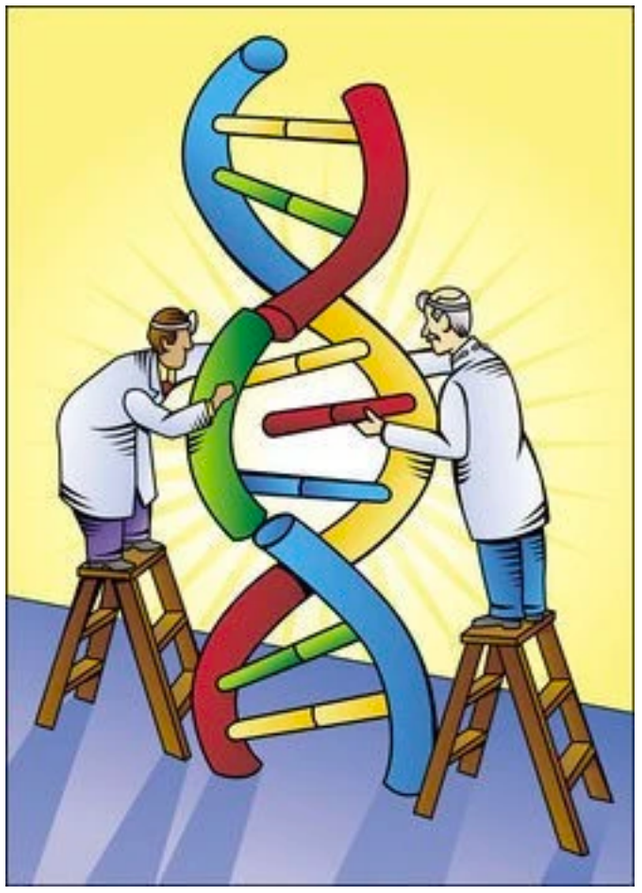
Introduction to Gene Therapy and AAV Therapeutics



Noah B. Bissonnette
MacMillan Group Meeting
Literature Review
March 1st, 2022

Outline

What is Gene Therapy?

History of Gene Therapy

AAV Biology

Challenges and Outlook

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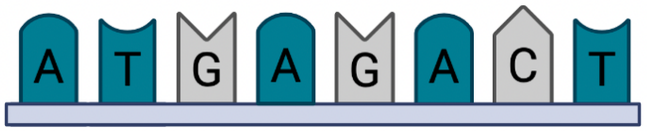
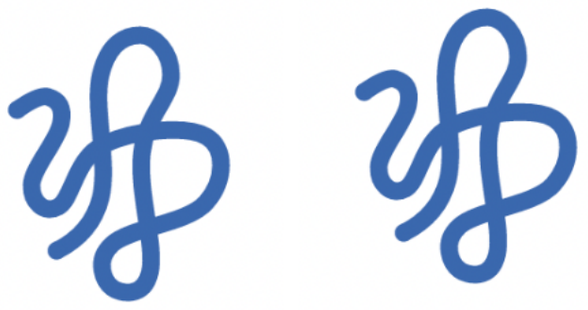
Introduction

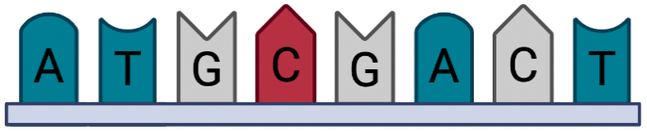
"The alteration of cell(s) genome with the goal of providing a therapeutic benefit"



Normal DNA



Folded proteins



Mutated DNA

Misfolded proteins

Monogenic diseases

Cystic Fibrosis	**Sickle Cell Anaemia**	**Hemophilia**	**Muscular Dystrophy**
Friedreich's Ataxia	**Amyotrophic Lateral Sclerosis (ALS)**	**Spinal Muscular Atrophy (SMA)**	**Huntington's Disease**

An estimated ~6,500 mongenic diseases affect humans

Prakash, V.; et. al.; *Mol Ther.* ***2016,*** *24,* 465.

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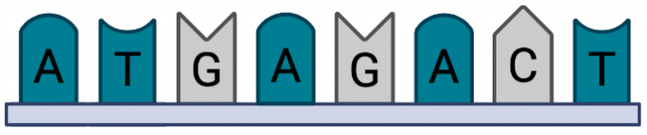

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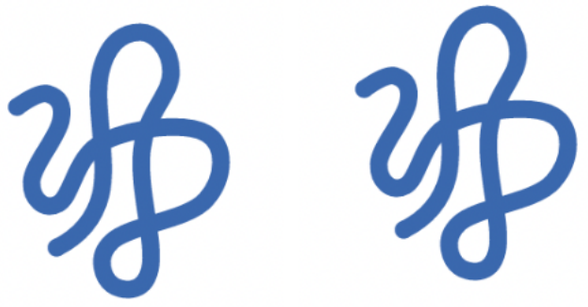

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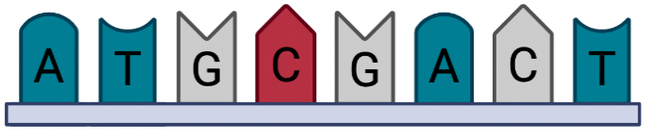

Mutated DNA

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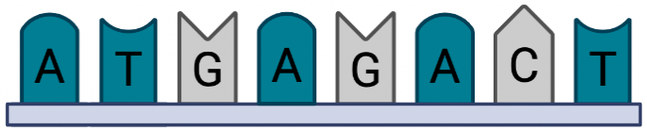
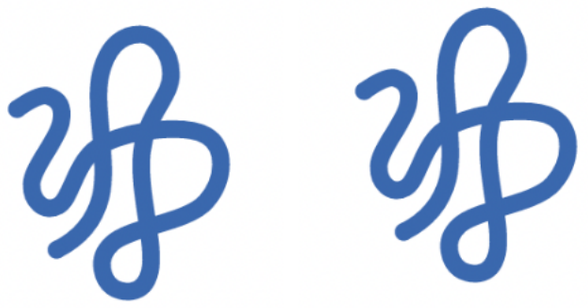
Insert or correct





Folded proteins = Cured monogenic disease

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"The alteration of cell(s) genome with the goal of providing a therapeutic benefit"

Gene Addition

Technologies:

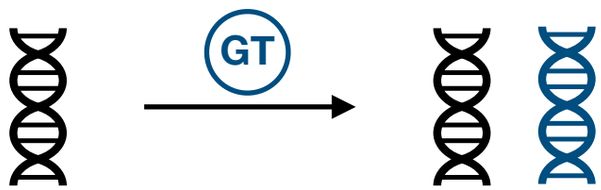
Adeno-associated viruses (AAVs)

Retroviruses

Chimeric antigen receptors (CARs)

Mechanism:

Expression of exogenous DNA



Target:

Modification of somatic cells

Dunbar, C. E.; et. al.; *Science.* ***2018,*** *359*.

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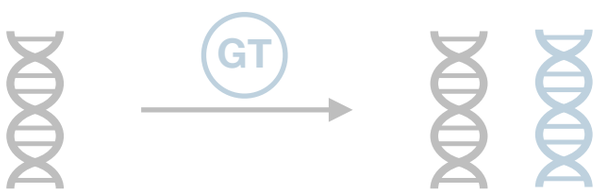

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Gene Insertion, Modification, and Deletion

Technologies:

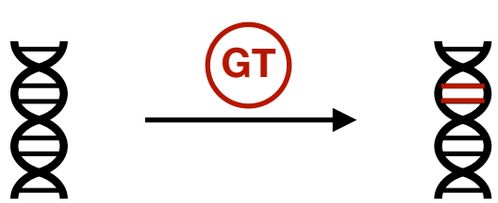
Zinc finger nucleases (ZFNs)

Transcription activator-like effector nucleases (TALENs)

CRISPR-Cas9

Mechanism:

Homology directed repair (HDR)
by inducing breaks in endogenous DNA



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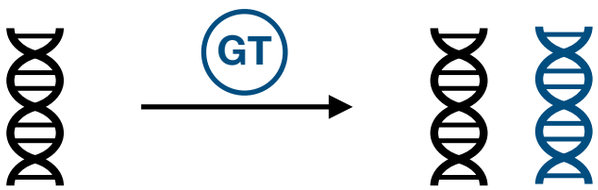

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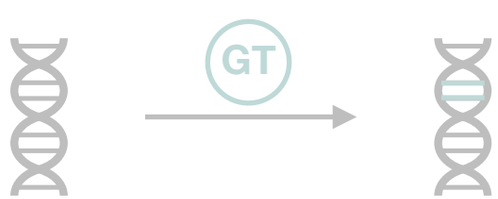

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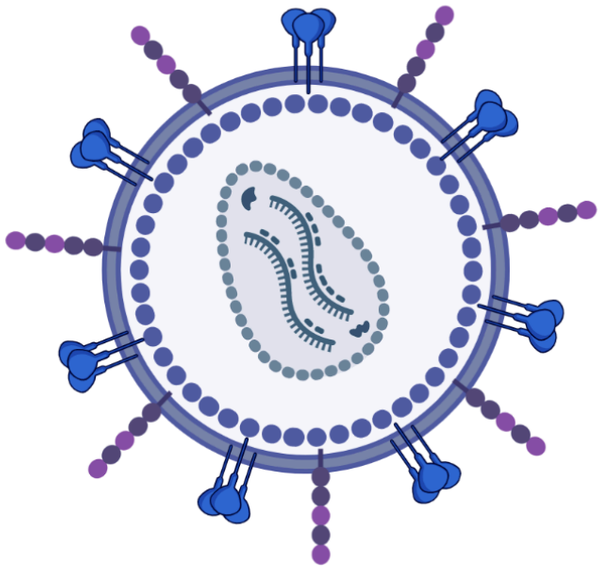
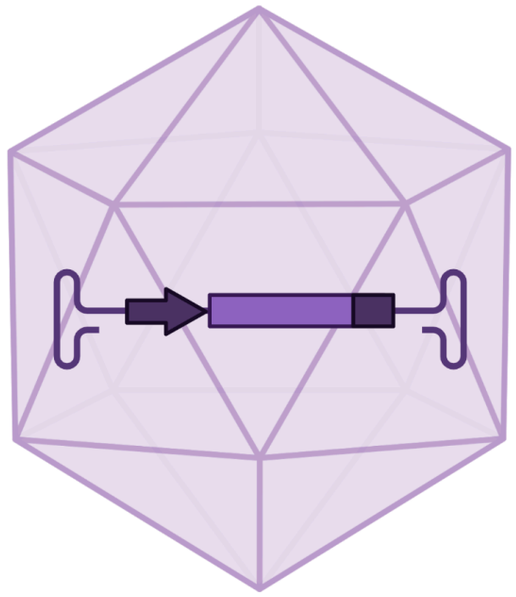
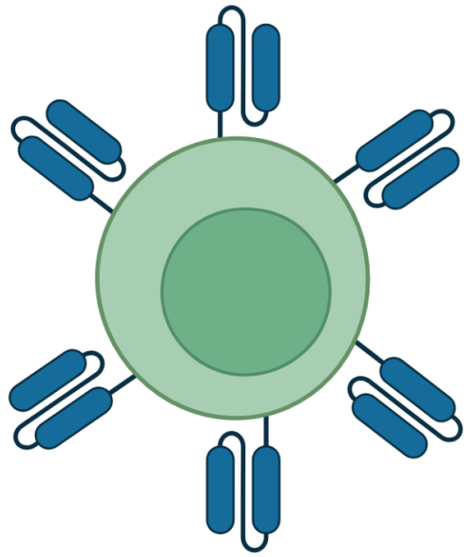


Adeno-Associated Virus



CAR-T Cell



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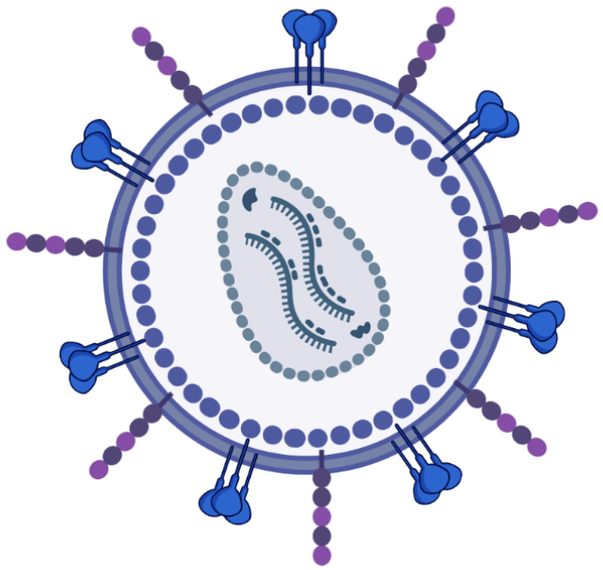

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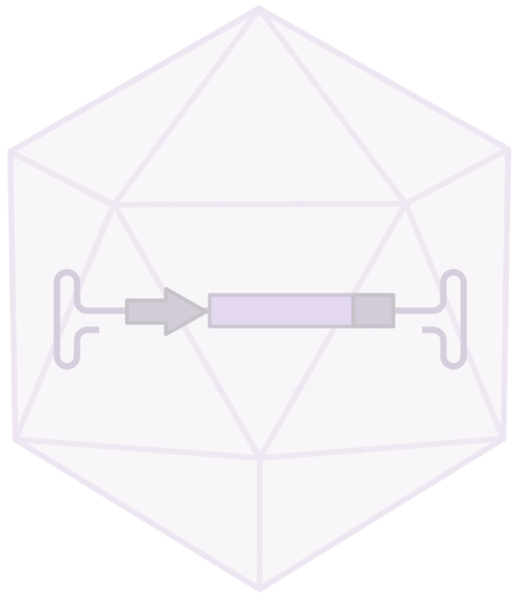

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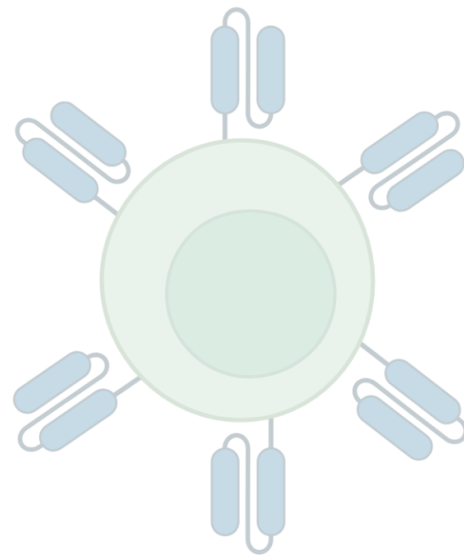
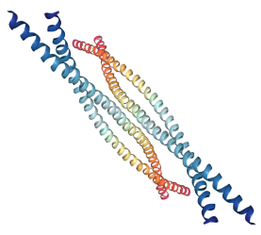
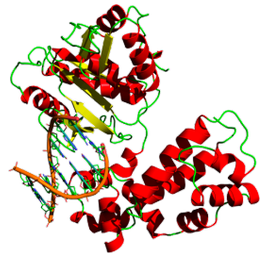
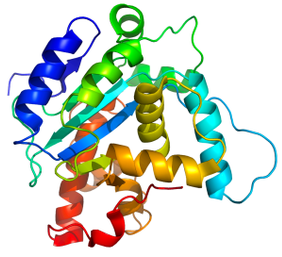




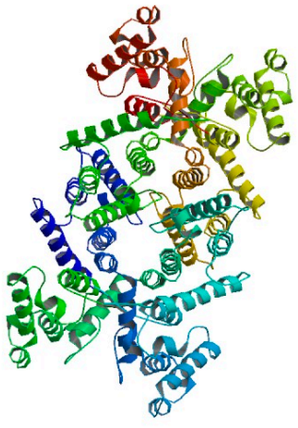
Keratin
(1.5 kb)



DNA polymerase
(2.8 kb)



Median protein
(26 kb)



Dystrophin
(2,400 kb)

Piovesan, A.; et. al.; *Database* ***2016,*** *2016,* baw153.

Dunbar, C. E.; et. al.; *Science.* ***2018,*** *359*.

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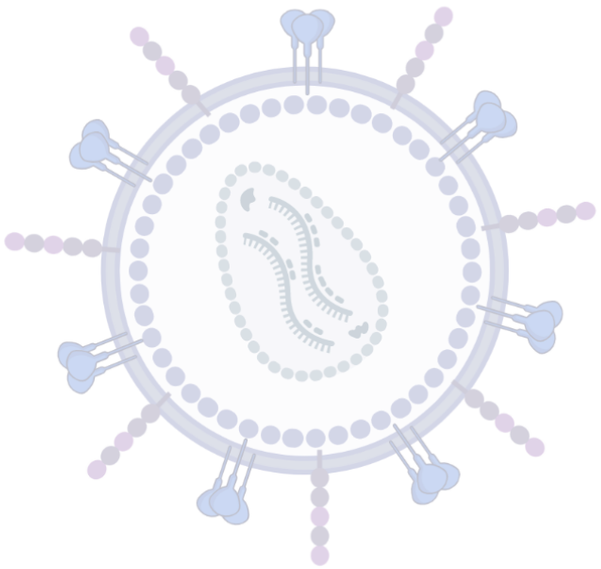

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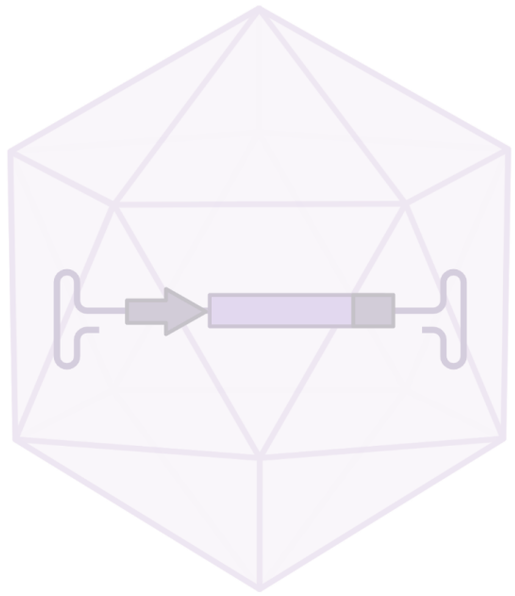

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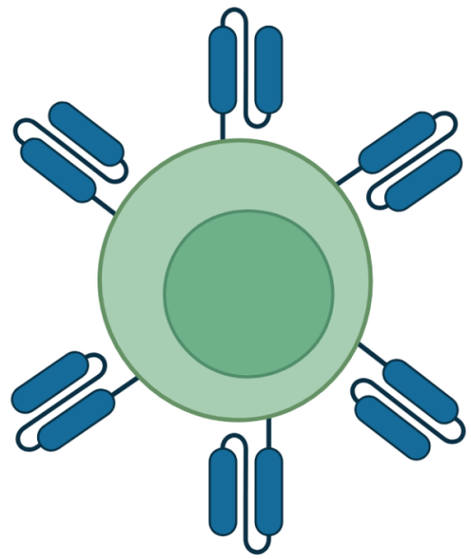

Modified patient T-cells

Installation of a novel antigen receptor

Transfection occurs in vitro

Requires extensive cell culture

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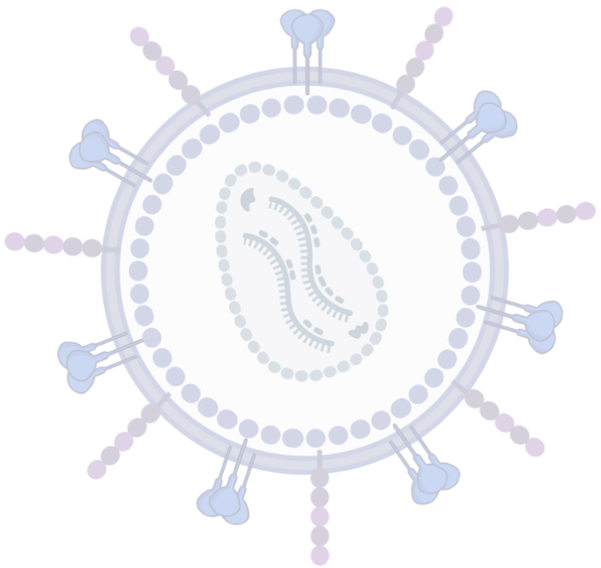

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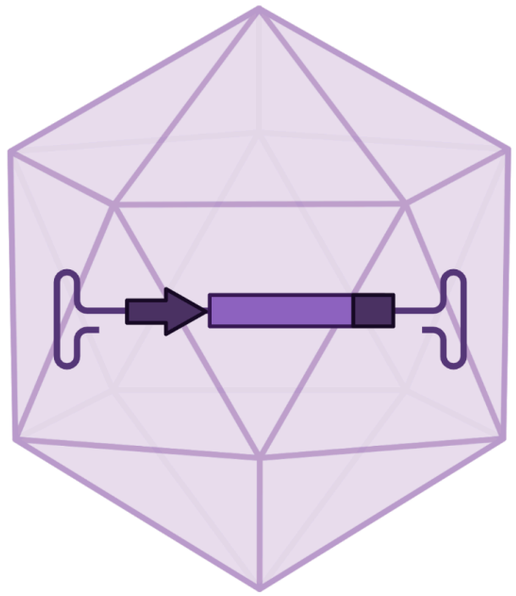

Parvoviridae Dependoparvovirus

Naturally occurring in humans and primates

AAVs alone do not cause human disease

Packages ~4.7 kb of ssDNA

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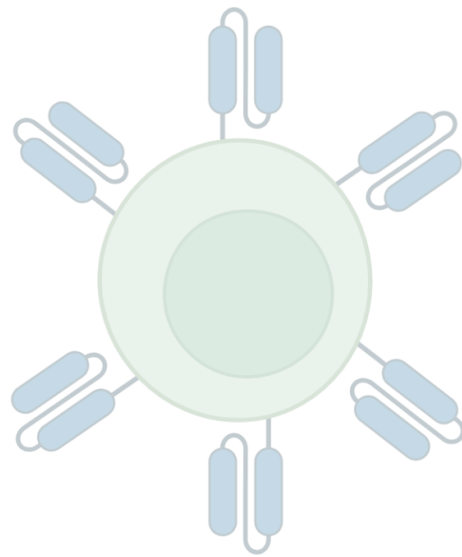

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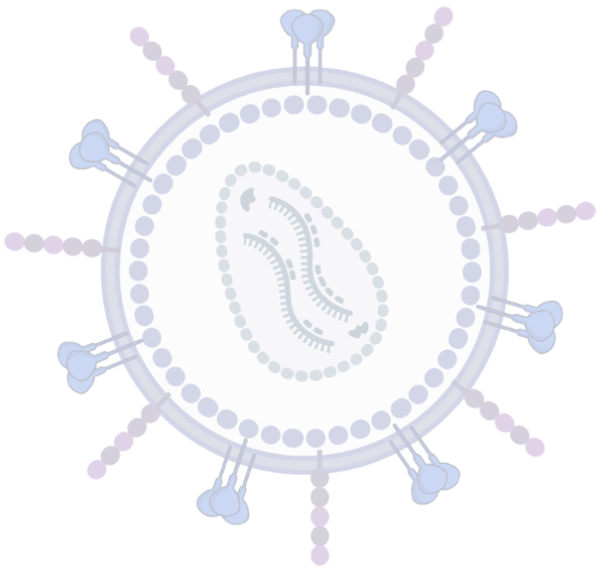

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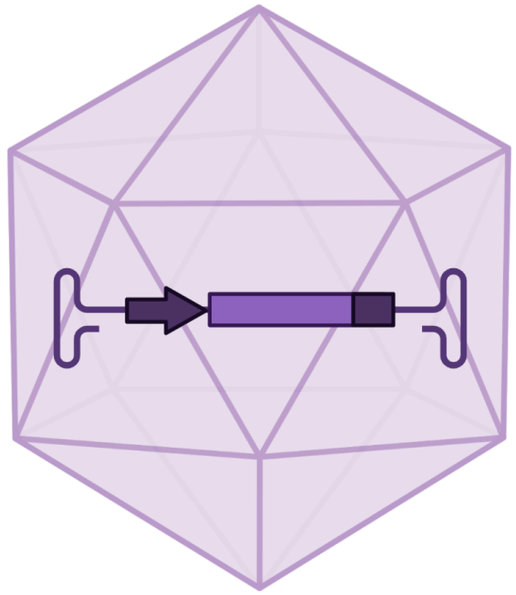

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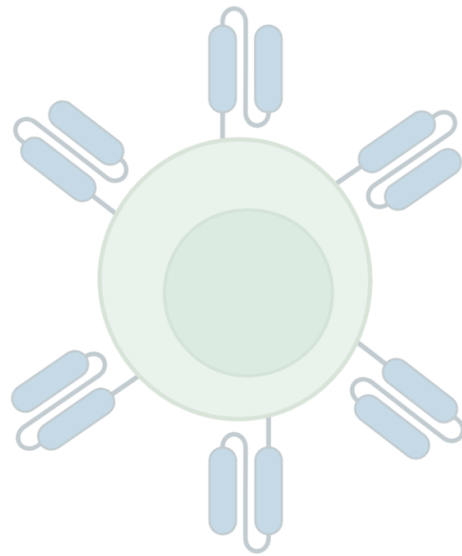

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AAVs have emerged as the "gold standard" for in-vivo gene addition therapies

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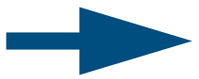
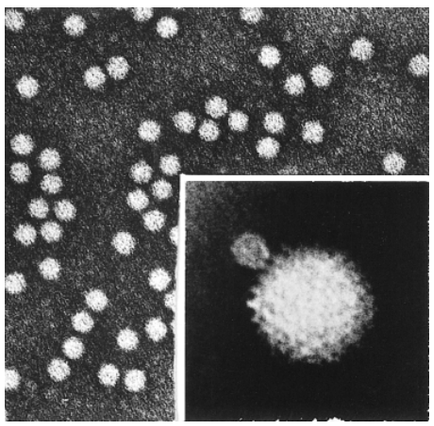


Timeline of Development

1965

Adeno-associated viruses are identified by electron microscopy



Atchison, R. W.; Castro, B. C.; Hammon, W. M.; *Science* **1965,** *149,* 754.

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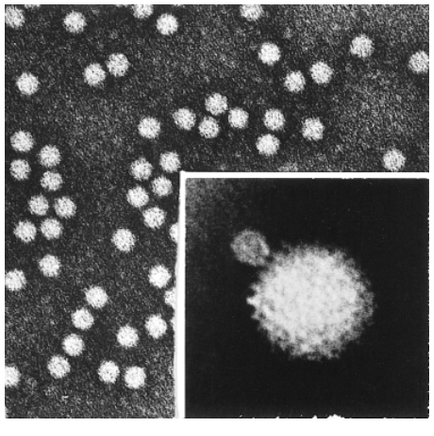

Friedmann, T.; Roblin, R.; *Science* **1972,** *175,* 949.

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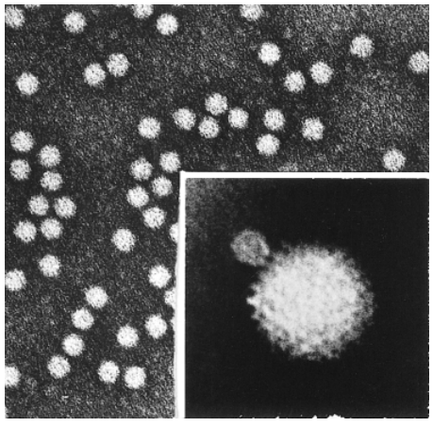

1980
Cline (UCLA) illegally attempts the first transfer of foreign genes to treat β-thalassemia

"I don't know of anyone in the country who has precisely the same type of skills that I have, with knowledge both in the animal systems and in clinical investigations in man. I think that in that sense I must be unique."
-Martin J. Cline

"It didn't work in mice, so I'm going to try it in a man"
-Martin J. Cline

"There is very little reason to believe, both from a molecular biology and cell biology standpoint, that an experiment like that would work"
-Philip Leder (NIH) about Cline's Experiment

Wade, N.; *Science* **1980,** *4469,* 509.

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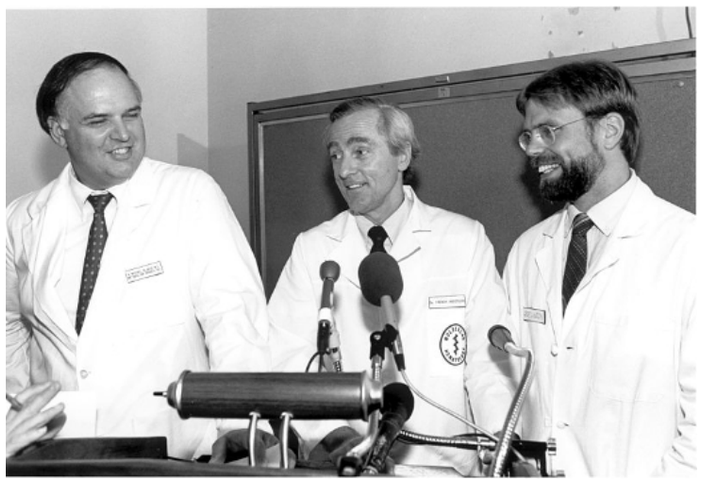

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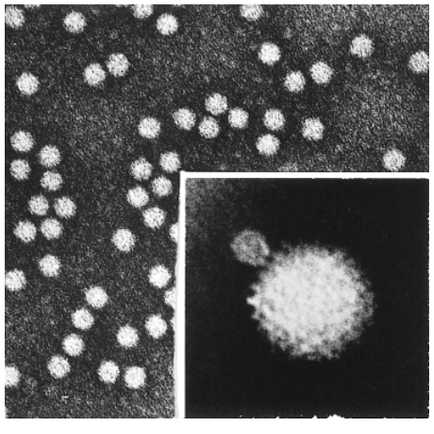

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Blase, R. M., et. al.; *Science* **1985,** *5235,* 475.

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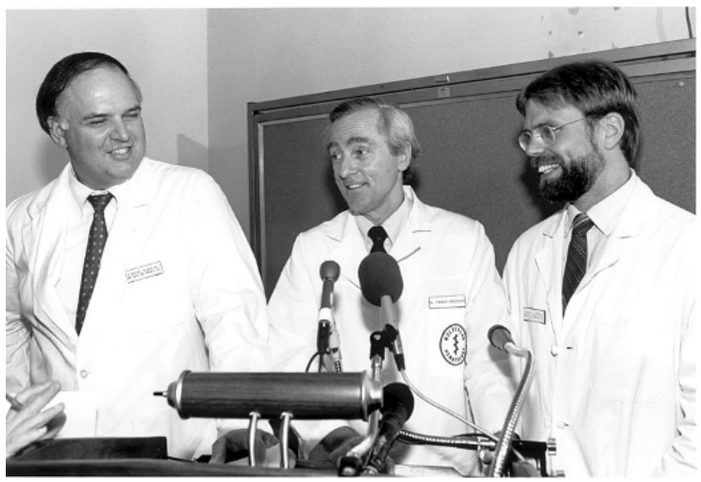

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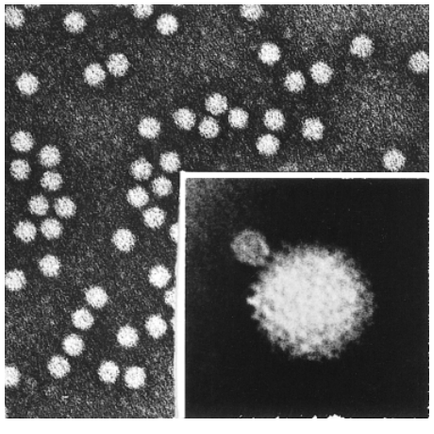

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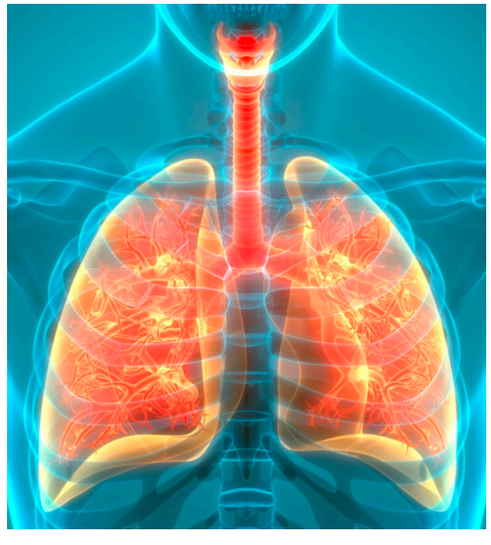
First in human trial of new rAAVs (Safer than wild type AAVs) to treat cystic fibrosis.



Flotte, T. R.; et. al.; *Hum. Gene Ther.* **1996,** *7,* 1145.

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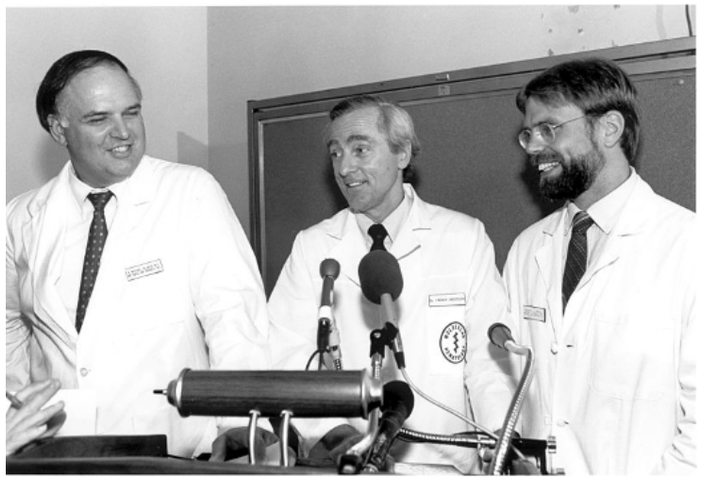

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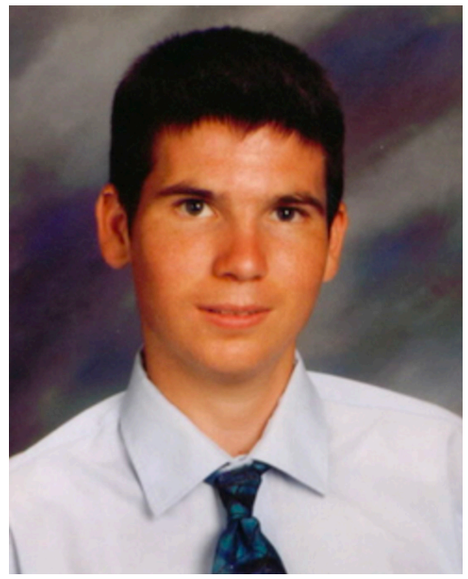

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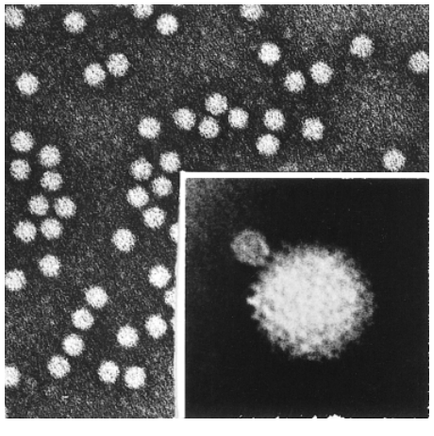
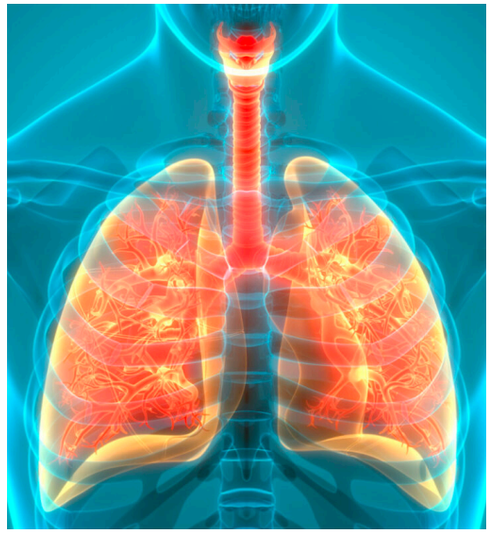
Jesse Gelsinger dies 4 days after being dosed with an experimental gene therapy for ornithine transcarbamylase (OTC) deficiency.

1965
Adeno-associated viruses are identified by electron microscopy



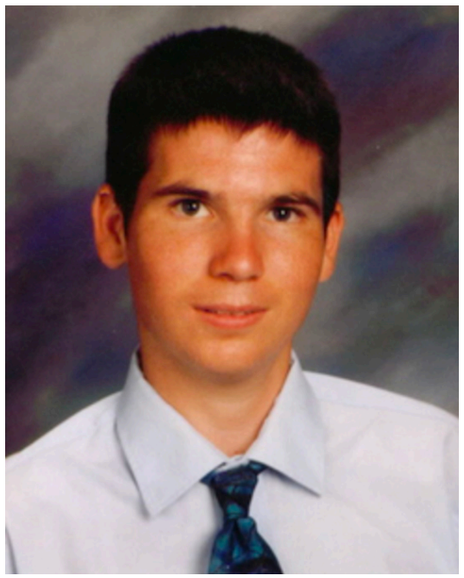
1980
Cline (UCLA) illegally attempts the first transfer of foreign genes to treat β-thalassemia

1995
First in human trial of new rAAVs (Safer than wild type AAVs) to treat cystic fibrosis.



Timeline of Development

Jesse Gelsinger



Jesse Gelsinger

Suffered from a very mild form of OTC deficiency

Unable to metabolize ammonia

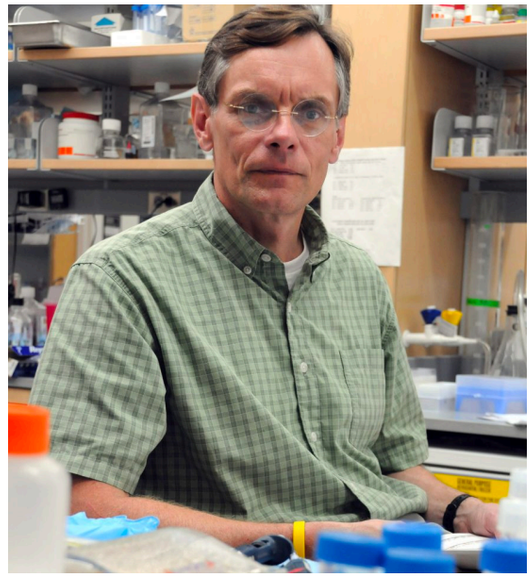
18th patient in the study

Received the largest dose (6 x 10^9 vg/kg)

Trigged a massive immune response leading to organ failure

Timeline of Development

Jesse Gelsinger



Jesse Gelsinger	*James Wilson, M.D. Ph.D.*
Suffered from a very mild form of OTC deficiency	Found to have violated FDA guidelines
Unable to metabolize ammonia	Included Jesse in the study despite high ammonia levels
18th patient in the study	Failed to disclose the deaths of monkeys in earlier phases
Received the largest dose (6 x 10^9 vg/kg)	University didn’t properly report 2 patient’s side effects
Trigged a massive immune response leading to organ failure	Barred from clinical trials for 10 years

Timeline of Development

Jesse Gelsinger

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

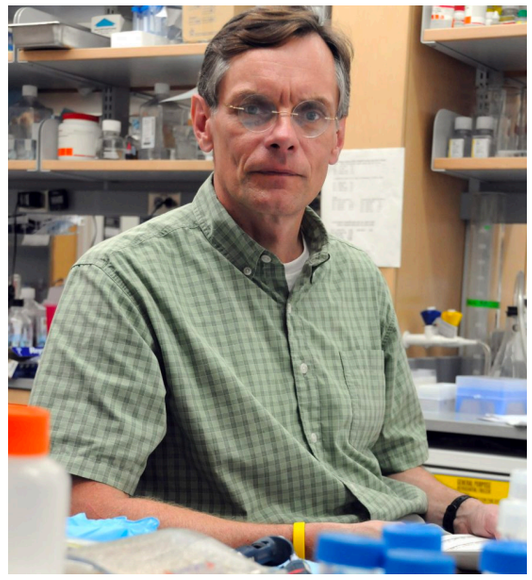
journal homepage: www.elsevier.com/locate/ymgme

Commentary

Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency

James M. Wilson *

Department of Pathology and Laboratory Medicine, University of Pennsylvania, Suite 2000 TRL, 125 S. 31st Street, Philadelphia, PA 19104-3403, USA



James Wilson, M.D. Ph.D.

Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy

Guang-Ping Gao, Mauricio R. Alvira, Lili Wang, Roberto Calcedo, Julie Johnston, and James M. Wilson*

c&en CHEMICAL & ENGINEERING NEWS TOPICS MAGAZINE COLLECTIONS VIDEOS JOBS

GENE THERAPY

Jim Wilson launches liver disease gene-editing company iECURE with $50 million

c&en CHEMICAL & ENGINEERING NEWS TOPICS MAGAZINE COLLECTIONS VIDEOS JOBS

BUSINESS MOVERS AND SHAKERS

The redemption of James Wilson, gene therapy pioneer

POLICYFORUM

MEDICINE

A History Lesson for Stem Cells

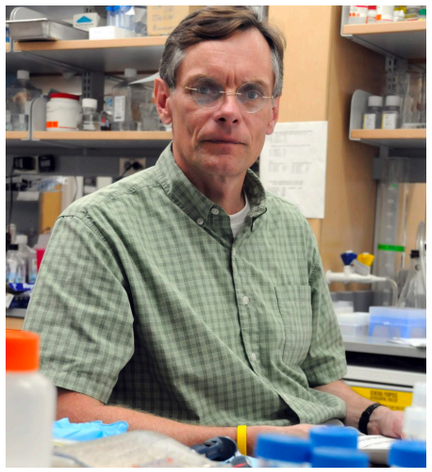
James M. Wilson

Factors that led to the decline of gene therapy at the turn of the century should be considered by the stem cell community to avoid a similar outcome.

Wilson continued his research and is now considered the "premier" authority in AAV gene therapy

42 companies, >100 clinical trials

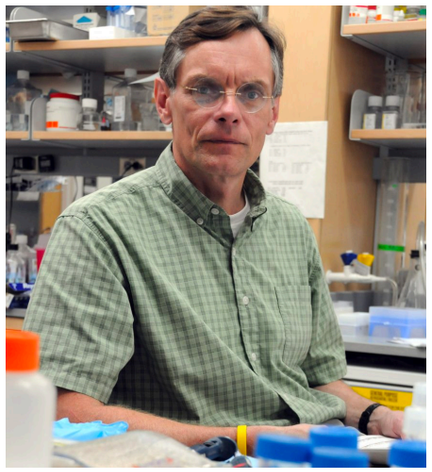
Timeline of Development



2002-2004
New, safer AAVs (7-9) are developed by Wilson (UPenn) enabling lower dosing requirements

Gao, G.; et. al.; *PNAS* ***2002,*** *99,* 11854.
Gao, G.; et. al.; *PNAS* ***2003,*** *100,* 6081.
Gao, G.; et. al.; *J. Virol.* ***2004,*** *78,* 6381.

Timeline of Development



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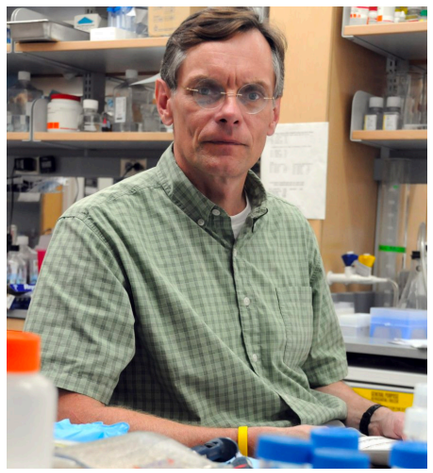
Clinical efficacy is achieved for the first time for the AAV treatment of inherited blindness

Hauswirth, W. W.; et. al.; *Hum. Gene Ther.* ***2008,*** *19,* 979.

Bainbridge, J. W.; et. al.; *N. Engl. J. Med.* ***2008,*** *358,* 2231.

Timeline of Development



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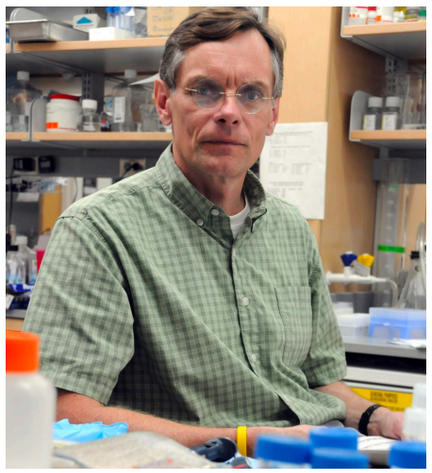

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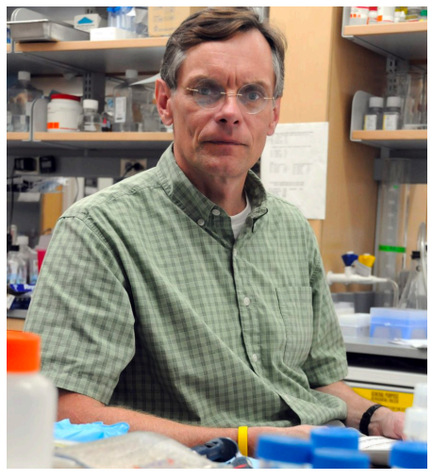

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EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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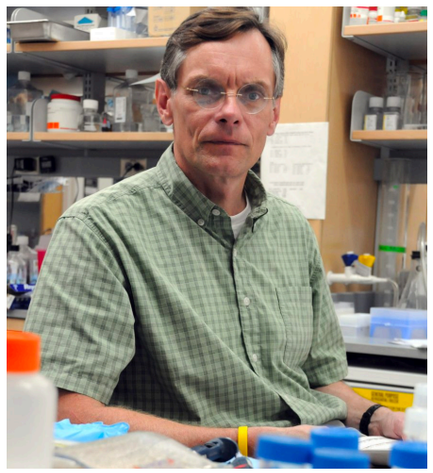
Children's National

Timeline of Development



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Children's National

2017-2022
21 more gene/cell therapy treatments are approved by the FDA.
As of 2021, >3,000 patients have received AAVs therapies through clinical trials

FDA

Kuzmin, D. A.; et. al.; *Nat. Rev. Drug Discov.* ***2021*****,** *20,* 173.

Outline

What is Gene Therapy?

History of Gene Therapy

AAV Biology

Challenges and Outlook

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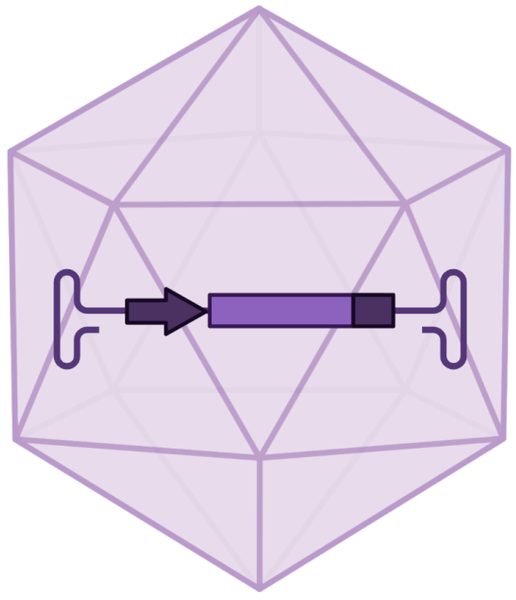
AAV composition



AAV Vector

AAV Biology

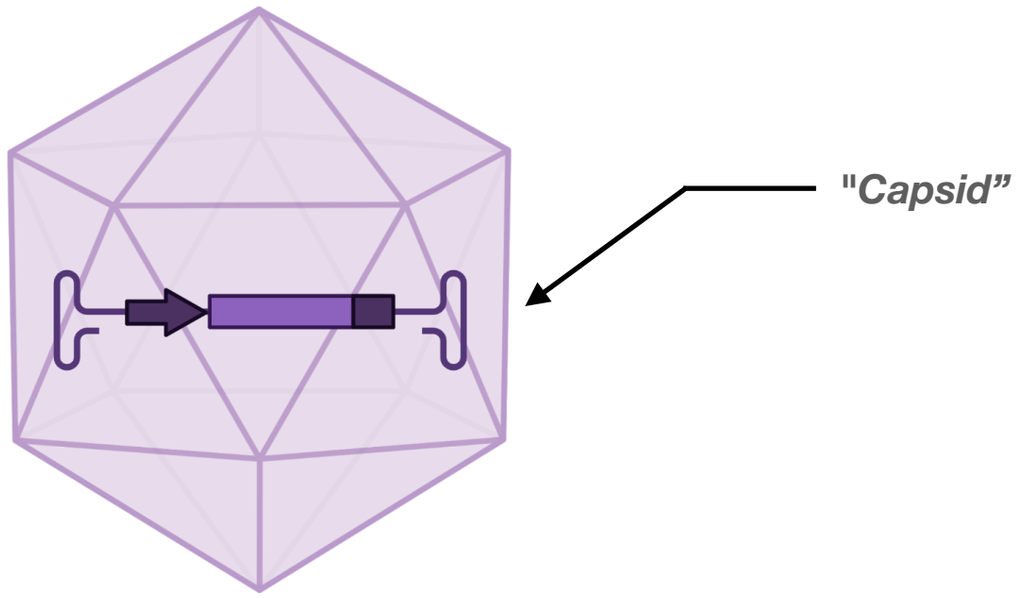
AAV composition

Capsid

-Oligomer of 3 proteins

-Protective “shell”

-Different serotypes enable targeting



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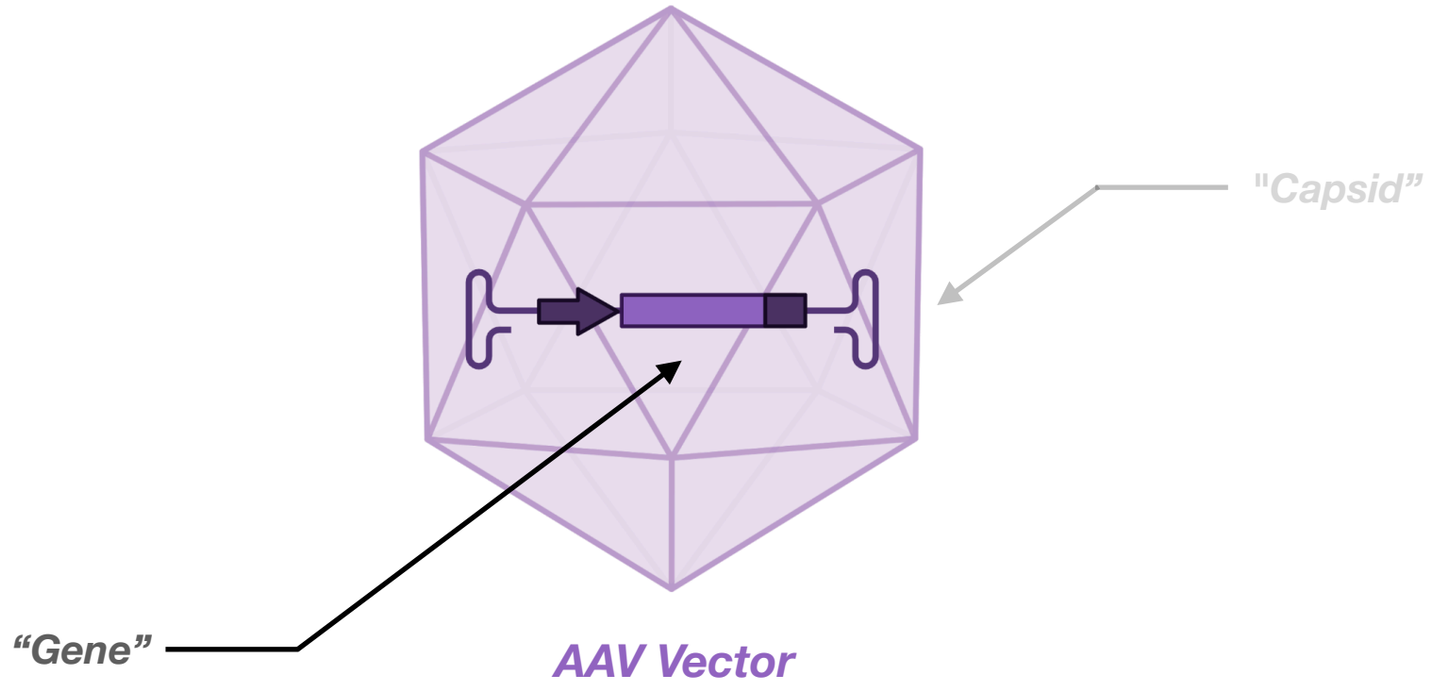

Gene

-*Rep* and *cap* in natural AAVs

-Therapeutic DNA in rAAVs

-4.7kb maximum size

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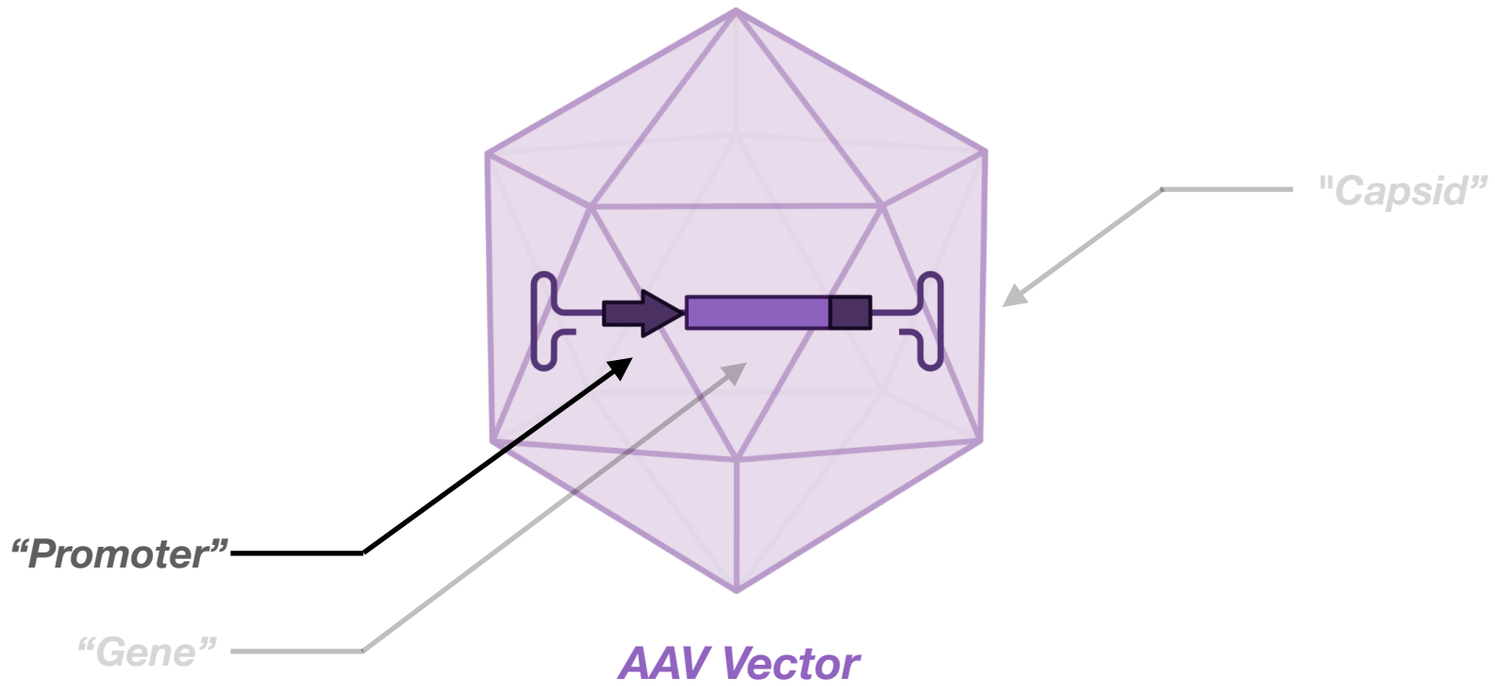

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-Not found in natural AAVs

-CMV or chicken β-actin promoter

-Improves gene expression

AAV Biology

AAV composition

Inverted Terminal Repeats (ITRs)

-Essential for downstream expression

-Enables circularization (episomal DNA)

-Only conserved ssDNA in rAAVs

Capsid

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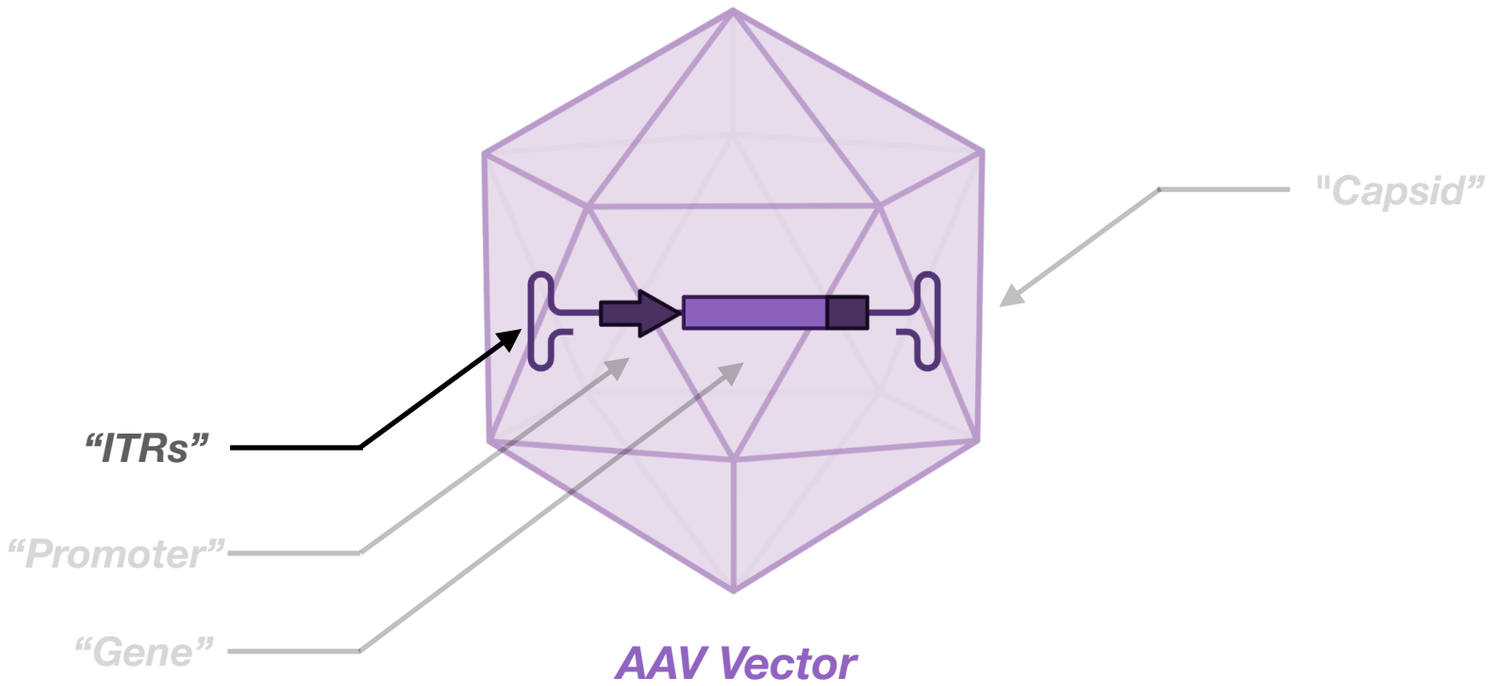

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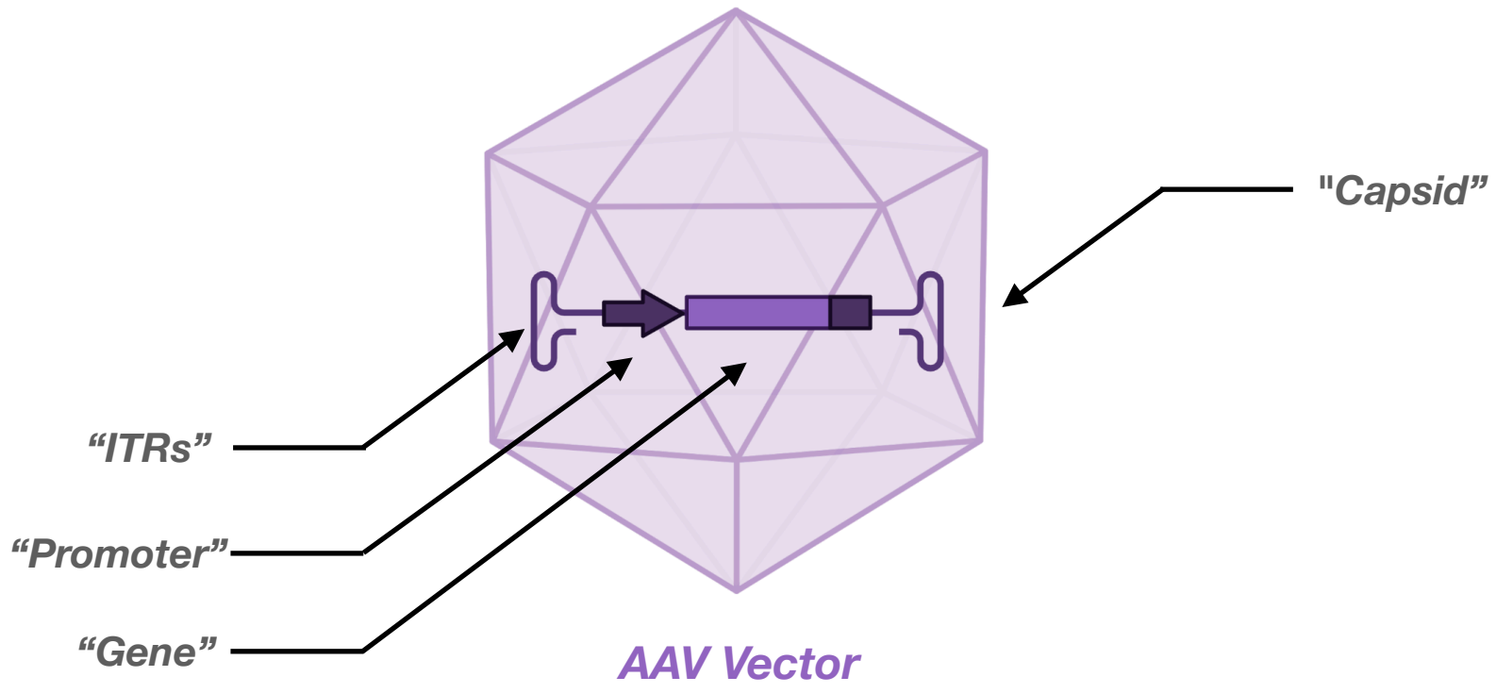

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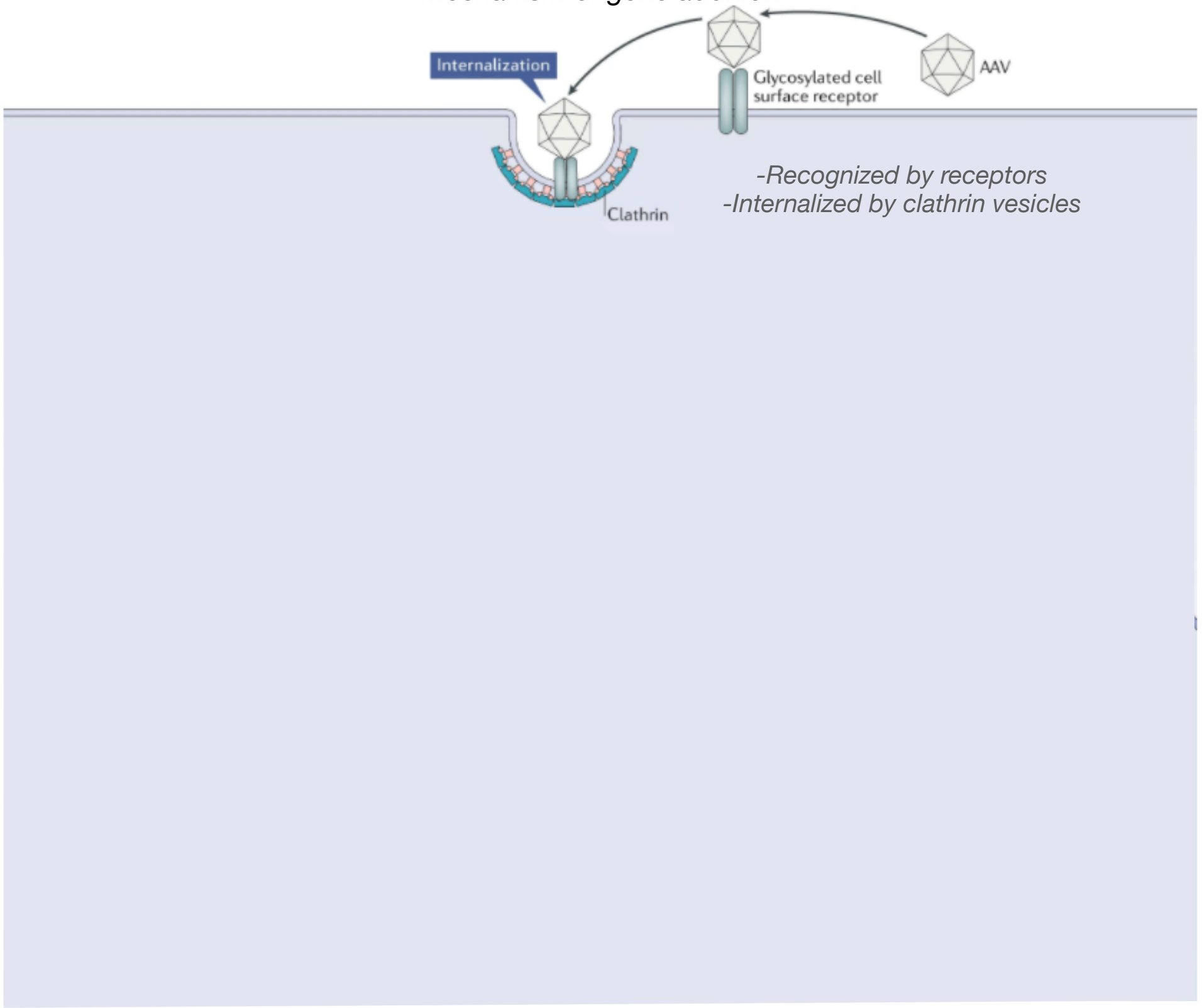
AAV Biology

Mechanism of gene addition

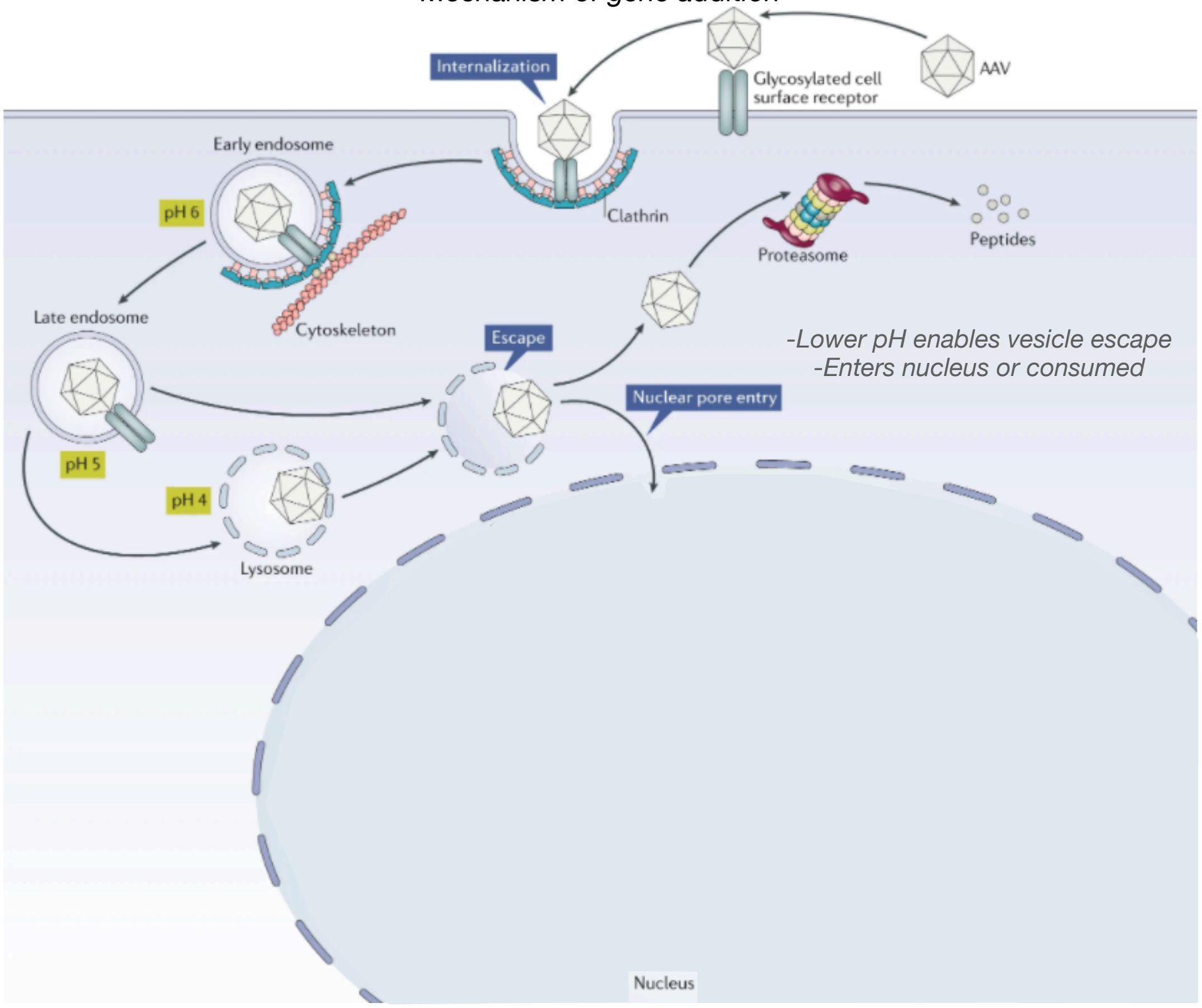


-Recognized by receptors
-Internalized by clathrin vesicles

Wang, D.; et. al.; *Nat. Rev. Drug Discov.* ***2019,*** *18,* 358.

AAV Biology

Mechanism of gene addition

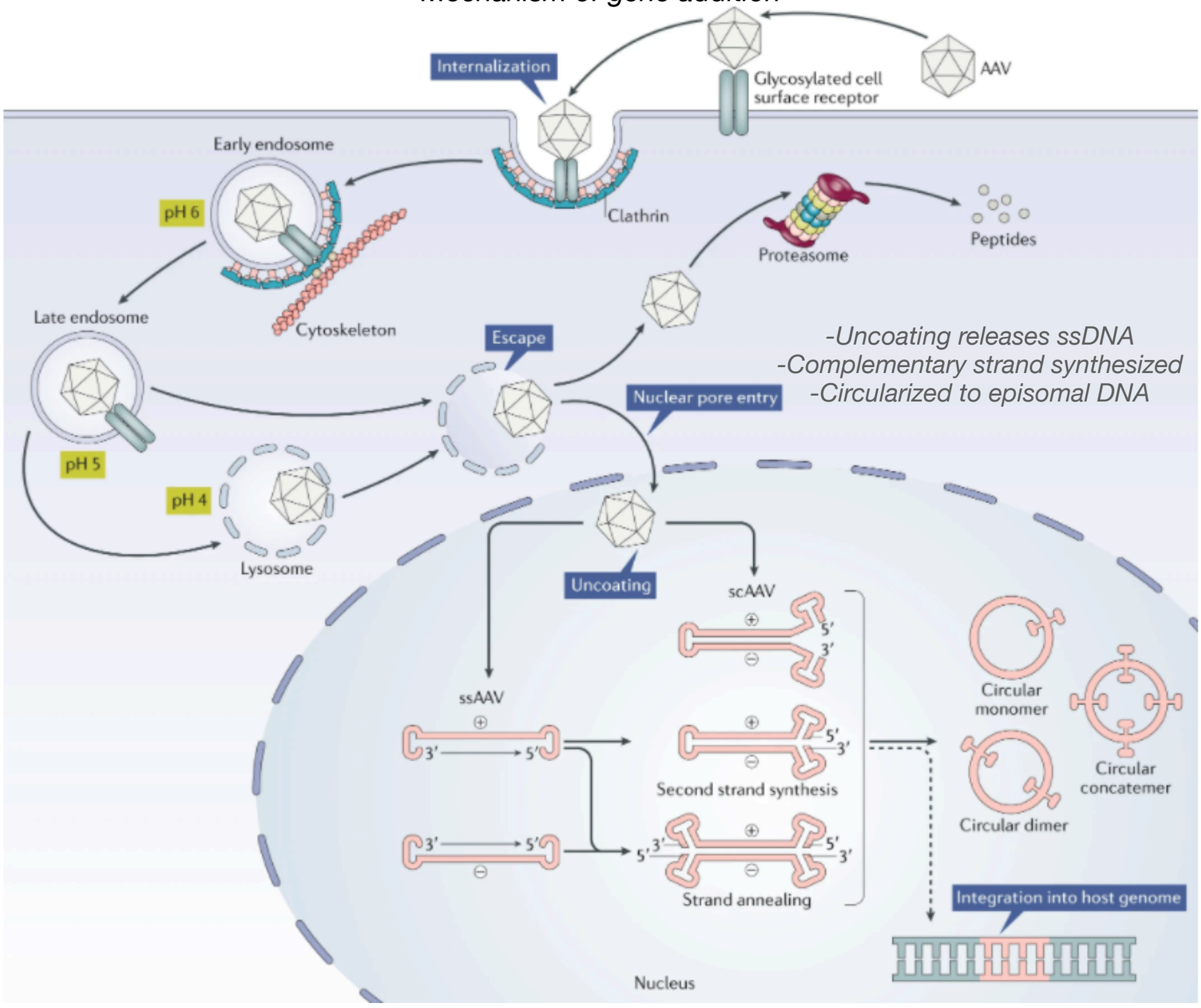


-Lower pH enables vesicle escape
-Enters nucleus or consumed

Wang, D.; et. al.; *Nat. Rev. Drug Discov.* ***2019,*** *18,* 358.

AAV Biology

Mechanism of gene addition

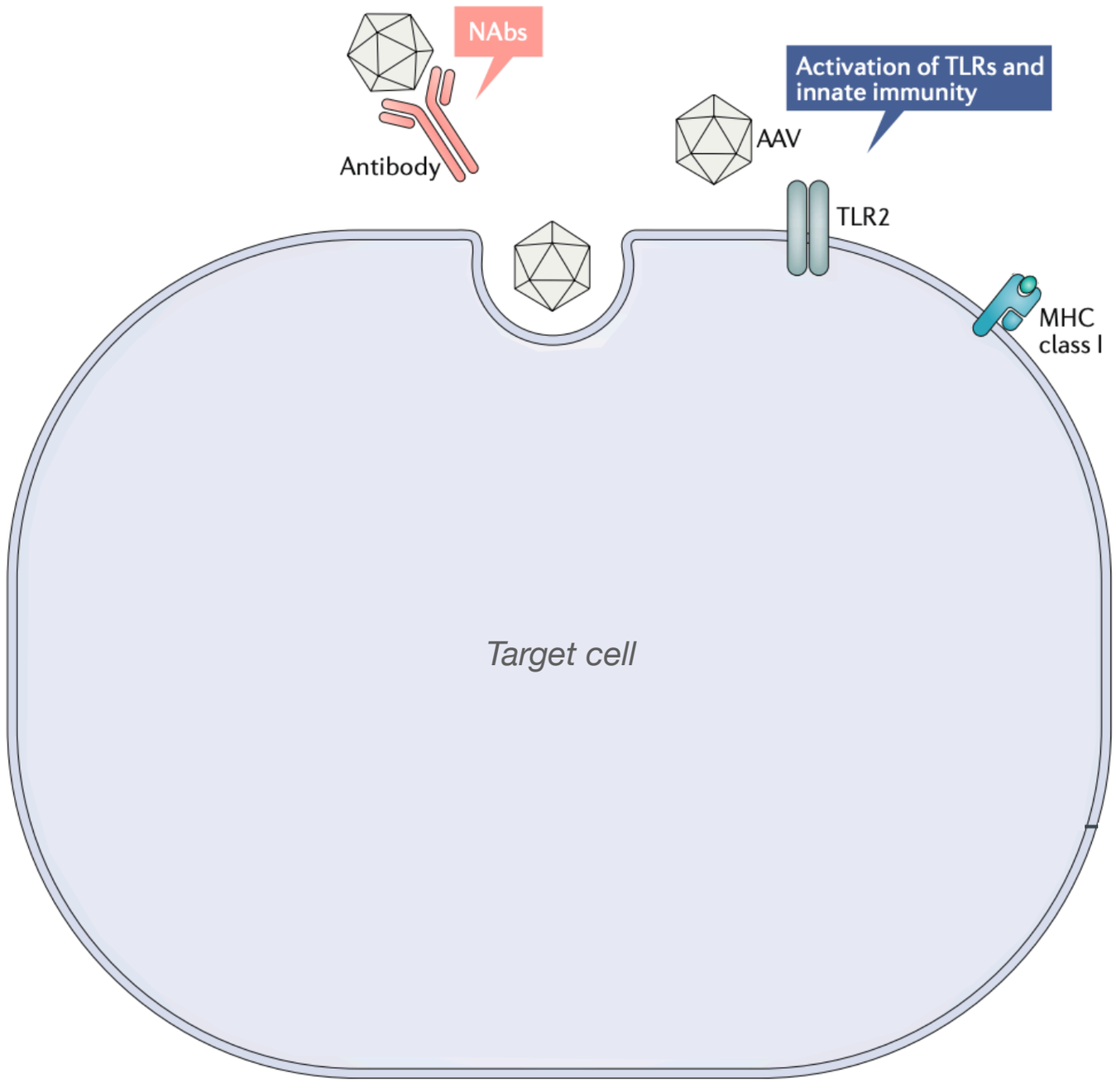


-Uncoating releases ssDNA
-Complementary strand synthesized
-Circularized to episomal DNA

Wang, D.; et. al.; *Nat. Rev. Drug Discov.* ***2019,*** *18,* 358.

AAV Biology

Immune response to rAAVs



Ronzitti, G.; et. al.; *Front. Immunol.* ***2020,*** *11,* 670.
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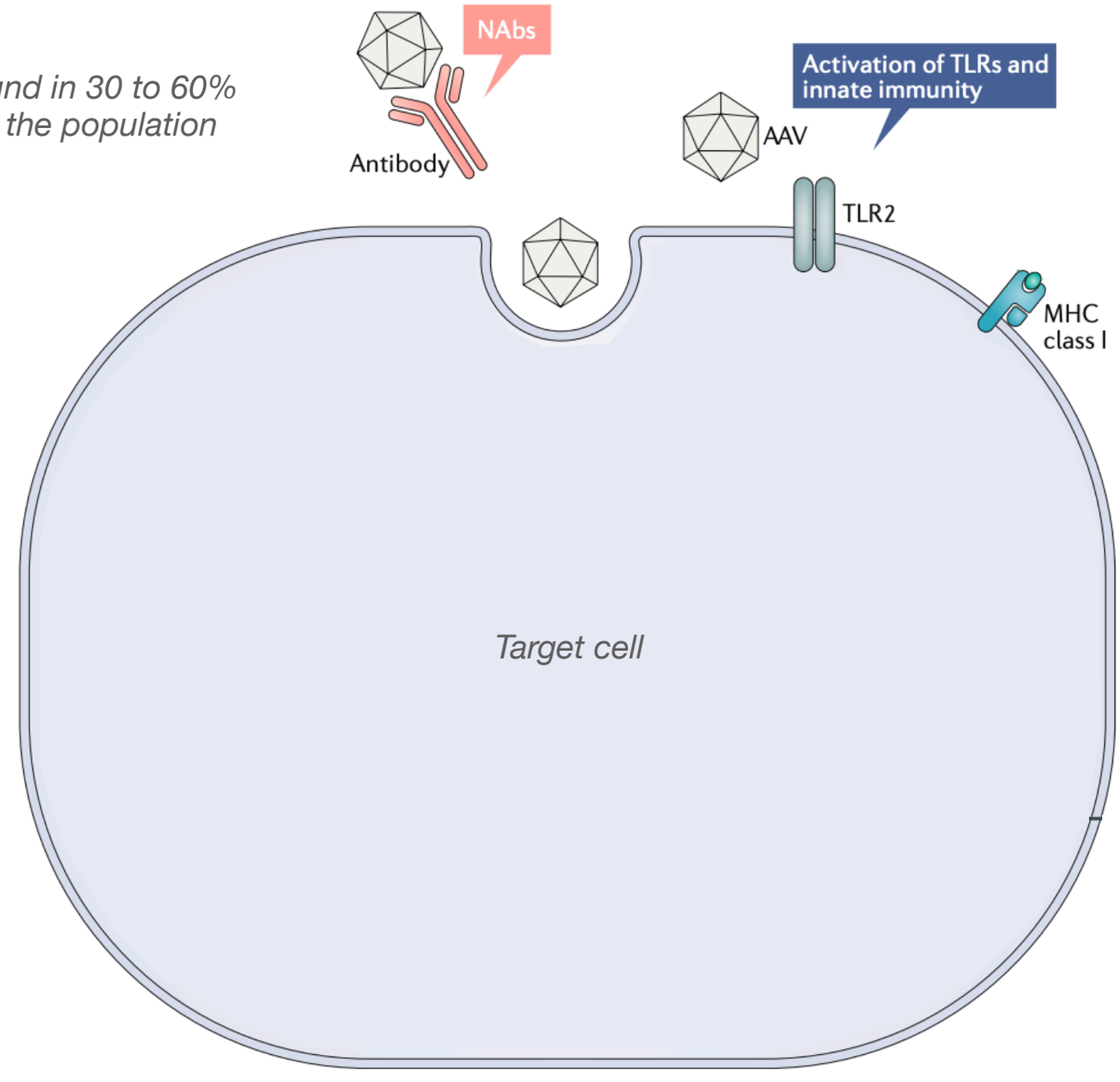
AAV Biology

Immune response to rAAVs

Found in 30 to 60% of the population



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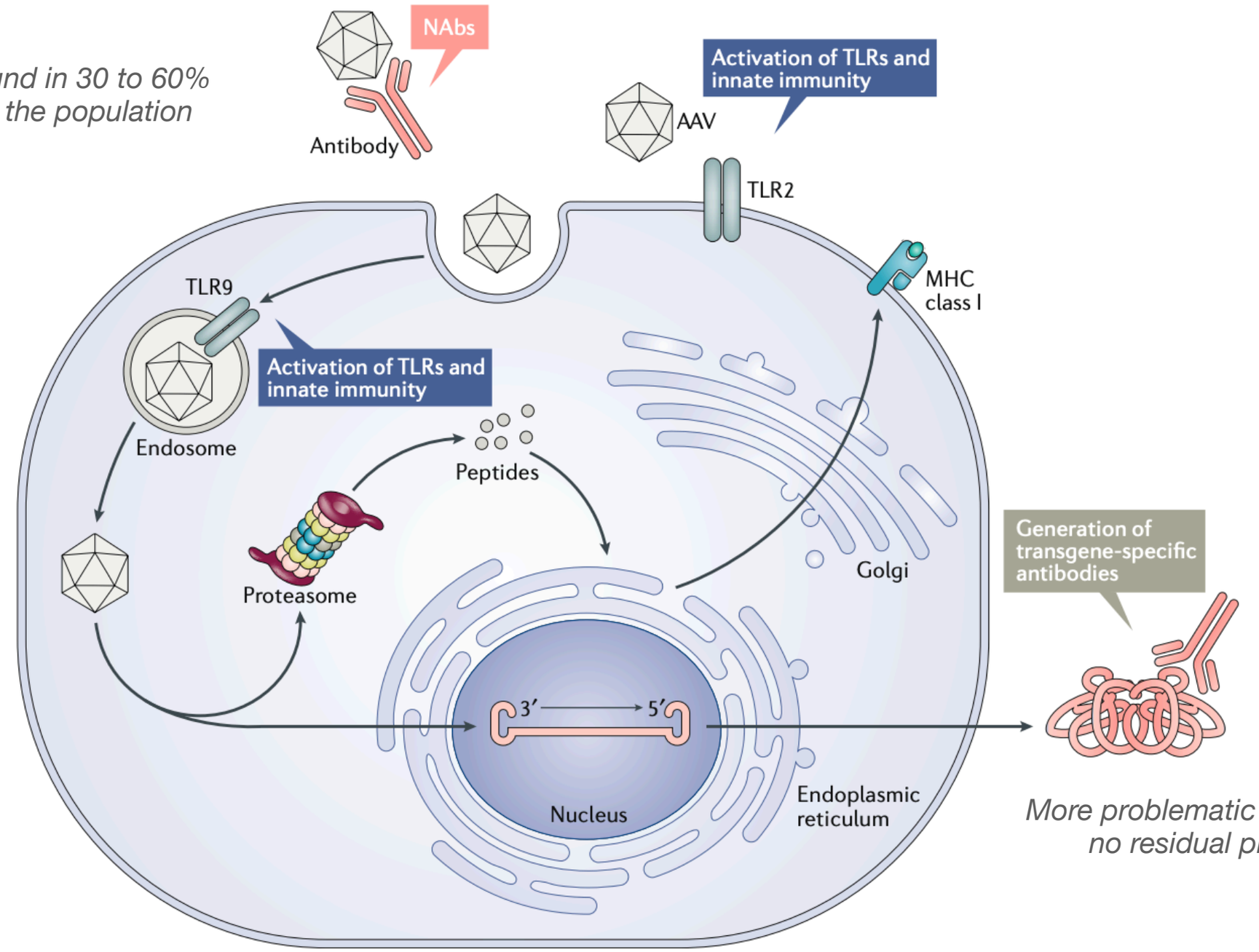

More problematic in disease states with no residual protein expression

Ronzitti, G.; et. al.; *Front. Immunol.* ***2020,*** *11,* 670.

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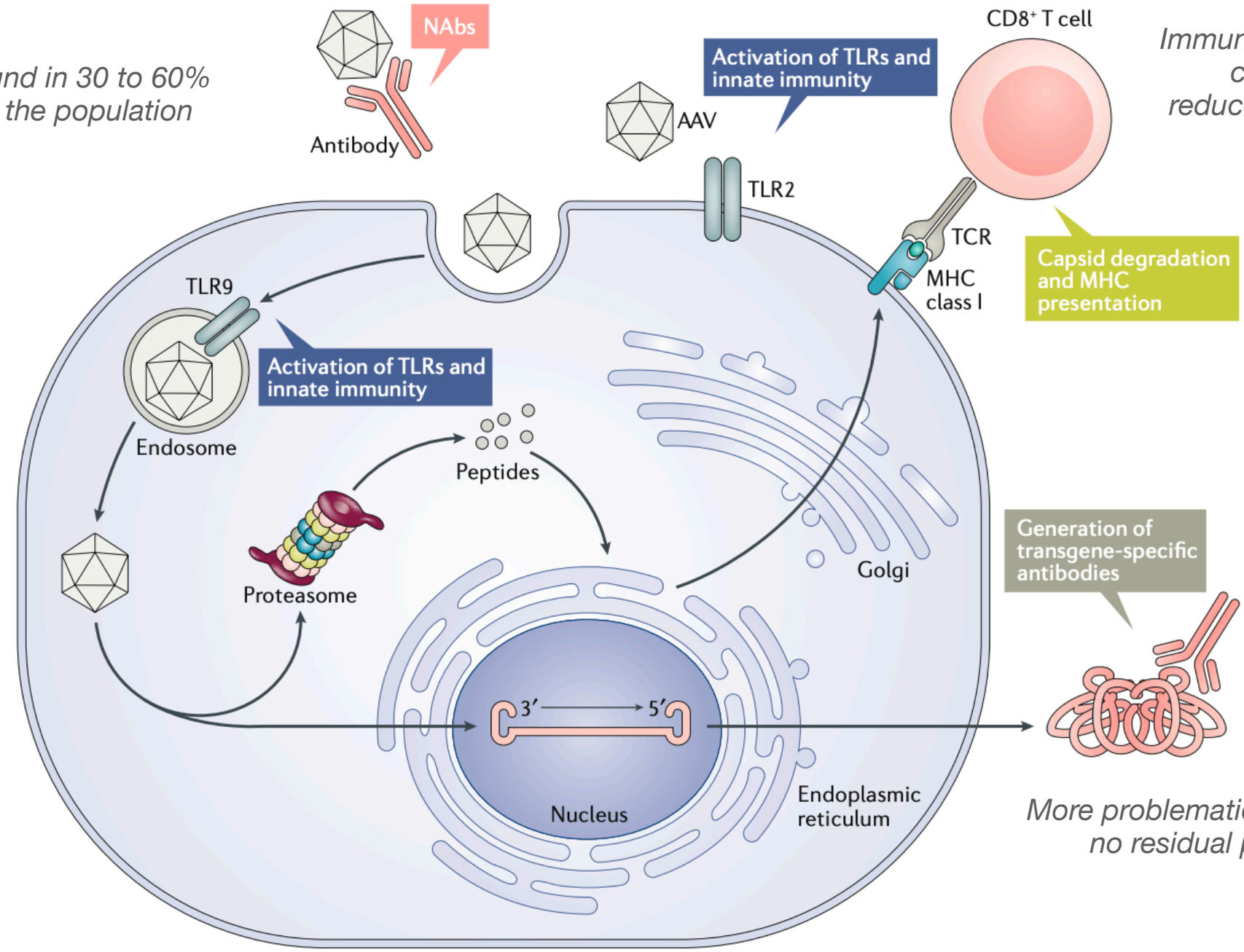


Immunosuppressant drugs can significantly reduce immune response

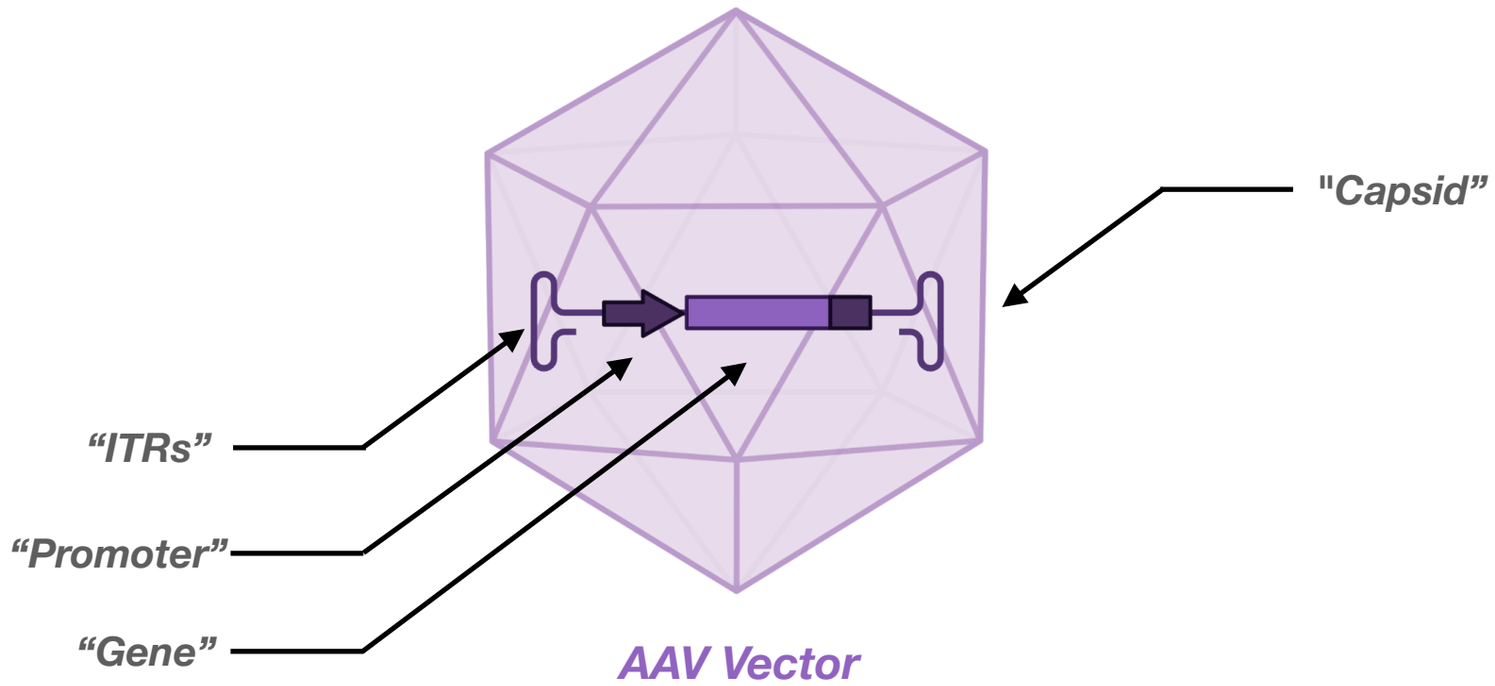
More problematic in disease states with no residual protein expression

Ronzitti, G.; et. al.; *Front. Immunol.* ***2020,*** *11,* 670.

Wang, D.; et. al.; *Nat. Rev. Drug Discov.* ***2019,*** *18,* 358.

AAV Biology

AAV serotypes



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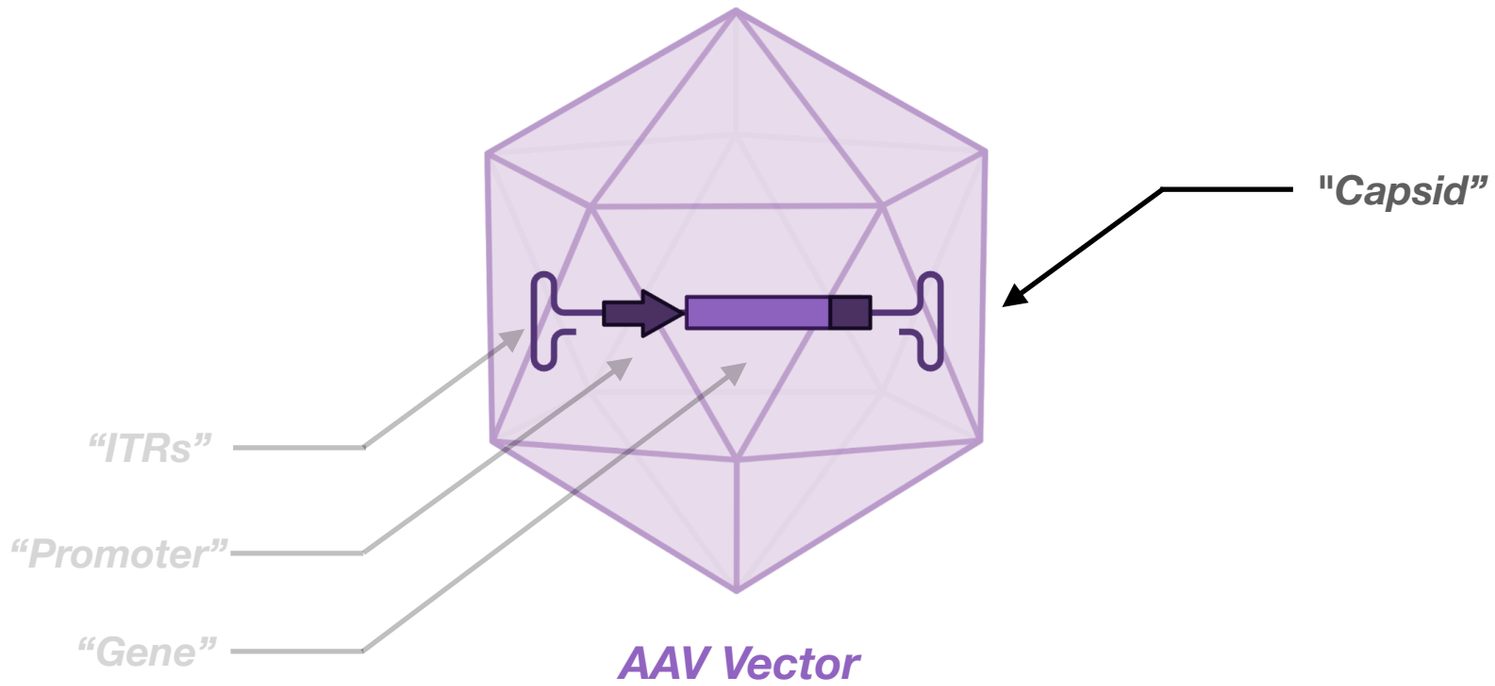
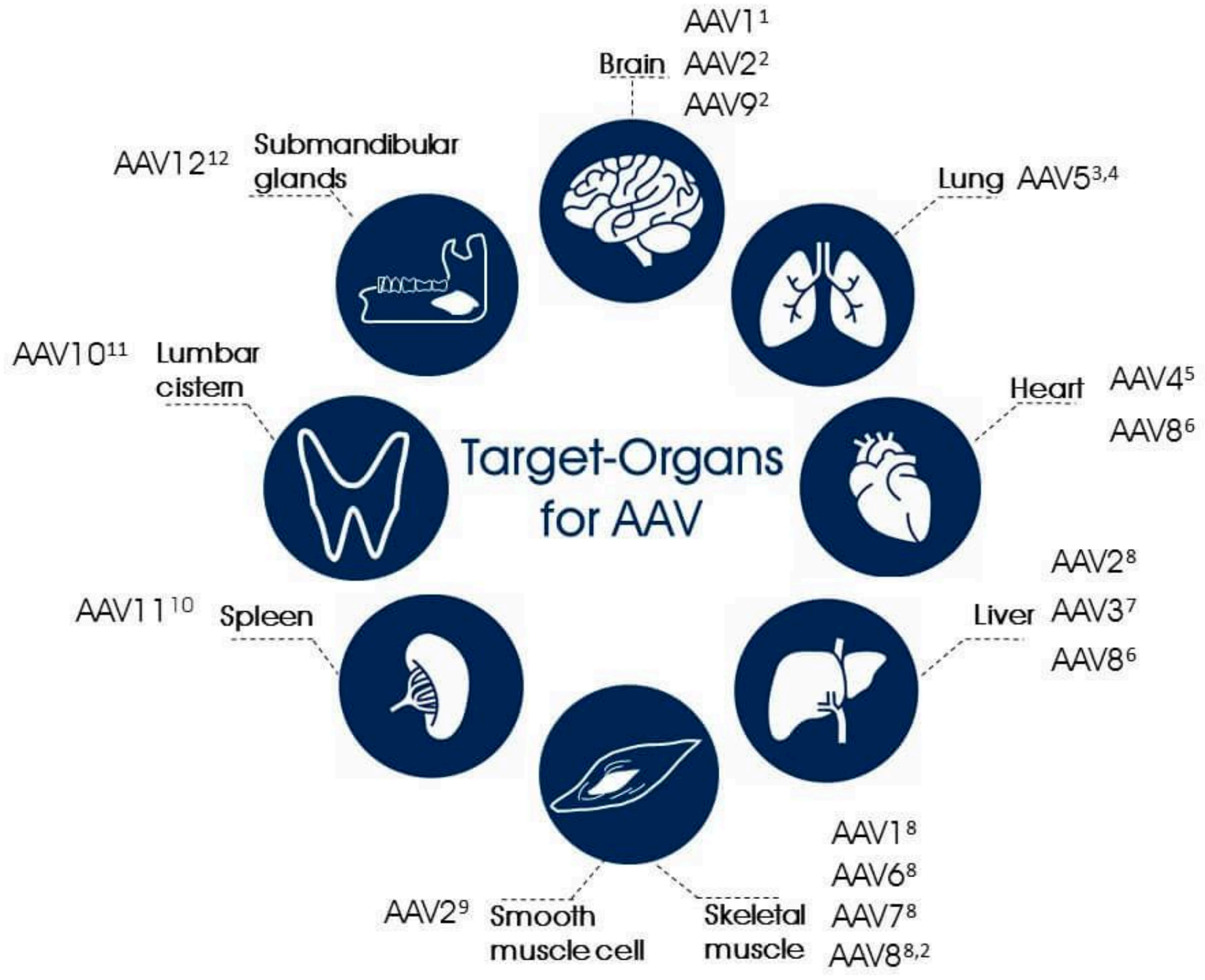


Novel capsid design enables discovery process

Optimizing the "cap" changes localization

AAV Biology

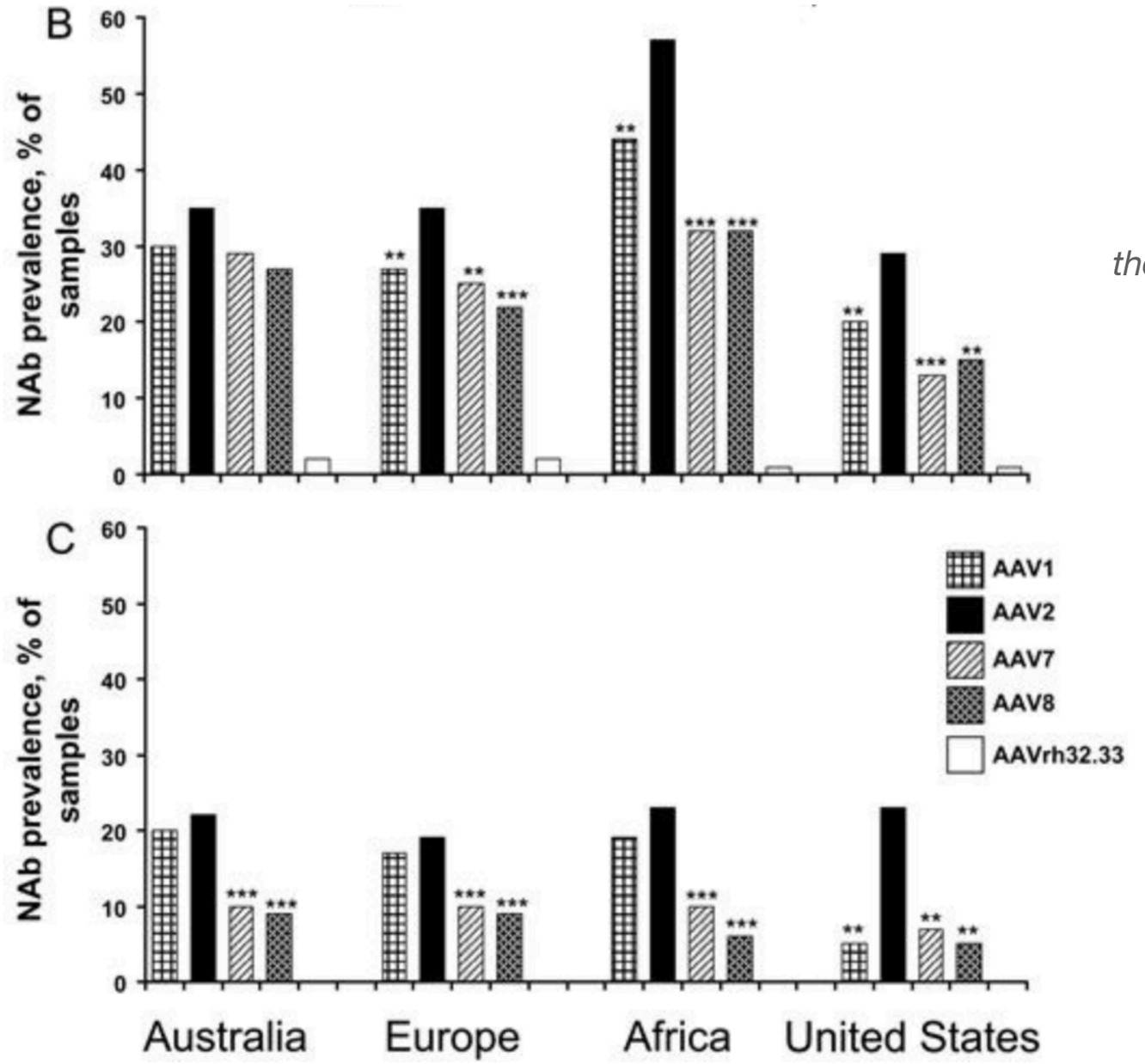
AAV serotypes



AAV Biology

AAV serotypes and immune response

1:20 serum dilution



1:80 serum dilution

% of samples with >50% inhibition of vector transduction
n = 889

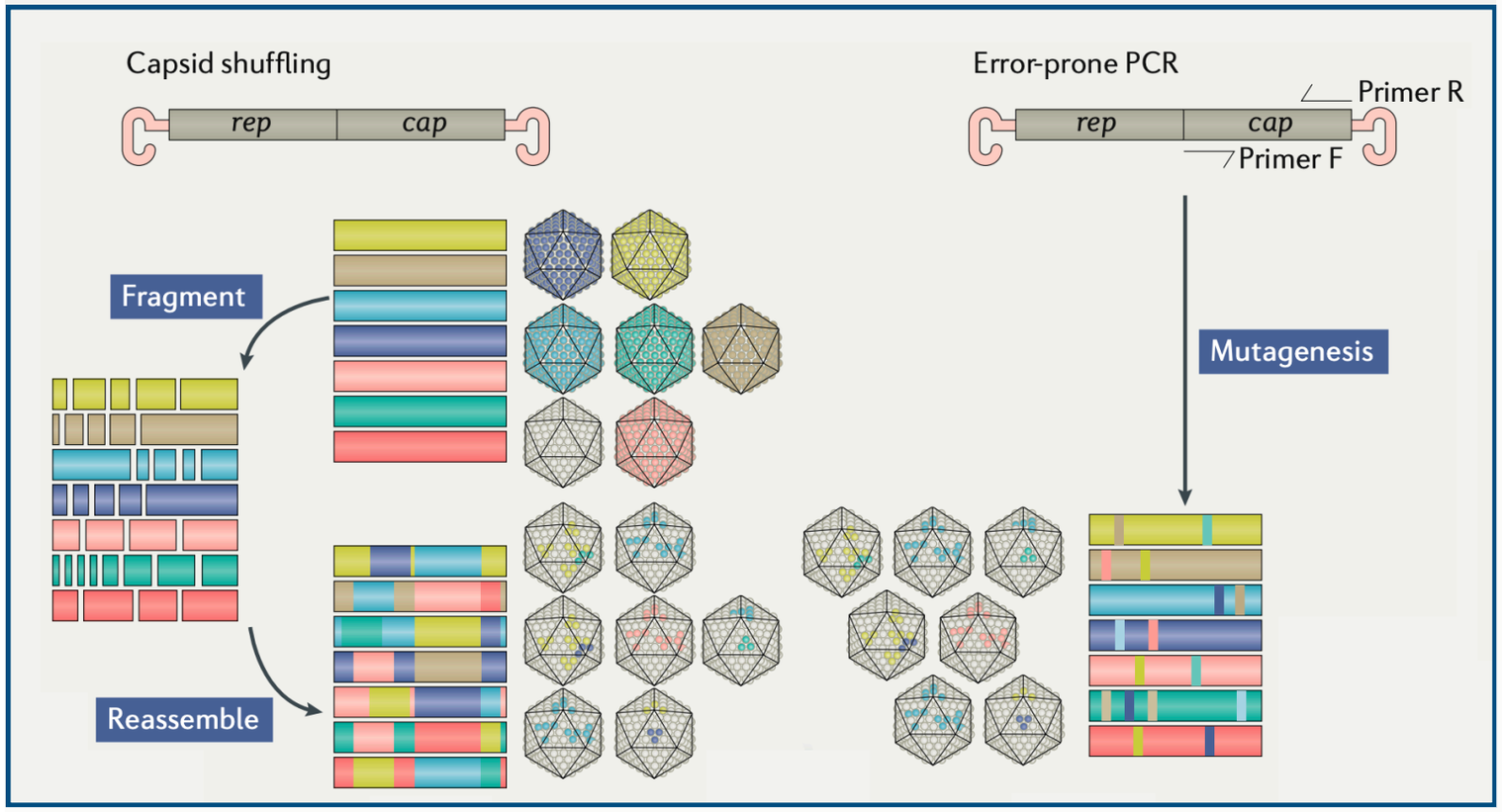
An estimated 30 to 60% of the population contains AAV NAbs

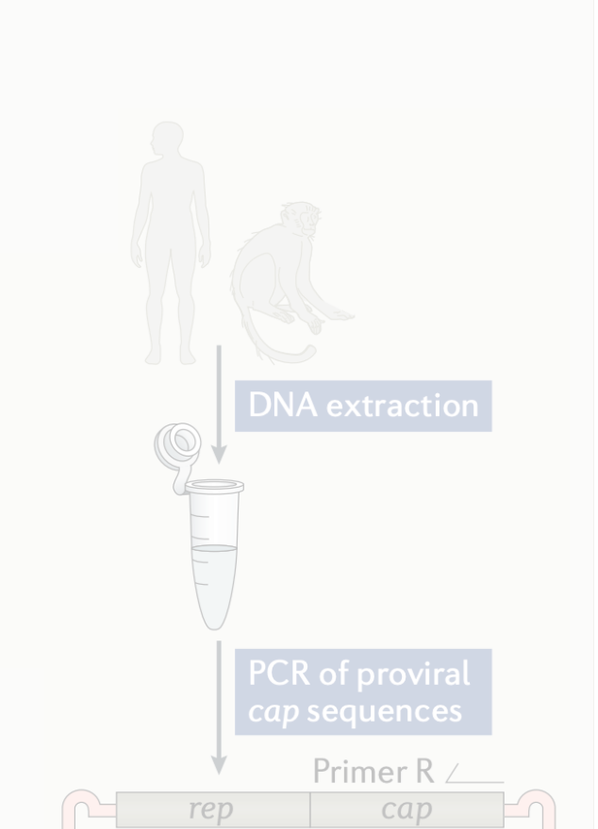
Novel AAV capsids may escape NAbs

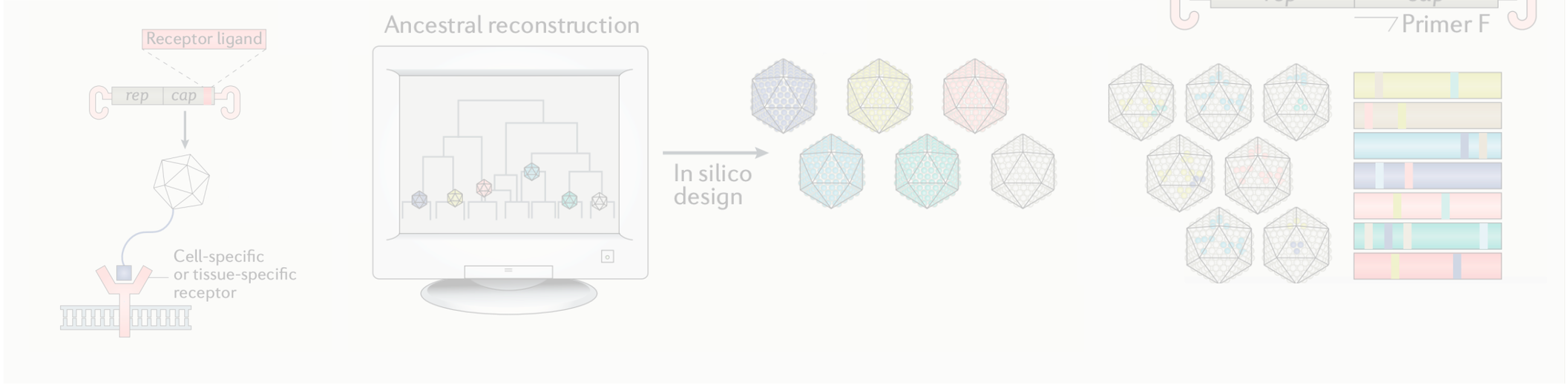
Calcedo, R.; et. al.; *J. Infect. Dis.* ***2009,*** *199,* 381.

AAV Biology

Discovery of AAV serotypes





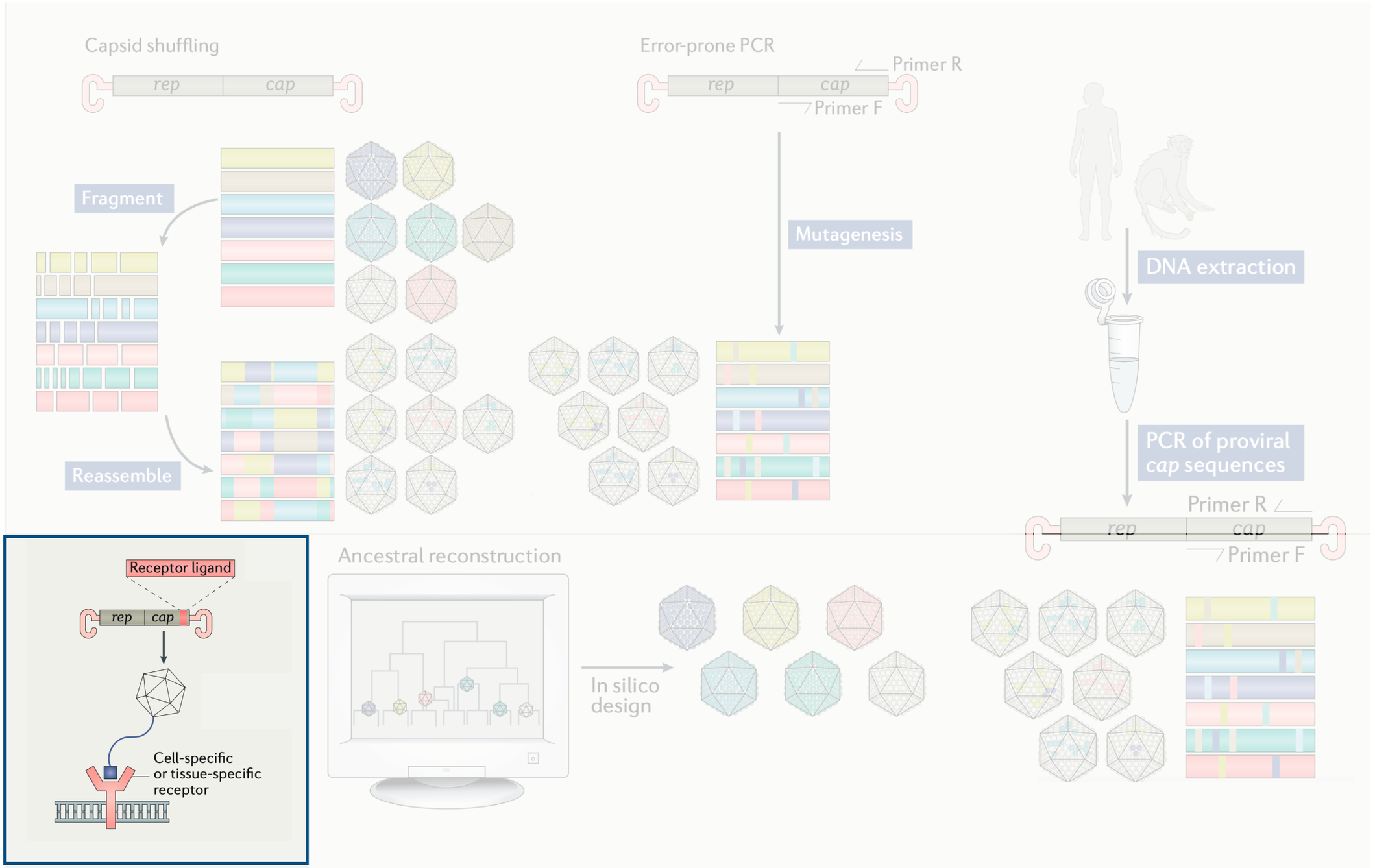


Directed Evolution

Wang, D.; et. al.; *Nat. Rev. Drug Discov.* ***2019,*** *18,* 358.

AAV Biology

Discovery of AAV serotypes

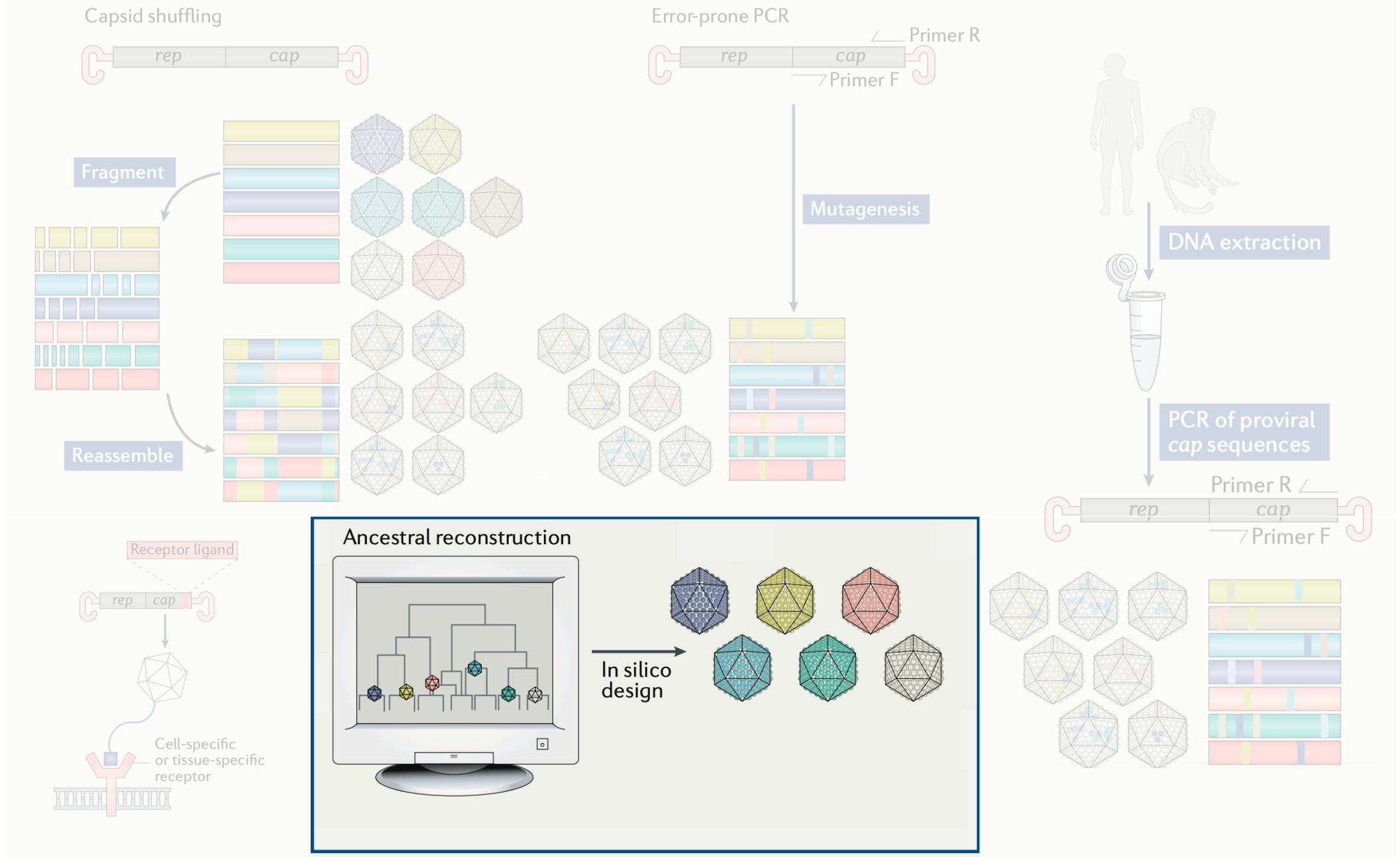


Rational Design

Wang, D.; et. al.; *Nat. Rev. Drug Discov.* ***2019,*** *18,* 358.

AAV Biology

Discovery of AAV serotypes



In Silico Design

Wang, D.; et. al.; *Nat. Rev. Drug Discov.* ***2019,*** *18,* 358.

AAV Biology

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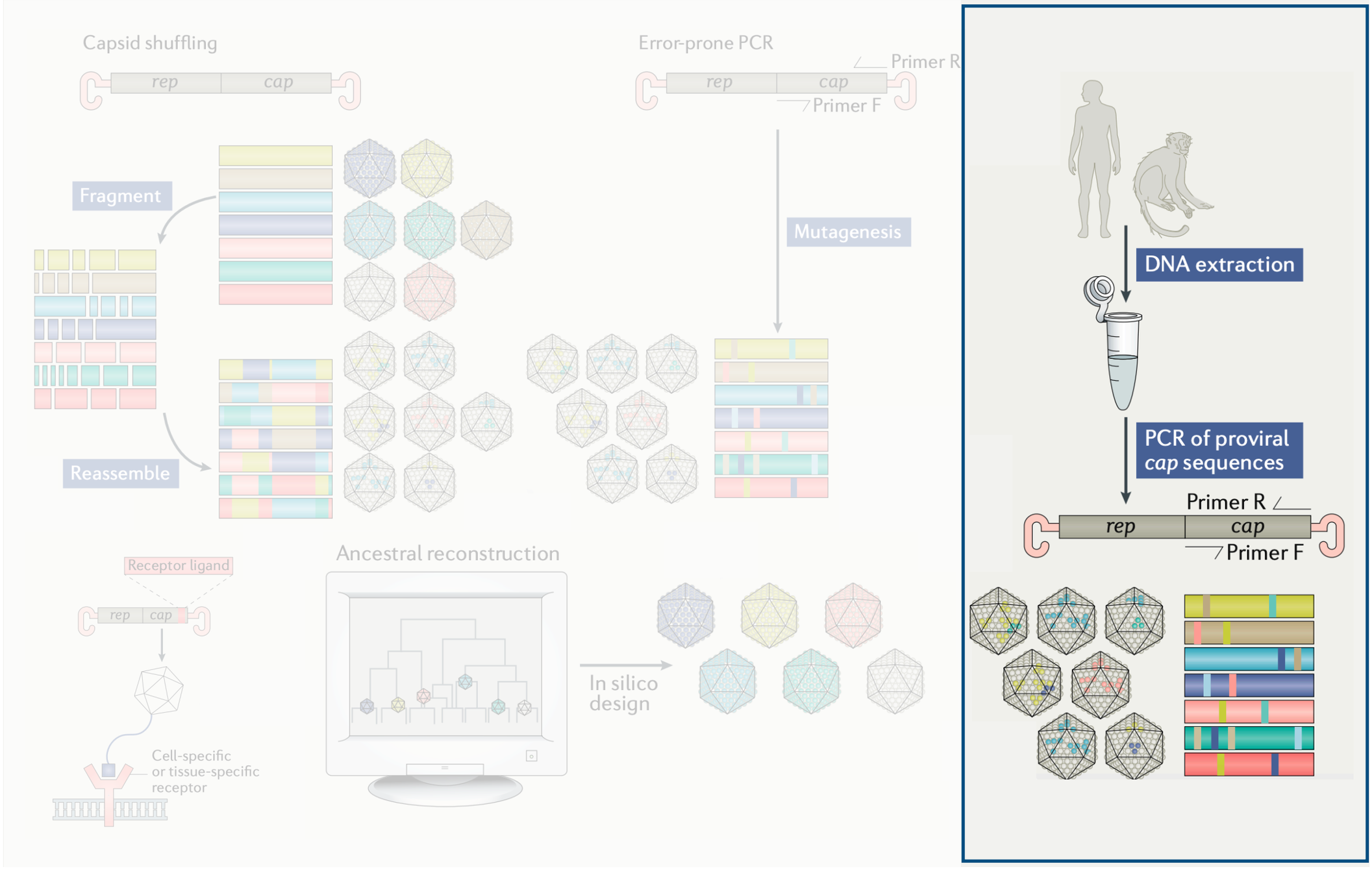
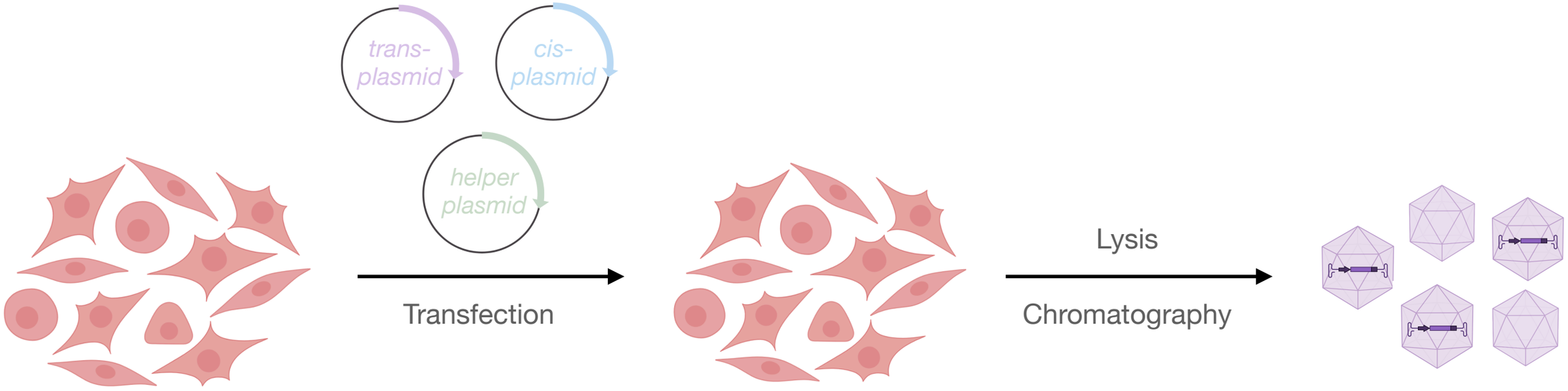


Naturally Occurring AAVs

Wang, D.; et. al.; *Nat. Rev. Drug Discov.* ***2019,*** *18,* 358.

AAV Biology

AAV production



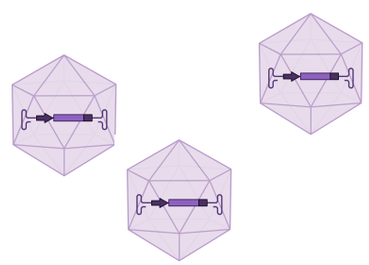
500 L Bioreactor

trans-plasmid — *AAV rep and cap genes*

cis-plasmid — *ITRs, promoter, and gene of interest*

helper plasmid — *Ad helper virus*

CsCl column or
Ion exchange



Filled AAVs

*On-scale AAV production is a **major challenge** to the field*

Srivastava, A..; et. al.; *J. Pharm. Sci.* ***2021,*** *110,* 2609.

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Approved therapeutics

Inherited blindness
2017 FDA approval
AAV 2

Lipoprotein lipase deficiency
2012 EMA approval
AAV 1
(Removed from market)

Spinal muscular atrophy
2019 FDA approval
AAV9

101 active clinical trials
Eye, liver, muscle and CNS are major targets
AAV2 is the most common serotype

Current Challenges and Outlook

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uniQure
The leader in gene therapy

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NOVARTIS

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AAV9

FDA

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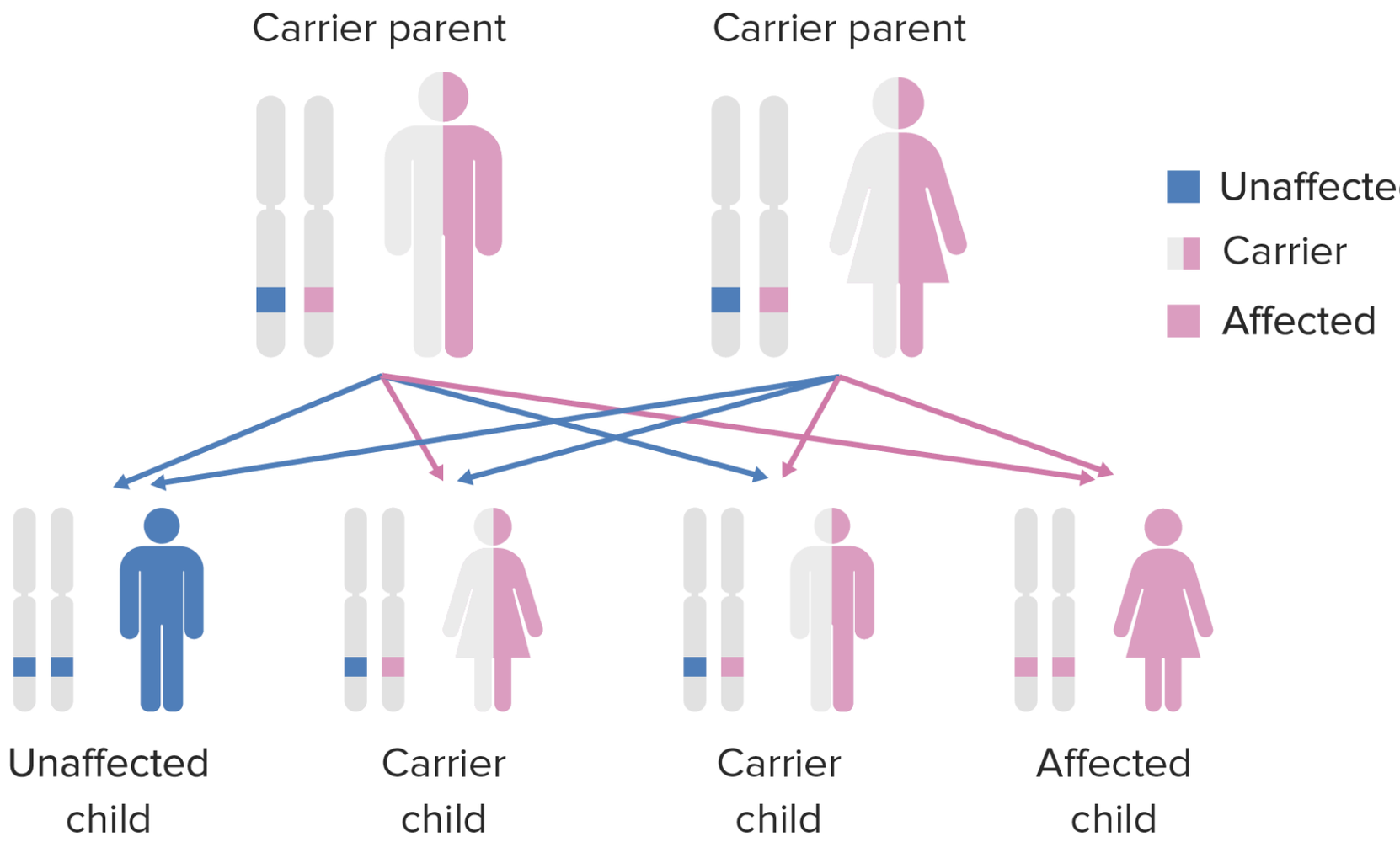
Current Challenges and Outlook

Case study: Leber's Congenital Amaurosis



Kumaranm, N.; et. al.; *Br. J. Opthalmol.* ***2017,*** *101,* 1147.

Current Challenges and Outlook

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Leber's Congenital Amaurosis

Autosomal recessive inherited blindess

Affects 3 out of 100,000 births

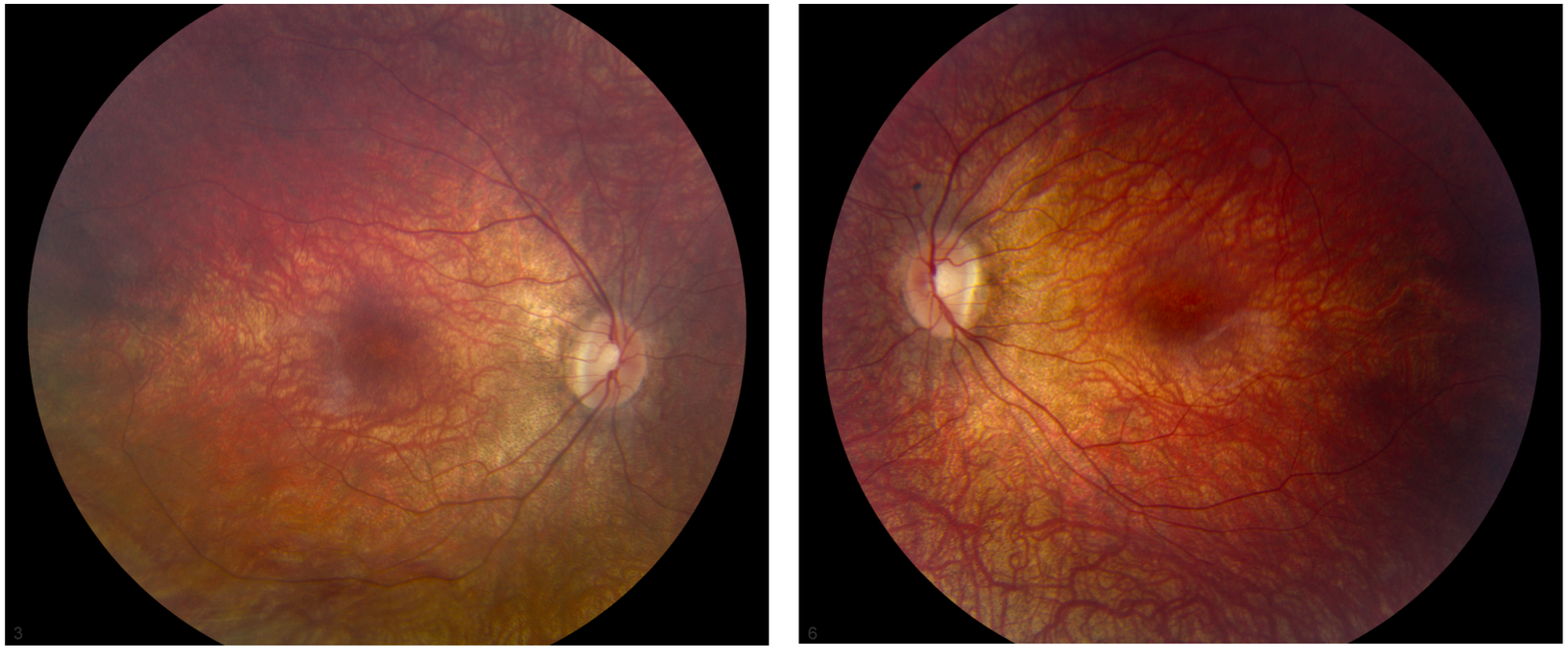
Accounts for ~20% of cases of inherited blindness

25 distinct mutation patterns identified

8% of patients suffer from RPE65 mutation

Left Eye

Right Eye



Fundus photos revealing punctuate yellow dots and pigment mottling associated with LCA

Kumaranm, N.; et. al.; *Br. J. Opthalmol.* ***2017,*** *101,* 1147.

Current Challenges and Outlook

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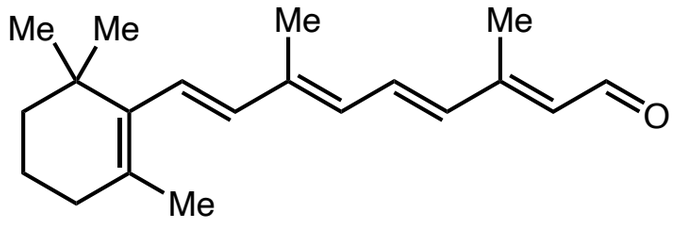

Rod outer segment

Retinal pigment epithelium

Tsin, A.; Betts-Obregon, B.; Grigsby, J.; *J. Biol. Chem.* ***2018,*** *294,* 13016.

Current Challenges and Outlook

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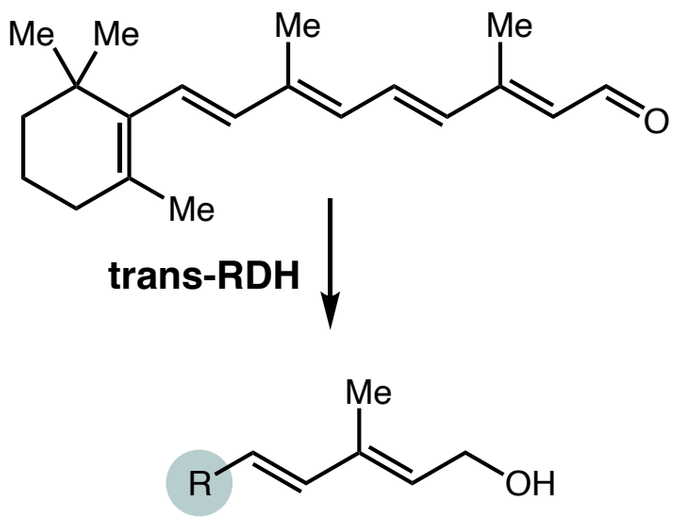

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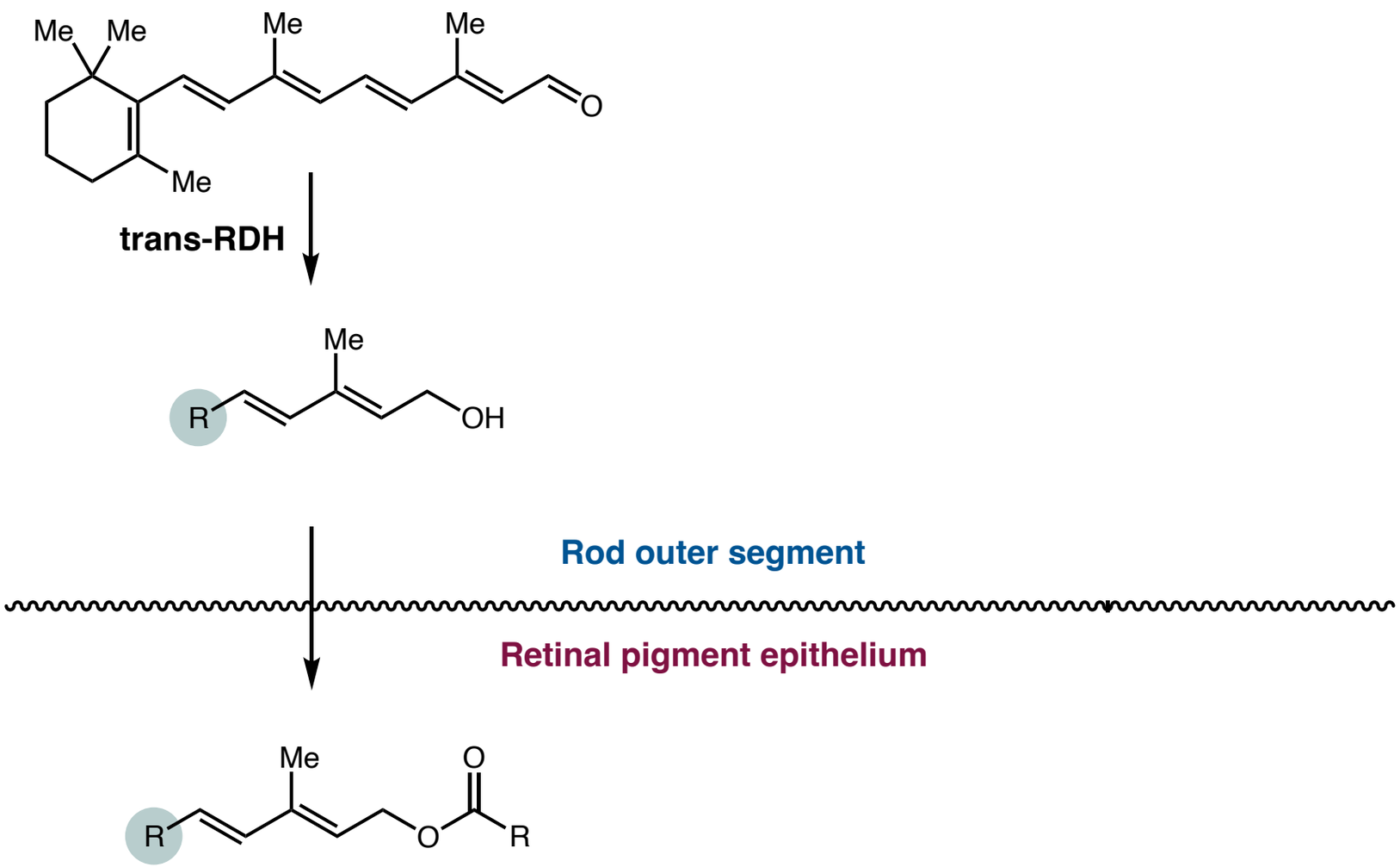

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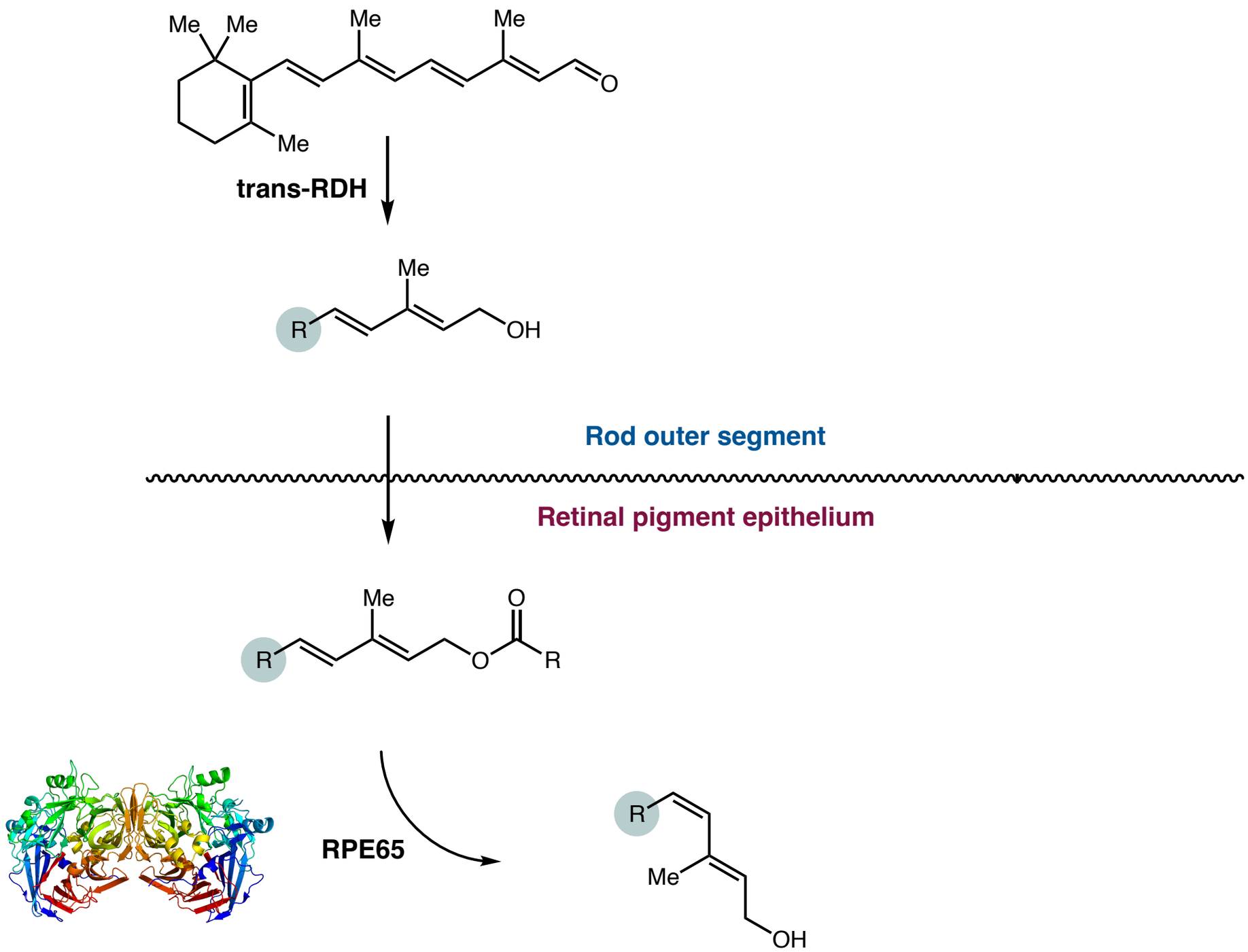

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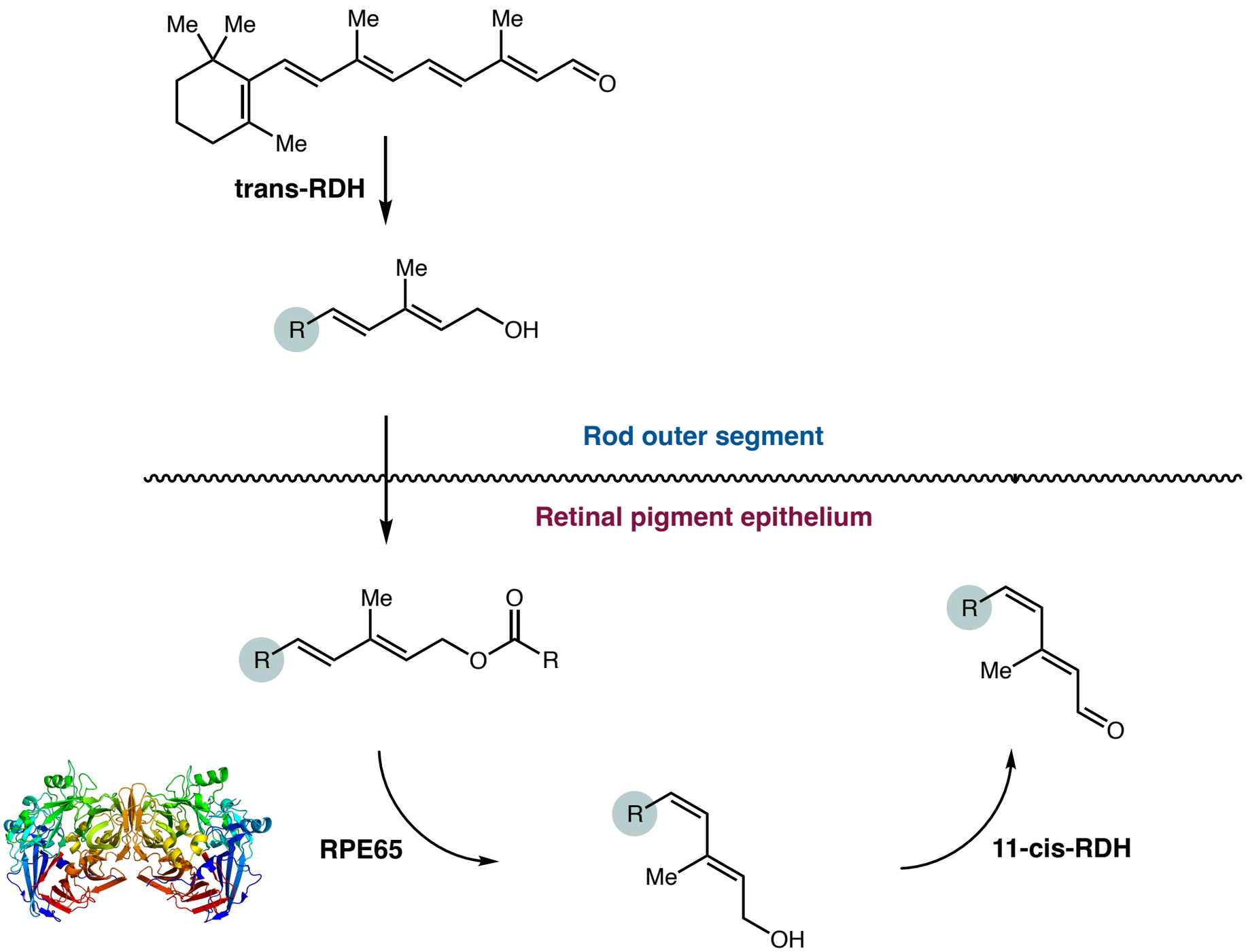

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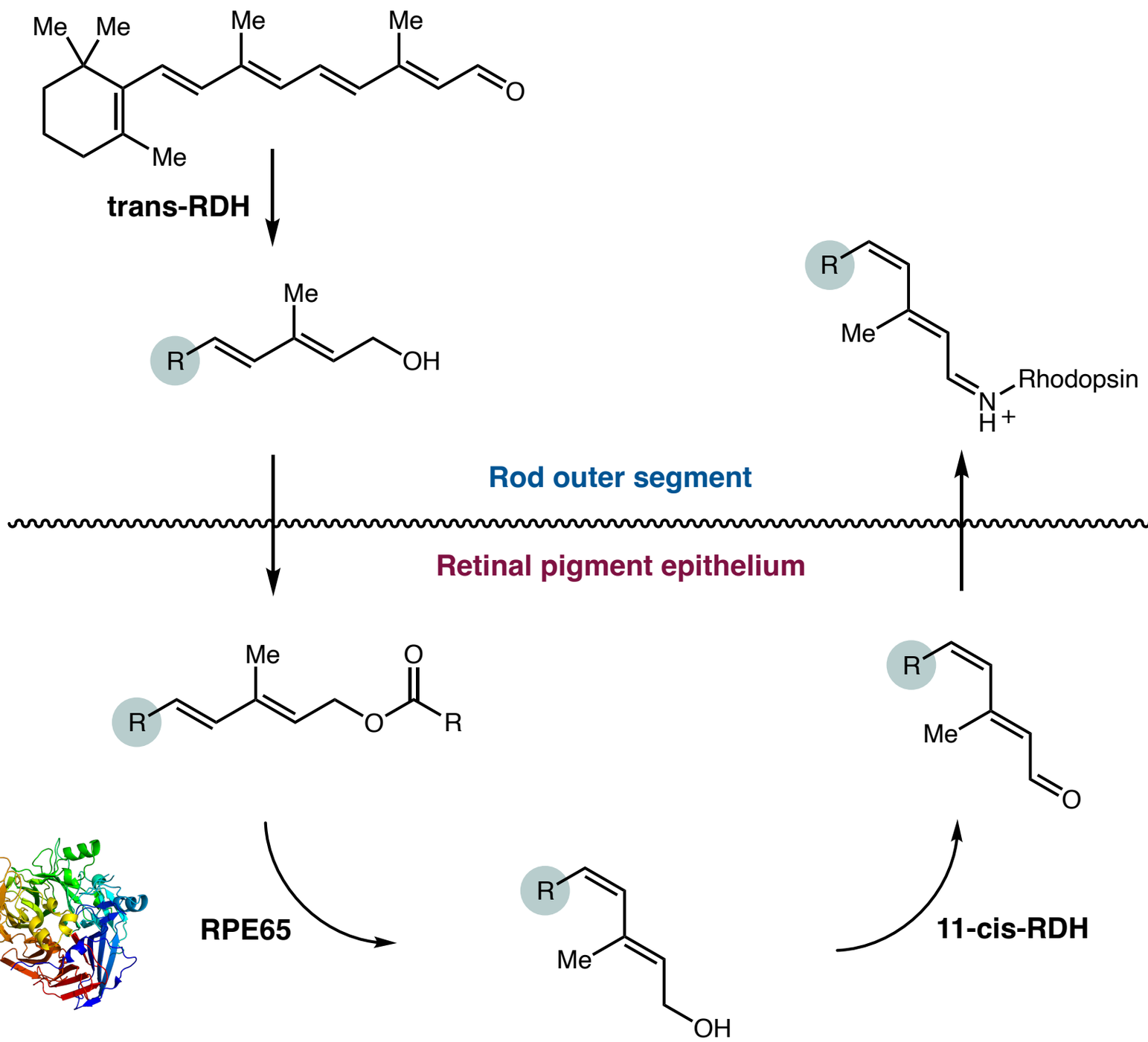

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Current Challenges and Outlook

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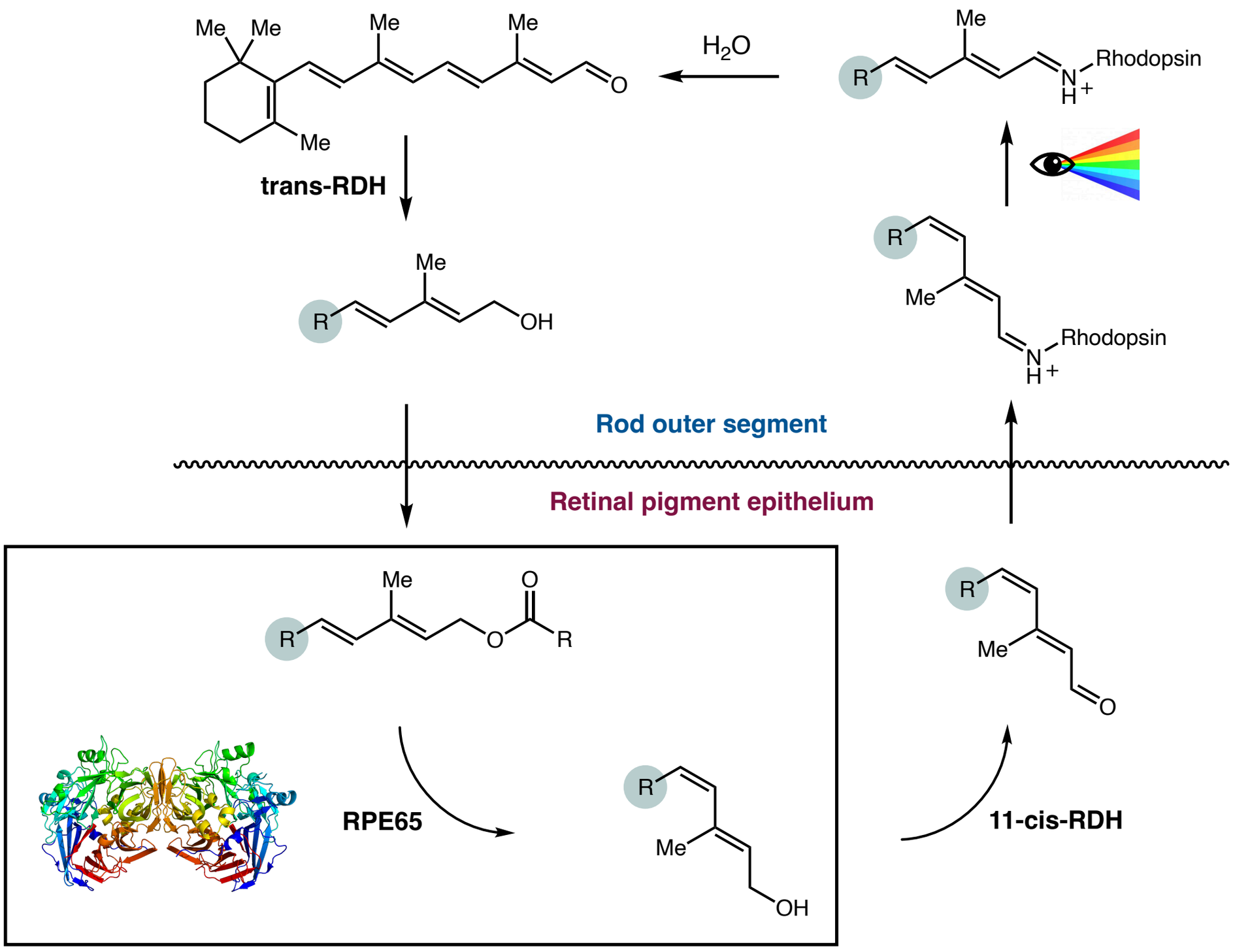

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Current Challenges and Outlook

Case study: Leber's Congenital Amaurosis

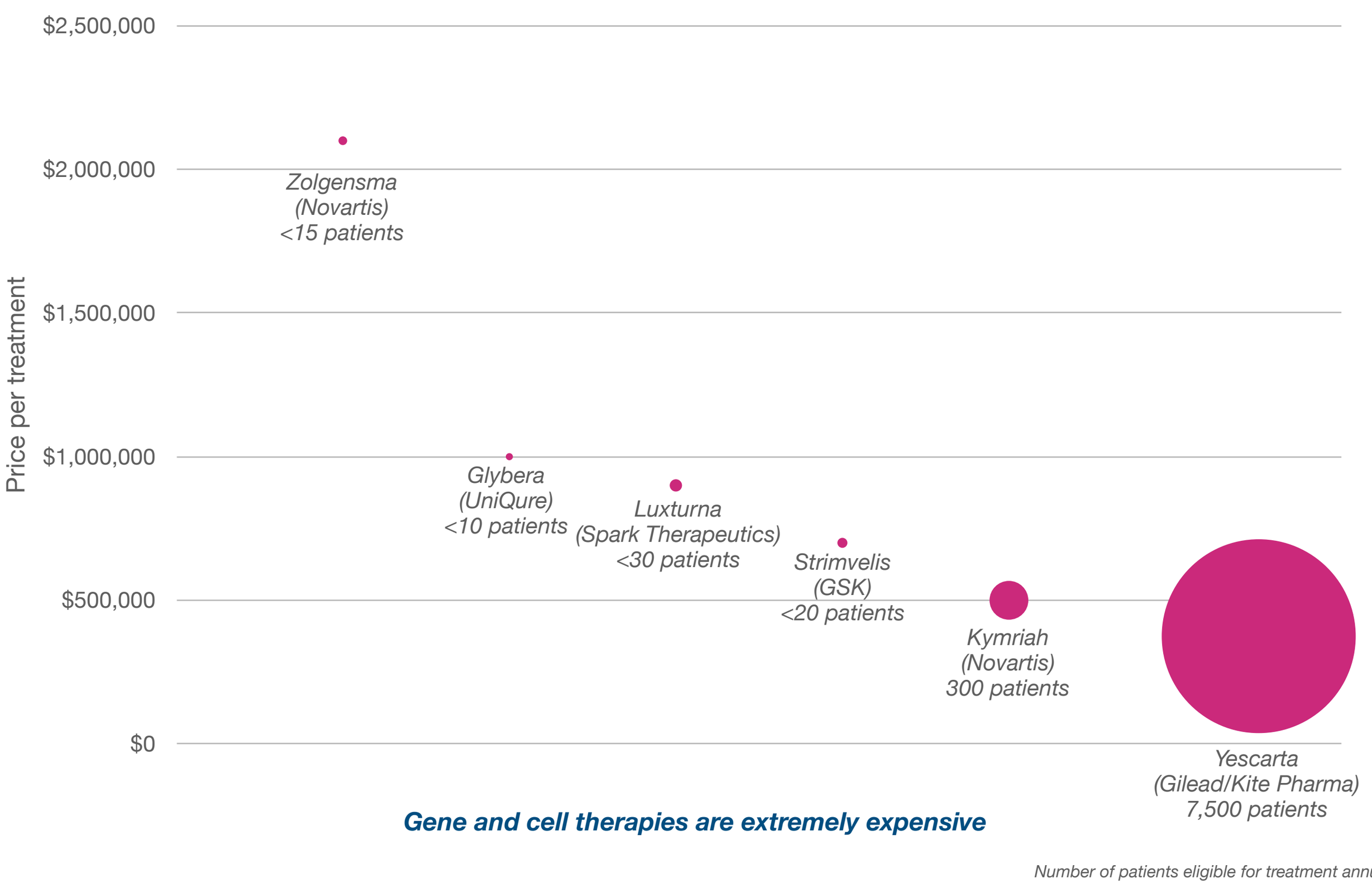
Sponsor	Perelman School of Medicine University of Pennsylvania	Spark Therapeutics	UCL
Gene	RPE 65	RPE 65	RPE 65
AAV Capsid	AAV 2	AAV 2	AAV 2
Outcome	Regression in visual function after 3 years	No regression in vision after 3 years	Regression in visual function after 3 years

"Subtle differences in manufacture processes, final formulation, design of expression cassette, or adjuvant immunomodulatory regimes could potentially affect long-term efficacy"

Dunbar, C. E.; et. al.; *Science* ***2018,*** *359*.

Current Challenges and Outlook

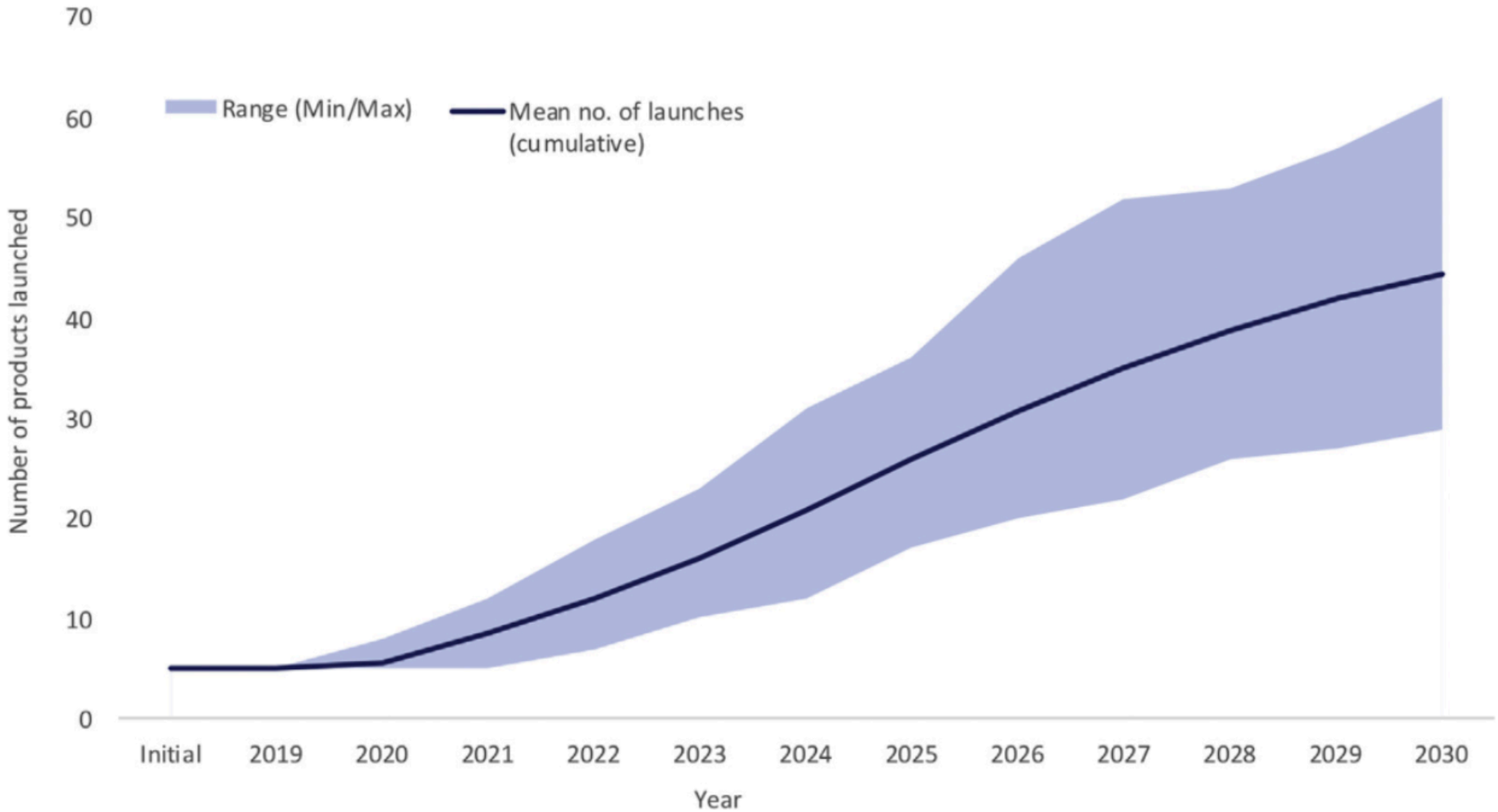
Case Study: Economics of "regenerative therapies"



Gene and cell therapies are extremely expensive

Current Challenges and Outlook

Case Study: Economics of "regenerative therapies"



- An estimated ~50 new cell and gene therapies could be launched by ~2030

- 650,000 individuals will most likely receive a treatment within the next 10 years

- Likely to become a $24.4 billion (annual) market by 2030

Quinn, C.; et. al.; *Value Health.* ***2019,*** *22,* 621.

Bainbridge, J. W.; et. al.; *Drug Discov.* ***2022,*** *27,* 17.

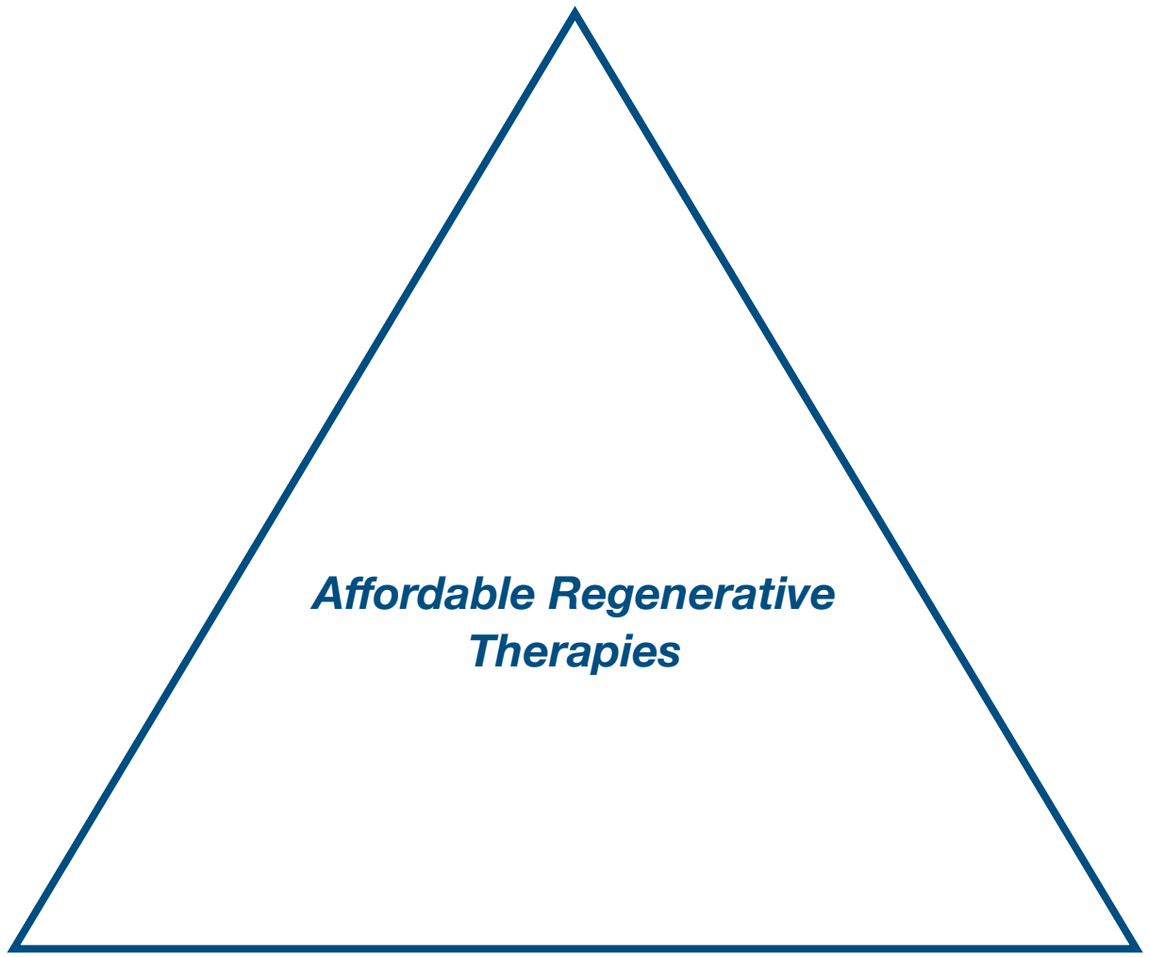
Current Challenges and Outlook

Case Study: Economics of “regenerative therapies”

Novel Payment Models and Regulation

Outcome based payments

Amortized pricing



Reducing Costs

Improvements in on-scale production

Inherent Drivers

Rare diseases have limited patient populations

Quinn, C.; et. al.; *Value Health.* ***2019,*** *22,* 621.

Bainbridge, J. W.; et. al.; *Drug Discov.* ***2022,*** *27,* 17.

Outline

What is Gene Therapy?

History of Gene Therapy

AAV Biology

Challenges and Outlook

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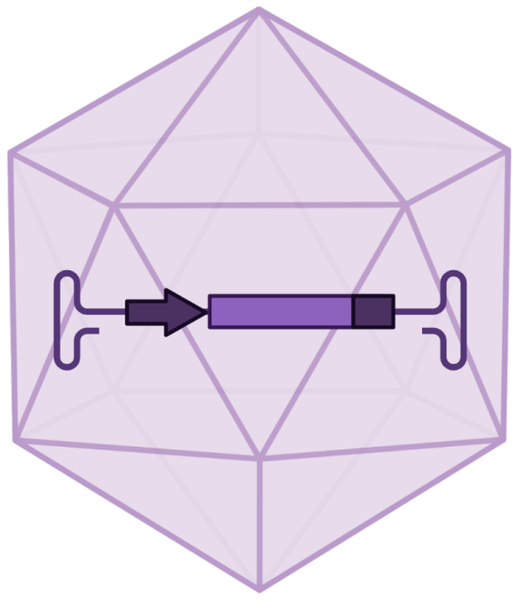
Challenges and Outlook

Introduction to Gene Therapy and AAV Therapeutics

Summary

Promising therapy for curing monogenetic diseases

Improvements in evading the immune system (NAbs in particular) enable dosing larger populations



AAV Vector

Further understanding of basic AAV biology (Novel capsids)

High cost of manufacturing must be reduced to increase adoption

Acknowledgments

Prof. David MacMillan

The MacMillan group

— **Advisory Committee** —

Prof. Erik Sorensen

Prof. Mohammad Seyedsayamdost