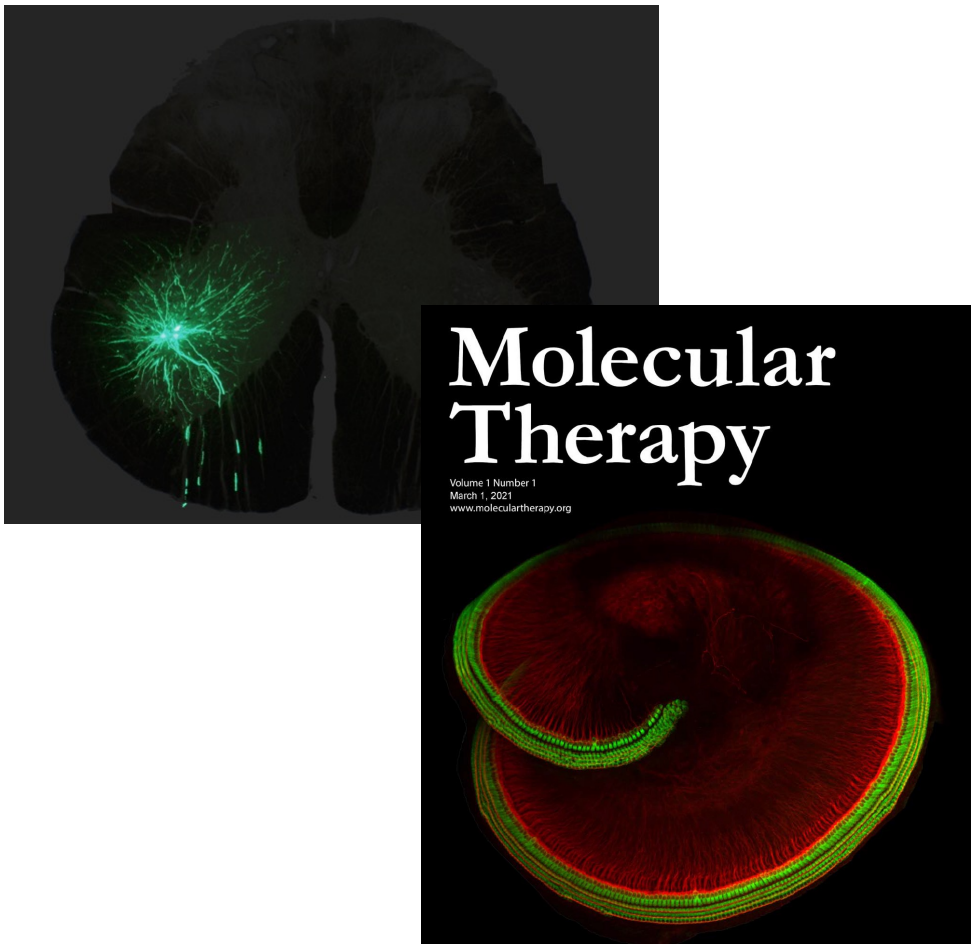
EPFL



Engineering and production of viral vectors for research and gene therapy

BIOENG-518, 2025

B. Schneider

March 14 2025

Lecture plan

- Why using viruses as '**vectors**'?

- Example of a broadly used vector: recombinant adeno-associated virus (AAV)
  - How is it produced?
  - How is it characterized?
  - How does it transduce cells?
  - How is AAV engineered to generate novel vectors with unprecedented features?

- Focus on some vector-based techniques used in research
  - Cell-type specific expression
  - Neuronal tracing

Lecture plan

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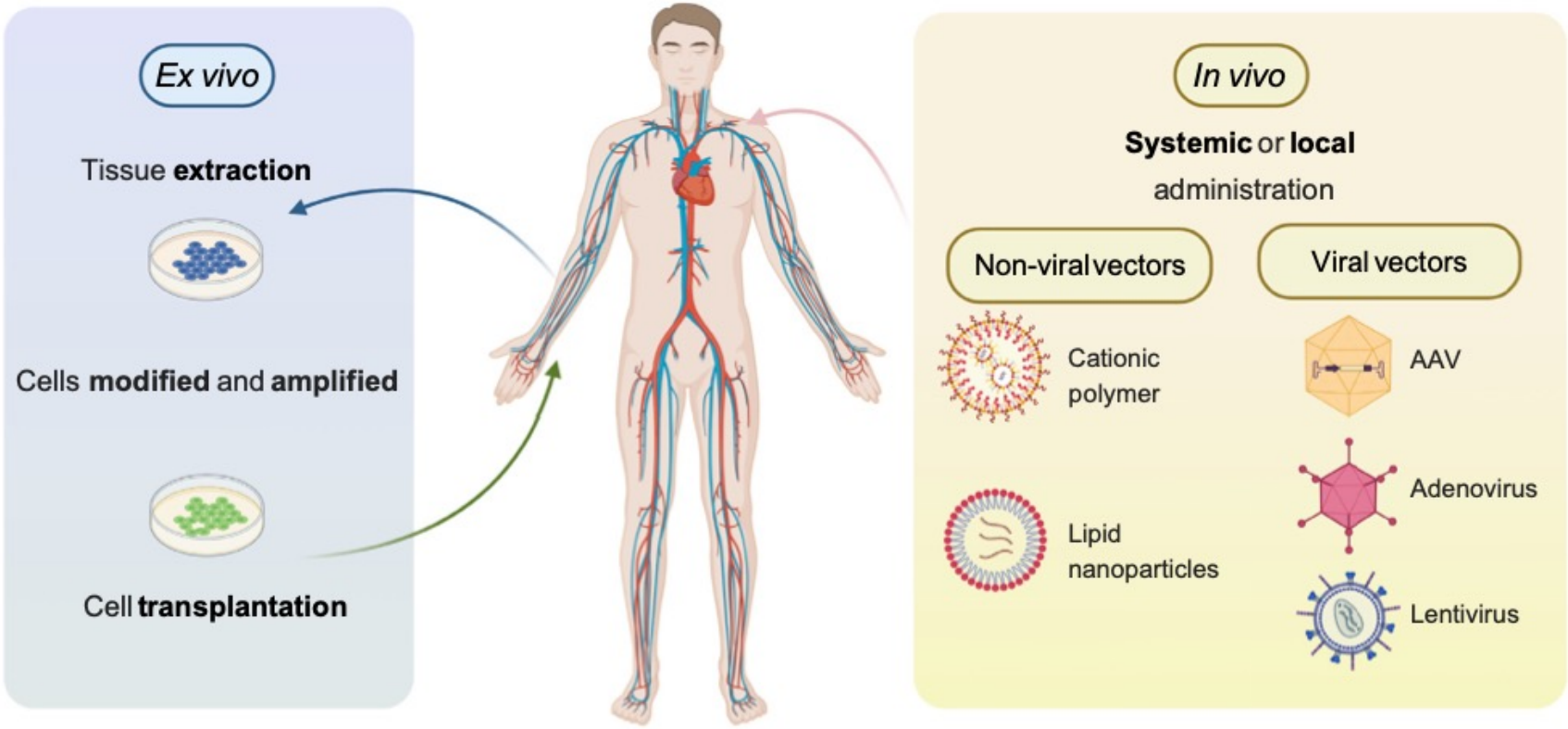
Gene therapy: nucleic acids as therapeutic modality



Development path to gene therapy:

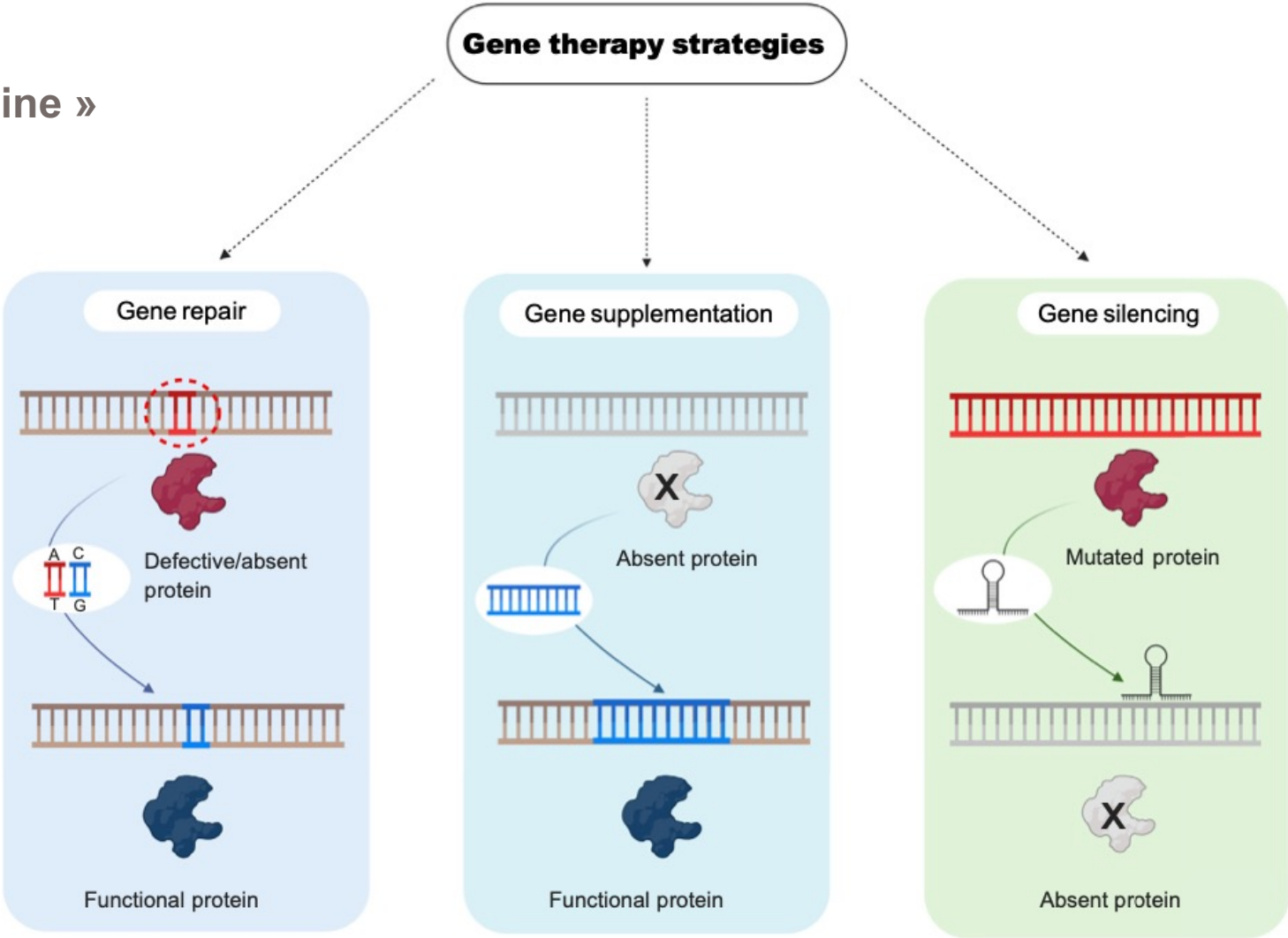
1- Disease model
2- Biodistribution
3- Safety/toxicity
4- Manufacturing

Therapy efficacy (clinical trial)

JHEP Reports 2021 vol. 3

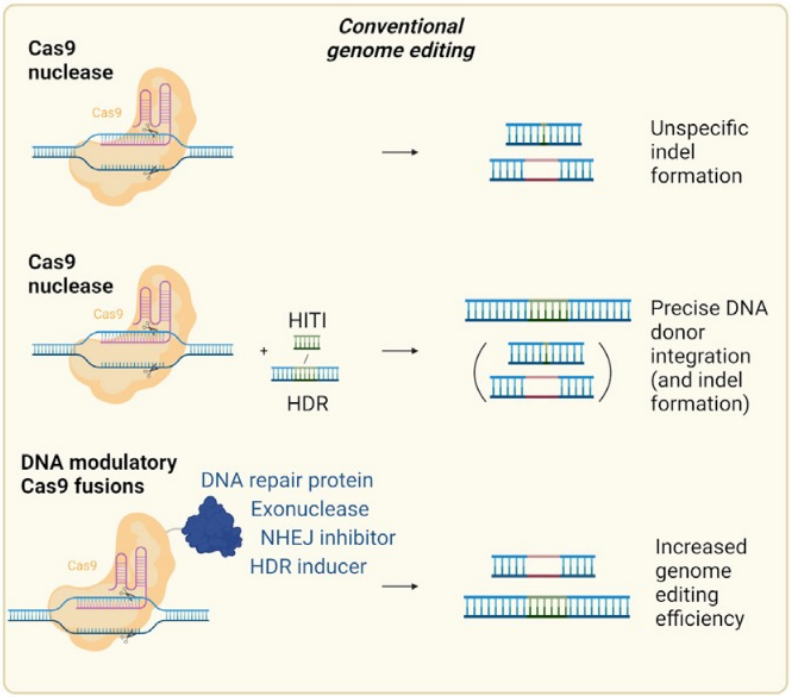
Gene therapy: nucleic acids as therapeutic modality

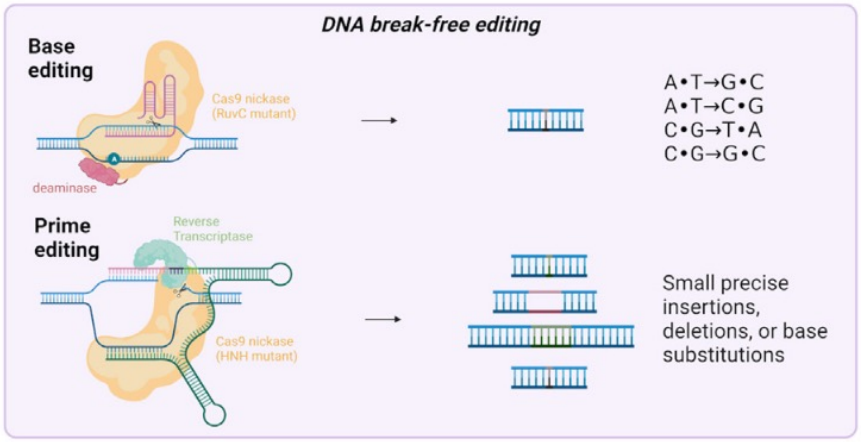
« Precision medicine »

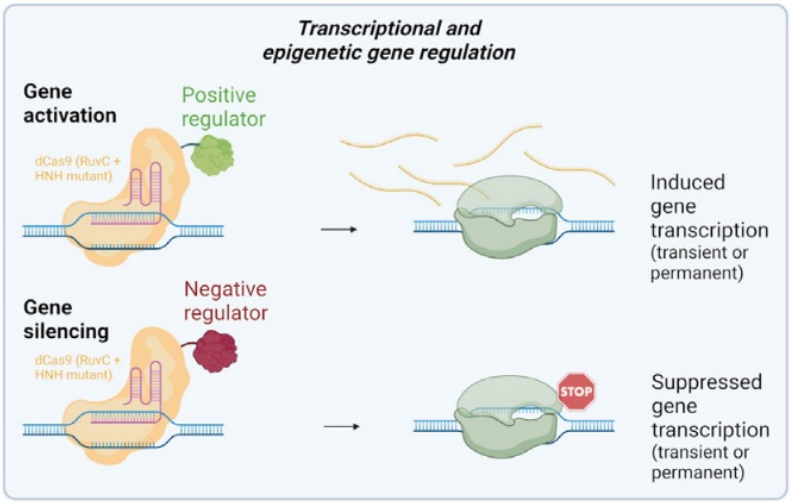


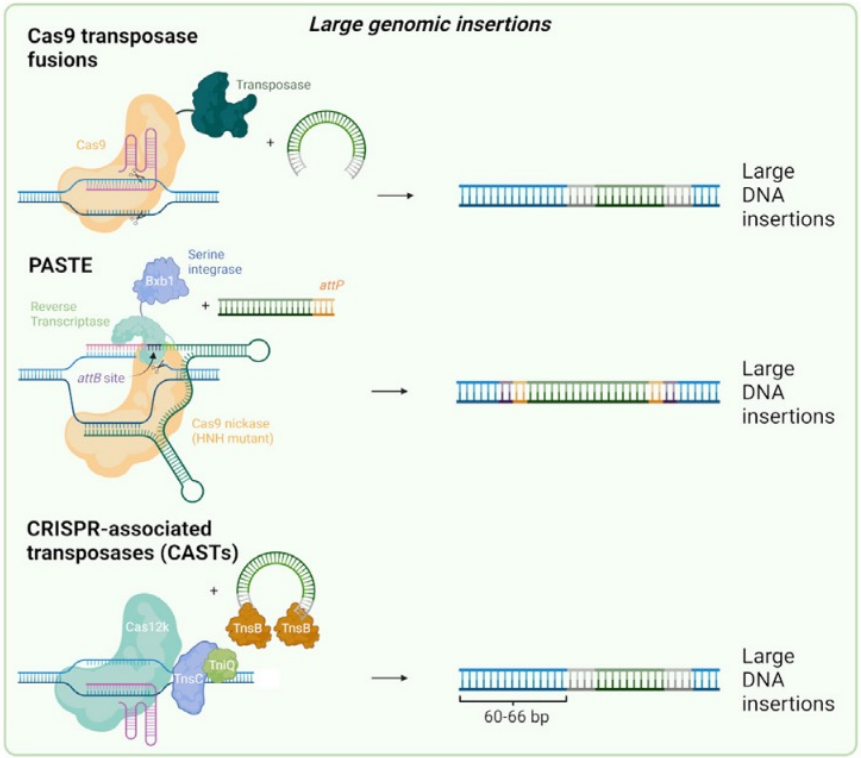
JHEP Reports 2021 vol. 3

Gene Editing Technologies



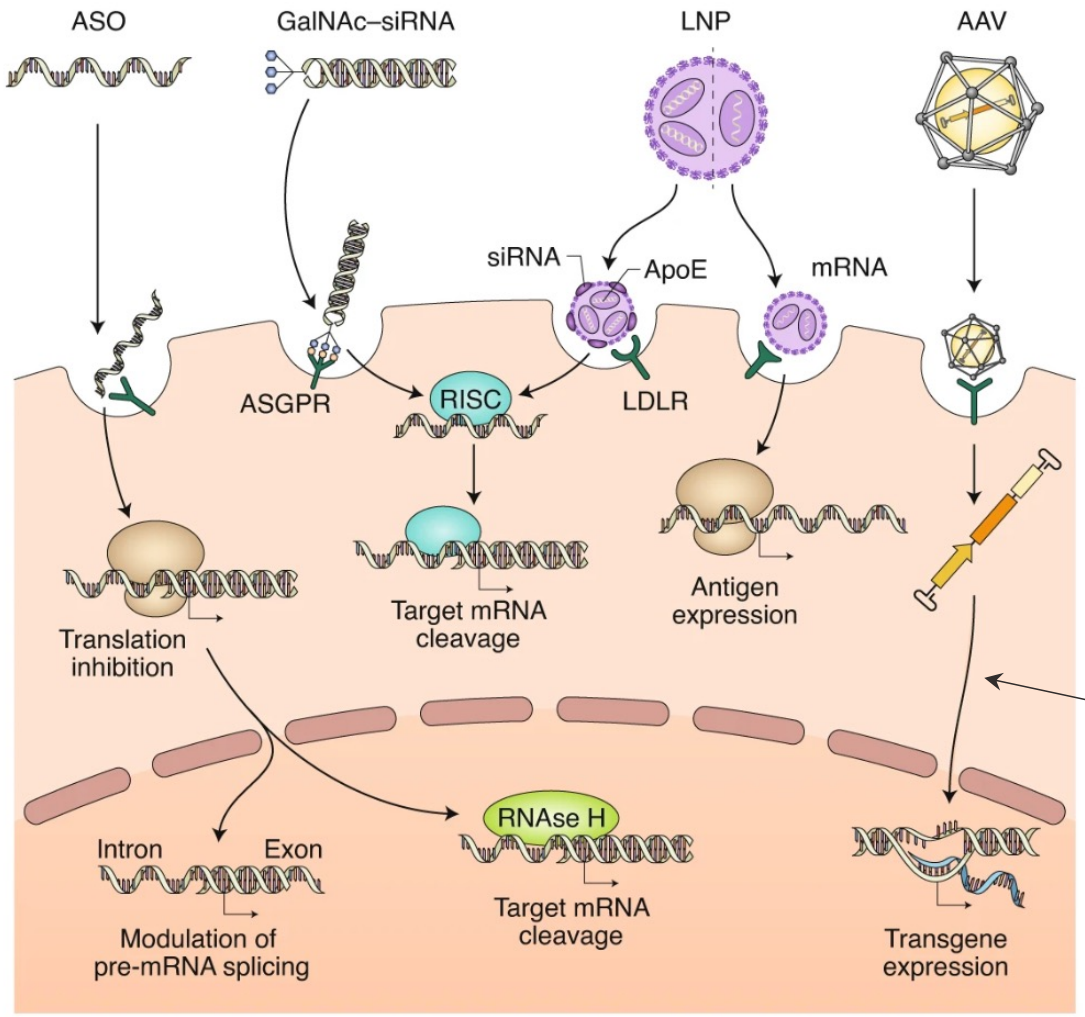






https://doi.org/10.1016/j.omtn.2023.102066

Delivery of nucleic acids



Viral vectors are particularly efficient at delivering a transgene into the nuclear compartment of post-mitotic cells.

ASO: antisense oligonucleotides
LNP: lipid nanoparticles

Nature Nanotechnology vol16, pp 630–643 (2021)

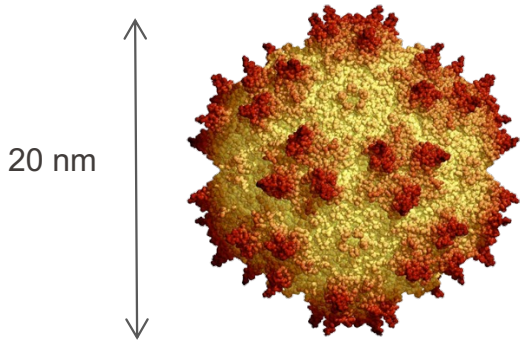
Gene therapy: modalities

	Advantage	Disadvantage	Application	Main obstacle
Antisense oligonucleotide	Manufacturability	Need for repeated administration	Gene silencing, splicing modifier	Cell targeting not controlled
mRNA	Manufacturability	Transient expression	Gene replacement, (vaccine), editing	Delivery
Viral vector	Long-term expression, efficacy	Dose finding is difficult, toxicity	Gene silencing, editing, gene replacement	Dose finding, manufacuring, immunity
Nanoparticles	Capacity, manufacturability	Toxicity	Gene silencing, editing, gene replacement	Delivery, efficacy in non-dividing cells
Genetically modified cells	Long-term effects, quality control	Manufacturability	Gene replacement, editing	Delivery, approach not compatible for neurons

Gene therapy: vectors

The main challenges of gene therapy: delivery, delivery and delivery

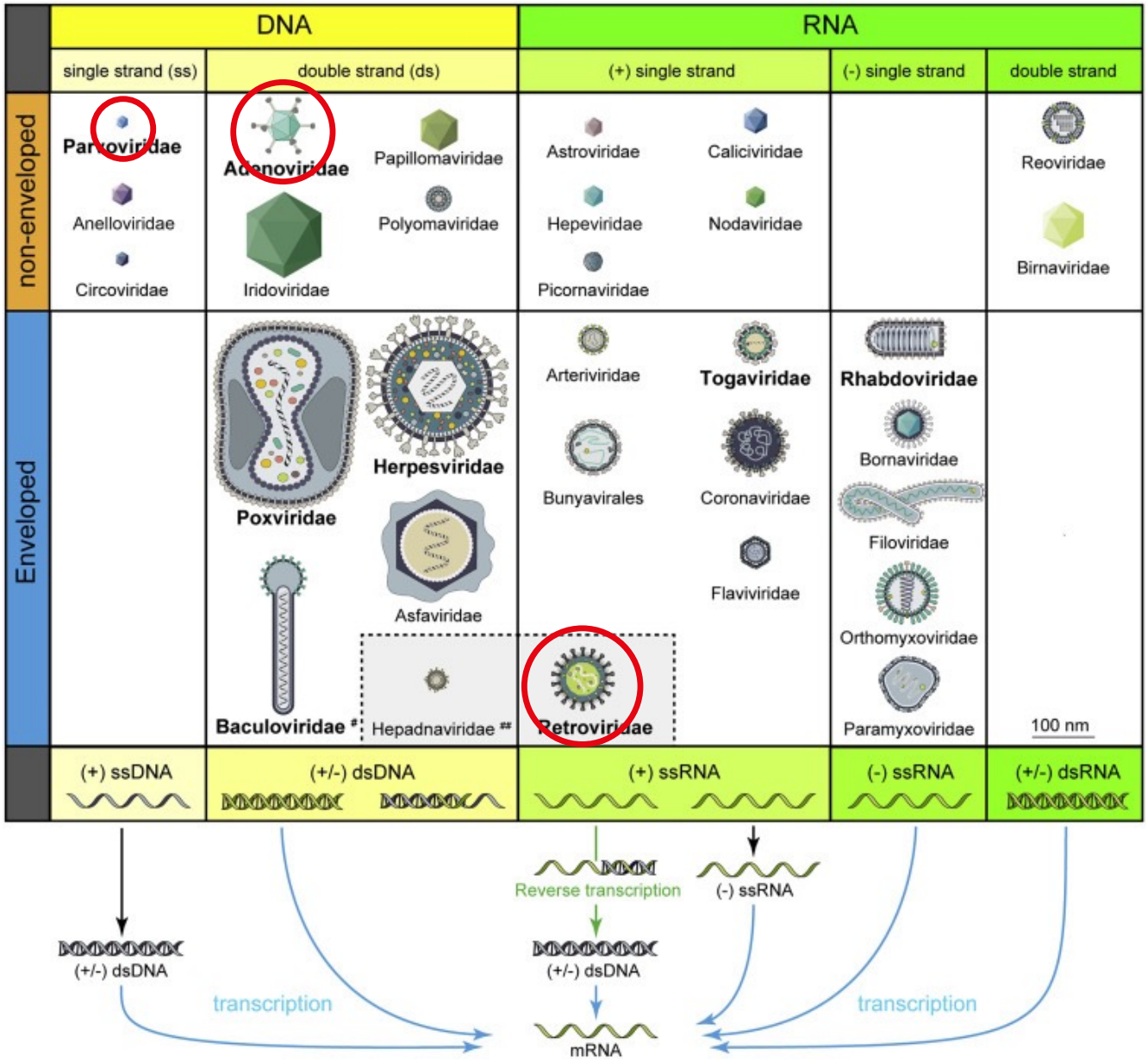
A Trojan horse for gene therapy: viral particles



Adeno-associated virus (AAV)

Major classes of animal viruses

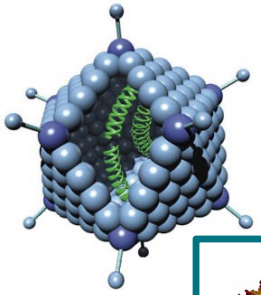
- Natural systems to deliver various forms of nucleic acids

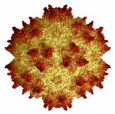


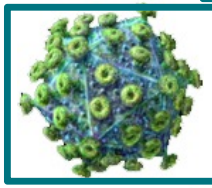
Neuron, Volume 107, Issue 6, 2020, pp 1029-1047

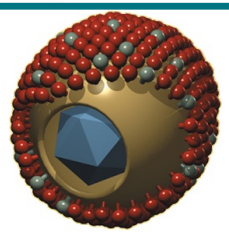
Viruses → vectors

Examples of viral vectors for use in the CNS









Virus	Size	Capacity	Pathogenic	Genomic insertion
Adenovirus	100 nm	8-30 kb	Yes	Rare
AAV	25 nm	4.7 kb	No	Rare (<1%)
Lentivirus	100 nm	9 kb	Yes (HIV)	Yes
Herpes	125 nm	20-150 kb	Yes	Rare

Lecture plan

- Why using viruses as vectors?
- Example of a broadly used vector: recombinant adeno-associated virus (AAV)
  - How is it produced?
  - How is it characterized?
  - How does it transduce cells?
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- Focus on vector-based research techniques
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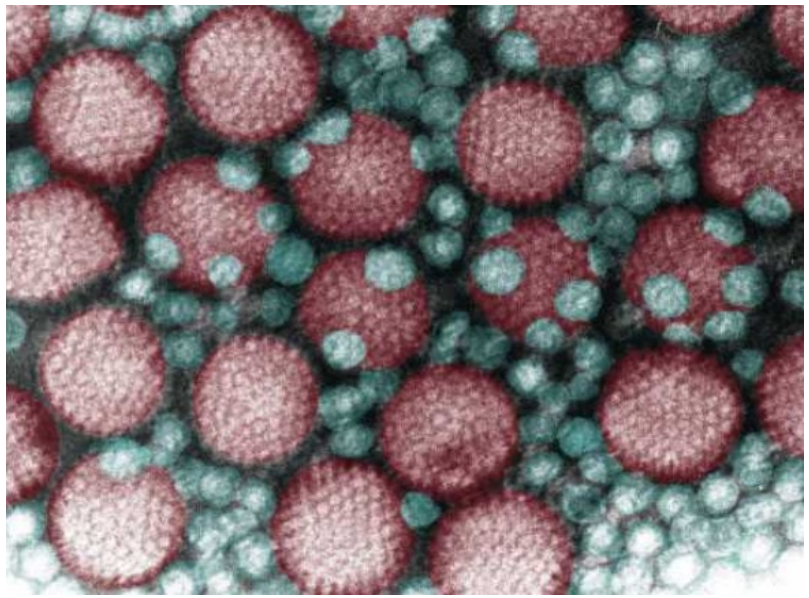
  - Neuronal tracing

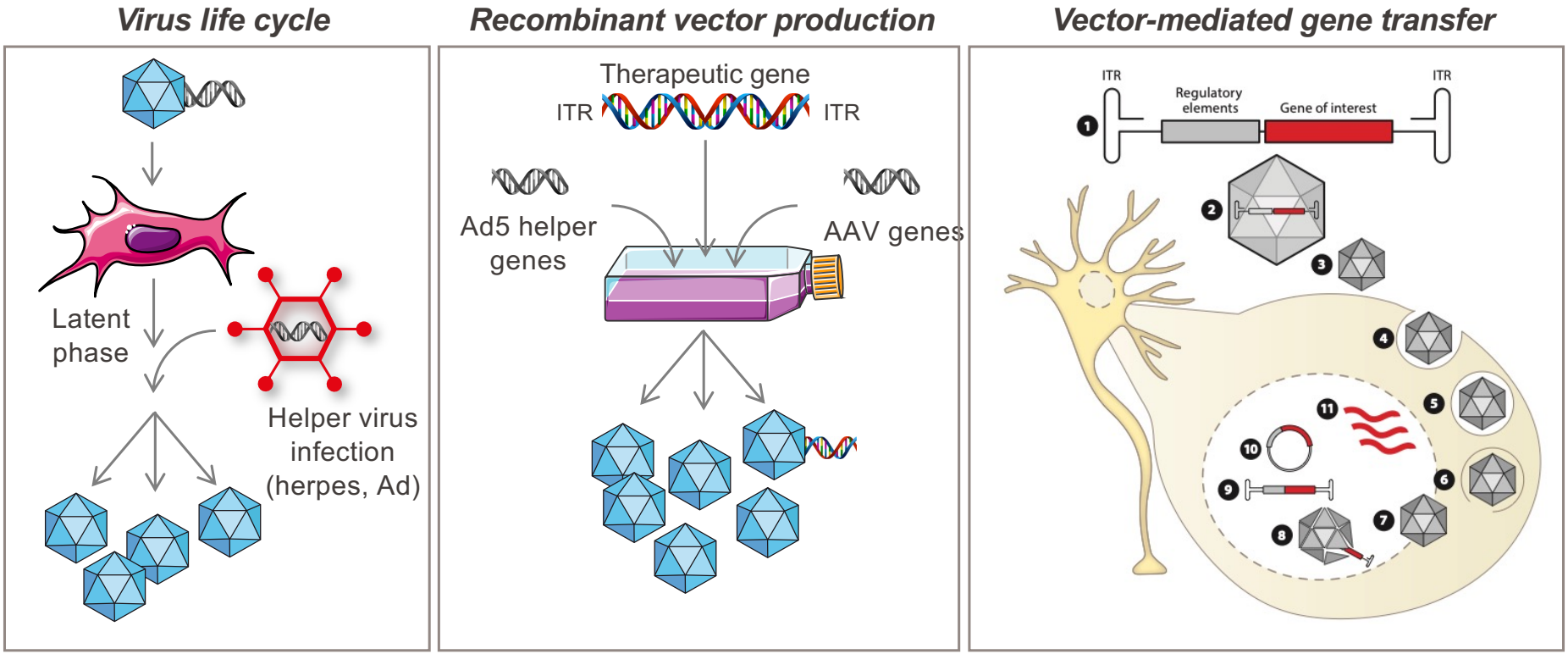
Adeno-Associated Virus: from a defective virus to an effective vector

- **Non-pathogenic**
- **60-mer protein capsid** (50 + 5 + 5 subunits)
- **4.75 kb genome**
- 20 nm, no lipid envelope
- "**gutless**" viral vector ⇒ does not code for any viral protein.
- Depends on adenovirus for replication.



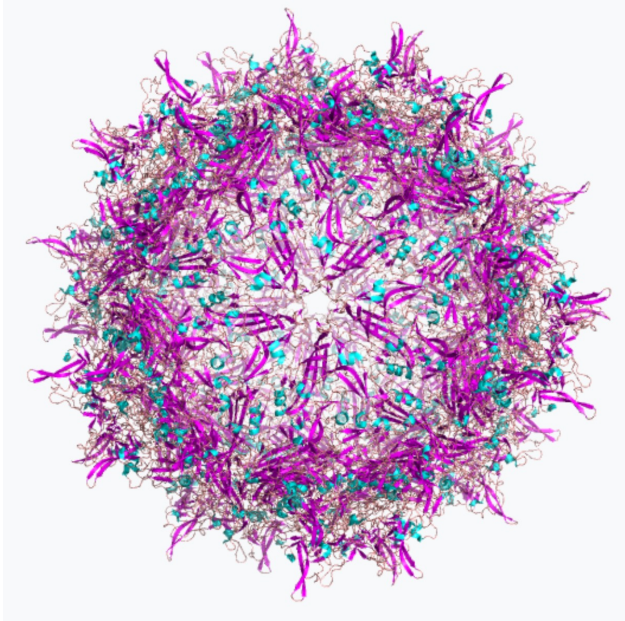
AAV: from a defective virus to an effective vector

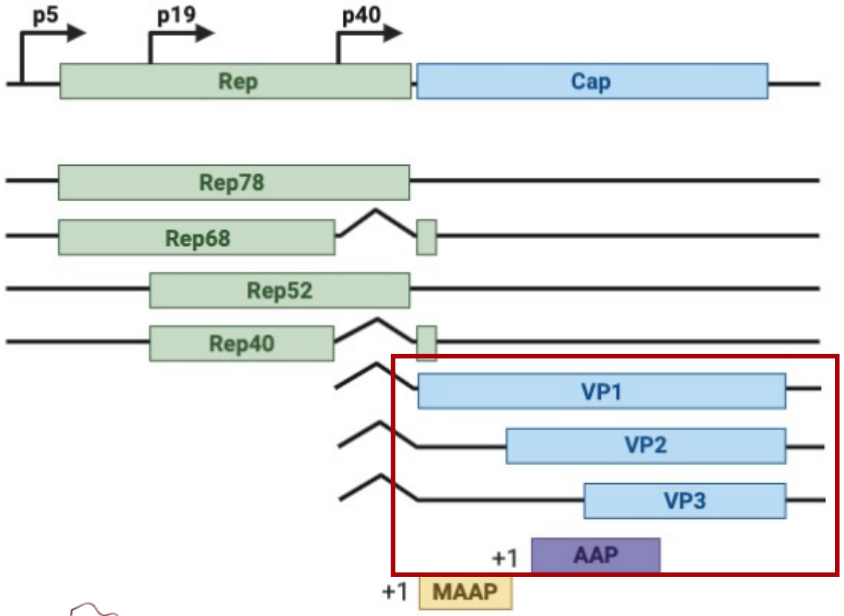
- Gene of therapeutic interest replaces part of the viral genome.
- AAV and helper genes are provided in trans: **the recombinant vector becomes defective for replication.**

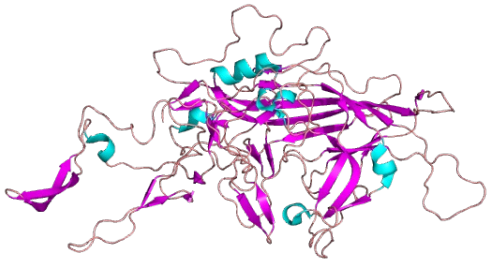


- Exploits **viral mechanisms for therapeutic gene transfer** into post-mitotic cells.

AAV capsid assembly

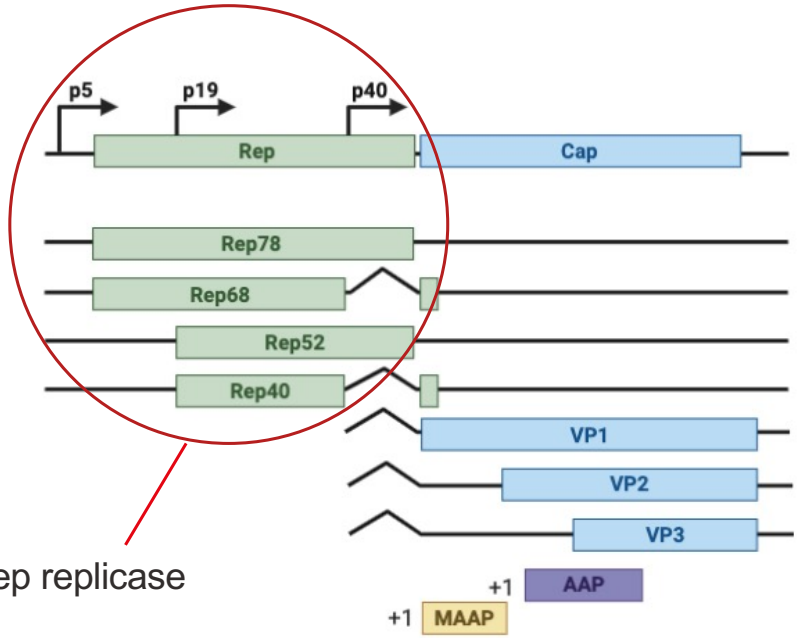




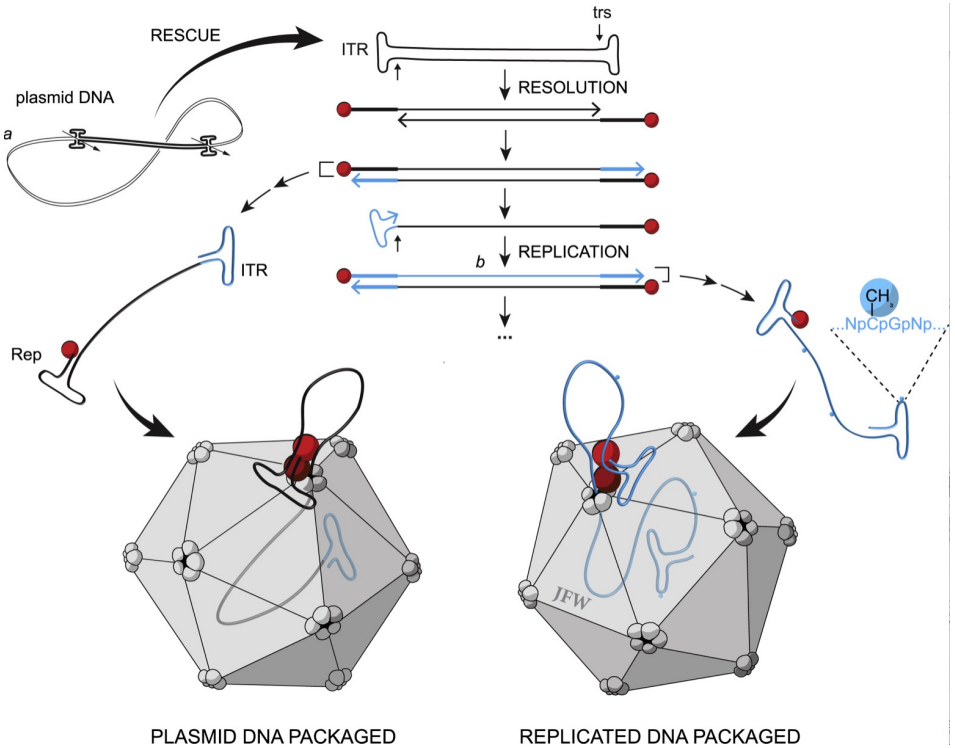


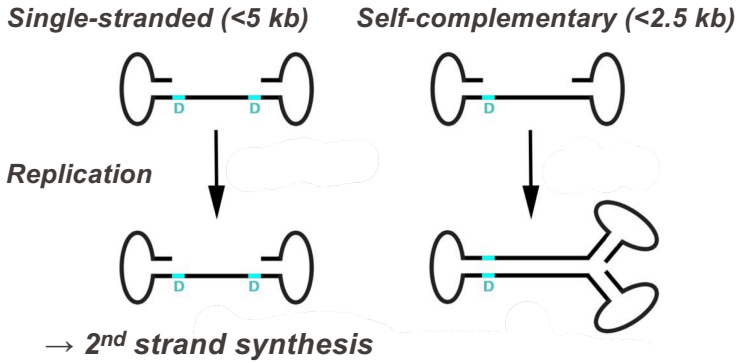
VP protein

AAV genome rescue, replication and packaging

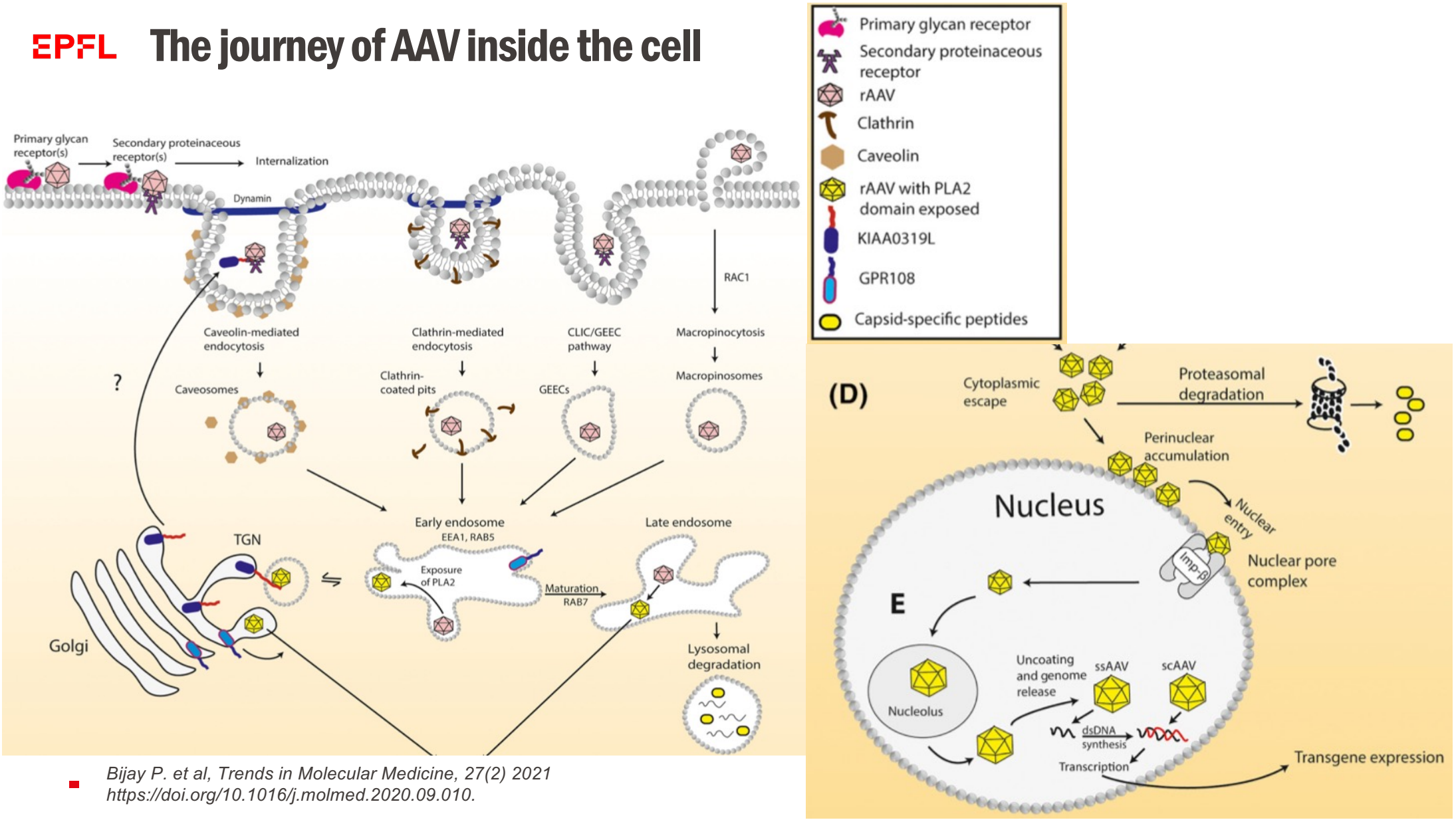


Rep replicase





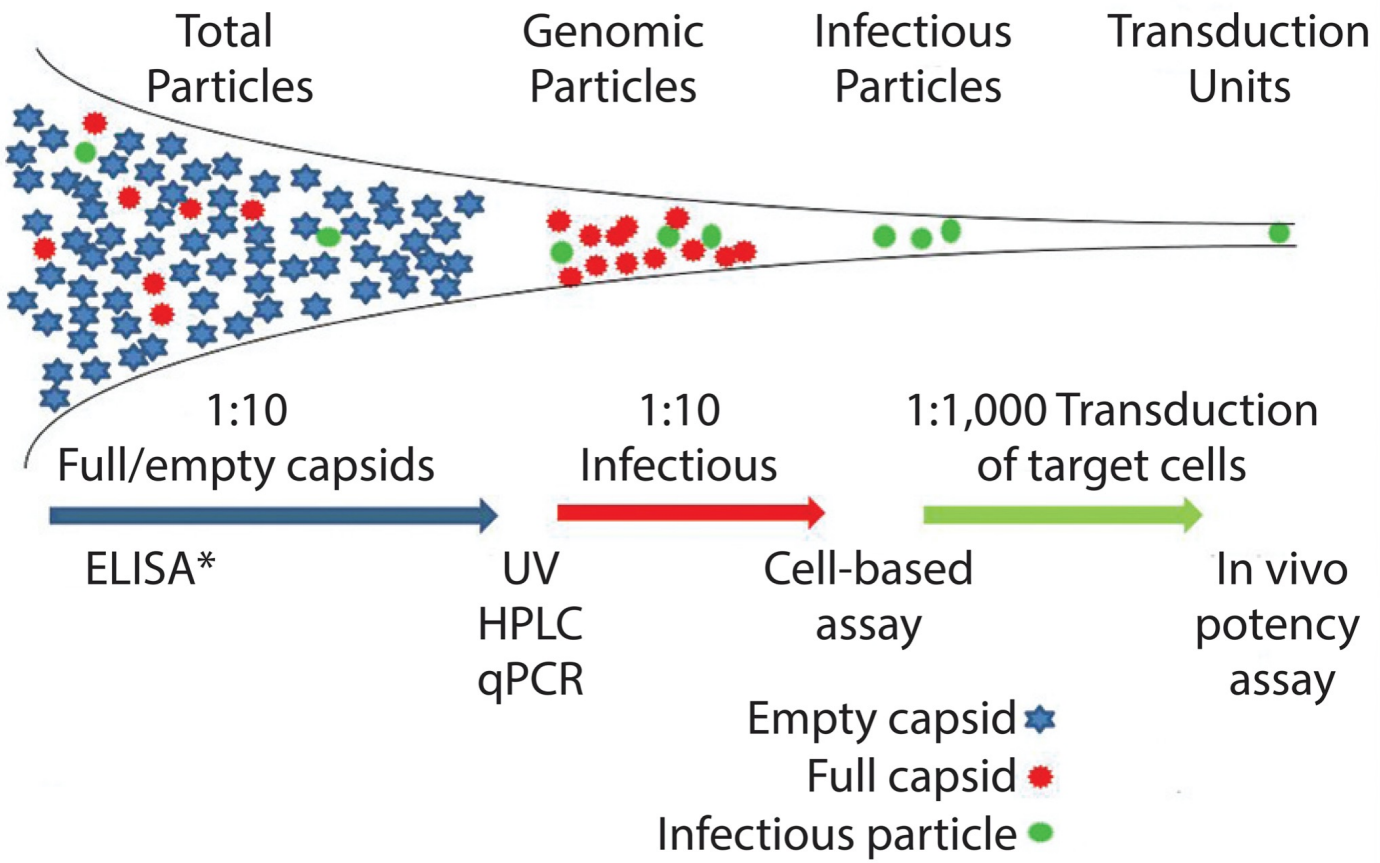
The journey of AAV inside the cell



- *Bijay P. et al, Trends in Molecular Medicine, 27(2) 2021 https://doi.org/10.1016/j.molmed.2020.09.010.*

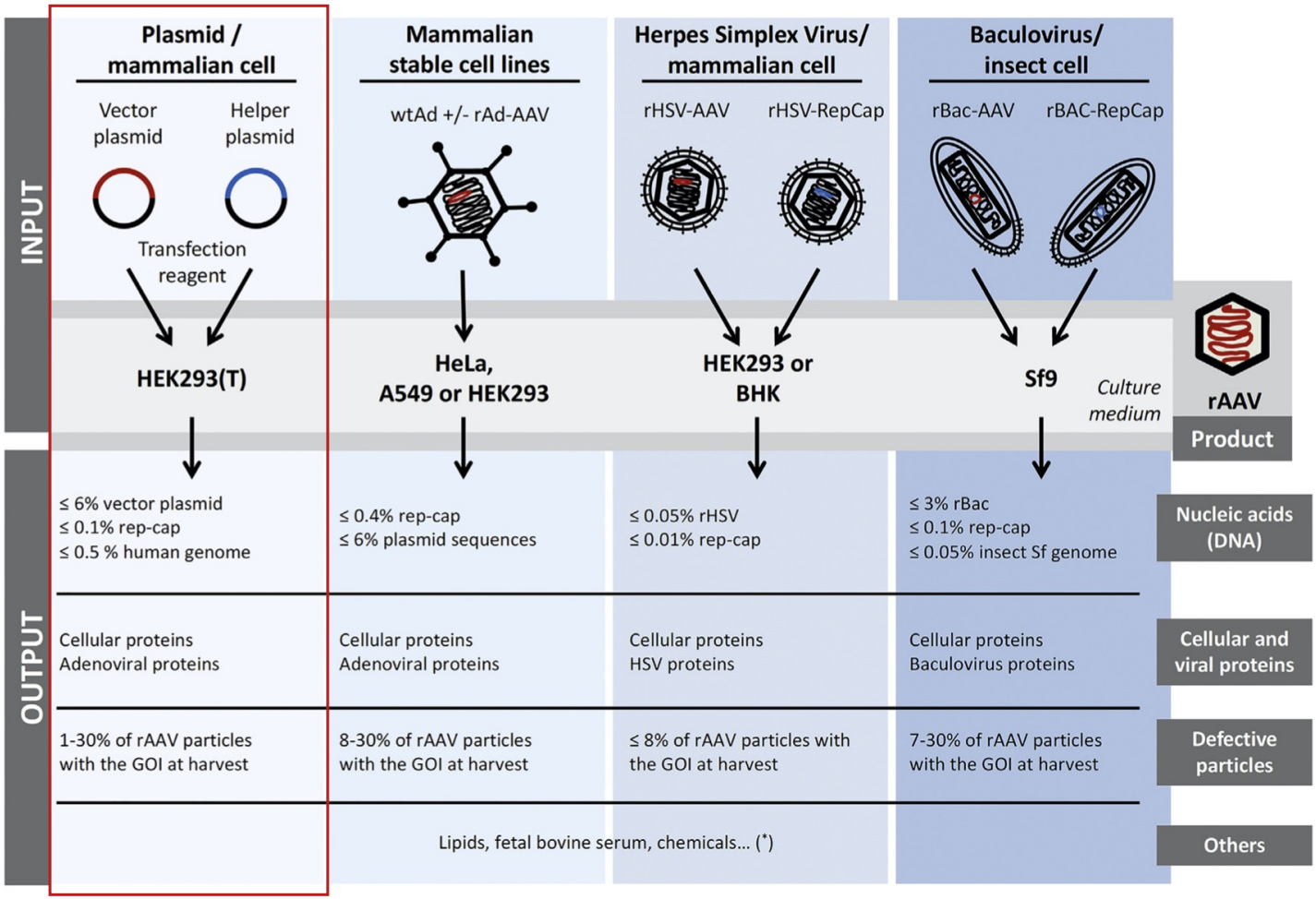
AAV vectors: the need for large vector quantities

Transient transfection



1:100,000 particles delivered will achieve the desired clinical output

Manufacturing platforms for AAV vectors



Next steps:

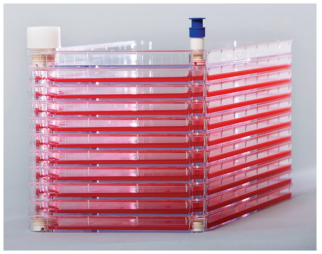
stable cell lines

for AAV manufacturing

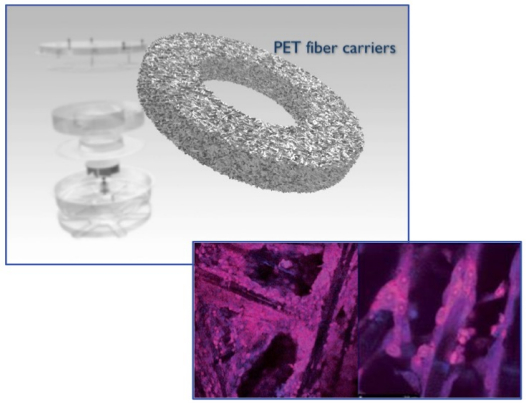
Penaud-Budloo M et al, Molecular Therapy - Methods & Clinical Development, 8, pp 166-180, 2018

Scalable solutions for AAV production

Adherent mammalian cells

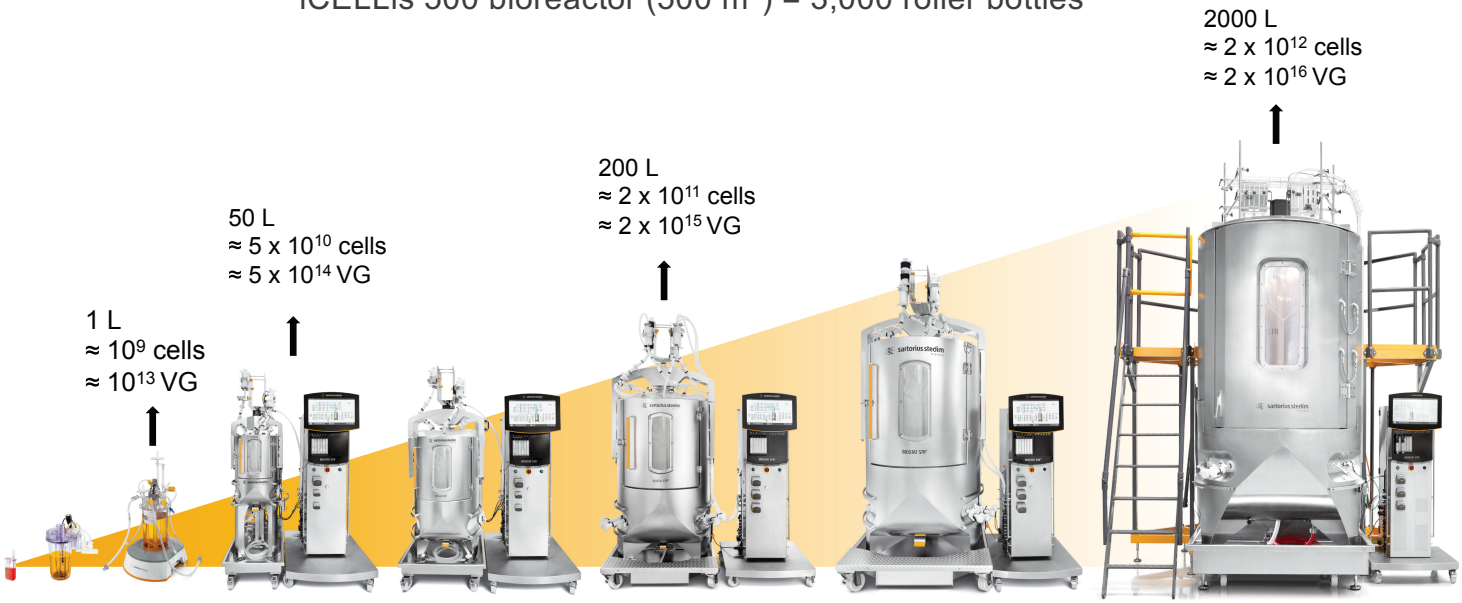


≈ 10^{9} cells
≈ 10^{13} VG



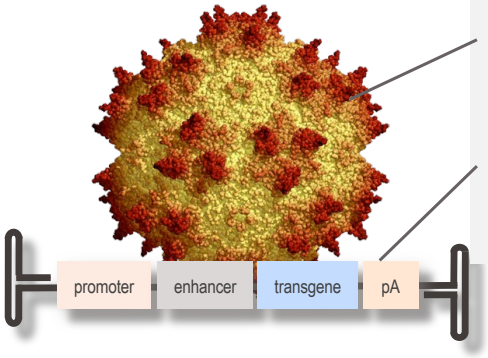
iCELLis 500 bioreactor (500 m^2) = 3,000 roller bottles

Mammalian cells in suspension



Gene therapy: vectors

Viral vectors: a challenge for biomanufacturing



- AAV capsid: **3 structural proteins** VP1, VP2, VP3:
  60 subunits in total, 3.9 MDa
- **AAV genome** (<5 kb)
- **5 additional proteins** have to be expressed during vector production

- Quality control
- Therapeutic dose determination
- Reproducibility
- One-time treatment

Biomanufacturing:

Contaminants | Full particles | Empty capsids

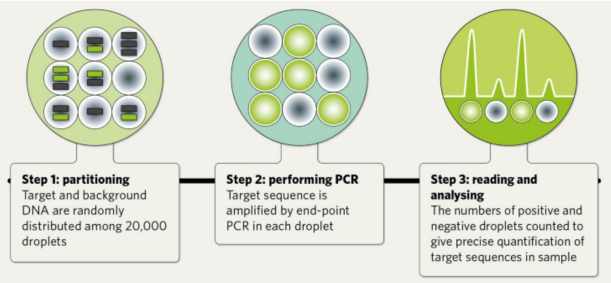
Functional genome | Defective genome

→ defines the therapeutic dose

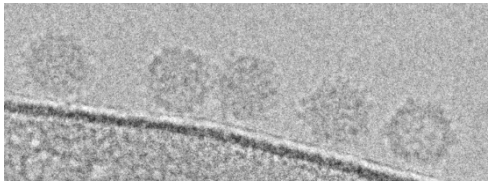
Often <10% of the particles

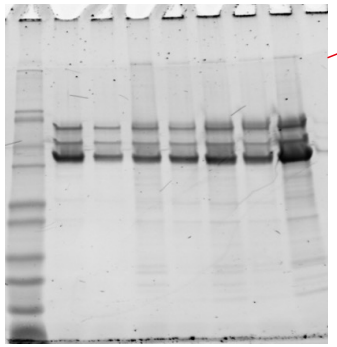
Required quantities of AAV for a gene therapy product: 1^E15 – 1^E20 genome-containing particles

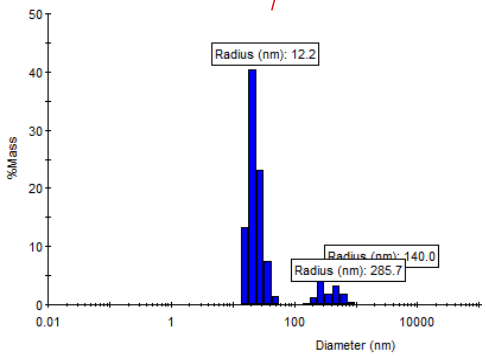
AAV analytics

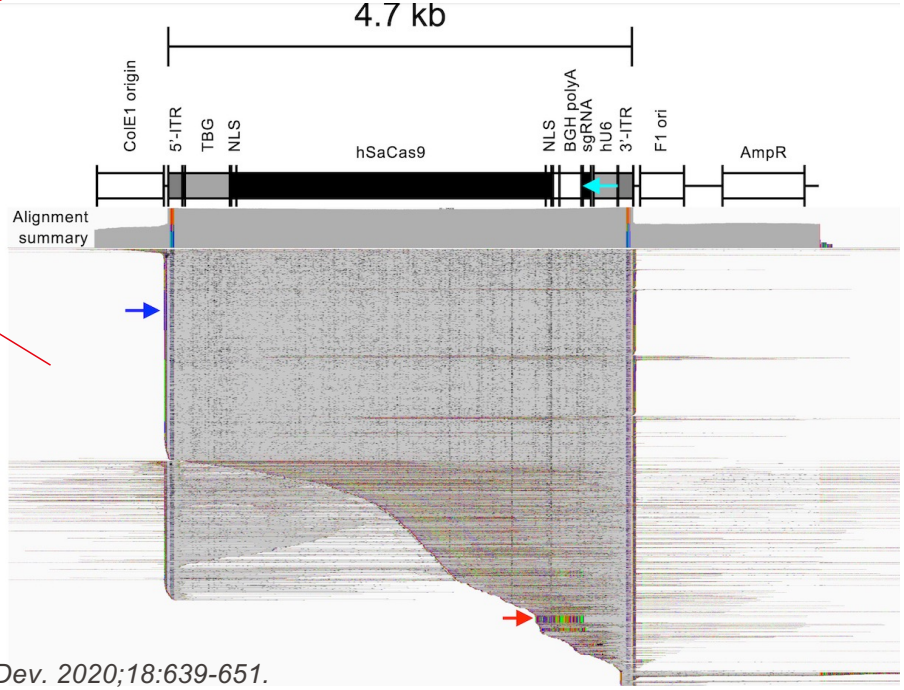


- Genome titer (digital PCR)
- Chromatography / UV260 280 absorbance ratio
- SDS-PAGE protein analysis
- Particle size
- Electron microscopy
- Formulation
- Genome sequencing









Mol Ther Methods Clin Dev. 2020;18:639-651.

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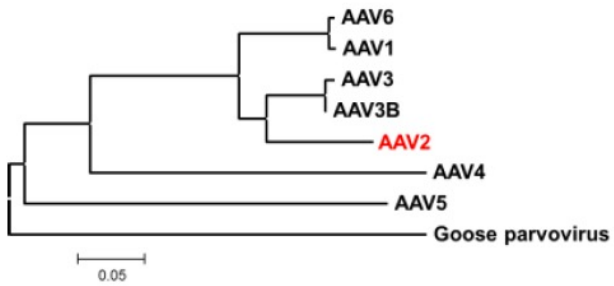
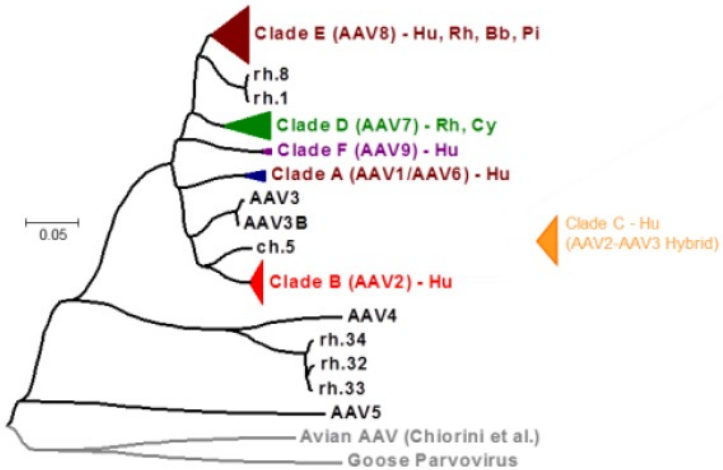
The toolbox of adeno-associated virus serotypes

AAV 1.0



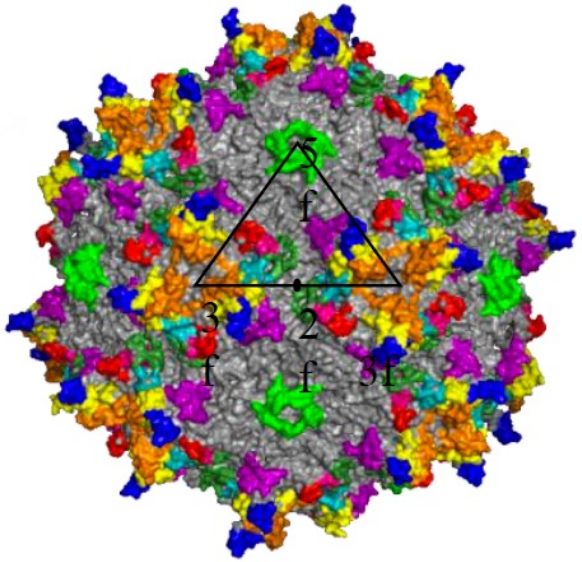
Initial AAV serotypes
(isolated in th 60's-70's)

AAV 2.0



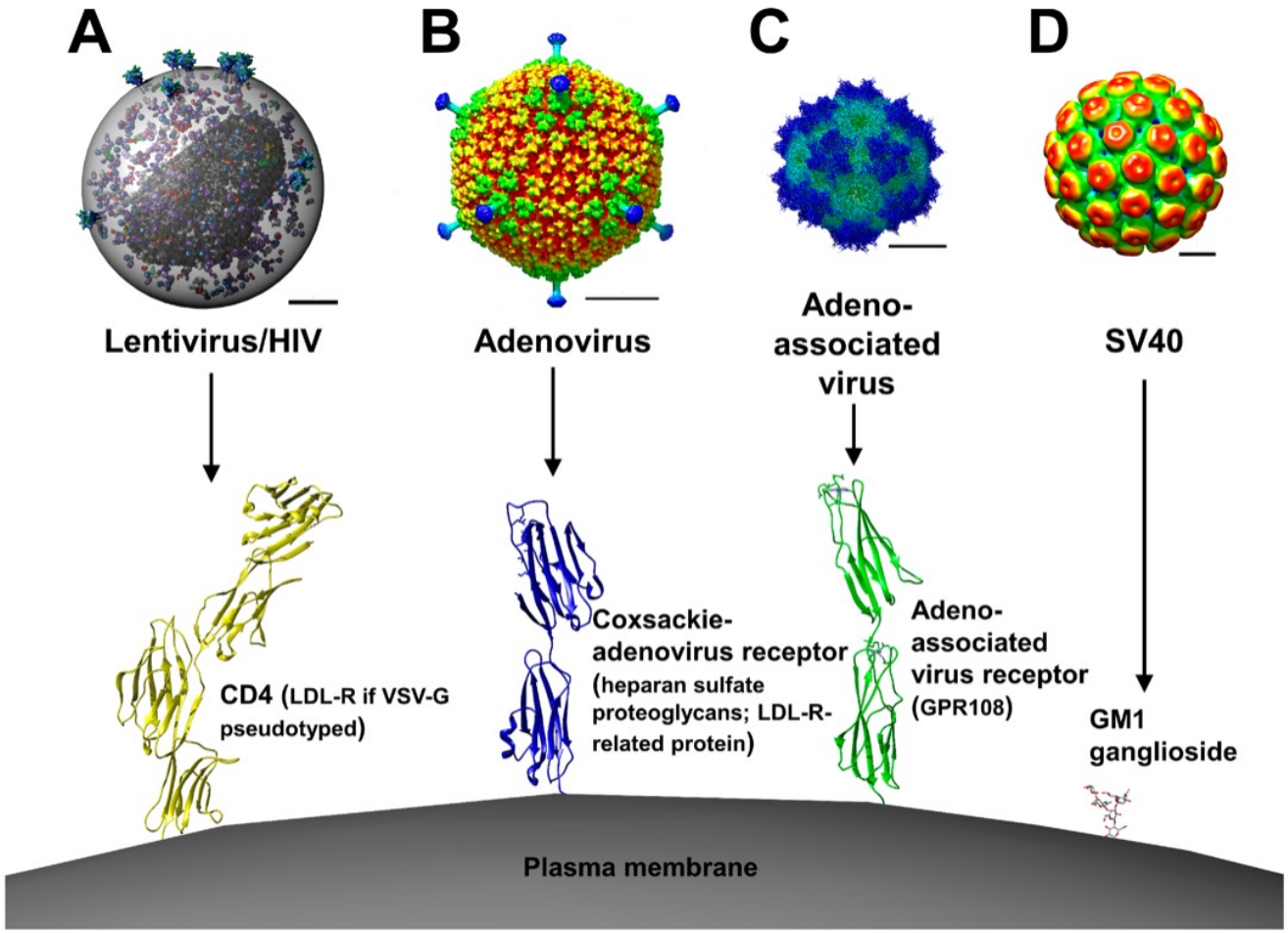
Expanded repertoire of natural serotypes
(identified from natural sources)
Most used in recent clinical trials

AAV 3.0



Synthetic serotypes
Libraries: >1x10^6 variants
Directed evolution

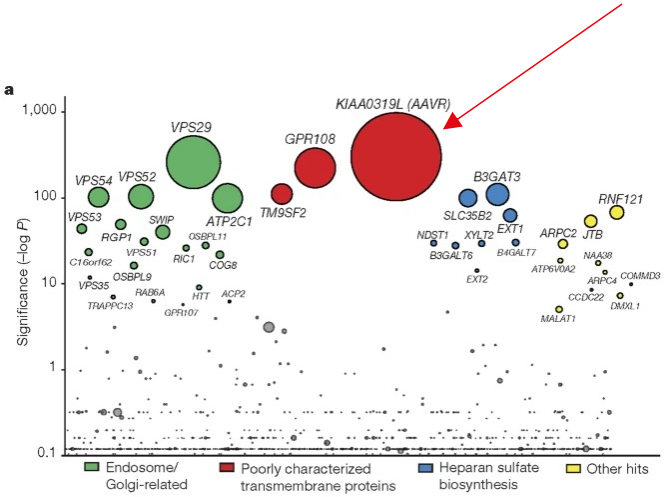
Virus receptors

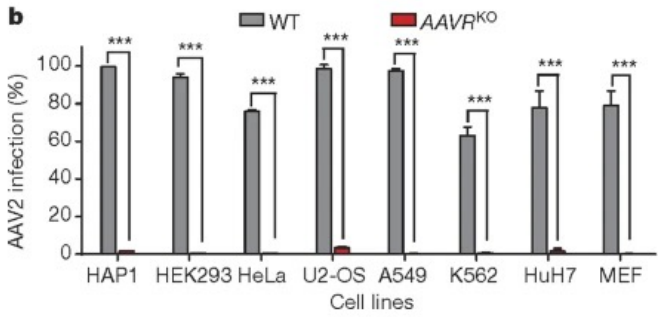


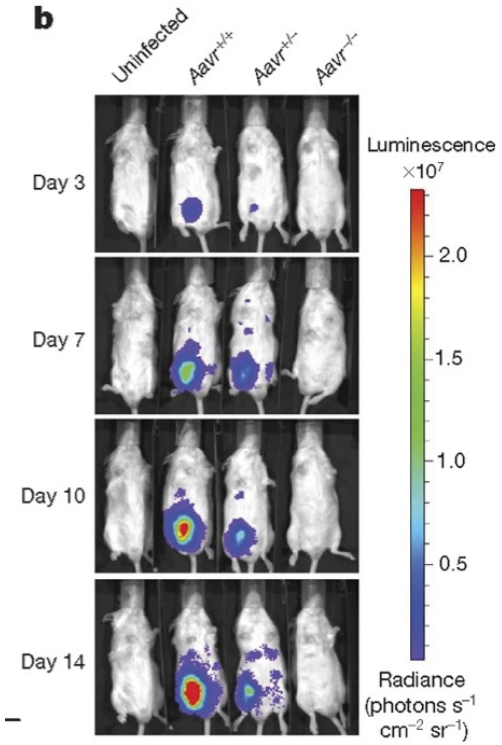
Genes **2020**, *11*(8), 915

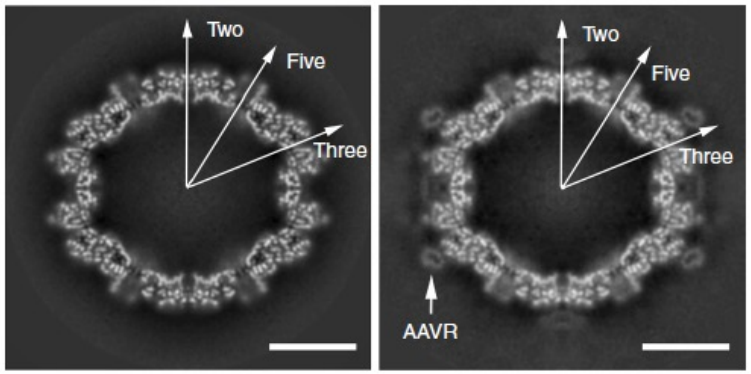
Essential AAV receptor: AAVR

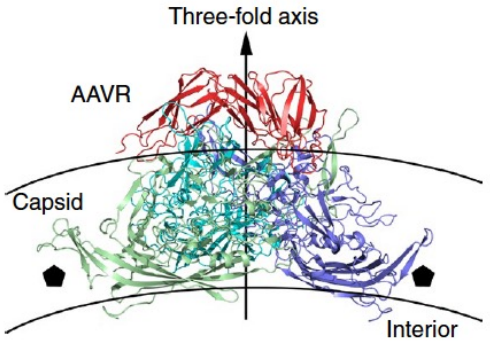
Genome-wide screening based on
cells resistant to AAV2 infection points to AAVR





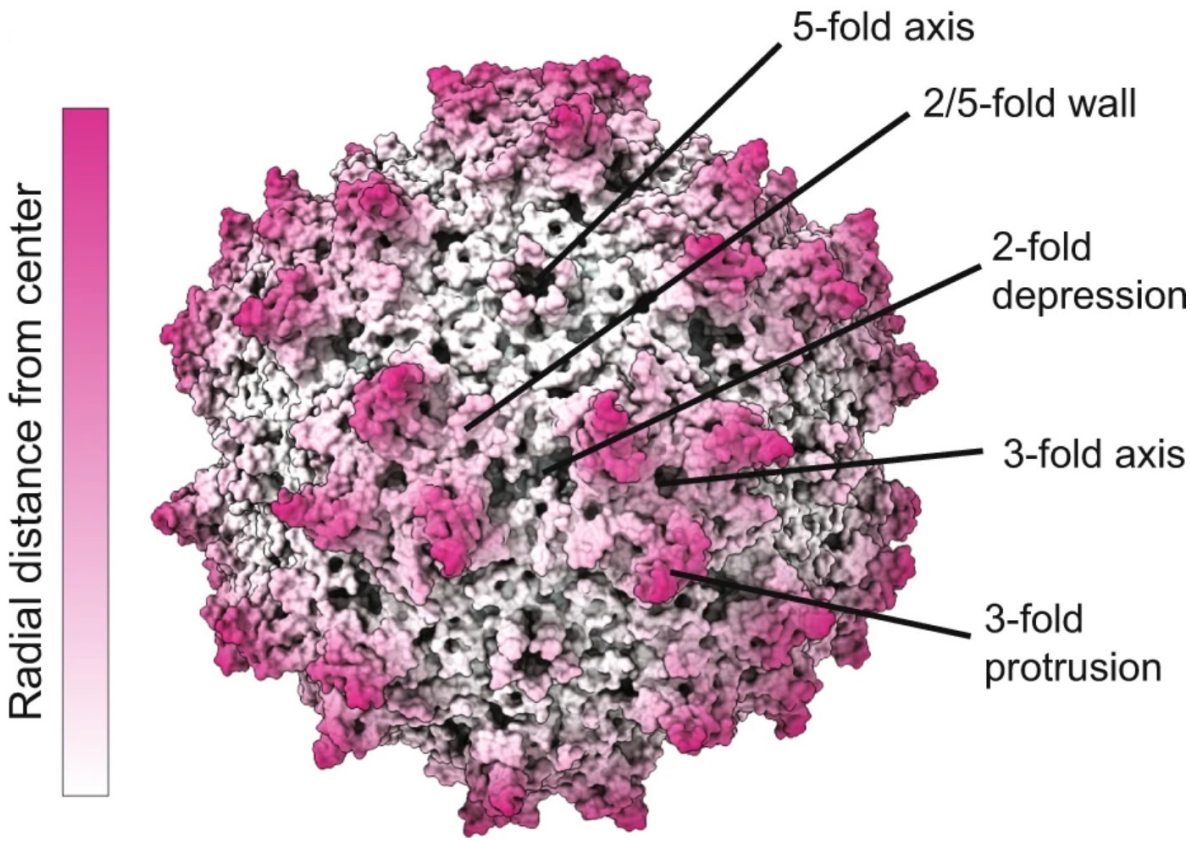




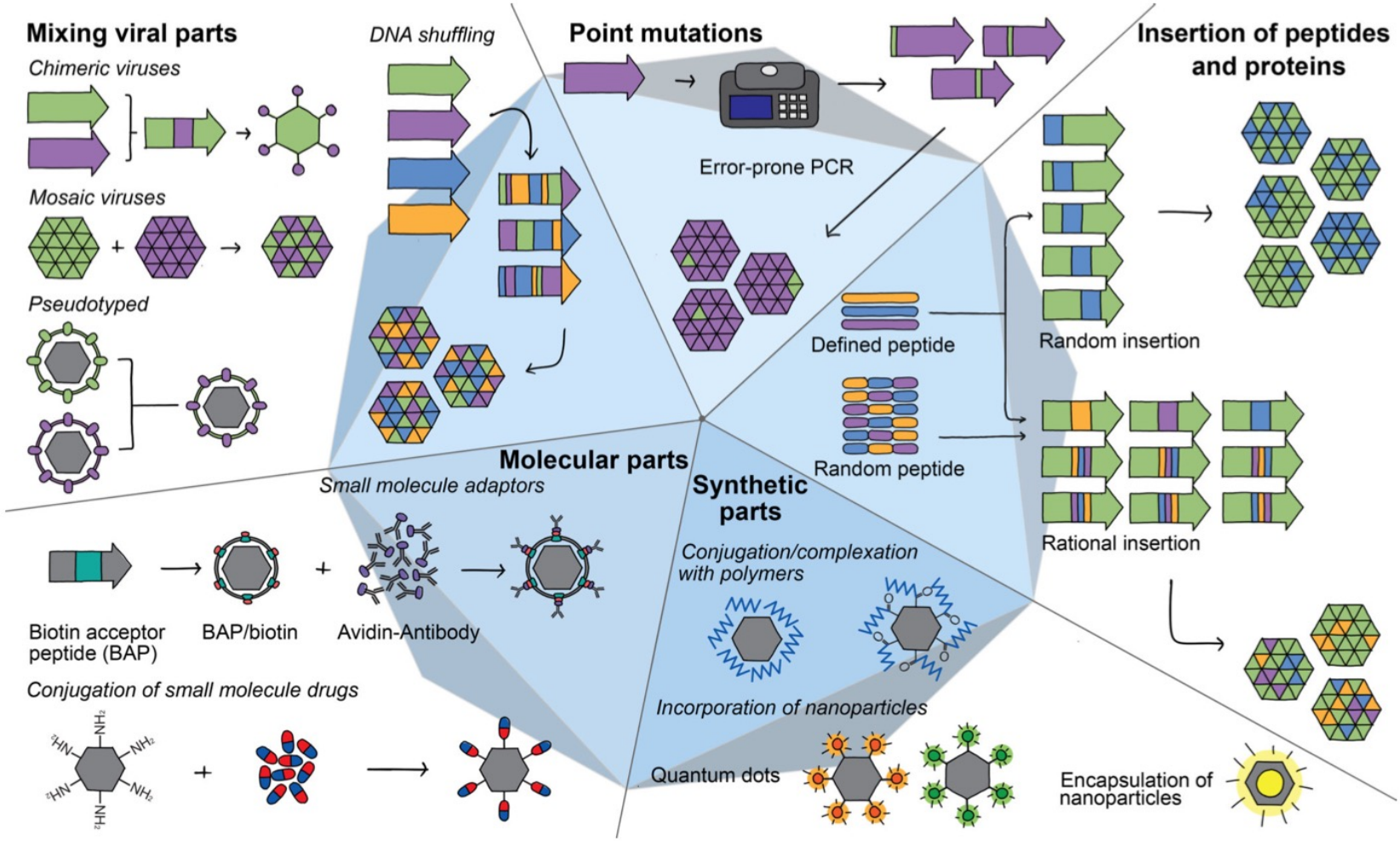


Nature 2016 530(7588), p108-12
Nature Microbiol 4 2019, p675–682

Structural insight into AAV capsids



Expanding the repertoire of AAV vectors



Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2014; 6(6): 548–558

Route of vector administration

CNS:

- **Intravenous**: systemic delivery
- **Intraparenchymal**: local stereotaxic injection(s)
- **Intracerebroventricular**
- **Intrathecal** (intracisternal)
- **Intrathecal** (lumbar)

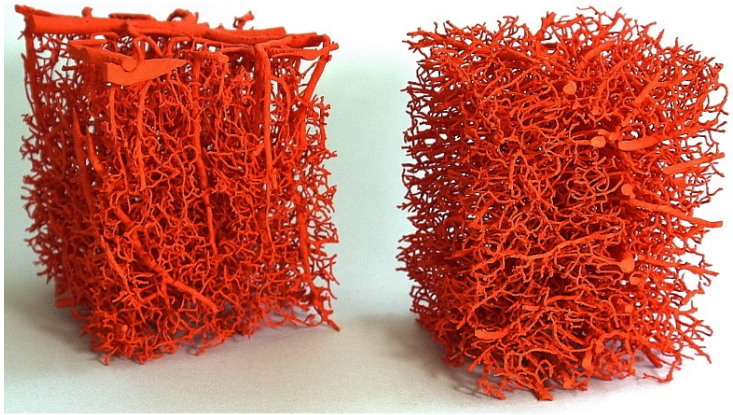
in the cerebrospinal fluid

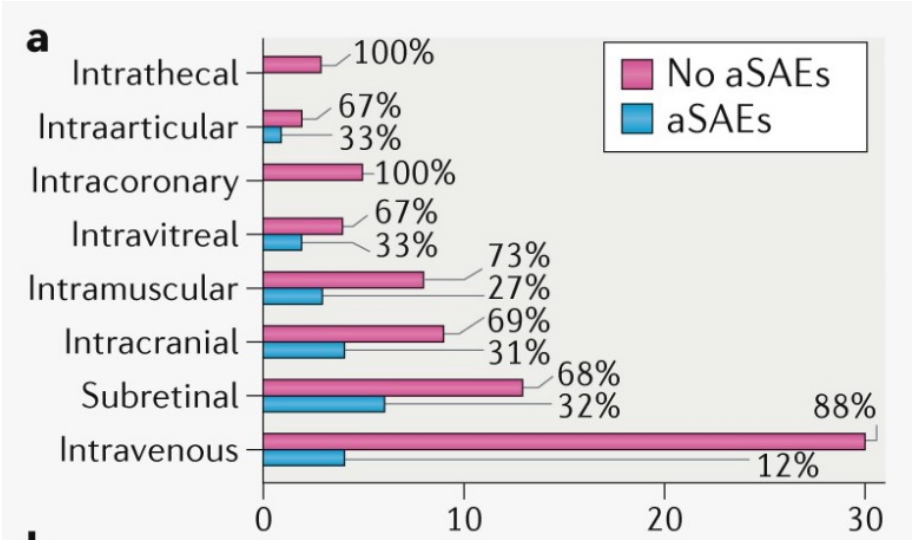
Eye:

- **Subretinal**
- **Intravitreal**

Kuzmin DA, Nat Rev Drug Discov, 2021

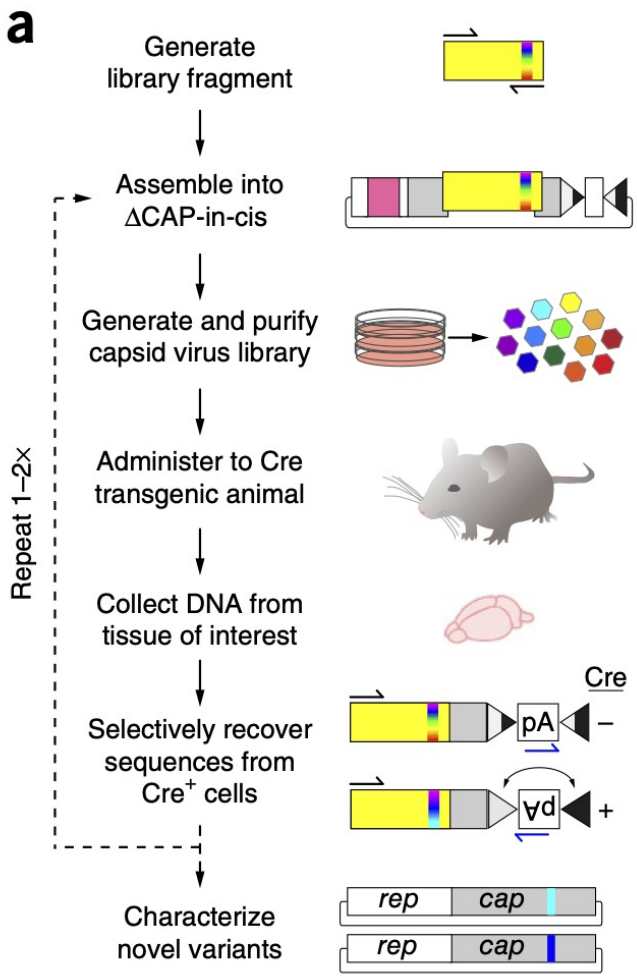
Brain vasculature



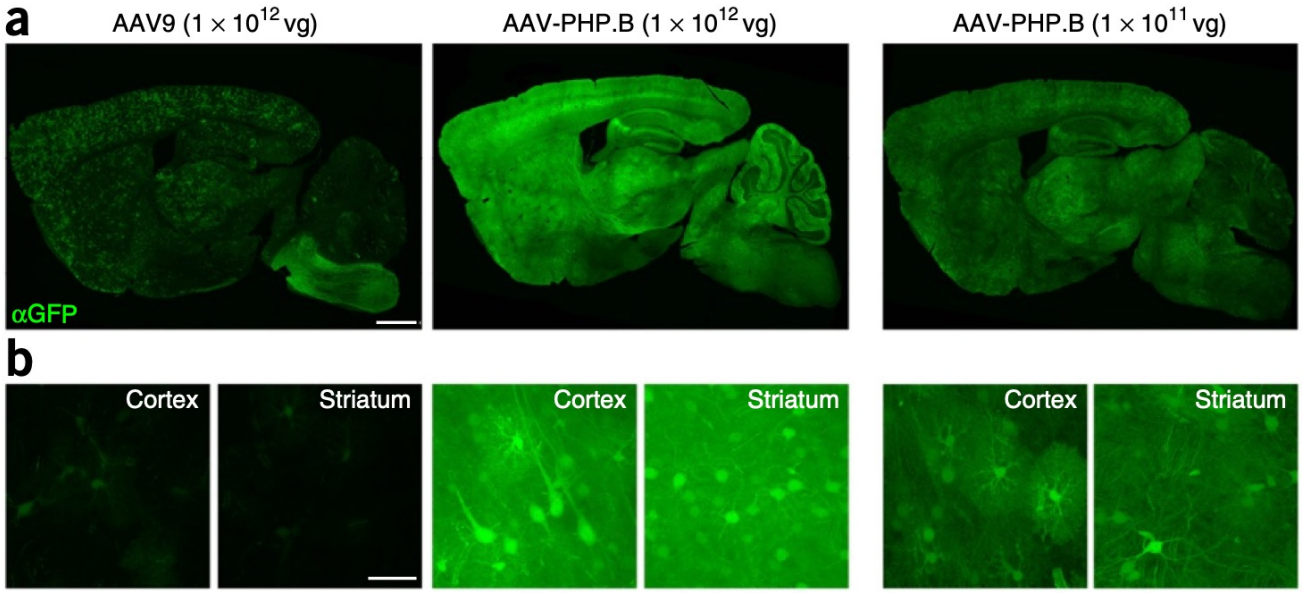


Administration-related severe adverse effects

Directed evolution of AAV able to pass the blood-brain barrier: AAV-PHP vectors



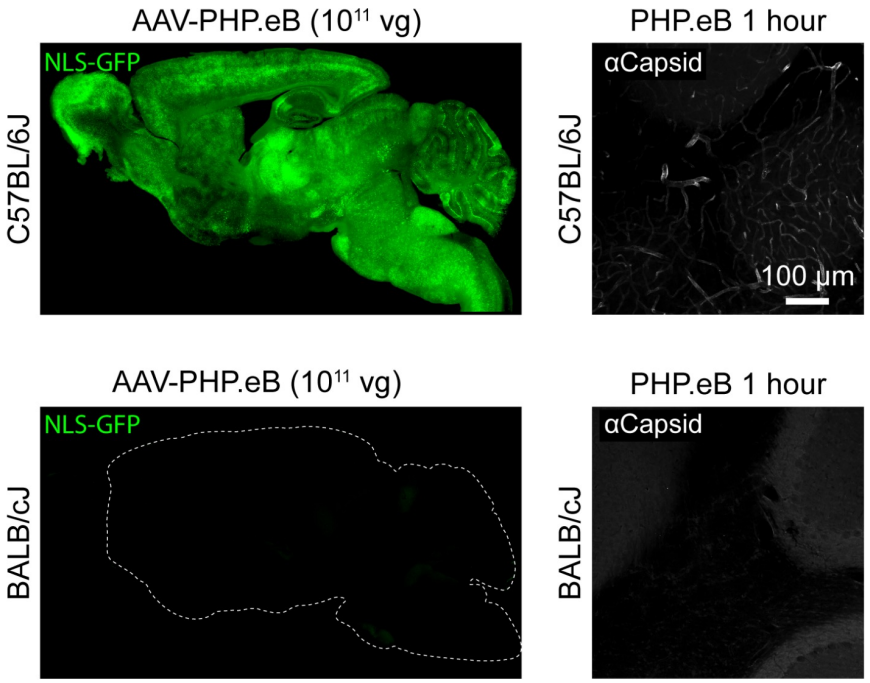
- Screening of AAV9 Cap variants.
- 7-mer random peptide inserted in loop VIII of AAV9 VP1.



Nature Biotechnology volume 34, pages 204–209 (2016)

Directed evolution of AAV vectors in the mouse species

AAV-PHP.eB variant selected for transport across the BBB binds the LY6A receptor expressed in the mouse endothelial cells of **C57BL/6 mice** (**no homologue in primates!**).



Delivering genes across the blood-brain barrier: LY6A, a novel cellular receptor for AAV-PHP.B capsids

Qin Huang, Ken Y. Chan, Isabelle G. Tobey, Yujia Alina Chan, Tim Poterba, Christine L. Boutros, Alejandro B. Balazs, Richard Daneman, Jonathan M. Bloom, Cotton Seed, Benjamin E. Deverman

Molecular Therapy
Original Article
AMERICAN SOCIETY of GENE & CELL THERAPY

The GPI-Linked Protein LY6A Drives AAV-PHP.B Transport across the Blood-Brain Barrier

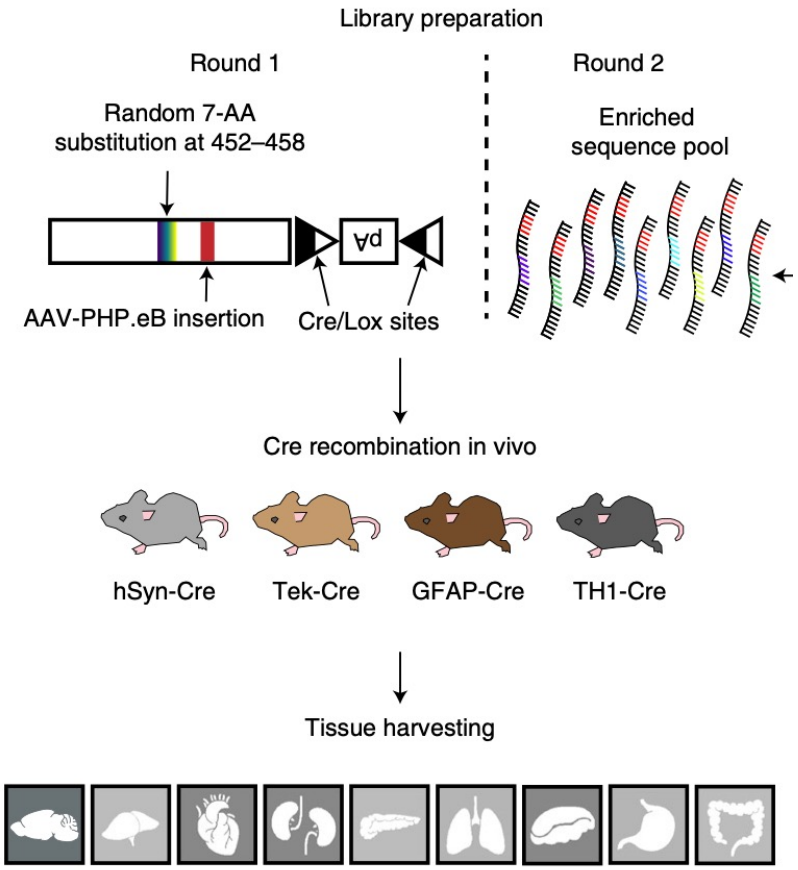
Juliette Hordeaux,[1,4] Yuan Yuan,[1,4] Peter M. Clark,[1] Qiang Wang,[1] R. Alexander Martino,[1] Joshua J. Sims,[1] Peter Bell,[1] Angela Raymond,[2,3] William L. Stanford,[2,3] and James M. Wilson[1]

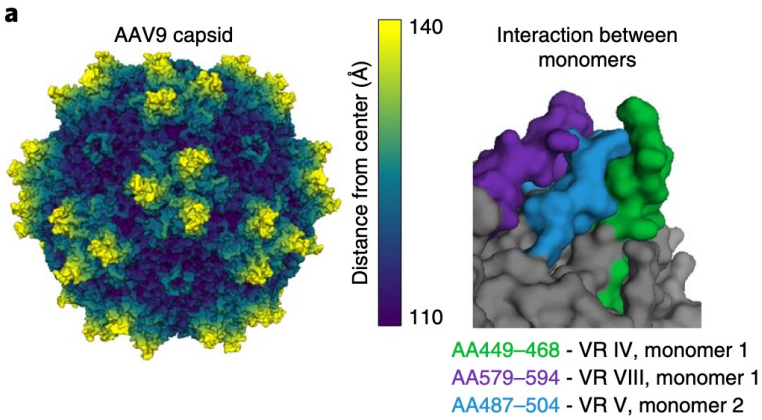
Human Gene Therapy, Vol. 31, No. 1-2 | Research Articles
Full Access

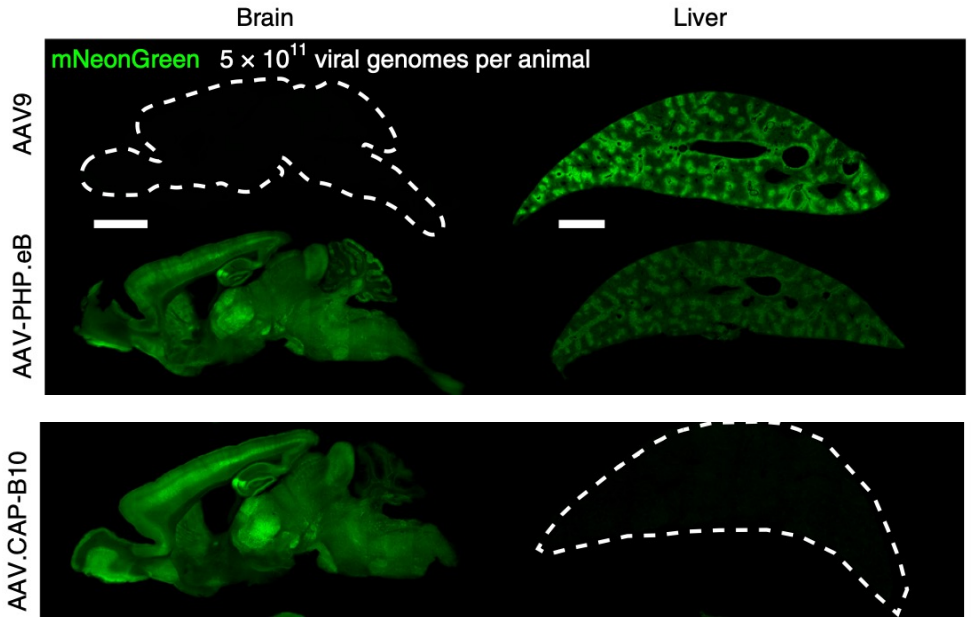
Ly6a Differential Expression in Blood–Brain Barrier Is Responsible for Strain Specific Central Nervous System Transduction Profile of AAV-PHP.B

Ana Rita Batista, Oliver D. King, Christopher P. Reardon, Crystal Davis, Shankaracharya, Vivek Philip, Heather Gray-Edwards, Neil Aronin, Cathleen Lutz, John Landers, and Miguel Sena-Esteves

Further evolution of AAV-PHP.eB vectors: modification of loop IV for liver detargeting.

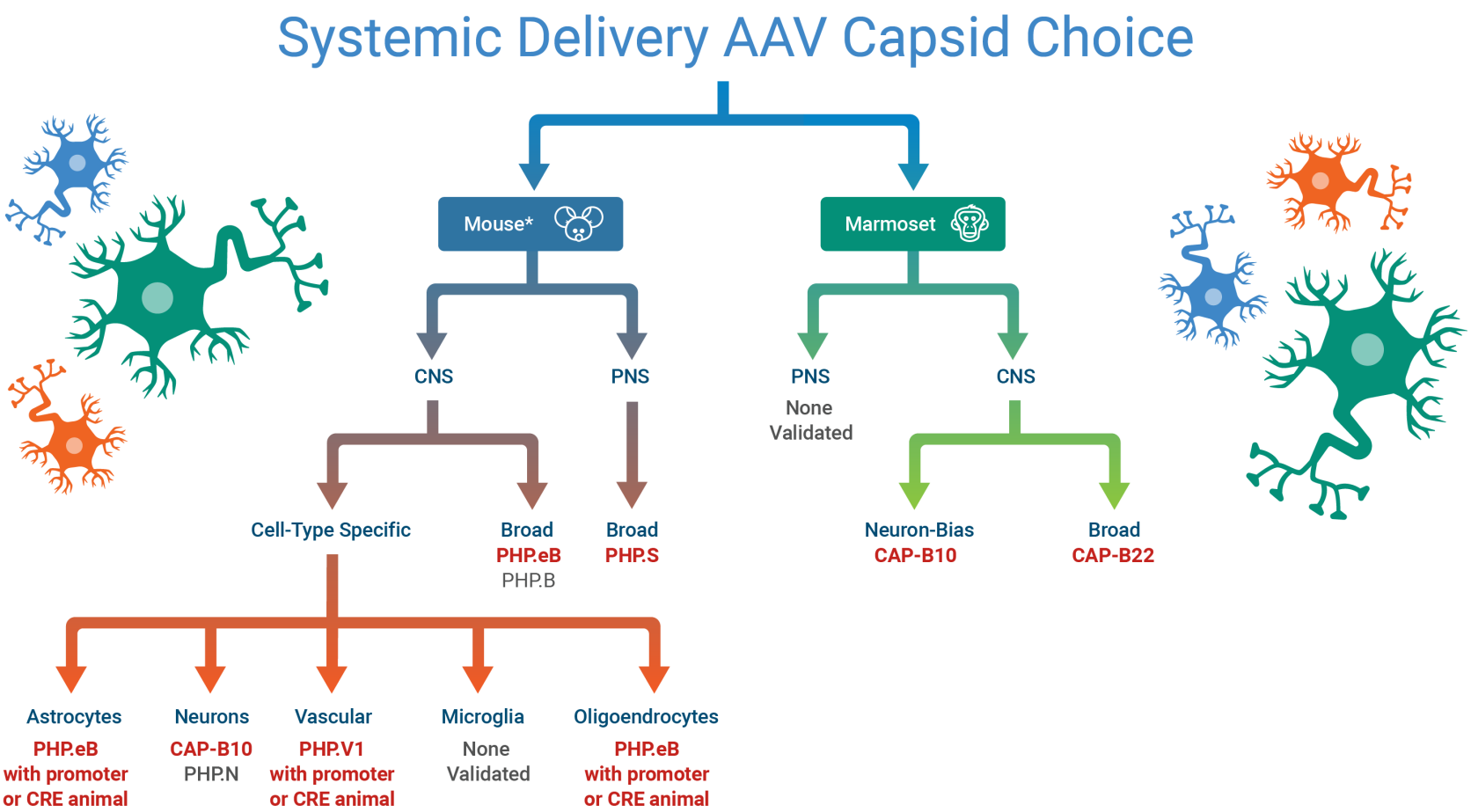




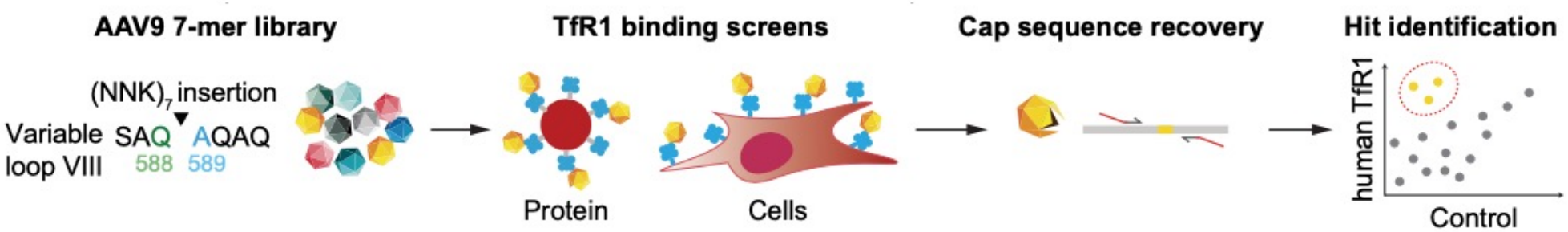


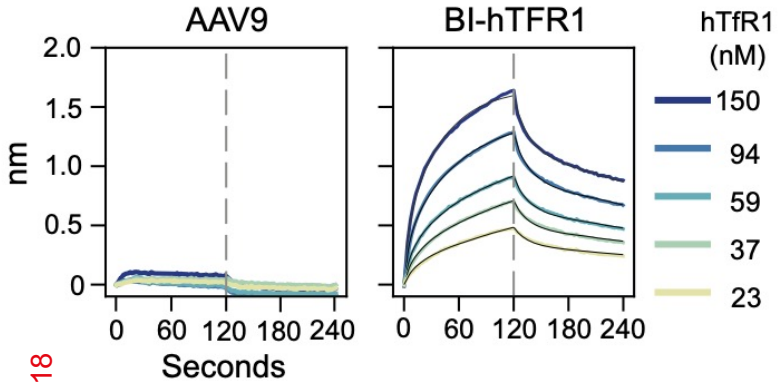
Nature NeuroScience | VOL 25 | JANUARY 2022 | 106–115

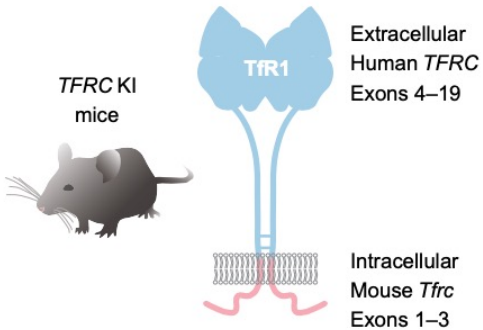
AAV-PHP vectors: decision tree

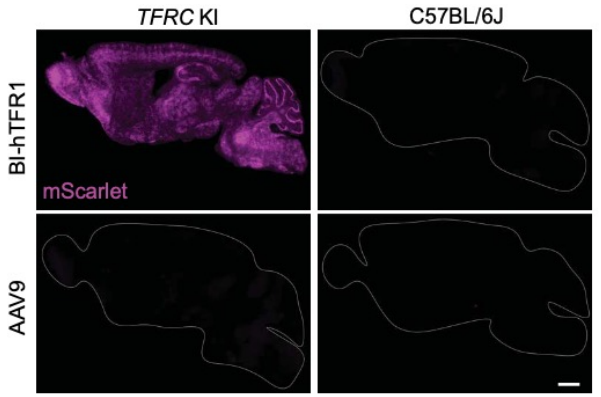


AAV binding the transferrin receptor for gene delivery to the brain





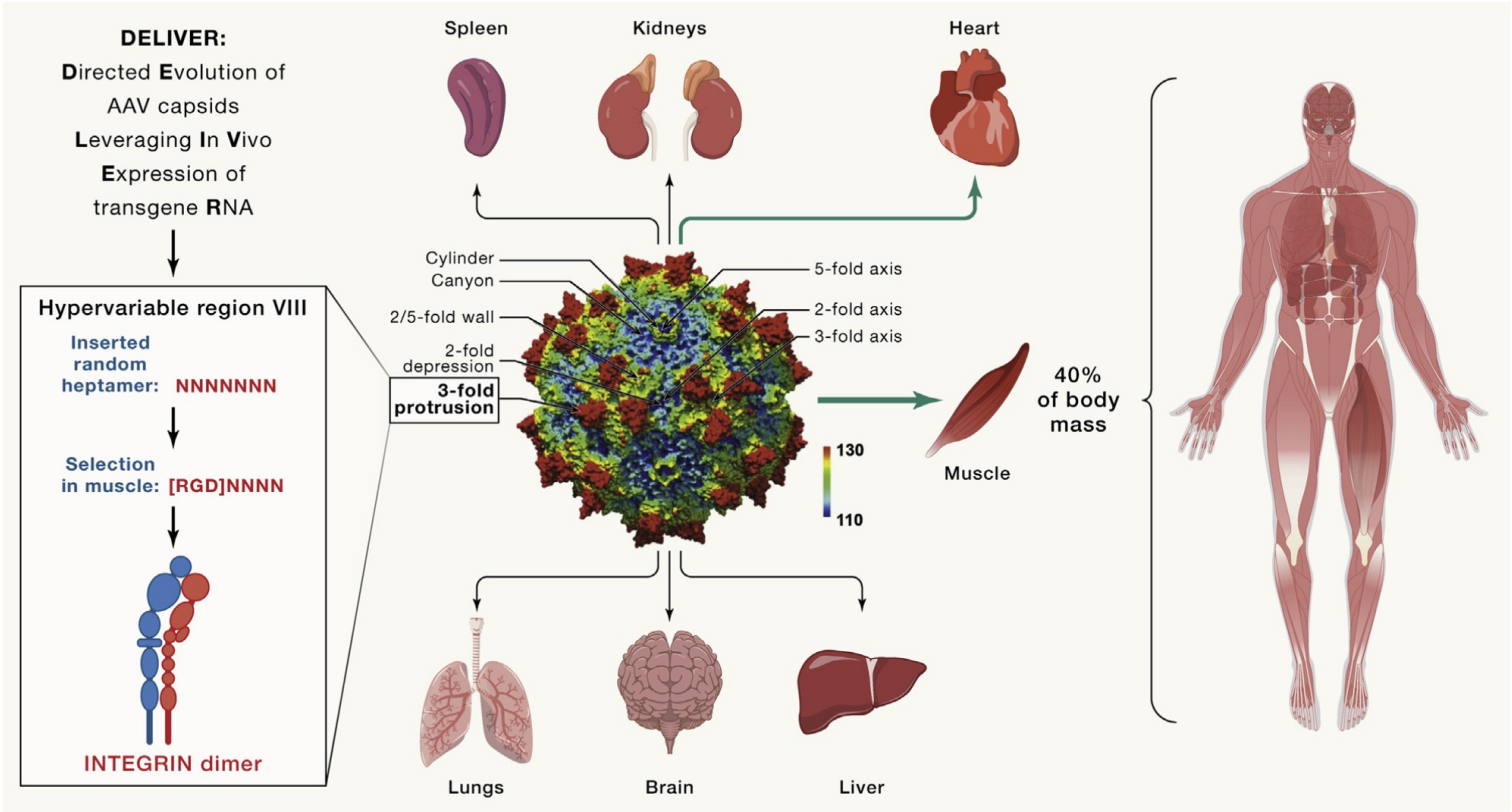


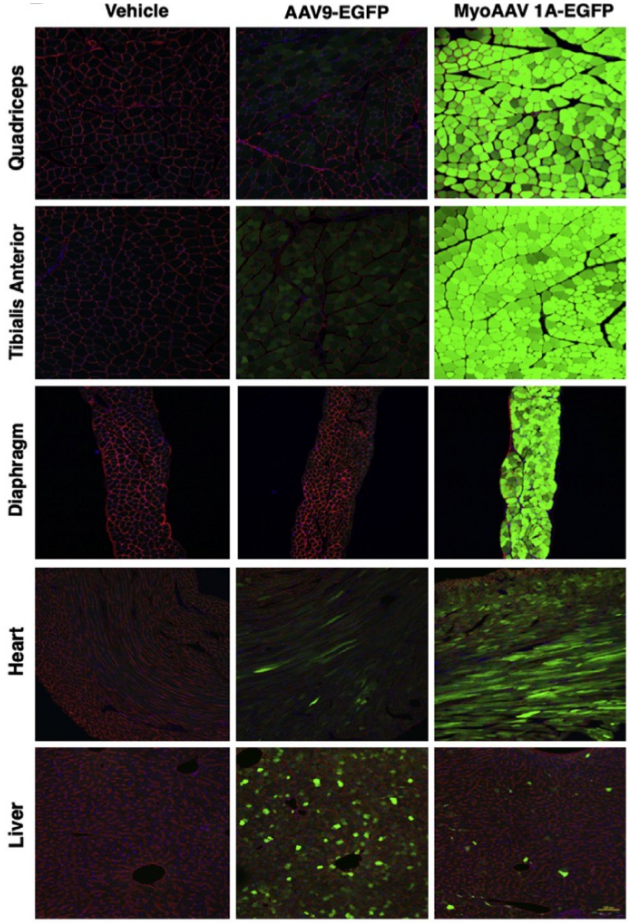


ENG-518

Huang et al., Science 384, 1220–1227 (2024)

Directed evolution of AAV9 for muscle targeting



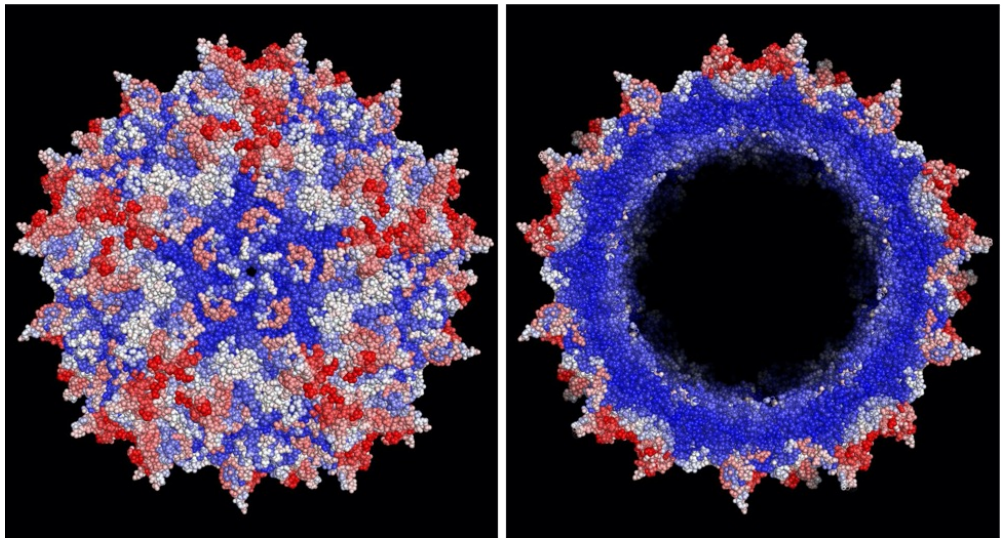


- *Tabebordbar et al., 2021, Cell184, 4919–4938*

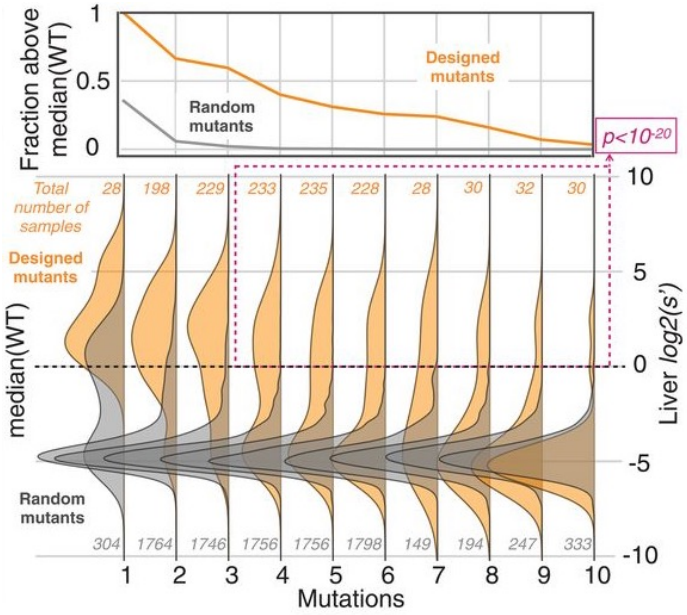
Machine-guided design of AAV capsids

Capsid ‘fitness’ for AAV production

Evaluation of the possibility to modify individual a.a. residues in the AAV2 capsid (red color = “fit for insertion”).



Improved efficacy of an approach based on ‘designed mutants’ over random mutagenesis to generate capsids for liver targeting



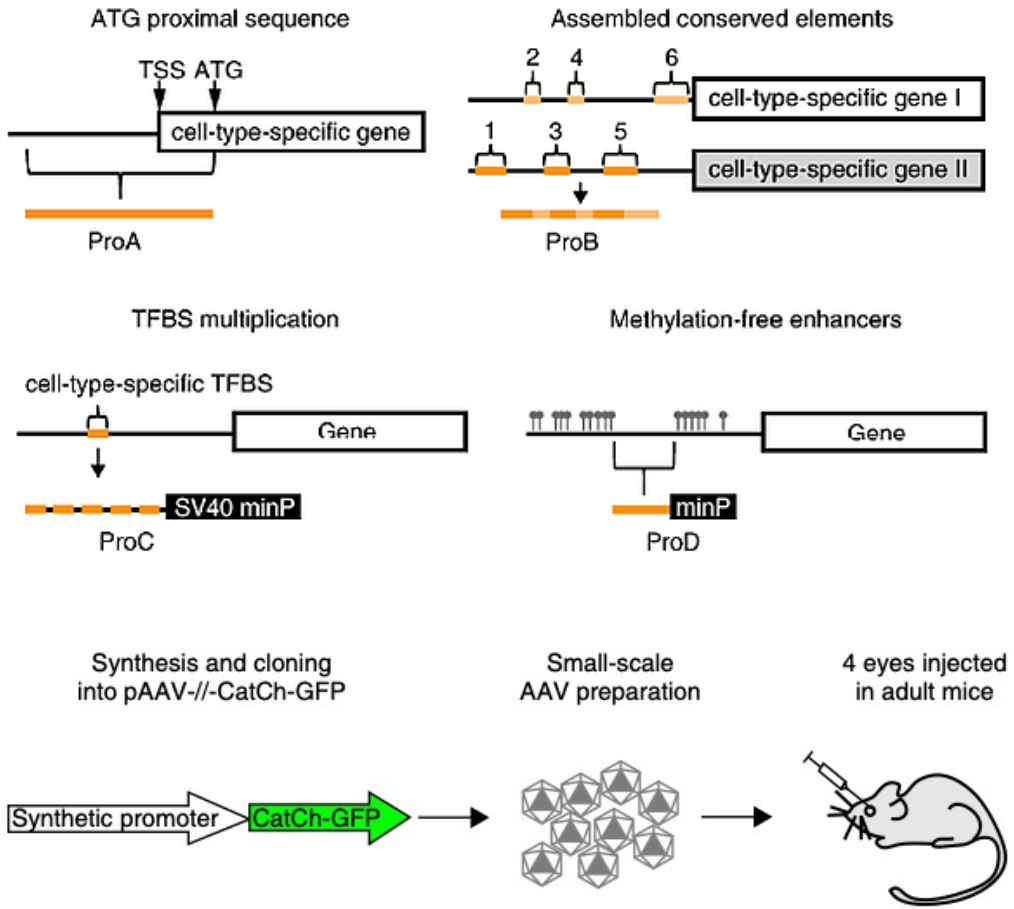
ENG-518

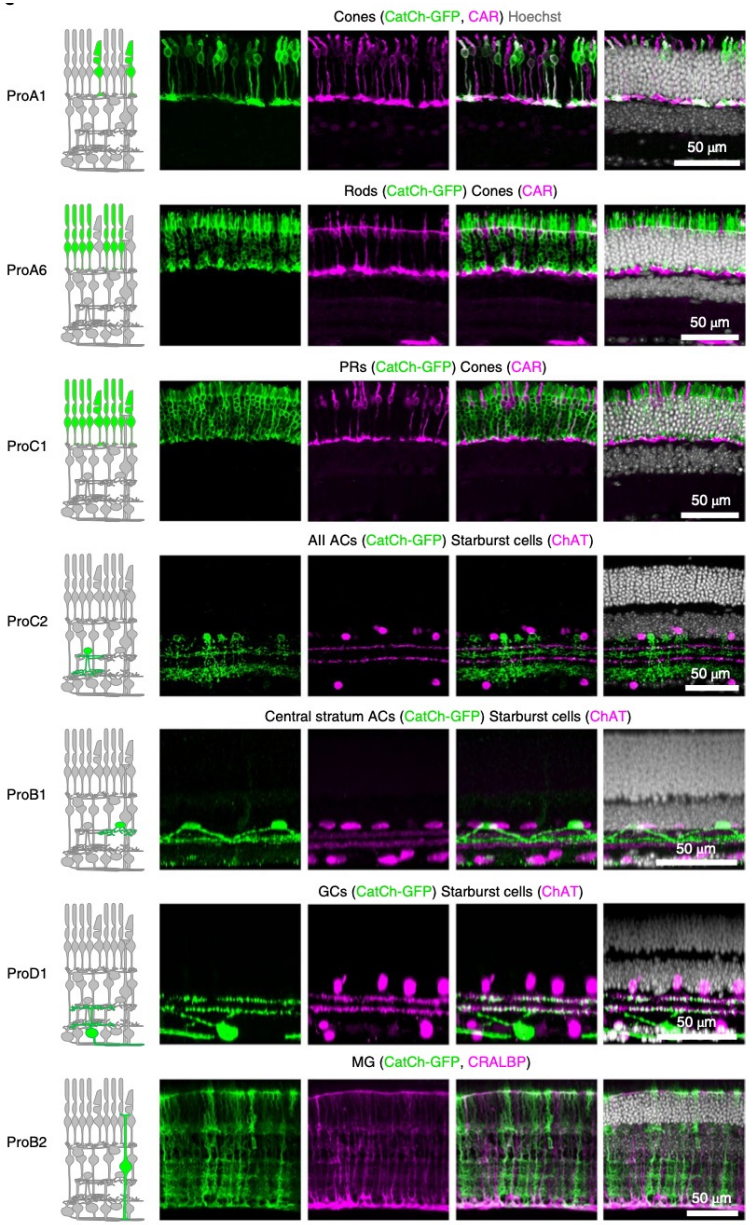
Science 2019: 366 (6469), pp.1139-1143

Lecture plan

- Why using viruses as vectors?
- Example of a broadly used vector: recombinant adeno-associated virus (AAV)
  - How is it produced?
  - How is it characterized?
  - How does it transduce cells?
  - How is AAV engineered to generate novel properties?
- Focus on vector-based research techniques
  - Cell-type specific expression
  - Neuronal tracing

Cell-type specific expression: promoters

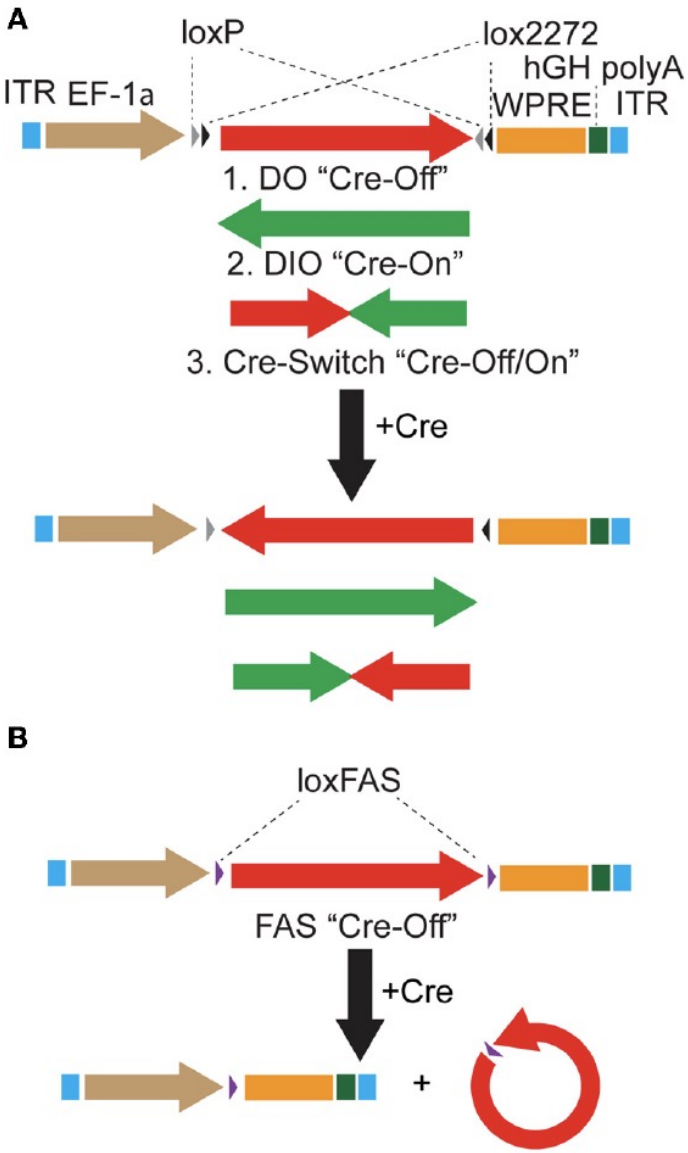




J. Jüttner et al, 22, Nat Neurosci 2019

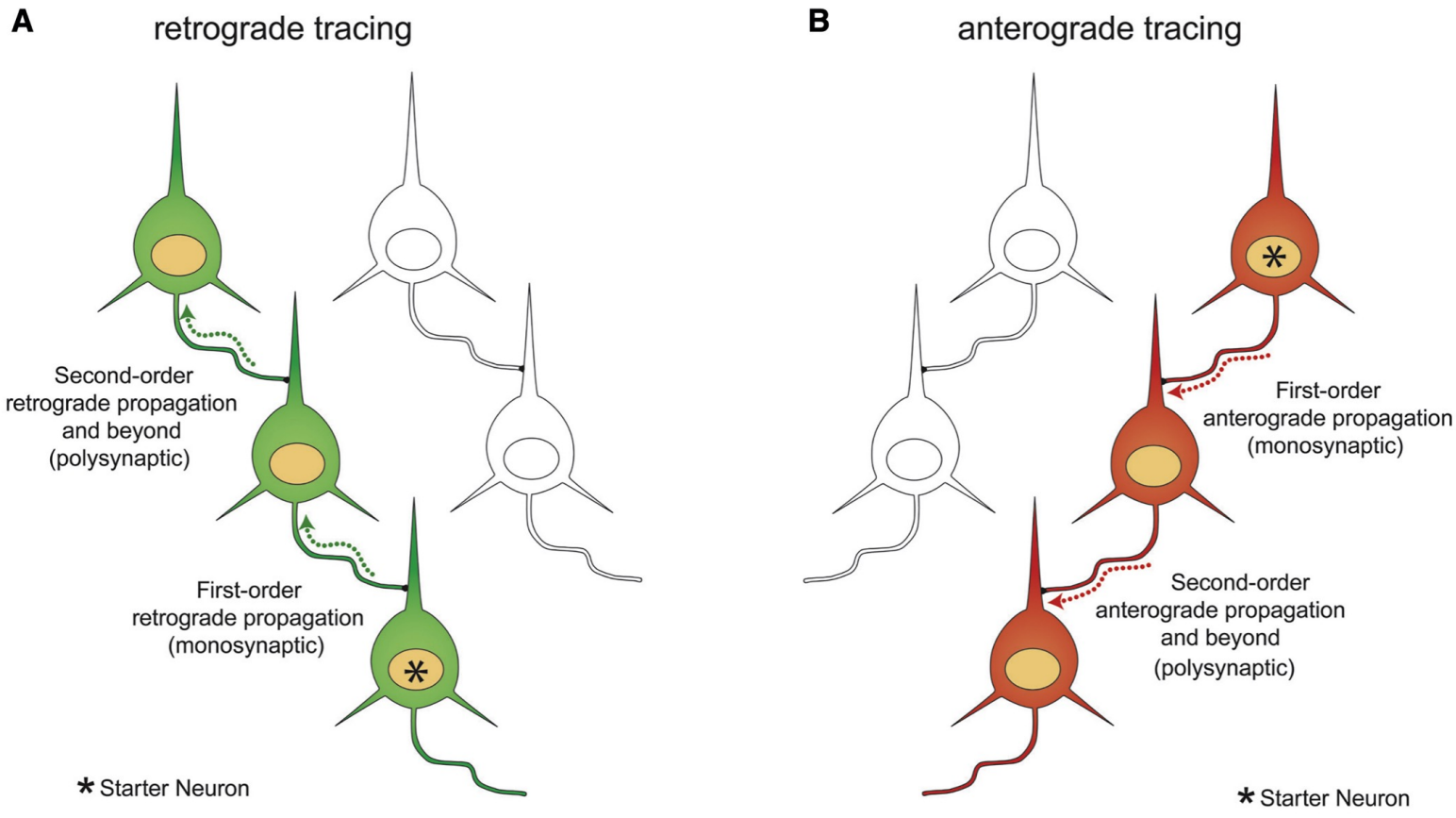
Cell-type specific expression: conditional systems

- **Site-specific recombinase dependent expression (e.g. Cre-Lox)**
  - DO: double-floxed open reading frame (Cre-Off)
  - DIO: double-floxed inverse open reading frame (Cre-On)
  - FAS: Cre-Switch



Front. In Neural Circuits, 6:47, 2012

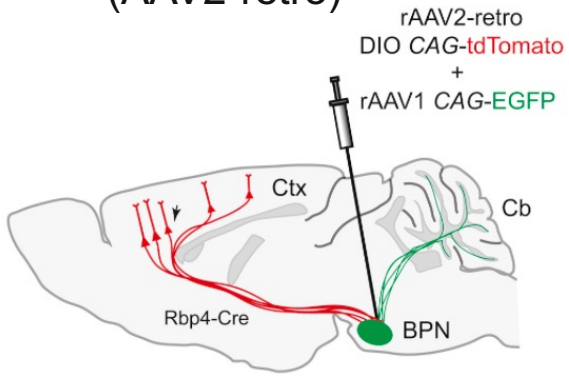
Neuronal tracing

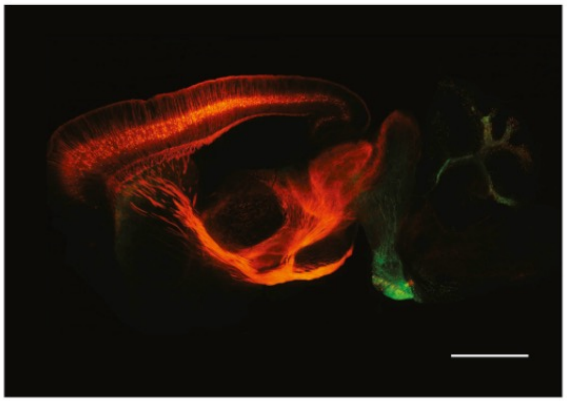


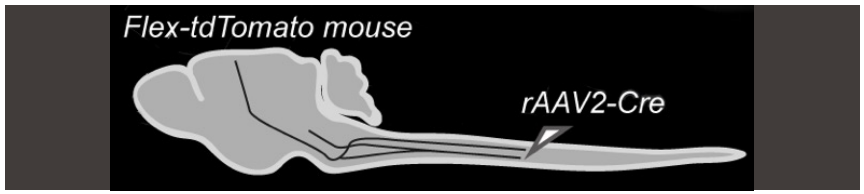
Neuronal tracing

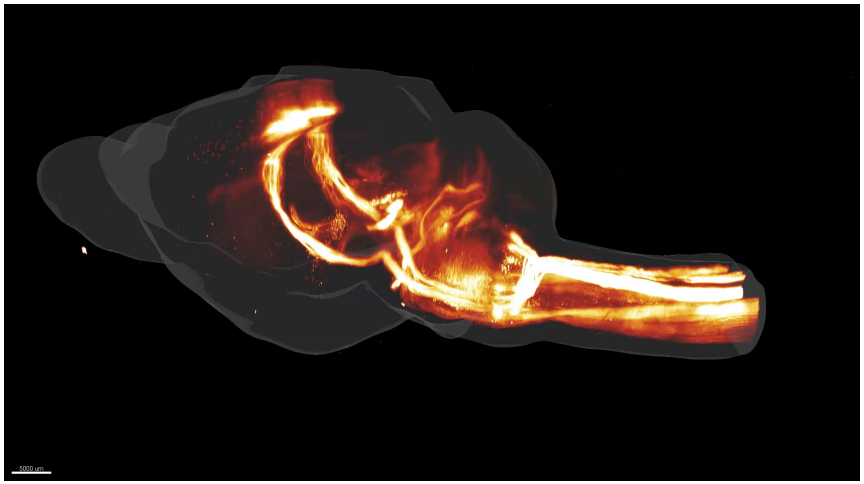
AAV vectors with optimized transduction properties

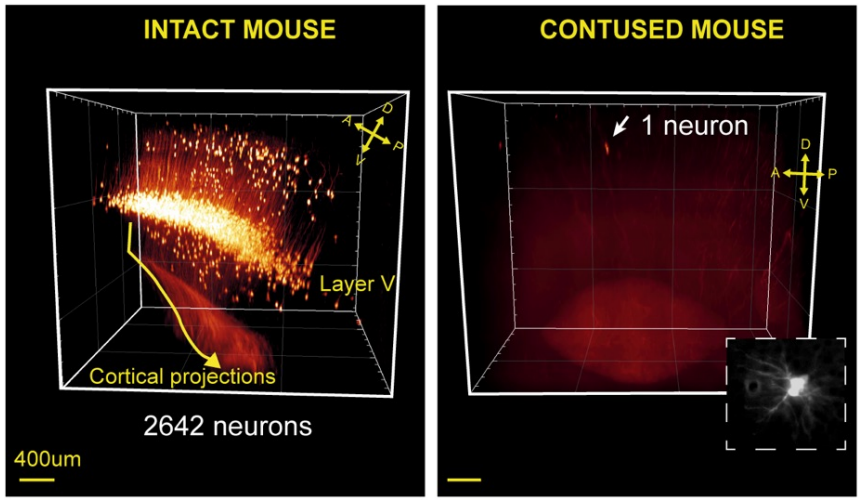
Retrograde transduction from axon terminals (AAV2-retro)











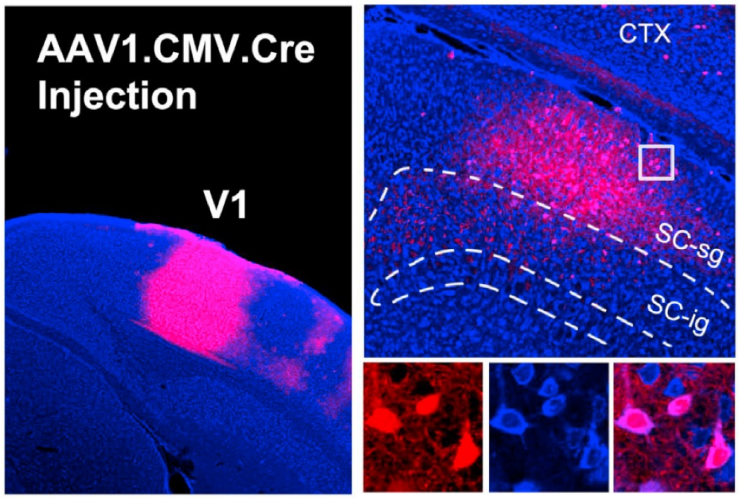
Tervo DG et al, Neuron 2016; Asboth L et al, Nat Neurosci 2018

Neuronal tracing

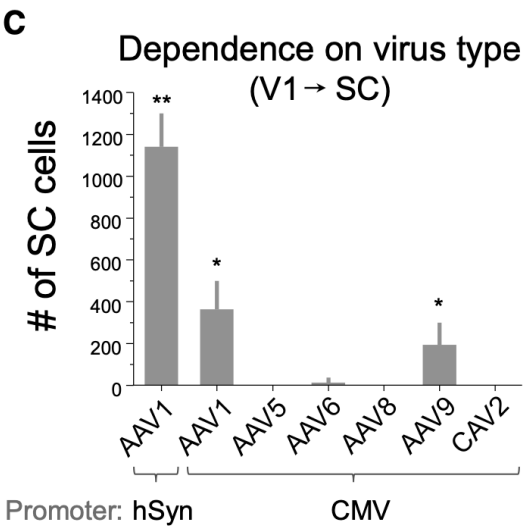
AAV vectors with optimized transduction properties

Transsynaptic anterograde transduction
(AAV1 or AAV9)

Visual cortex ➜ superior colliculus

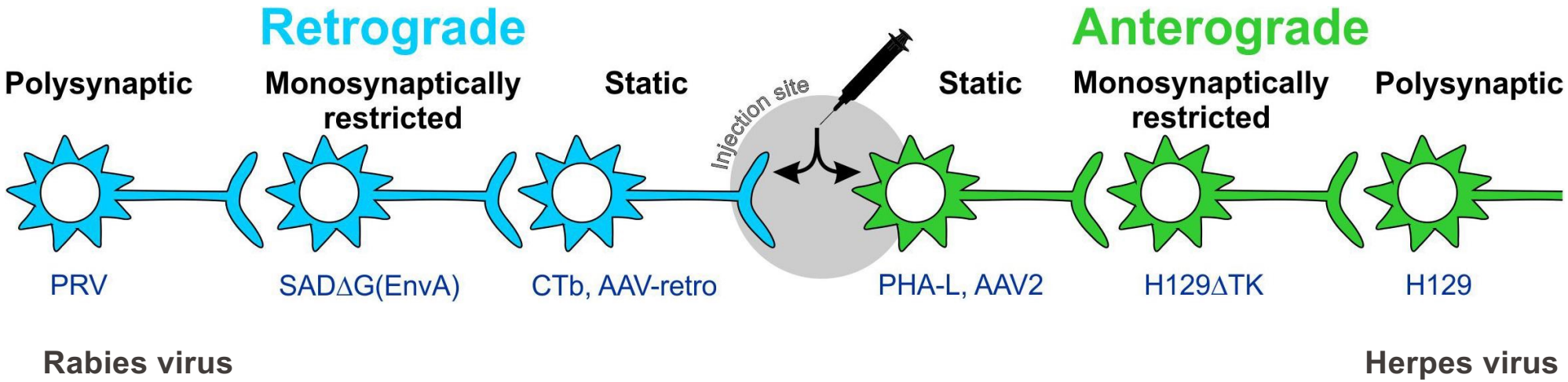


Transynaptically
transduced neurons
(Ai14 tdTomato reporter mice)



Zingg B et al, Neuron 93, 33-47, 2017

Neuronal tracing



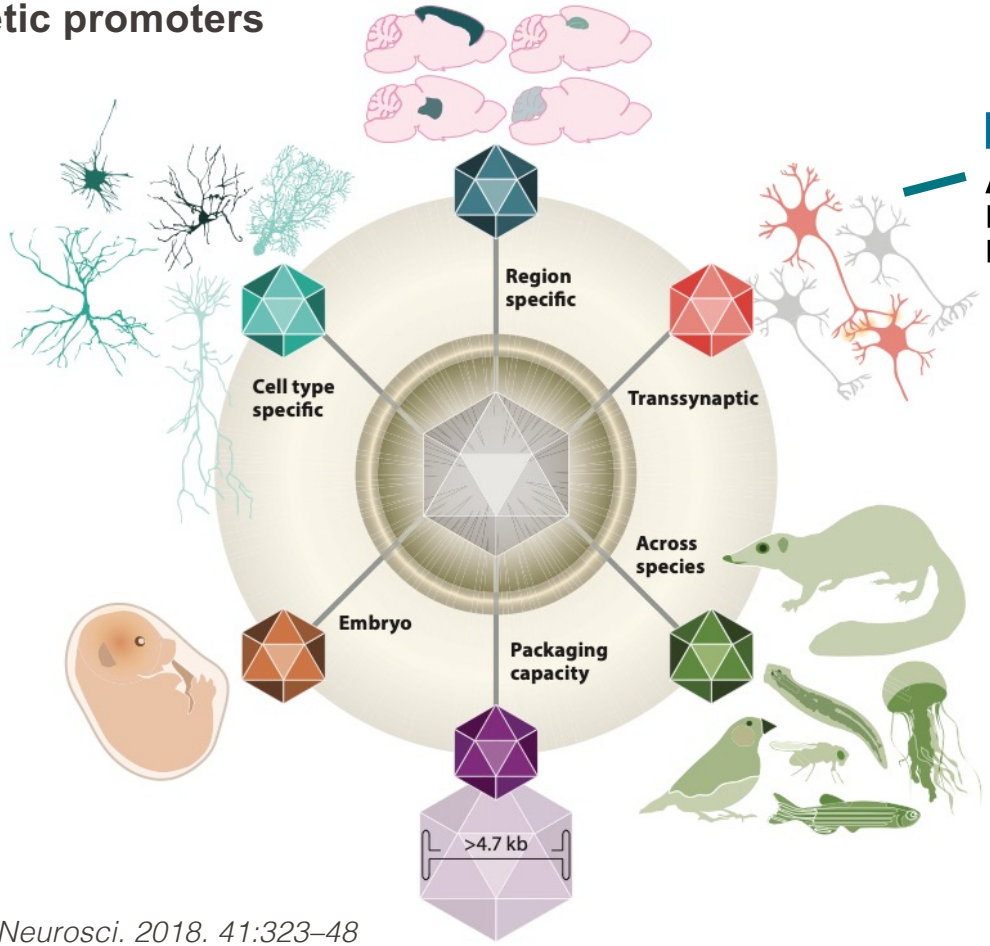
Viruses:
H129: Herpes simplex
PRV: pseudorabies virus
SADΔG: rabies virus, G protein deleted

Tracers:
PHA-L: phytohemagglutinin-L
CTb: cholera toxin subunit B

Saleeba C et al, Frontiers Neurosci, 13, 2019

Future of AAV vector engineering

Gene expression control
e.g. synthetic promoters



Annu. Rev. Neurosci. 2018. 41:323–48

NeuroResource

Neuron

AAV-Mediated Anterograde Transsynaptic Tagging: Mapping Corticocollicular Input-Defined Neural Pathways for Defense Behaviors

+ design of AAV vectors avoiding pre-existing immunity

Take-home message…

Engineering and production of AAV vectors for research and gene therapy

Rapid progress over the past few years in research labs…

- AAV and lentiviral vectors have become standard ‘research tools’.
- Multiple applications, in particular in Neuroscience.
- System adapted to over-expression, cell type-specific expression, tissue specific expression, RNAi, gene editing.
- Rapid progress in the development of new vectors by ‘directed evolution’.

Viral vector manufacturing

- AAV and lentiviral vectors have reached industrial-scale manufacturing.
-