

Engineering viral vectors for non-invasive and specific gene delivery to the brain and body (via machine-learning assisted directed evolution)

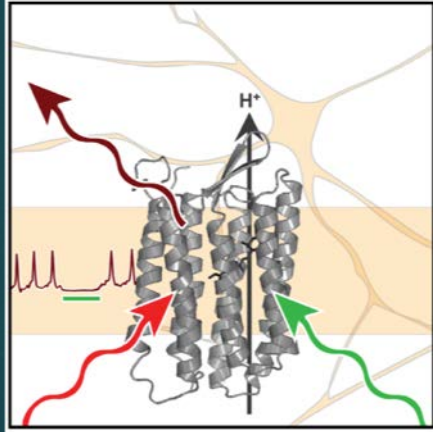
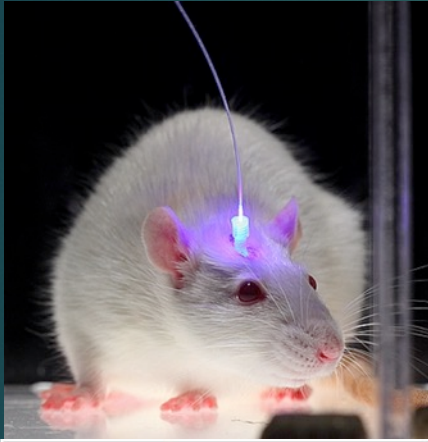


Viviana Gradinaru, **CALTECH**

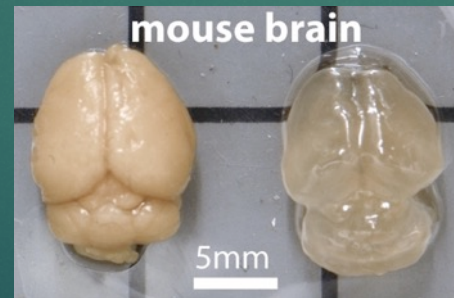
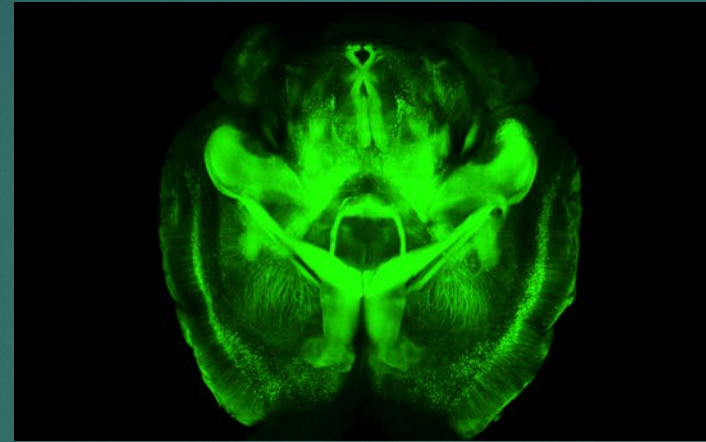
Director of the Caltech's Center for Molecular and Cellular Neuroscience

Professor of Neuroscience and Biological Engineering

Heritage Principal Investigator

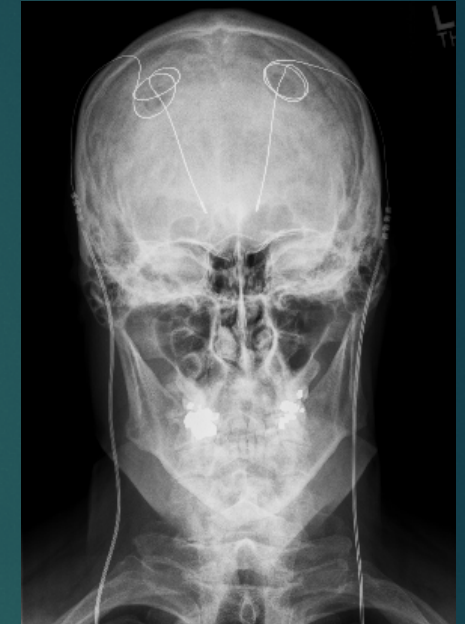
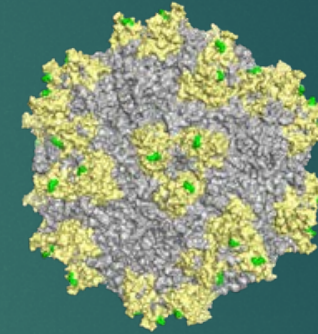


Optogenetics



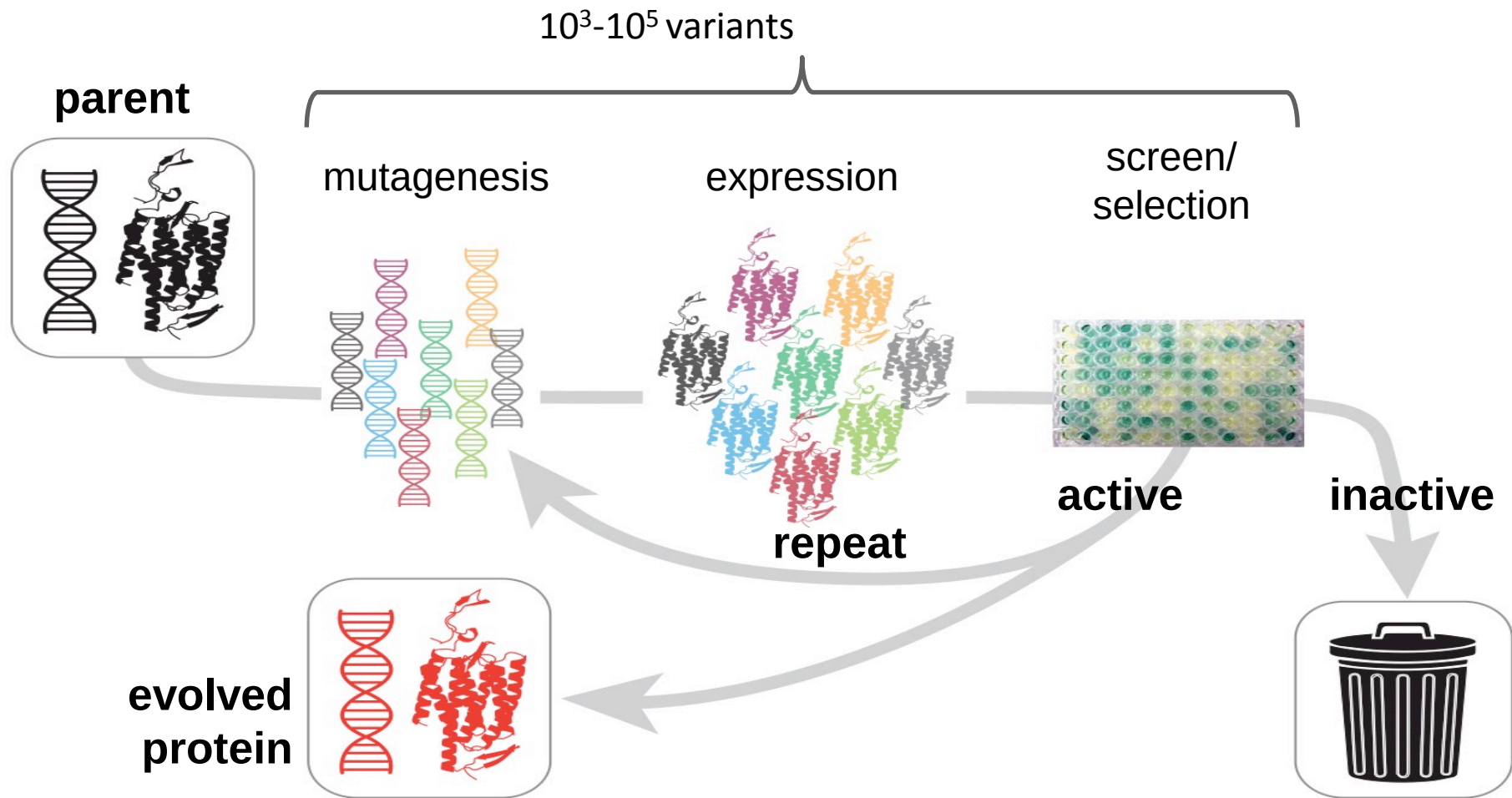
**CLARITY &
delivery vectors**

**delivery
vectors**

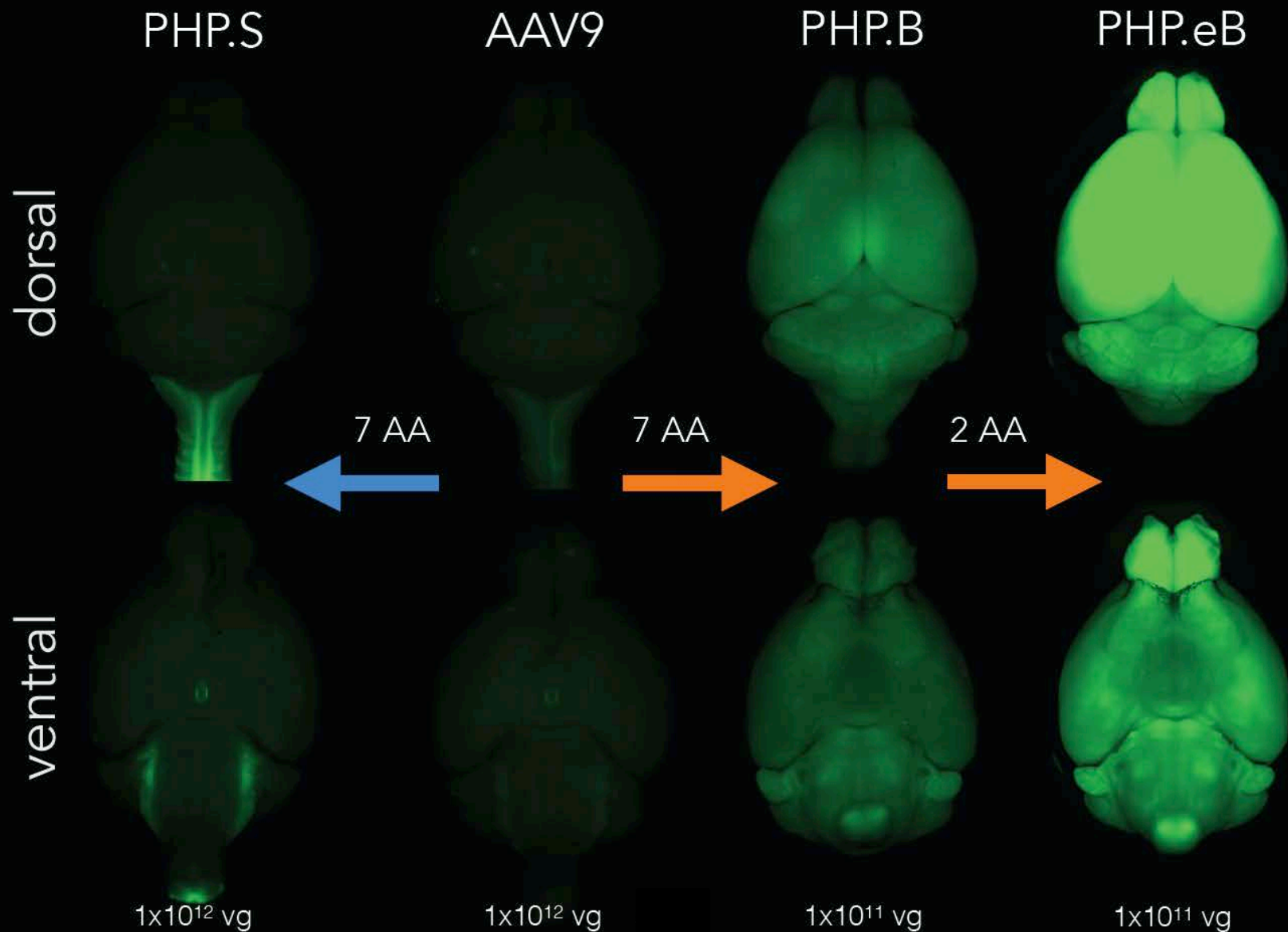


Deep Brain Stimulation

We are able to engineer natural proteins for novel functionality through directed evolution



AAV capsids are versatile and can be evolved to select for specific features



Deverman BE, Pravdo P, Simpson B, Banerjee A, Kumar, S.R., Chan K, Wu WL, Yang B, Gradinaru V.

Cre-Dependent Capsid Selection Yields AAVs for Global Gene Transfer to the Adult Brain.

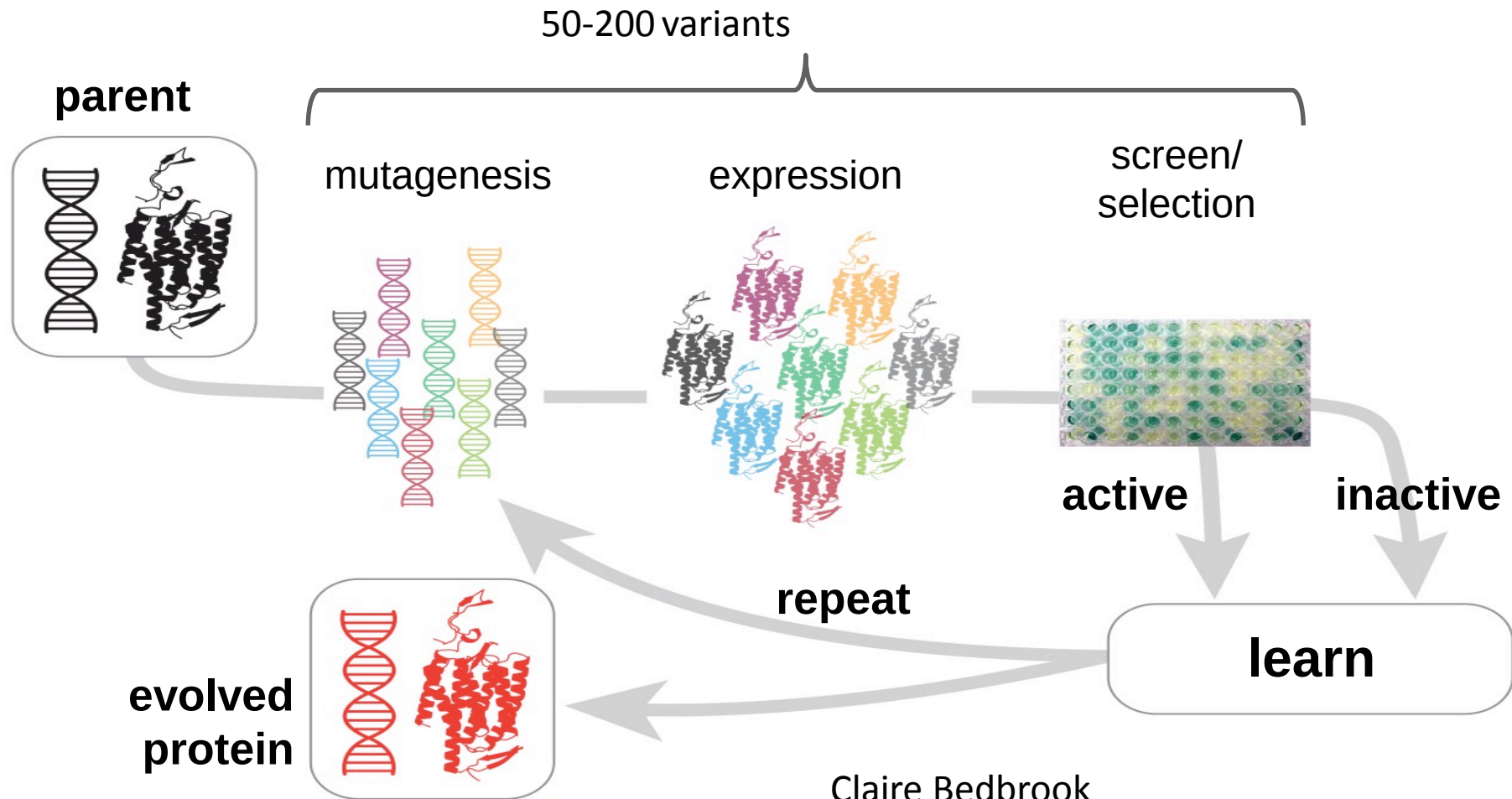
Nature Biotechnol. 2016

K Chan, M Jang, B Yoo, A Greenbaum, N Ravi, W Wu, L Sánchez-Guardado, C Lois, S Mazmanian, B Deverman, V Gradinaru.

Engineered adeno-associated viruses for efficient and noninvasive gene delivery throughout the central and peripheral nervous systems

Nature Neuro 2017

To convert data into knowledge...
we can use **machine learning**

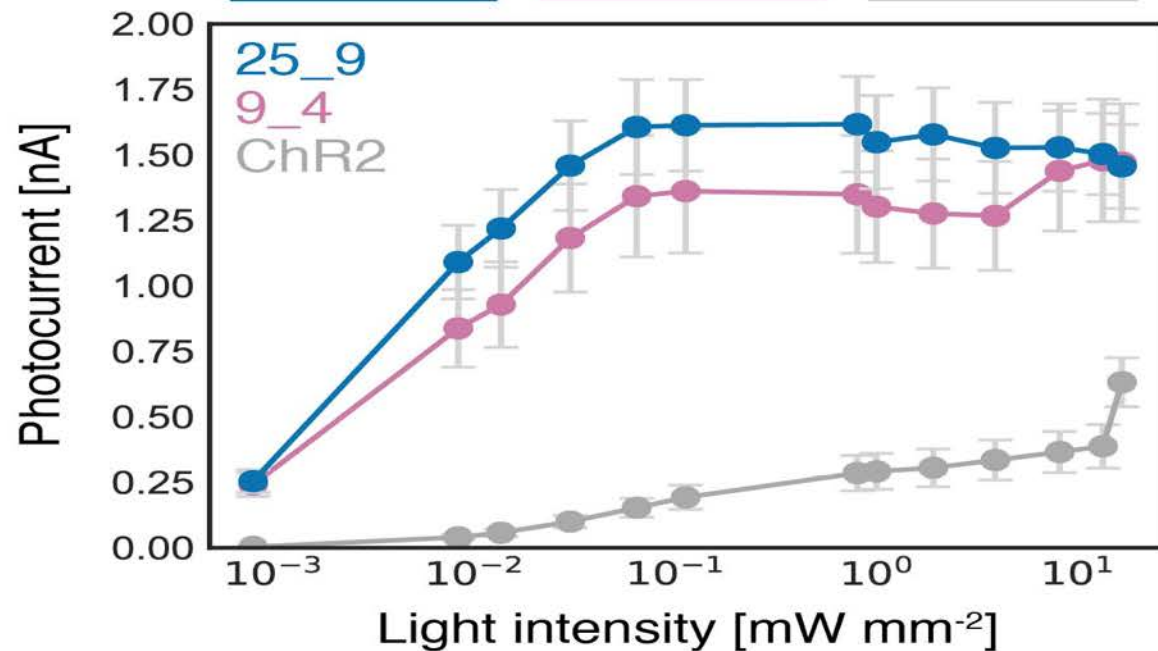
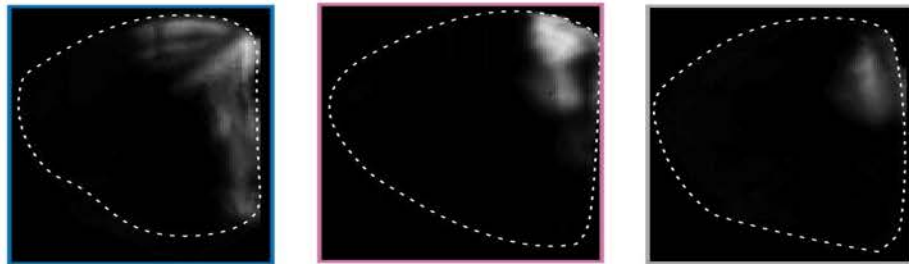


Claire Bedbrook
(collaboration with Frances Arnold Lab)

Improved opsins for systemic delivery

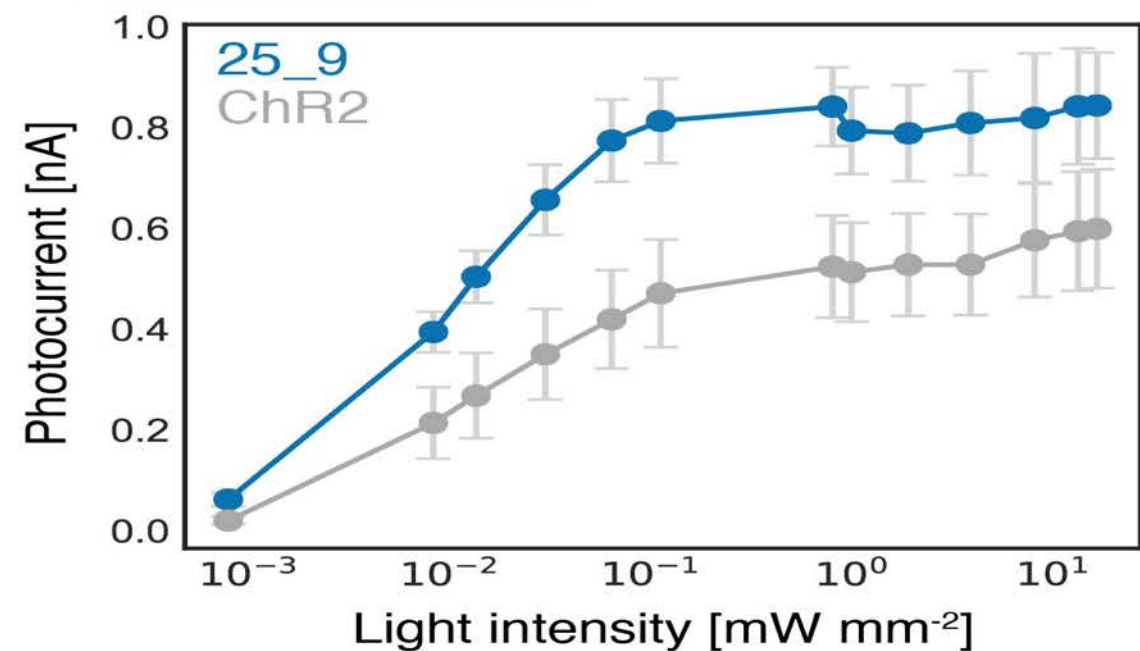
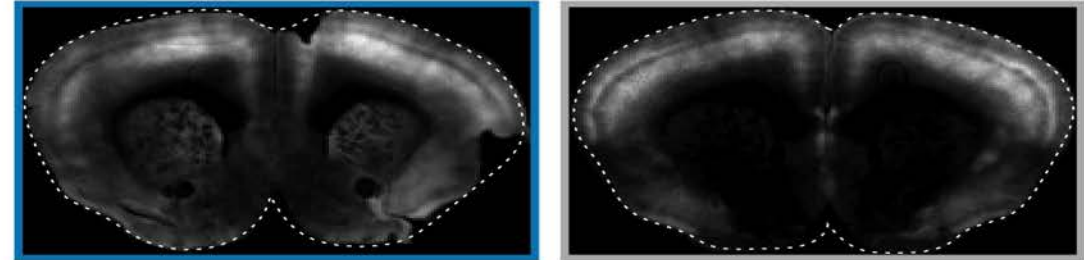
D

Direct intracranial injection



E

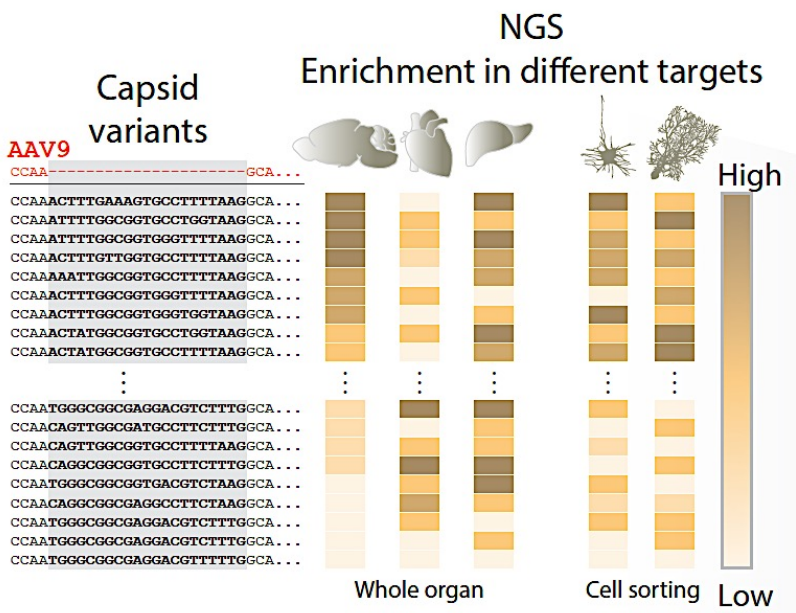
Systemic delivery (5x10¹¹ VG)



"Machine learning-guided channelrhodopsin engineering enables minimally-invasive optogenetics." Bedbrook, C. N., Yang, K. K., Robinson, J. E., Gradinaru[@], V., & Arnold[@], F. H. bioRxiv, 2019; 10.1101/565606

Engineering designer AAVs for neuroscience

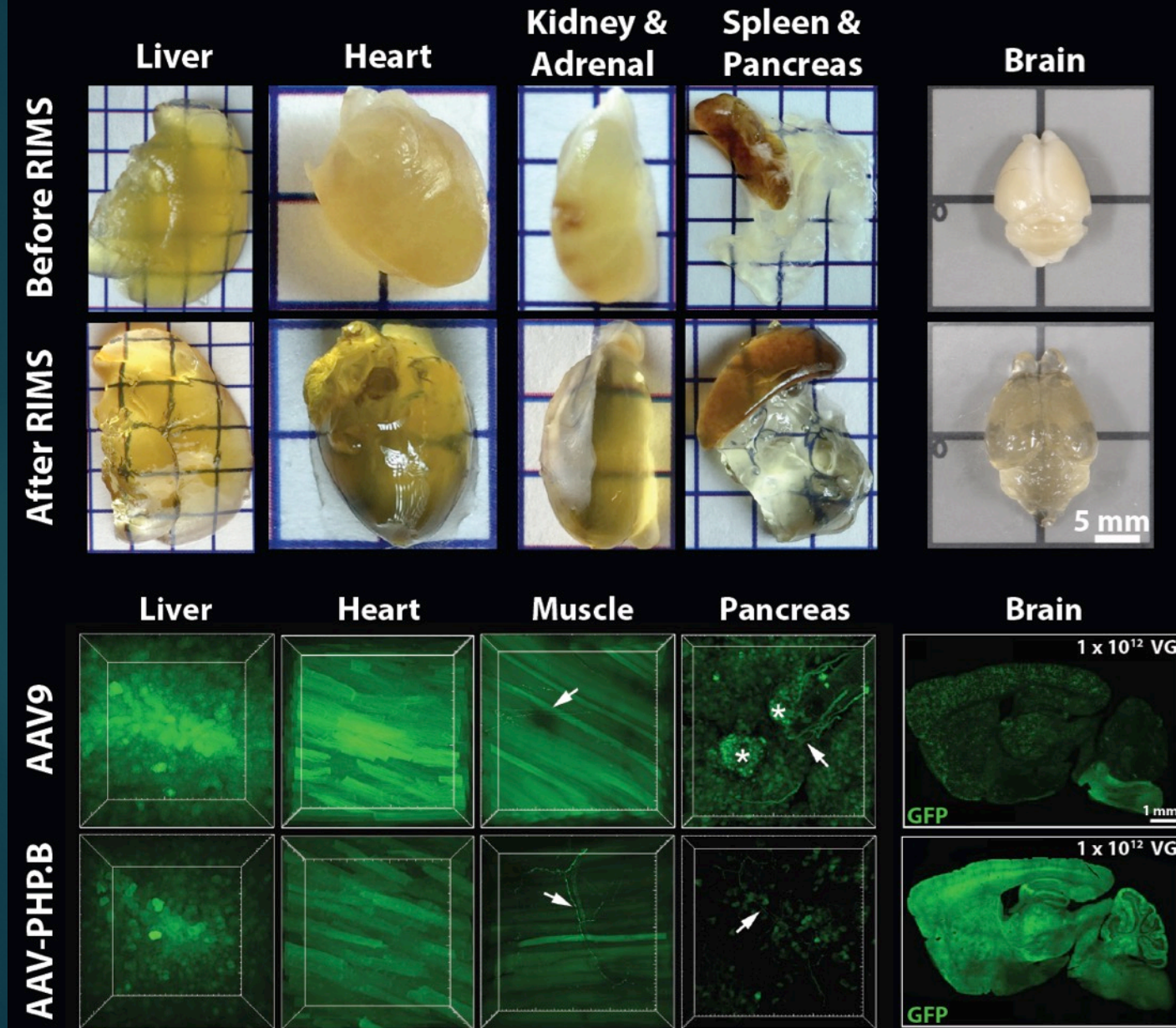
Bypassing limits of biological selection – NGS next generation sequencing followed by selection and learning *in silico*



AAV-PHP.V1 capsid shows brain-wide efficient and specific transduction of endothelial cells upon systemic delivery in adult Tek-Cre mouse brain



All current systemic AAVs for CNS also hit other organs (e.g. liver)



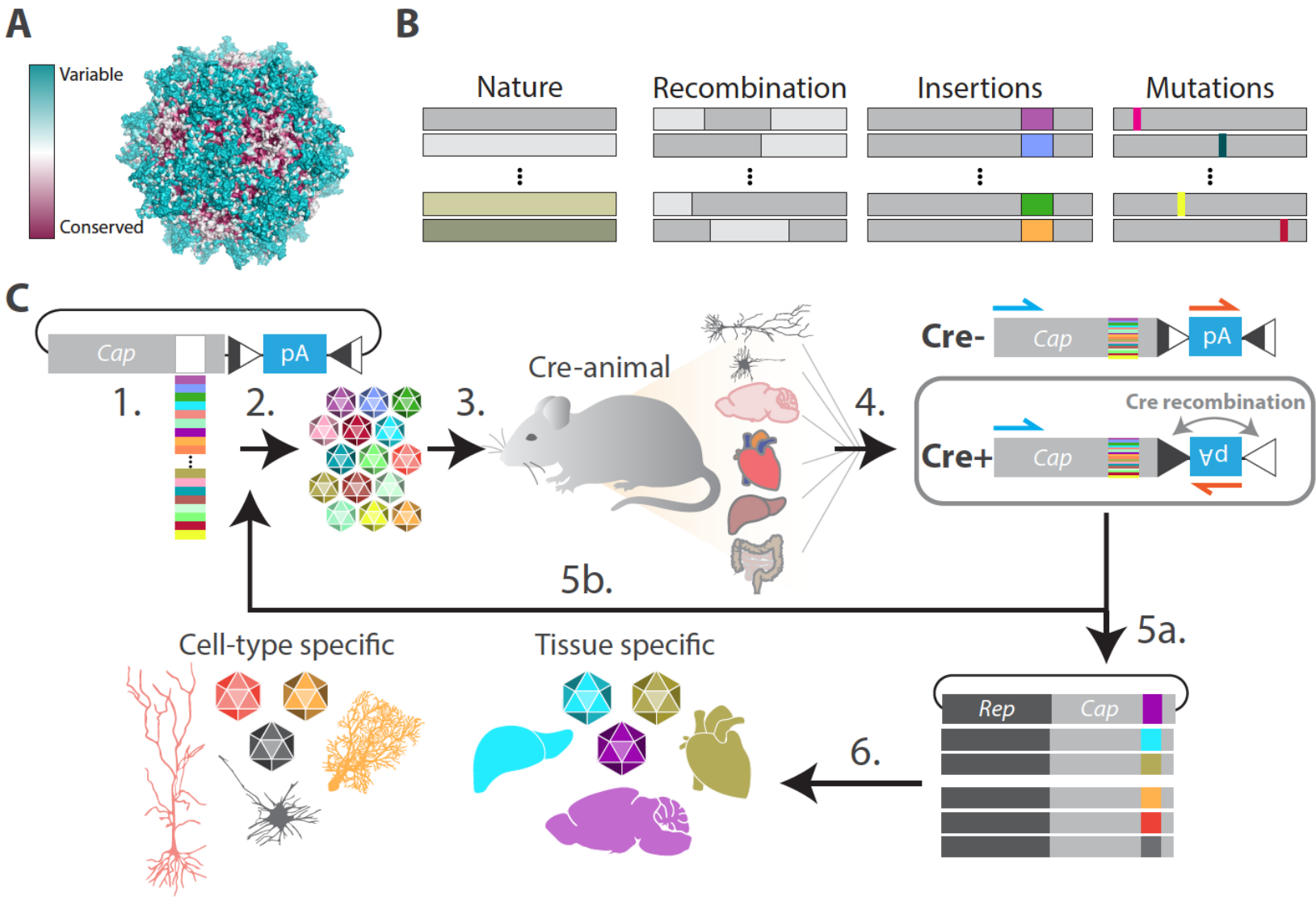
Whole-body viral biodistribution
By tissue clearing with PARS-CLARITY

Yang *et al.* (Cell, 2014)
Treweek *et al.* (Nat.Prot, 2015)
Deverman *et al.* (Nat.Biotech, 2016)

LiverTox: NIH database launched 2012
on liver injury associated with drugs

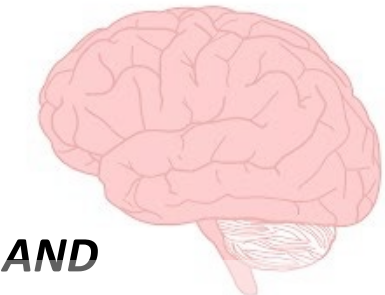
“Drug-induced liver injury is the leading cause of acute liver failure in the US, accounting for at least half of cases.”

Novel approach for specific and selective novel vectors via capsid alteration



We can generate viral diversity and apply selective pressure for desired properties

e.g. delivery that is strong in the brain

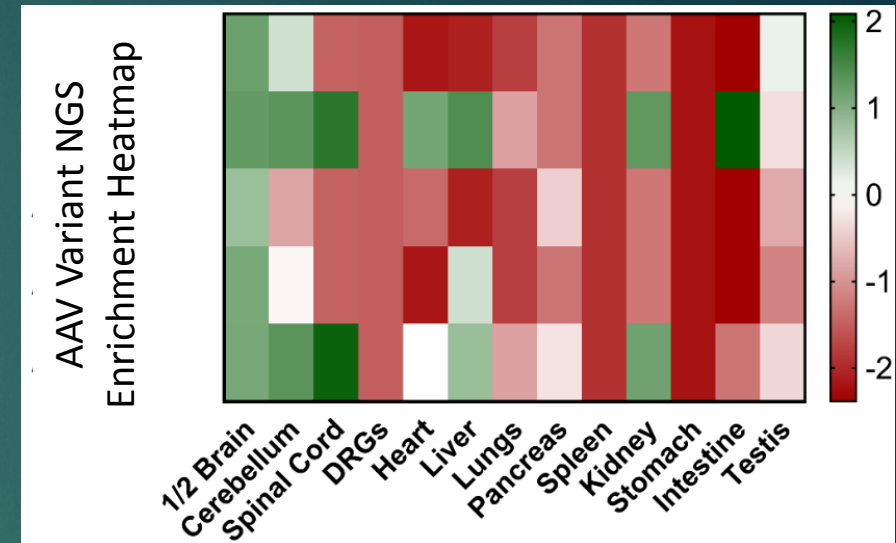
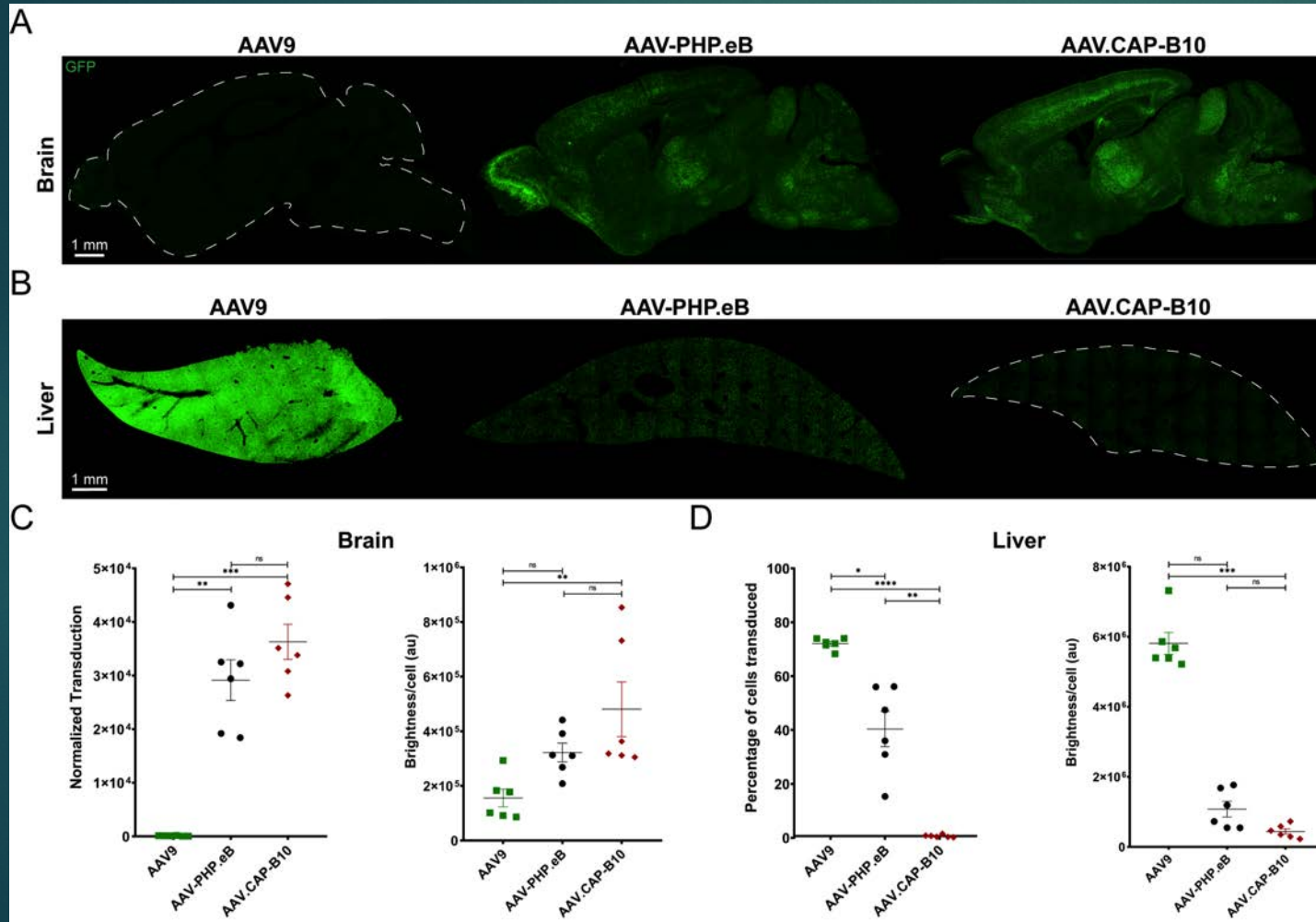


AND



weak in the liver

A gene delivery vector that targets the brain noninvasively *and* spares the liver



- Many variants efficiently cross BBB
- *in vivo* mouse selection
- Variability in peripheral organ de-targeting
- Some variants - higher neuronal specificity

Does it work in NHPs?

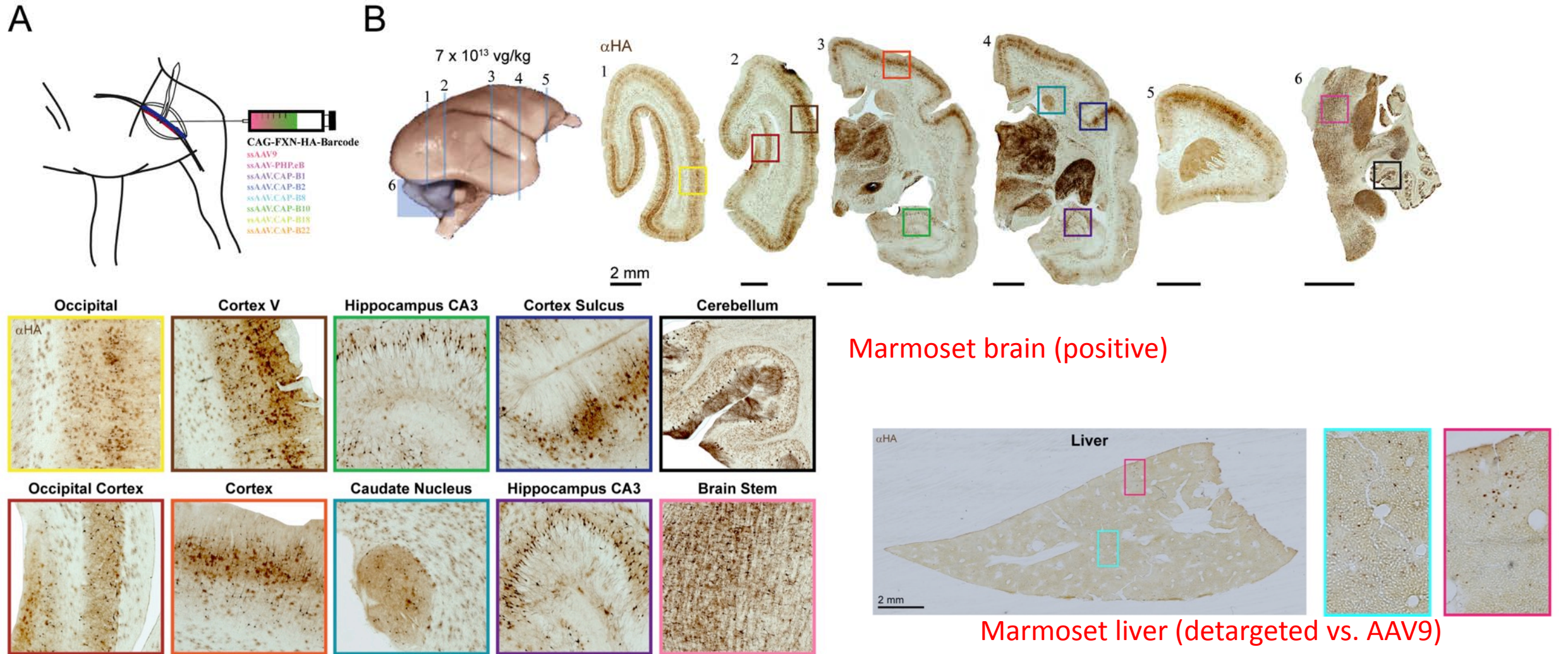
Bottleneck: cost and time for testing one designer capsid at a time

Approach for optimizing the platform for specificity and targeting in NHPs

1. Select candidates first *in vivo* in rodents by CREATE directed evolution
2. Screen for confirmation of expression in NHPs
 - To save time and cost we designed a multiplex screening approach where 10-20 virus candidates can be evaluated at the same time (by RNA barcoding)
 - The only downside is that there will be fewer copies of each individual virus, but this still allows us to see expression if they cross the BBB
3. Confirmation of 2-3 top candidates.

Does it work in NHPs?

(ongoing collaboration wt NIH/NIMH Transgenic Marmoset Core led by Dr. Jim Pickel)

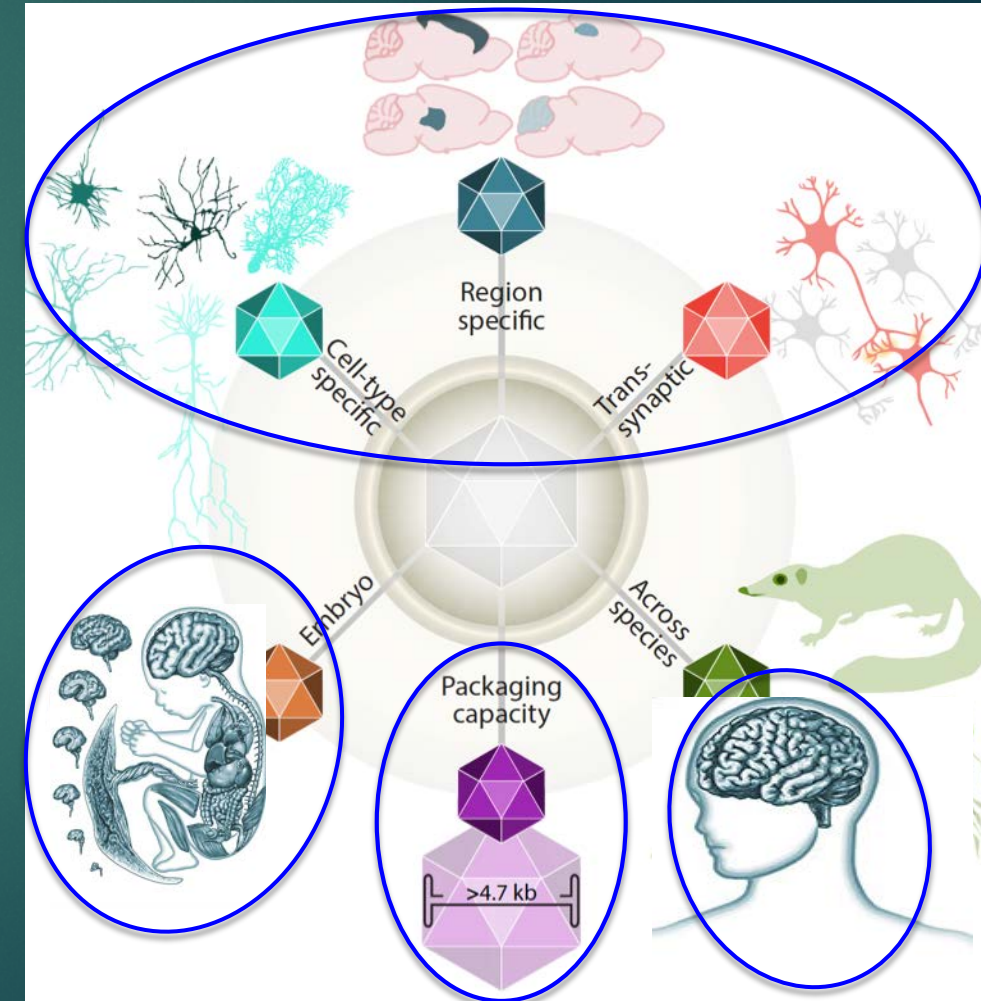


Marmoset brain (positive) and liver (detargeted) expressing pooled, barcoded FXN-HA tag viruses (7 of new variants + AAV9) 6 weeks after systemic delivery (no health pbs noticed).

Note: each variant has low MOI therefore individual strong variants are expected to have even stronger expression (in progress)

Key features required for safe, effective, and minimally invasive gene delivery

- ▶ Vectors capable of needed specificity
 - ▶ cell-type, circuit, organ, region
- ▶ Vectors that can bypass natural body barriers to access key tissues:
 - ▶ across the blood-brain-barrier
 - ▶ across the placenta to embryo
- ▶ Vectors capable of large cargo delivery
 - ▶ AAV limit is ~4.7kb
 - ▶ 5-8kb needed for many genetic tools
 - ▶ *High efficiency for co-delivery helps (e.g. for CRISPR/Cas)*



Visualizing the Activity and Anatomy of Brain Circuits: Optogenetics, Tissue Clearing, Viral Vectors

Caltech

Gradinaru Lab, Caltech

Graduate Students: Ryan Cho, Xiaozhe Ding

Priya Kumar, Michael Altermatt, Gerry Coughlin
Tatyana Dobрева, David Brown

Miggy Chuapoco, Xinhong Chen

Postdocs: Collin Challis, Min Jee Jang, Rose Challis,
Elliott Robinson, Anat Kahan, Nick Goeden

Recent PI alumni: Jenny Treweek (USC),
Alon Greenbaum (UNC/NC)

Clover Director: Nick Flytzanis

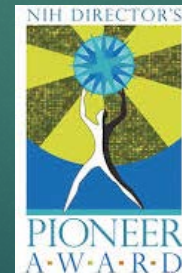
Lab Alumni:

Claire Bedbrook (Stanford), Ken Chan (Broad)
Ben Deverman (Broad), Bin Yang (Caltech)
Cheng Xiao, Lindsay Bremner (Caltech), Chunyi Zhou

Financial support:

NIH: R01 NIA, NINDS BRAIN, R24&UC4 NIDDK,
NIH Director's New Innovator, PECASE, SPARC, Pioneer
NSF: Neuronex

Foundations: Moore, Beckman, Curci, Sloan, Pew,
Heritage Medical Foundation and **DARPA**



www.glab.caltech.edu
viviana@caltech.edu

**DISSEMINATION BY CLOVER
CLARITY, OPTOGENETICS AND VECTOR ENGINEERING
RESEARCH CENTER @ THE BECKMAN INSTITUTE CALTECH**



clover.caltech.edu

