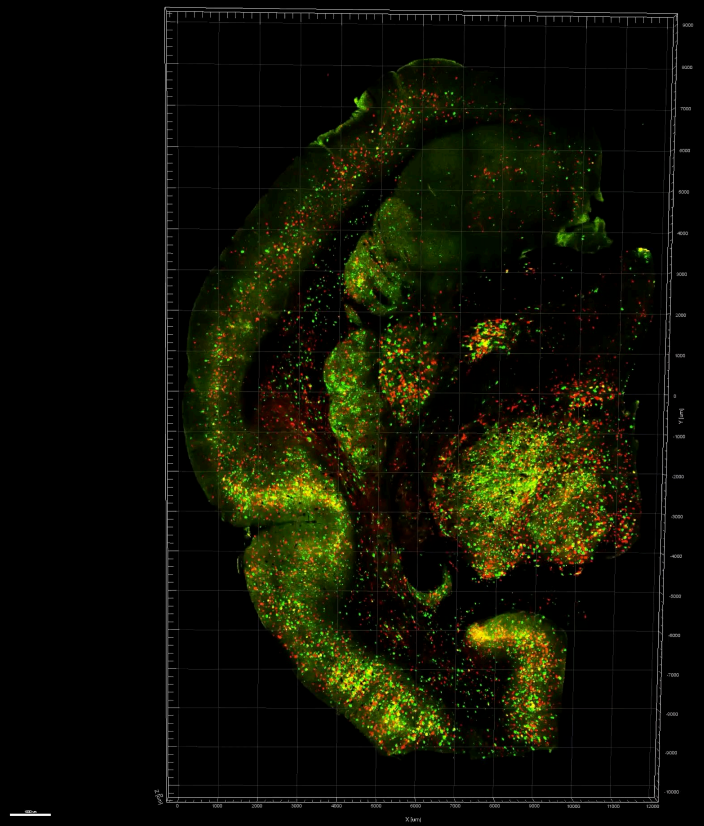
New World monkeys
~35 Mya

rodents
~75 Mya

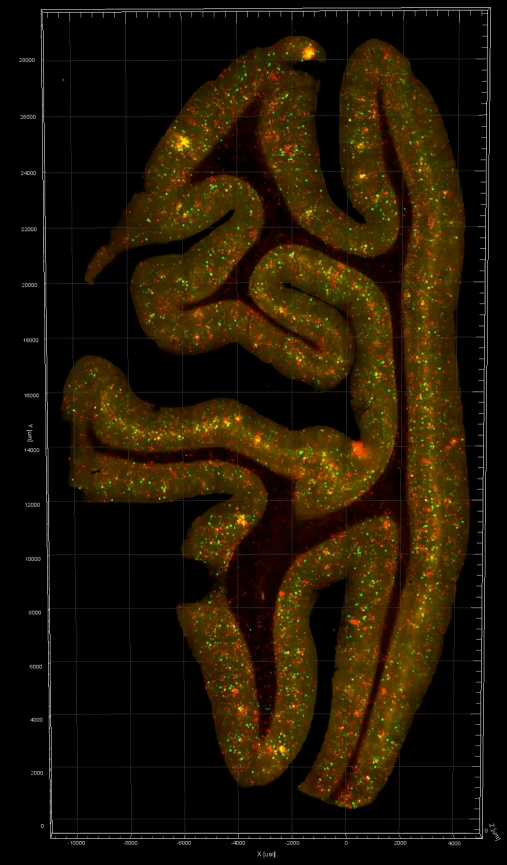
mouse
adult weight ~0.025 kg
brain weight ~0.4 g

Engineered AAV variants cross the blood-brain-barrier in **non-human primates**.

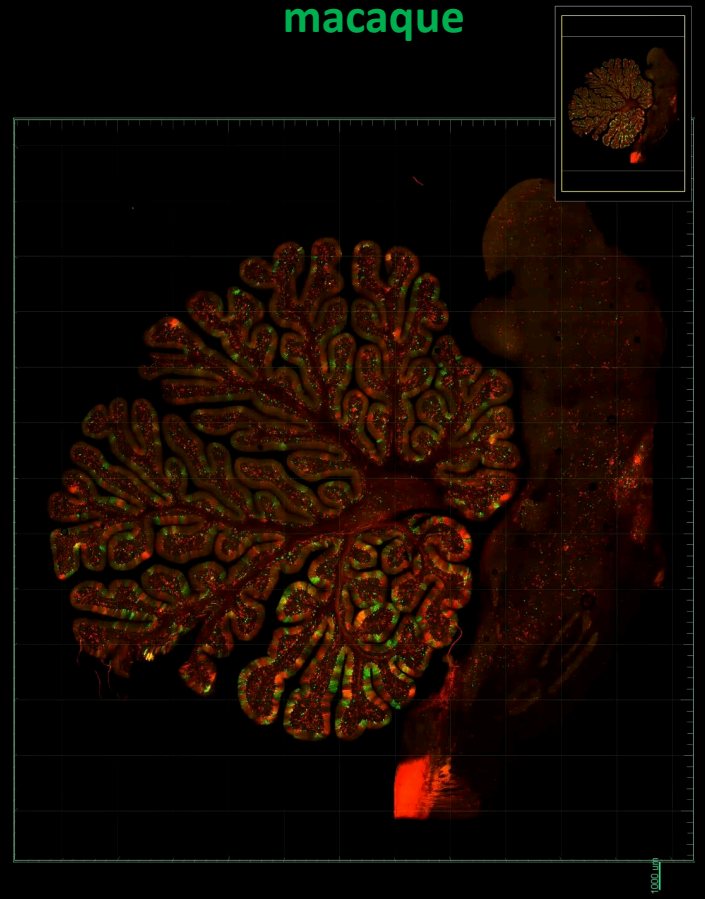
marmoset



macaque



macaque



Xinhong Chen & Priya Kumar…**Gradinaru V**, 2022

Engineered AAVs for non-invasive gene delivery to rodent and non-human primate nervous systems

Caltech

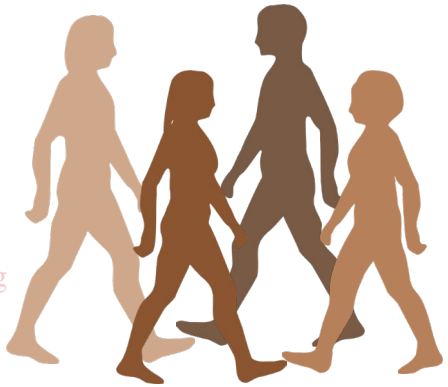
With
UC Davis: Drew Fox
UCSD: Cory Miller

The BBB varies across species, strains, and biological variables

Rhesus macaque
adult weight ~6 kg
brain weight ~95 g

Old World
monkeys
~25 Mya

human
adult weight ~70 kg
brain weight ~1350 g



hominids

Age

Disease State

Sex

Ancestry

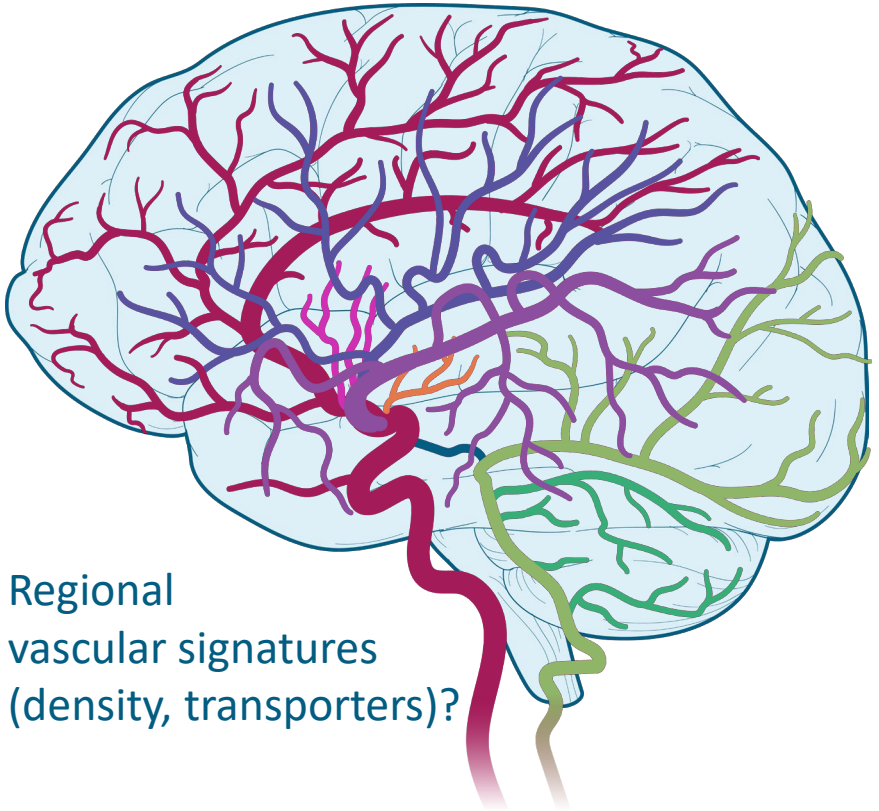
New World
monkeys
~35 Mya

marmoset
adult weight ~0.24 kg
brain weight ~8 g

rodents
~75 Mya

mouse
adult weight ~0.025 kg
brain weight ~0.4 g

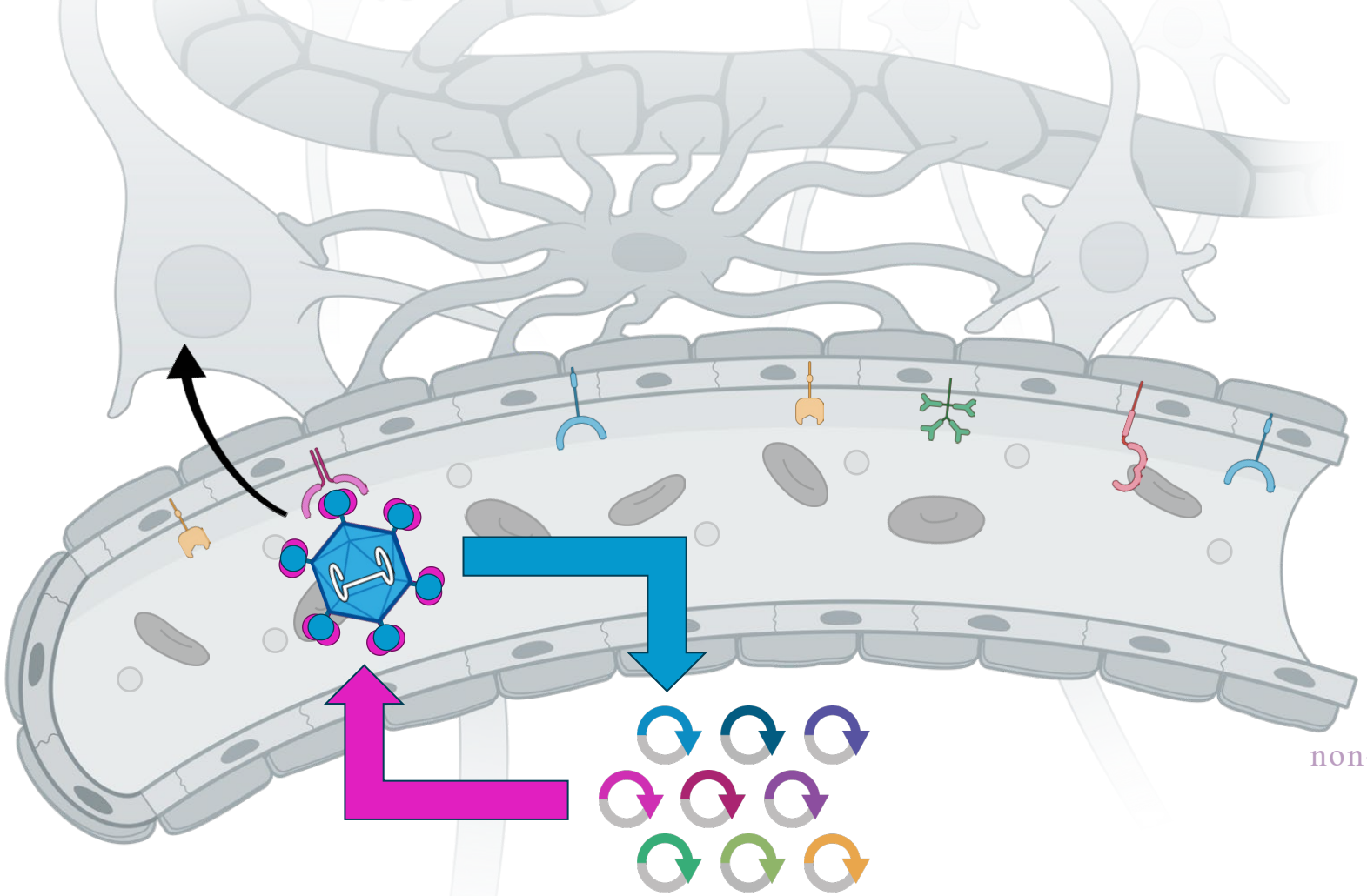
Major goal: understand the mechanisms underlying BBB transport specificities both across and within brains

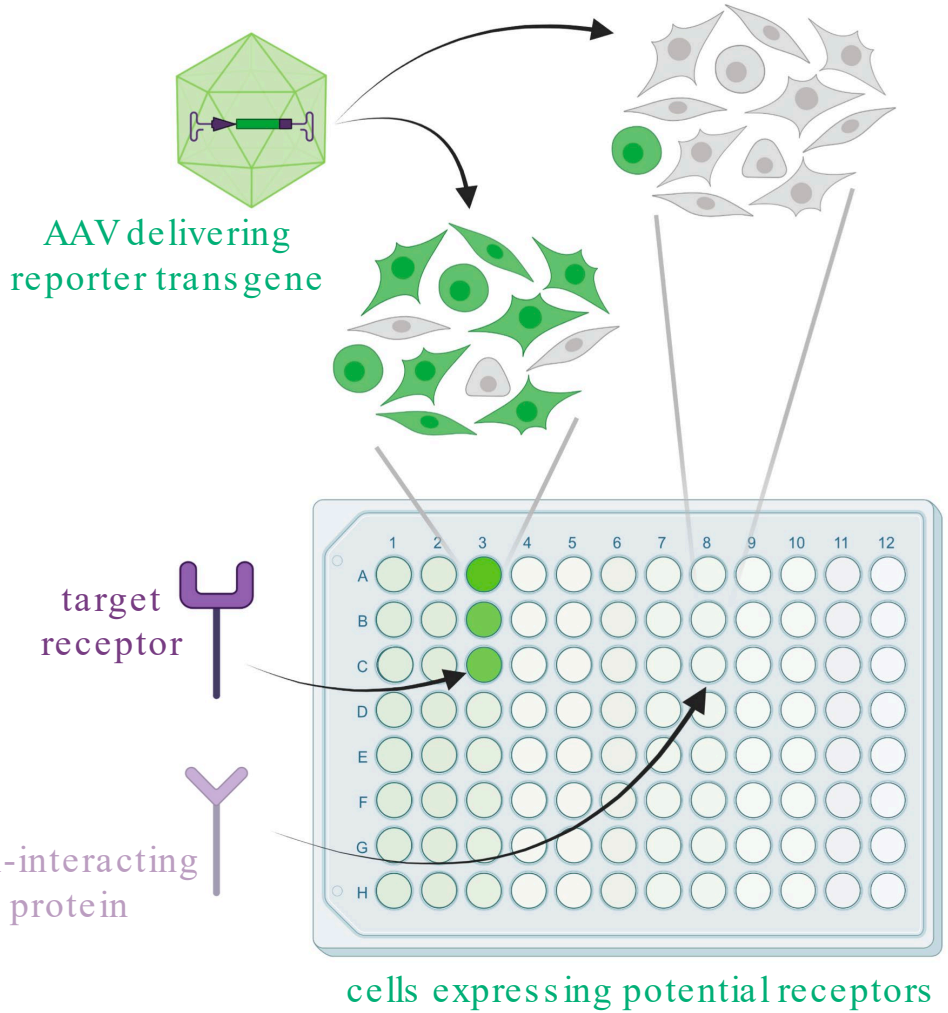


Regional
vascular signatures
(density, transporters)?

and we've already built the tools to do it

Our approach: ask BBB-penetrant AAVs how they get across

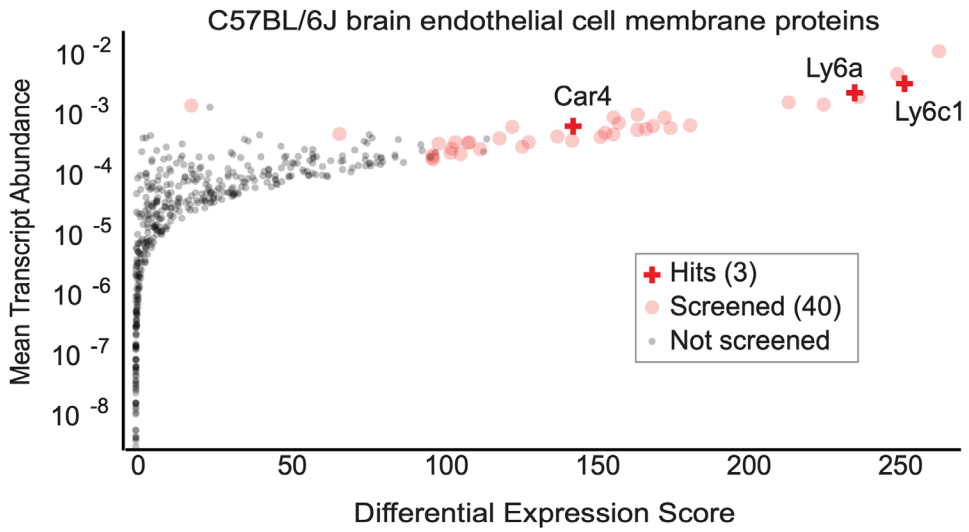


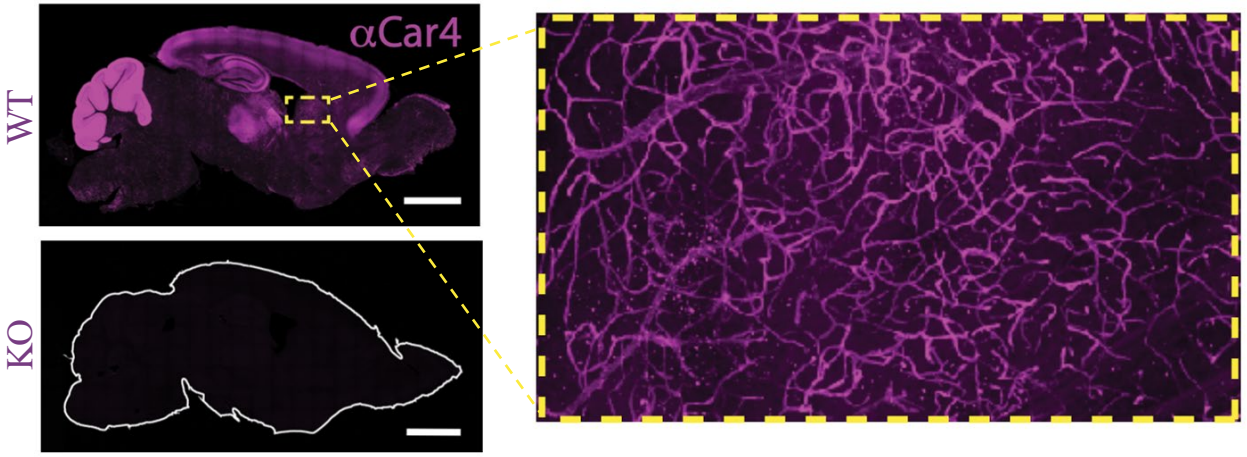


Transforming mechanistic studies of BBB penetration into a series of solvable problems in receptor biology

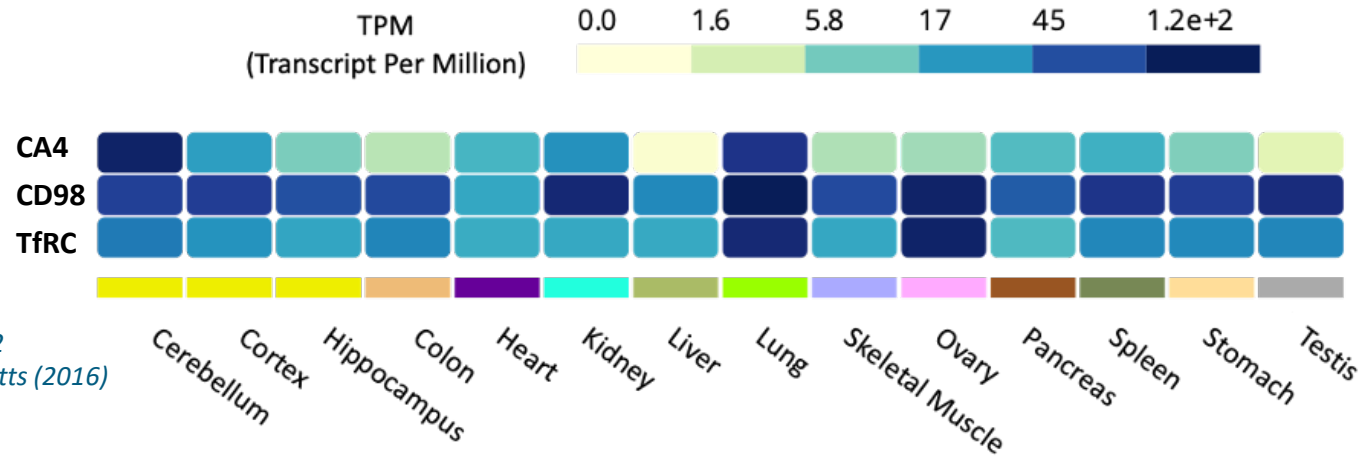
Shay TF, Sullivan EE, Ding X..Gradinaru V (2023) Primate-conserved carbonic anhydrase IV and murine-restricted LY6C1 enable blood-brain barrier crossing by engineered viral vectors

An unexpected basic science discovery: an ancient conserved enzyme, Carbonic Anhydrase IV (mouse Car4, human CA4), can mediate transport across the BBB



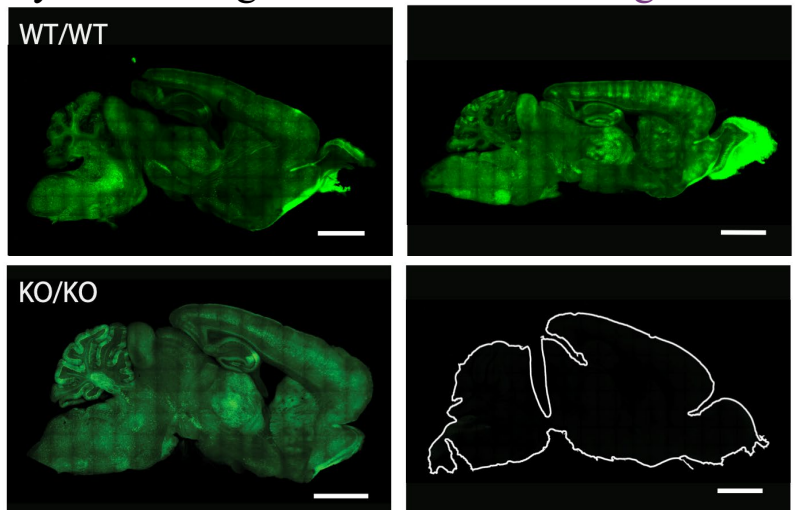


Less off-target expression vs. other human BBB transporters



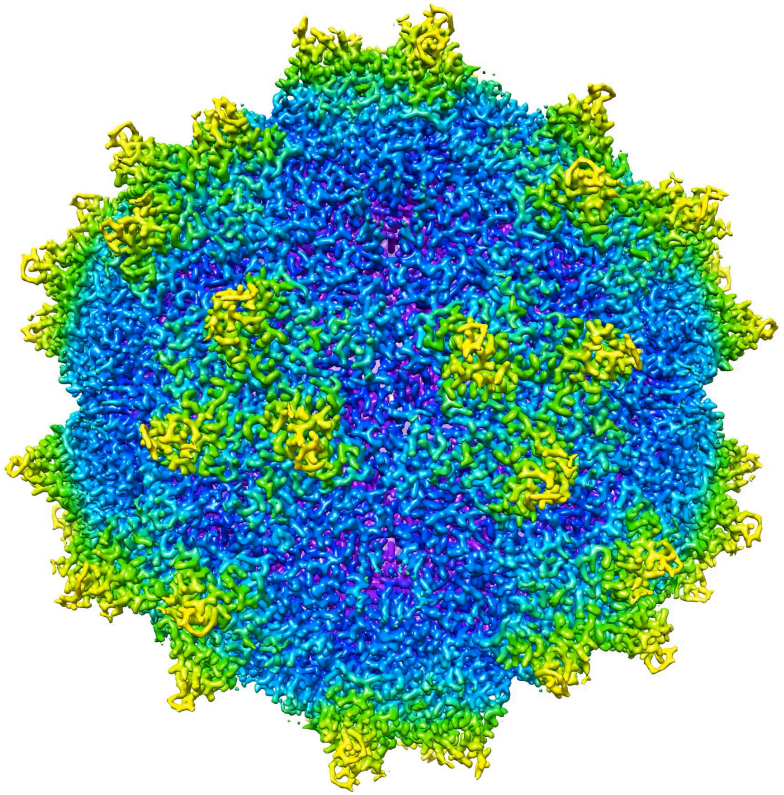
CD98/SLC3A2
Zuchero…Watts (2016)

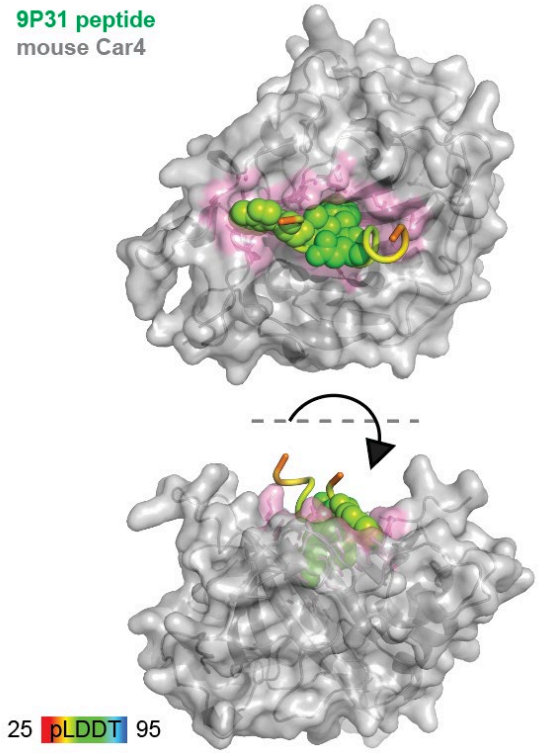
Ly6a-binding AAV | Car4-binding AAV

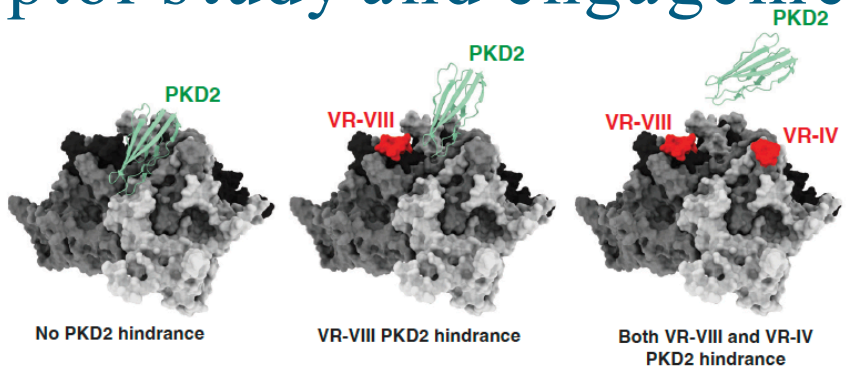


Shay TF, Sullivan EE, Ding X..Gradinaru V (2023) Primate-conserved carbonic anhydrase IV and murine-restricted LY6C1 enable blood-brain barrier crossing by engineered viral vectors

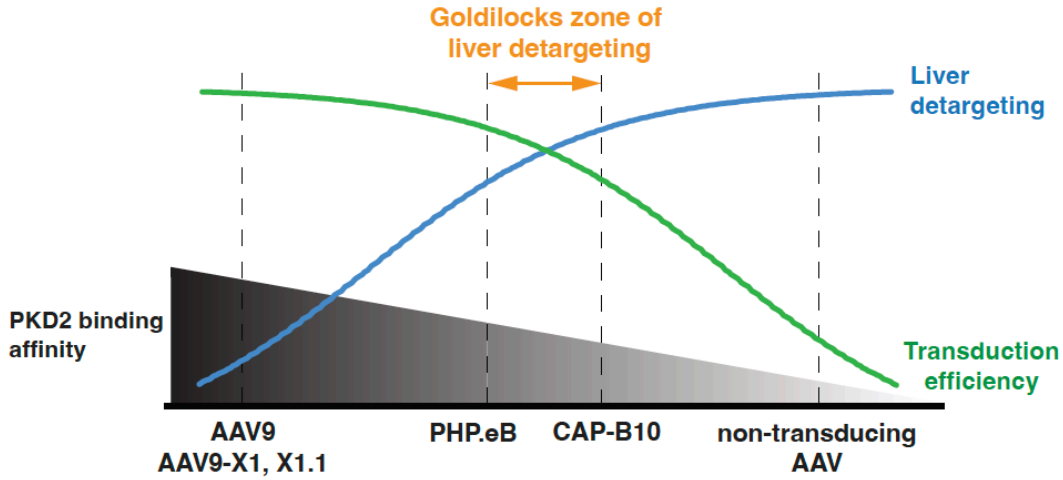
AI-enhanced structural biology to guide BBB receptor study and engagement







under review, biorxiv



high-res structures of AAVs and putative receptors -> enable *in silico* modeling of ***AAV–receptor binding*** with AlphaFold-Multimer

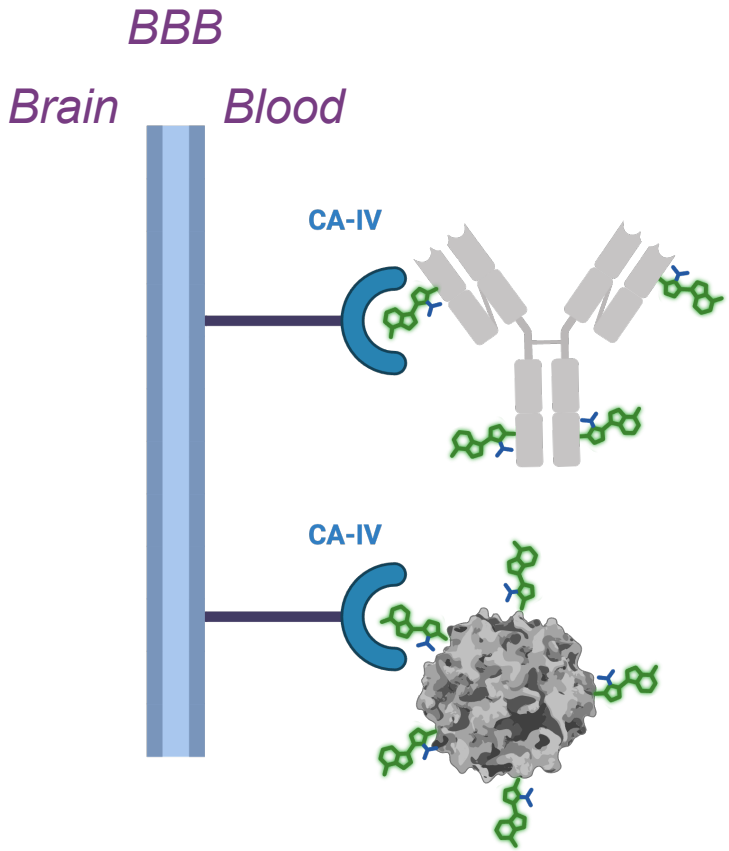
Bypassing limitations of mechanism-blind screens for receptor-targeted engineering of diverse modalities with refined tropism and predictability across species

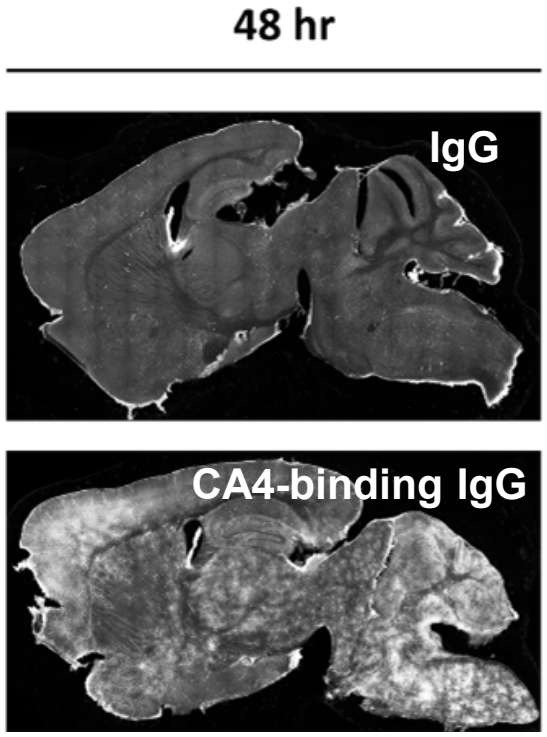
Jang S..Gradinaru V (2022) Structural basis of receptor usage by the engineered capsid AAV-PHP.eB

Shay TF, Sullivan EE, Ding X..Gradinaru V (2023) Primate-conserved carbonic anhydrase IV and murine-restricted LY6C1 enable blood-brain barrier crossing by engineered viral vectors

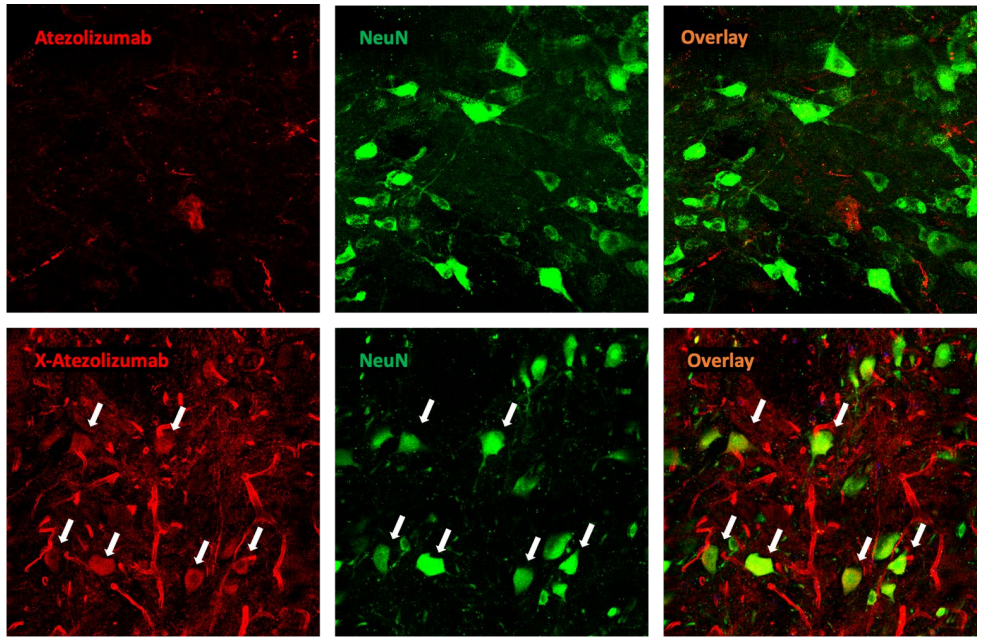
Beyond viral vector-based delivery across the BBB: expanding to other modalities

CA4-binding therapeutic antibodies cross the BBB and reach brain, neurons





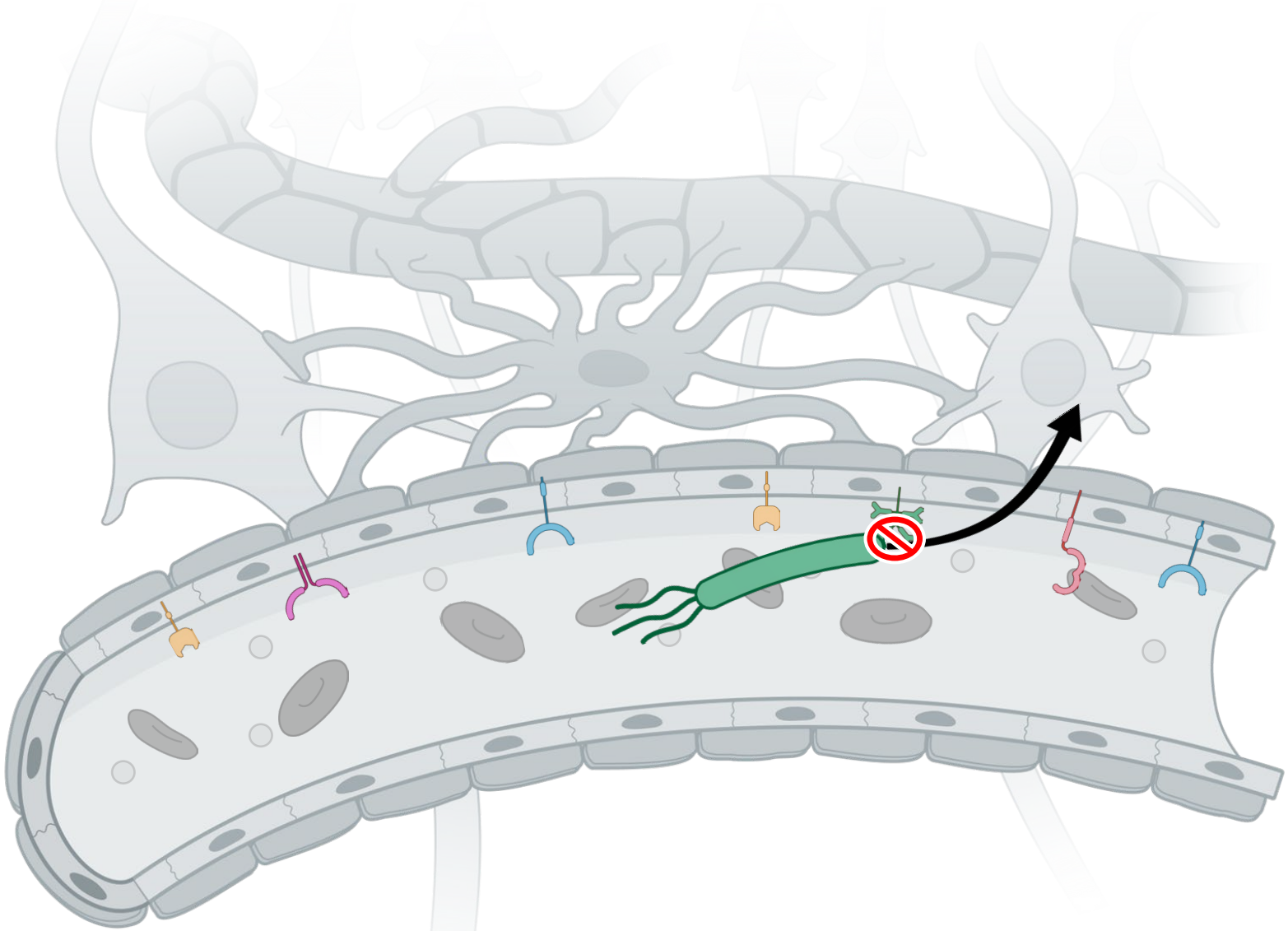
7 days post intravenous injection, Brain Stem



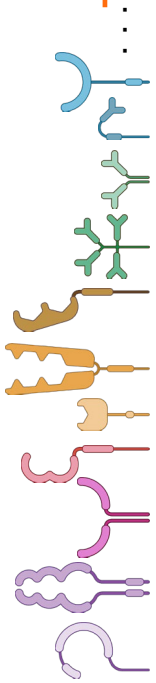
Atezolizumab (Tecentriq):
used in the clinic for non-small cell lung cancer
(30-64% of patients have brain metastases)

Under revision

By identifying BBB receptors, we can anticipate/block new threats



Novel match with BBB Receptors ?



Including a pathogen that evolved to use TfR!

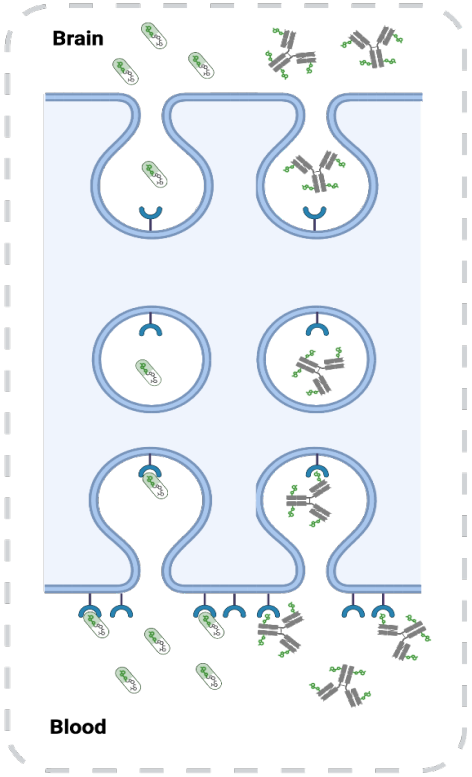
"Pathogenic bacteria exploit transferrin receptor transcytosis to penetrate the blood–brain barrier"

Cheng Z…Wang L (2023)

Pathogens can access the brain and cause severe disease (as some retroviruses, including HIV-1, already do)

Understanding BBB transport biology in diverse evolutionary contexts can help:

Deliver Brain Therapeutics

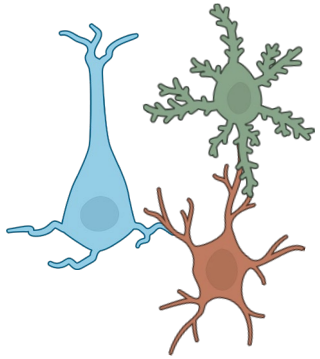


Nanoparticles

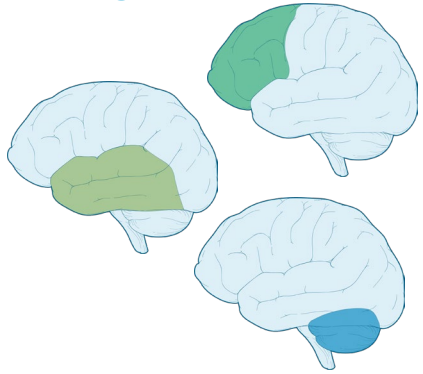
Antisense oligos

Antibodies

Cell specific

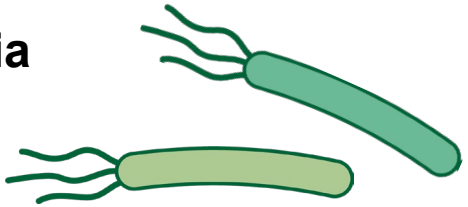


Region specific

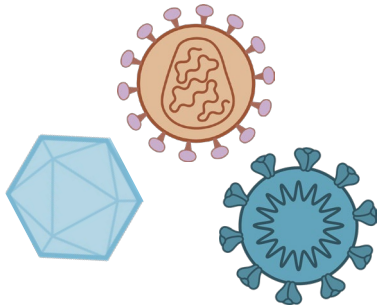


Navigate Health Threats

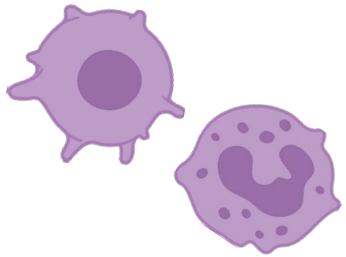
Bacteria



Viruses



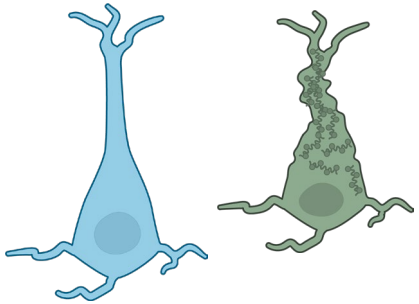
Inflammation



Cancer
(brain metastasis)

Encompass Biological Variation

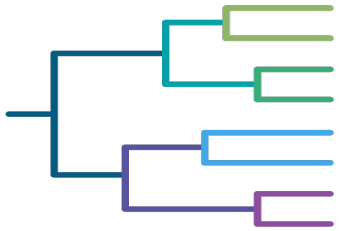
Disease state



Sex

Age

Ancestry



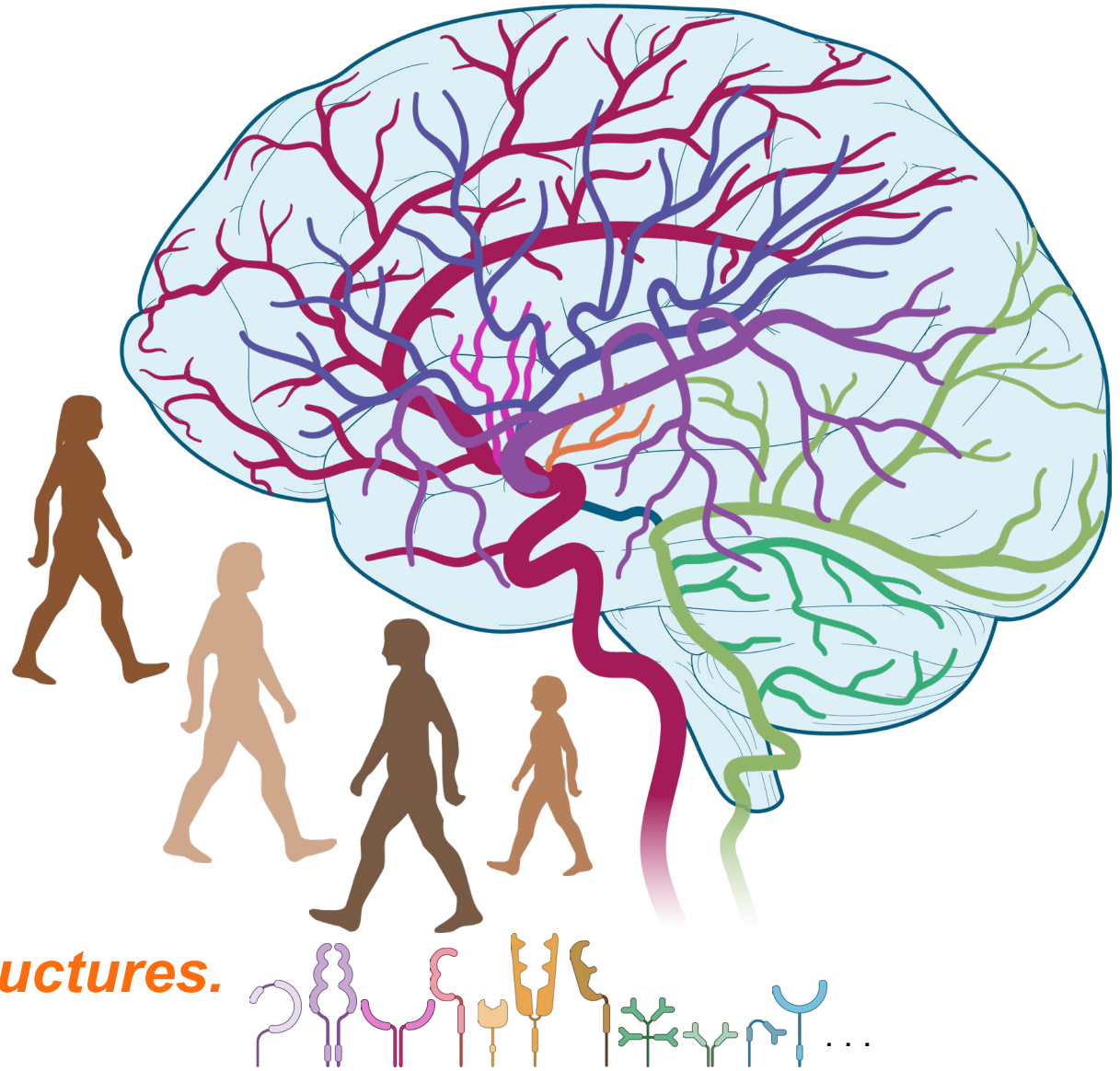
Major impact of fundamental BBB biology research for defense and transport:

Leverage Blood-Brain Portals for a Better Understood and More Resilient Brain

Understand and Fight Brain Disease with
targeted access of neuromodulators

and **anticipate emerging threats,**
pathogens that may evolve to cross the BBB

across biological variables
across individuals and lifespans



Generalizable approach to
understand physiological defense structures.

Thank you, this team has been working hard to Deliver!

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hhmi Howard Hughes Medical Institute

NIH The BRAIN Initiative

NSF

THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

Neurodegeneration Challenge Network

asap Aligning Science Across Parkinson's

DARPA

FARA Friedreich's Ataxia Research Alliance

NIH DIRECTOR'S NEW INNOVATOR AWARD

NIH DIRECTOR'S PIONEER AWARD

Unlocking Blood-Brain Portals

for enabling targeted delivery
across the blood-brain barrier
for research and clinical purposes.