# AnelloBricks®: Development of a Scalable, Low-Cost, In-Vitro Assembled, Anellovirus-Derived Platform for Gene Therapy Applications


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  *Chief Scientific Officer, Ring Therapeutics*



**ASGCT 2025**

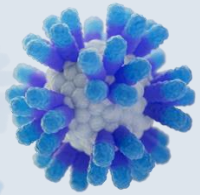
# DISCLOSURES

- Work was performed at Ring Therapeutics by Yue Zhang, Nidhi Mukund Acharekar, Andrew Keezer, Lynn Zeheb, Rajendra Boggavarapu, Hyun Jung Jun, Kurt Swanson, Konstantin Konstantinov, Geoffrey Parsons, Timsi Rao, Erik Hansen
- Ring employees receive salary and equity-based compensation.

# What if the ideal vector is already inside us?

## Commensal

A relation between two kinds of organisms in which one obtains food or other benefits from the other without harm or benefit



## Anellovirus (ANV)

*Anelloviridae* family
~30 nm in diameter

Ubiquitous, persistent DNA viruses with high genetic diversity that appear to have coevolved with their mammalian hosts, including humans, over a long evolutionary timescale

## Pathogenic viruses[1]

**Zika Virus**
*Flaviviridae* family
~50 nm in diameter

**Adenovirus**
*Adenoviridae* family
~100 nm in diameter

**Influenza A Virus**
*Orthomyxoviridae* family
~100 nm in diameter

**Coronavirus**
*Coronaviridae* family
~100 nm in diameter

**HIV**
*Retroviridae* family
~120 nm in diameter

**Ebola Virus**
*Flaviviridae* family
~80 nm x 800 nm in diameter

[1]HHMI Biointeractive Virus Explorer: https://media.hhmi.org/biointeractive/click/virus-explorer/index.html





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# Anelloviruses have evolved and lived in harmony with humans for millennia[1]

## Intrinsic traits

**Ubiquitous biodistribution**

**Natural immune evasion**

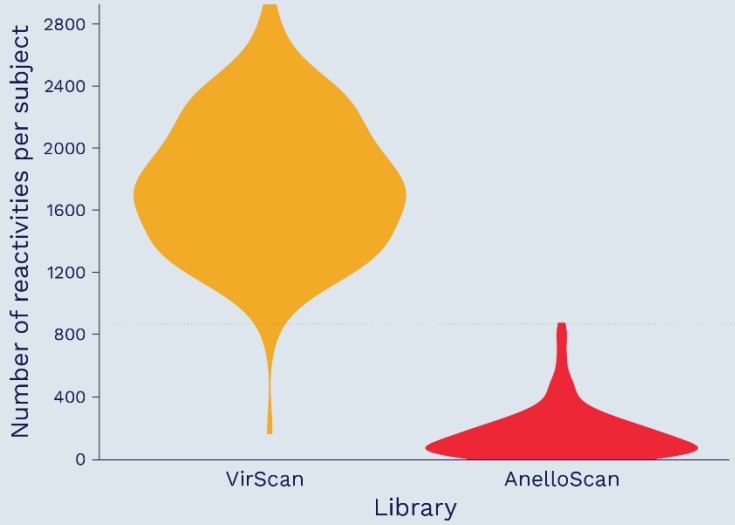
**Potentially unique tissue tropism**

## Human virome

**97.7%**
*Anelloviridae*[1]

**2.3%**
Other[1]*

## Immune Evasion





1. Cebriá-Mendoza et al., 2021





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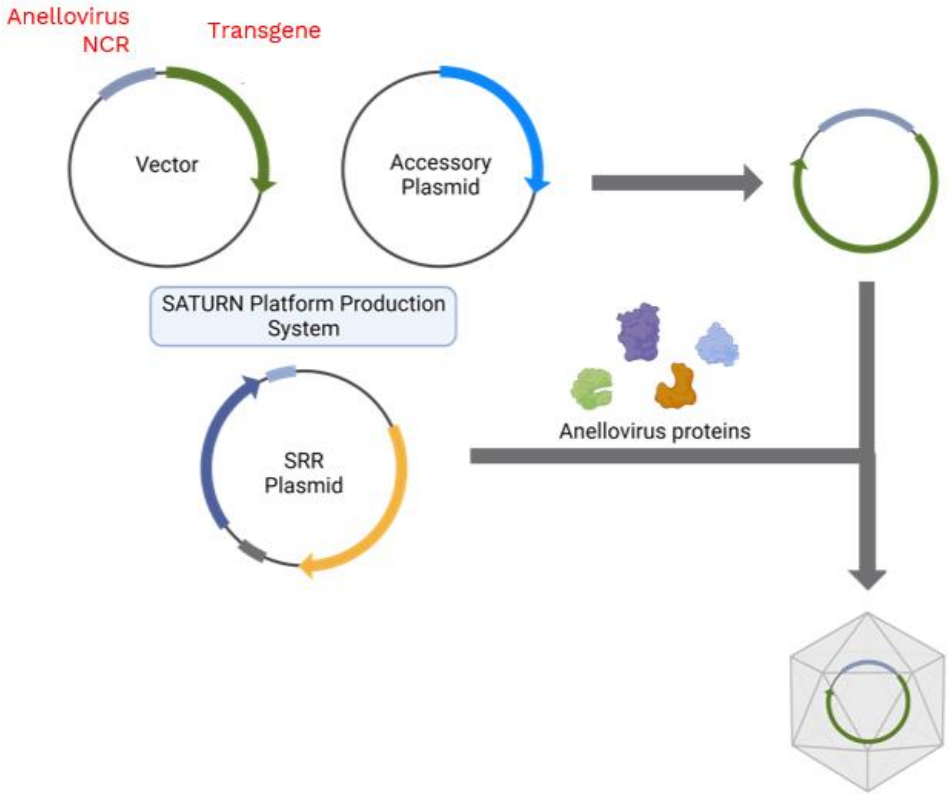
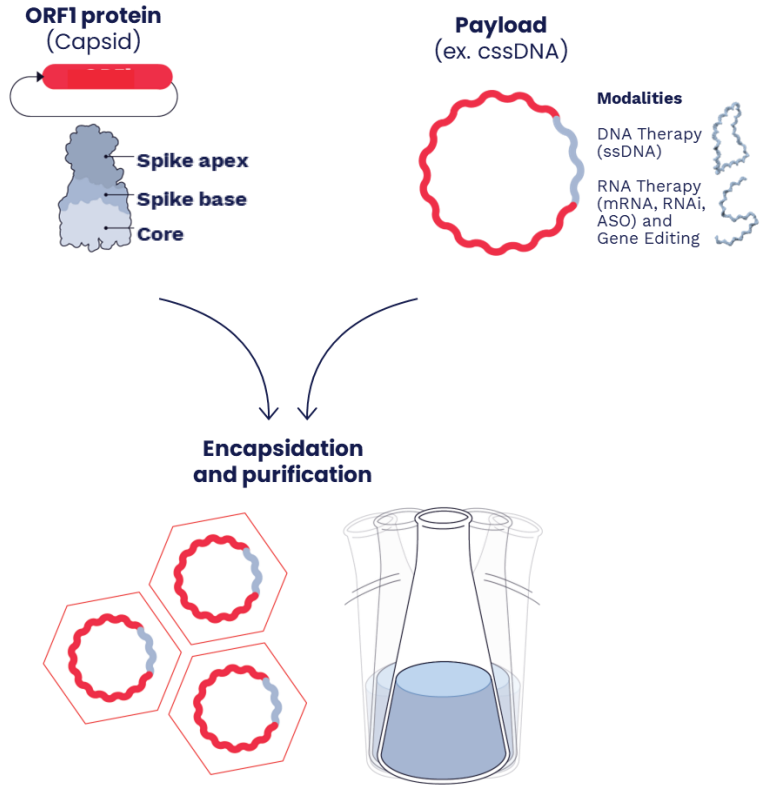
# Two methods of producing Anellovectors

## **RingX**: cell-based system



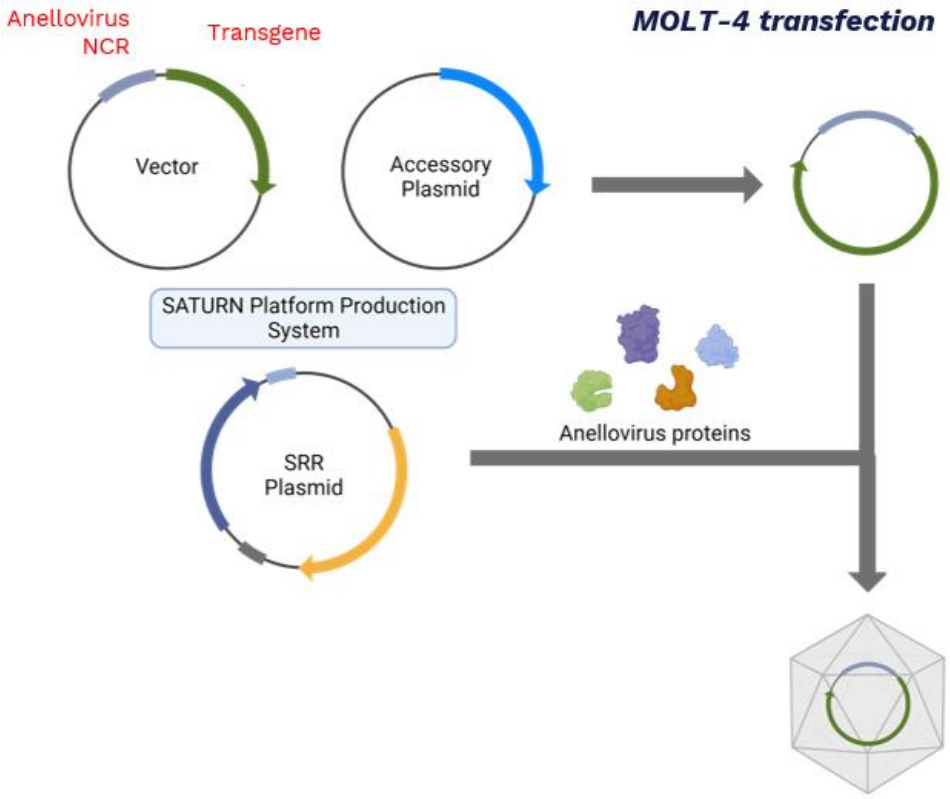

*Should yield AnelloVectors® with similar phenotypic properties*

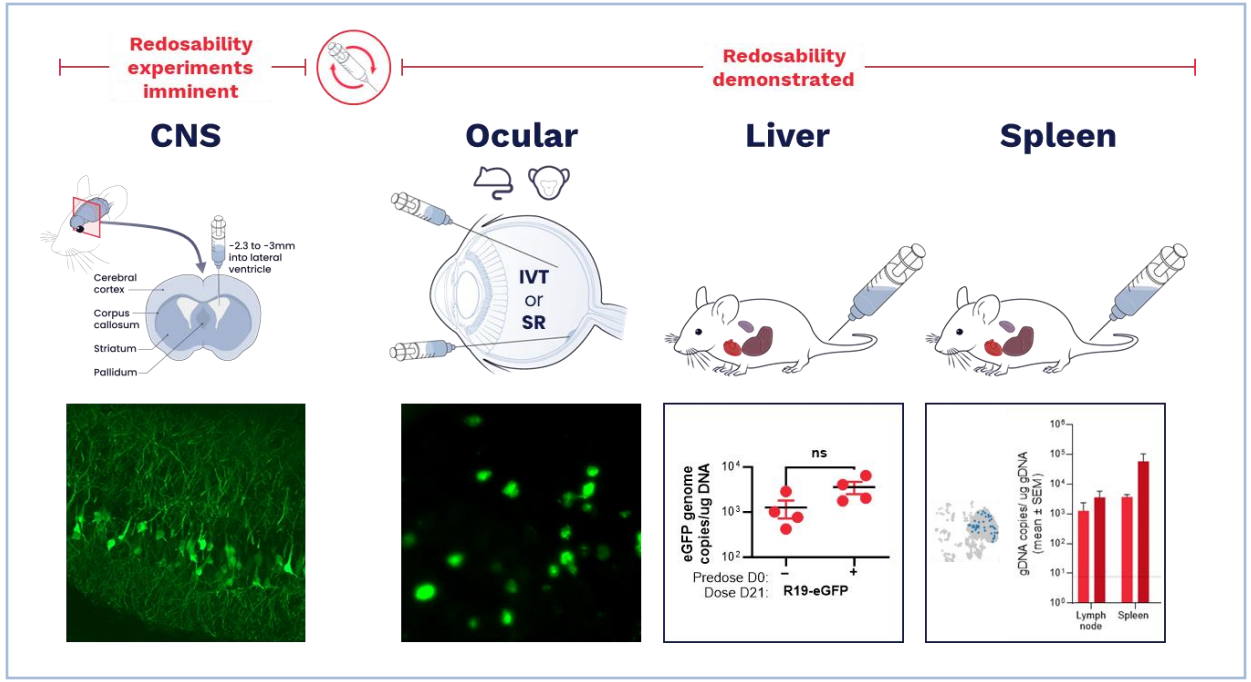
## **AnelloBricks®**: *in vitro* assembly



# RingX validated biology with *in vivo* demonstration across several tissues

**RingX**: cell-based system

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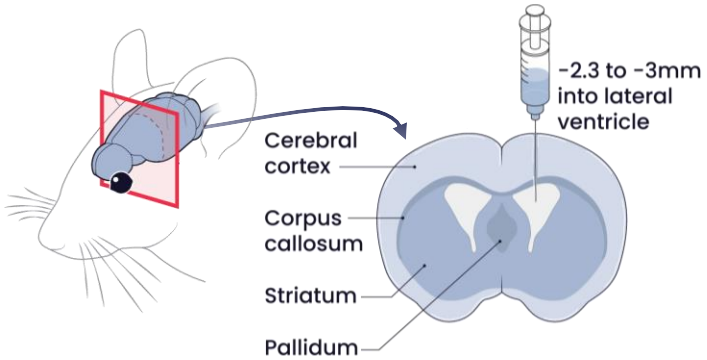
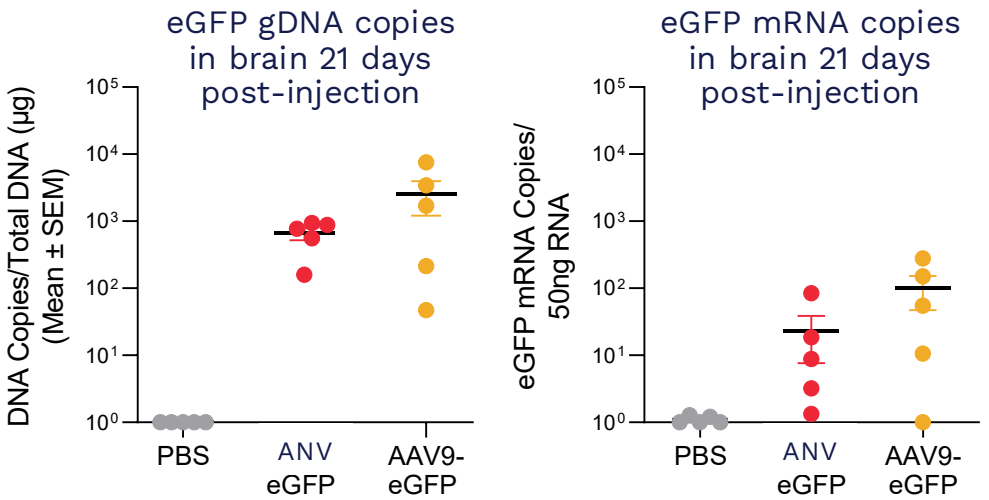
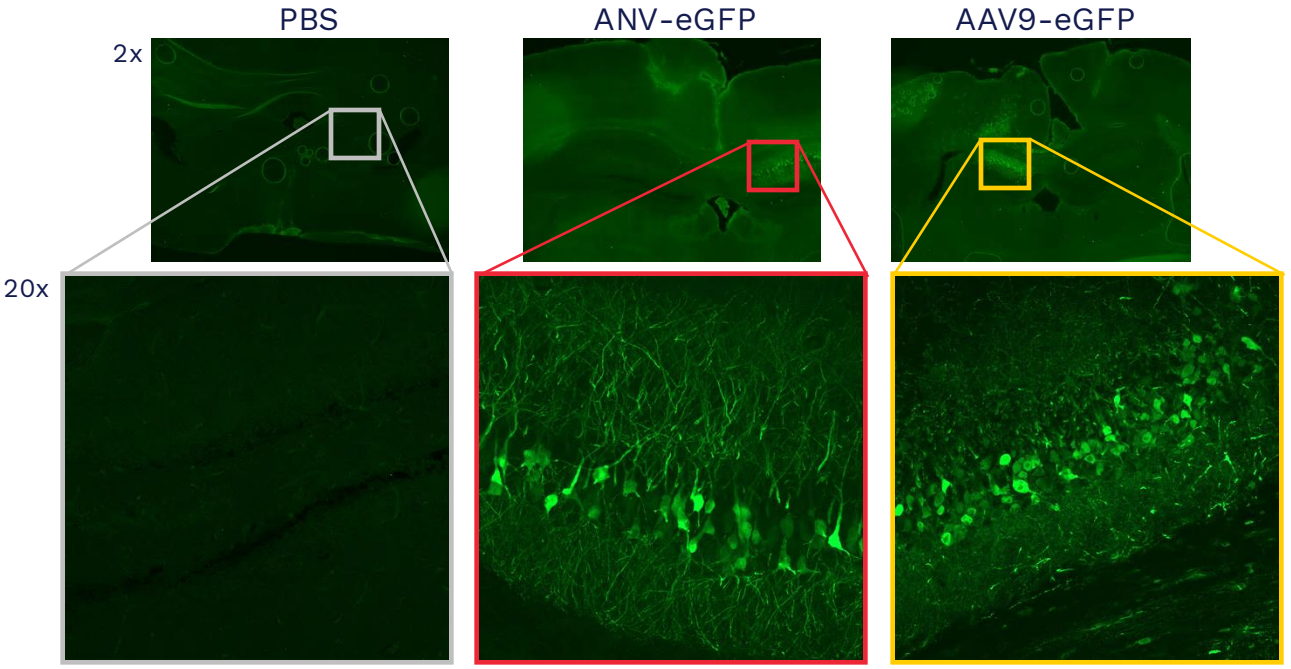
# RingX: Robust CNS transduction, potential tox benefits




RingTx® differentiation

**Safety:** AnelloVector neurons presented potentially "healthier" morphology. AAV9 toxicity known from literature.[1]

Study Designs: CNS/ICV: D0 Intracerebroventricular injection» D21 Harvest Tissue (ANV-fCMV-eGFP; AAV9-eGFP: AAV9-fCMV-eGFP).
eGFP, enhanced green fluorescent protein; qPCR, quantitative polymerase chain reaction.
1. References available upon request





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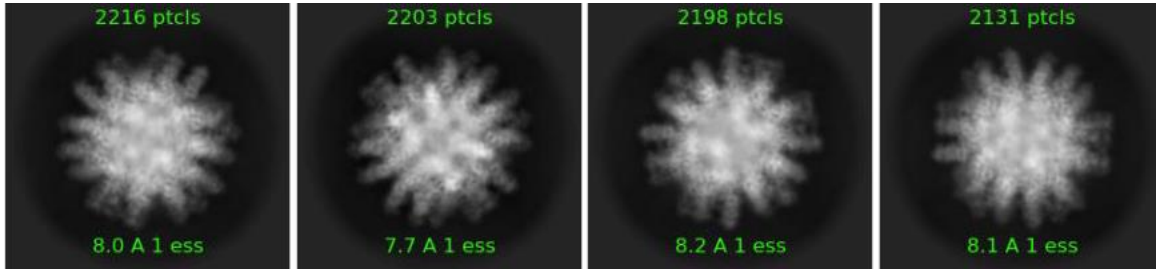
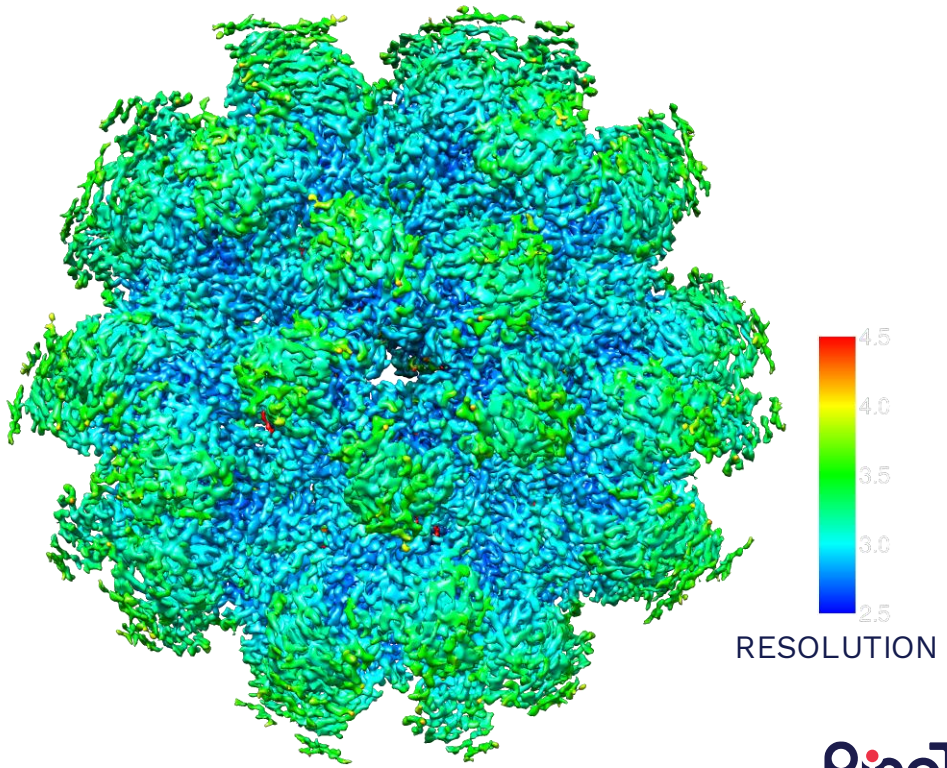
# The first structure of an anelloviral-like particle

Cryogenic electron microscopy




## A single protein forms the ANV particle

- ORF1 is the anellovirus capsid protein
- 60 monomers of the anellovirus capsid protein (ORF1) assemble into an icosahedral particle core
- Spike domains extend to form the particle surface
- The hypervariable region (HVR) resides at the tops of the spikes




**Liou, Sh., Boggavarapu, R. et al. Nat Commun 15, 7219 (2024)**



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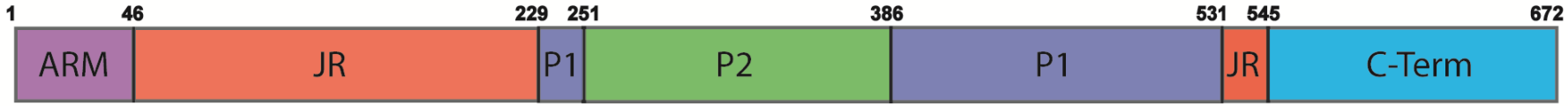
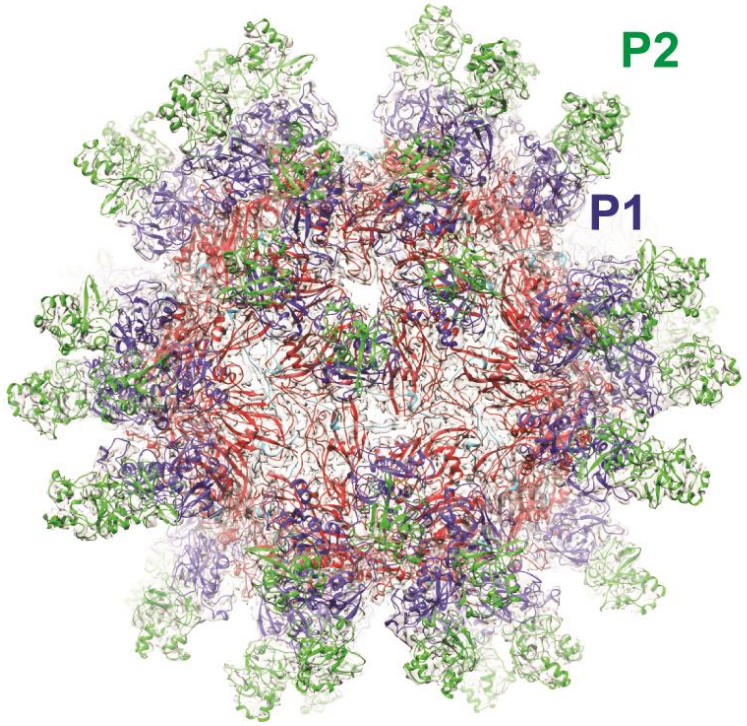
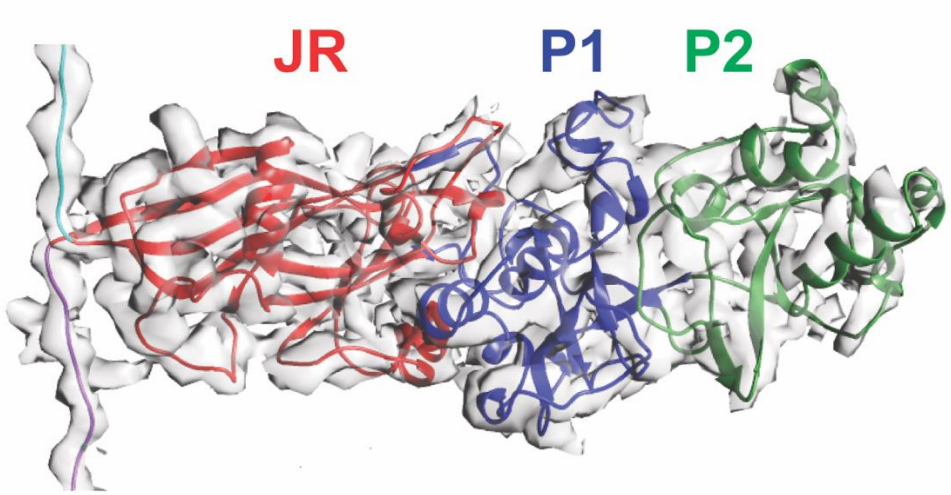
# ORF1 is the capsid protein




- The particle core is comprised of jelly role domains that bind the viral genome
- An unstructured N-terminal Ariginine-rich motif (ARM) domain is also thought to bind the viral genome

Liou, Sh., Boggavarapu, R. et al. Nat Commun 15, 7219 (2024).



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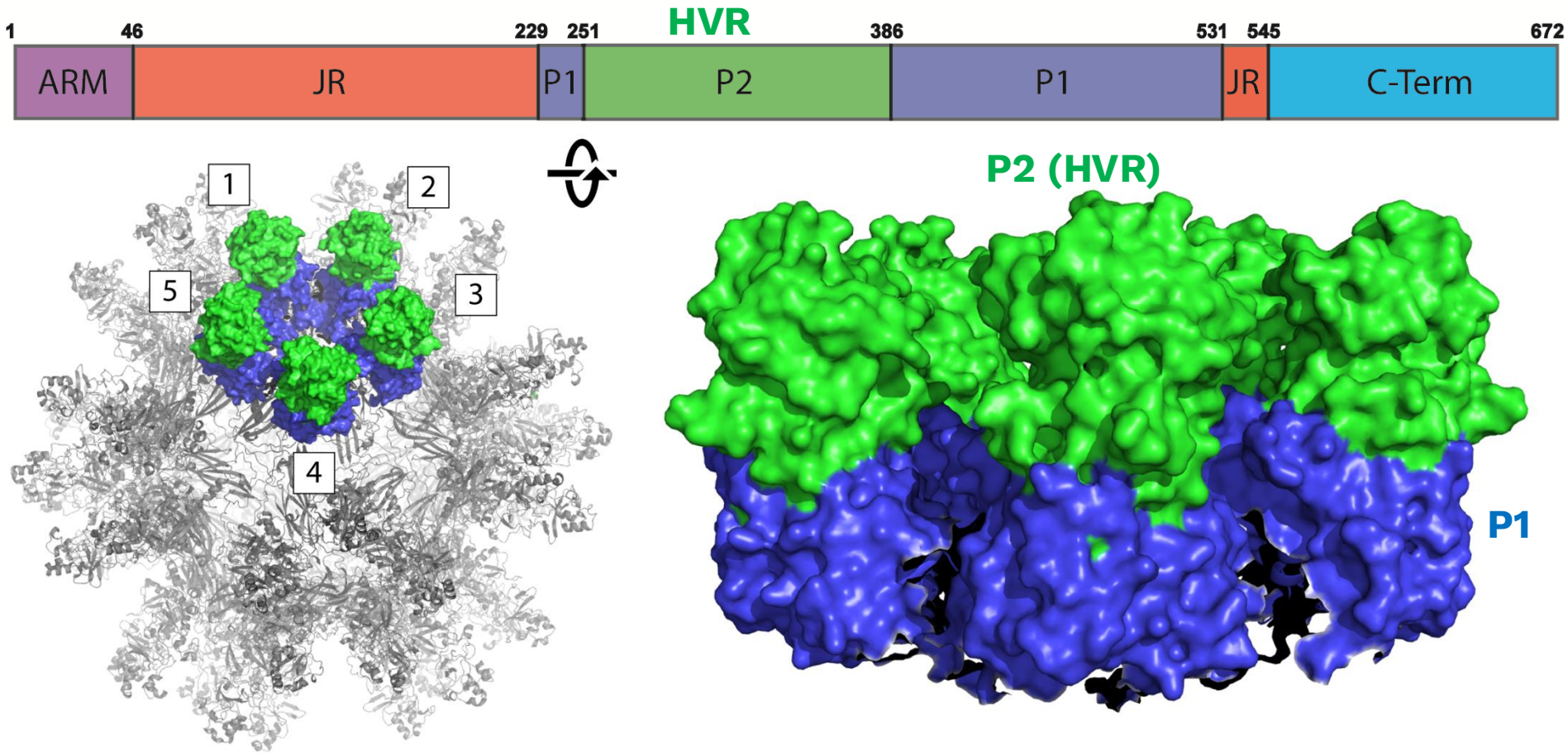
# The anellovirus capsomere is a pentamer of ORF1




Five spike domains sit on the 5-fold axis forming a “crown” structure
**Hypervariable Region** forms the apex of the crown, more conserved sequences of the **P1** subdomain and **JR** below.



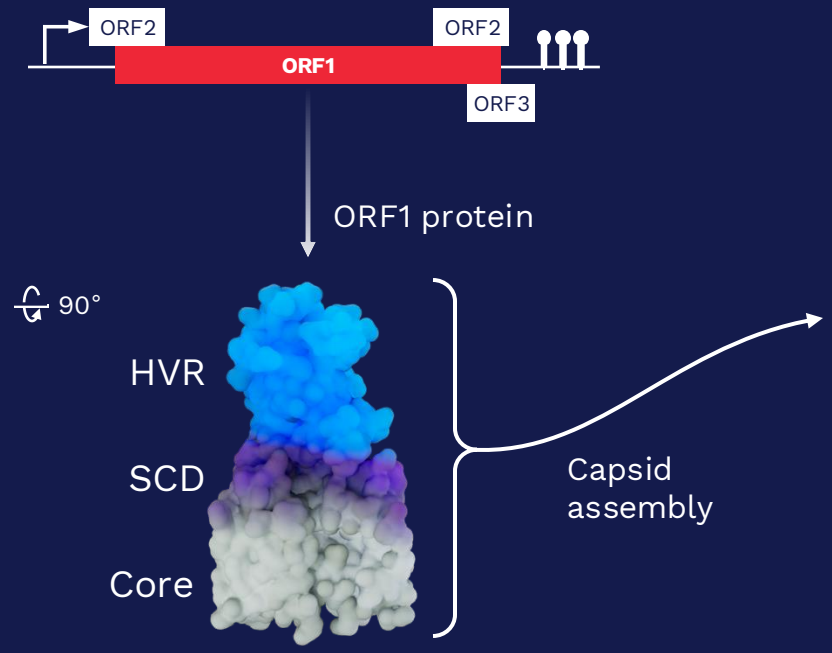
Liou, Sh., Boggavarapu, R. et al. Nat Commun 15, 7219 (2024).

# AnelloBricks® – an *in vitro* process to assemble gene therapy vectors

Inspired by the structure of the anelloviral particle

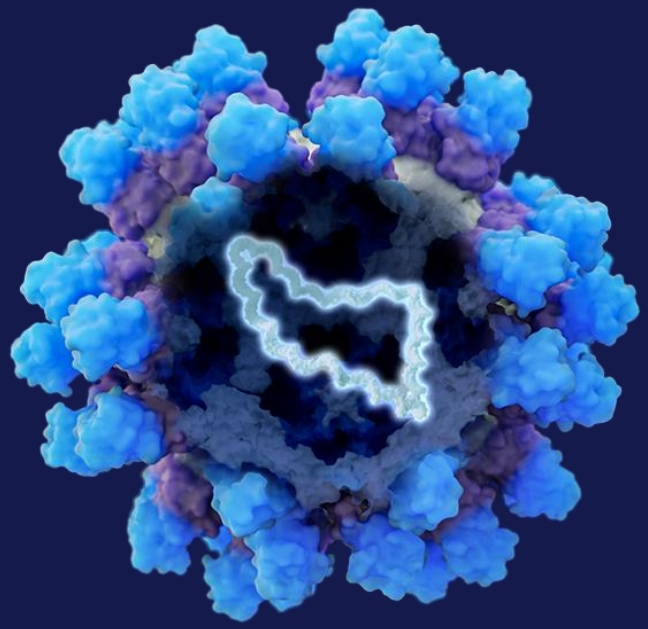
## Capsid defines key properties

(single capsid protein – ORF1)




## *In vitro* assembly platform enabling payload versatility

>30,000 ORF1 sequence library available for assembly



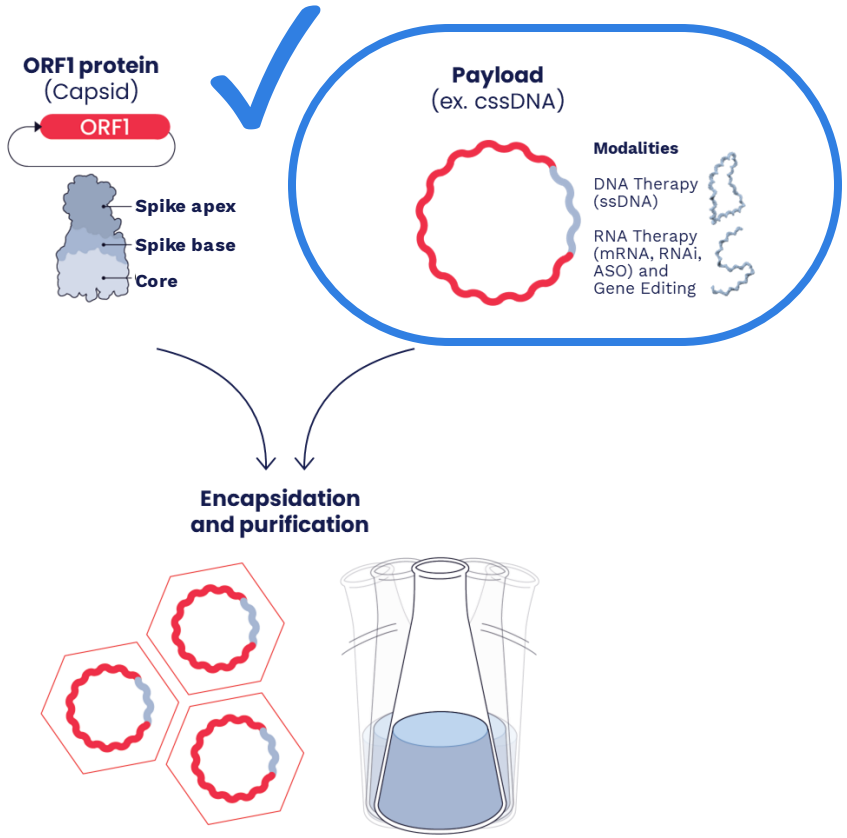
**AnelloBricks®**

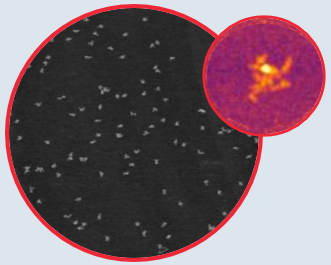
- Can control whether particles are empty or not
- Ability to package different payloads


Abbreviations: HVR, hypervariable region; ORF, open reading frame; ORF1, capsid protein; SCD, semi-conserved domain.

# cssDNA production and characterization

## AnelloBricks®: *in vitro* assembly

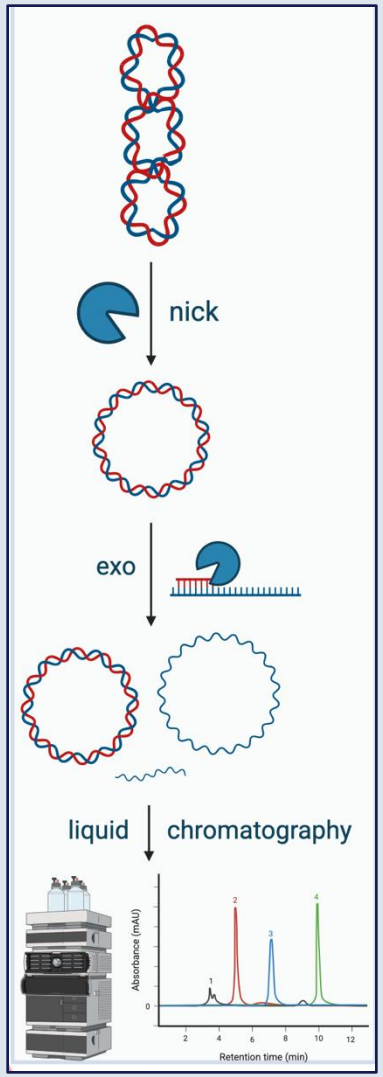


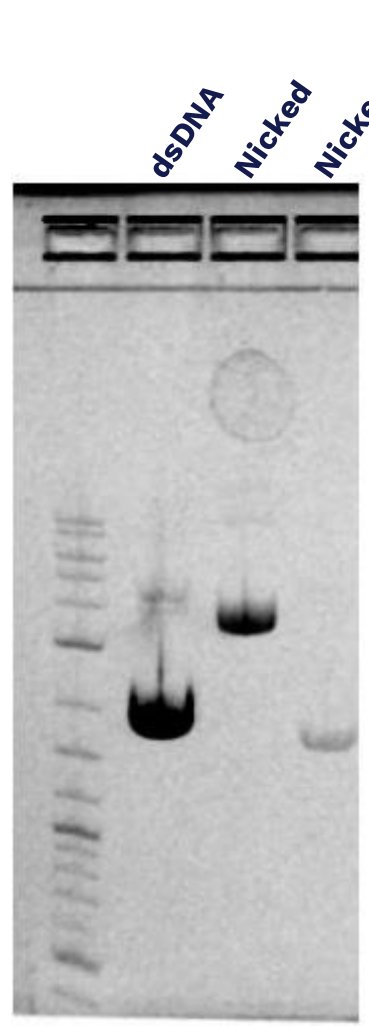



**cssDNA produced in-house from dsDNA**

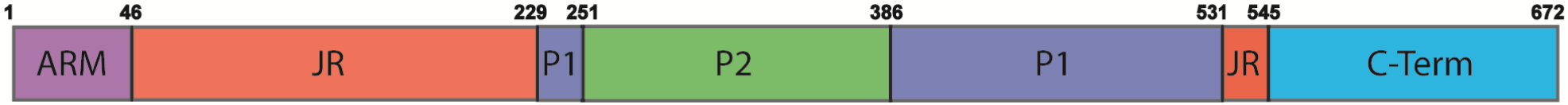
Highly scalable

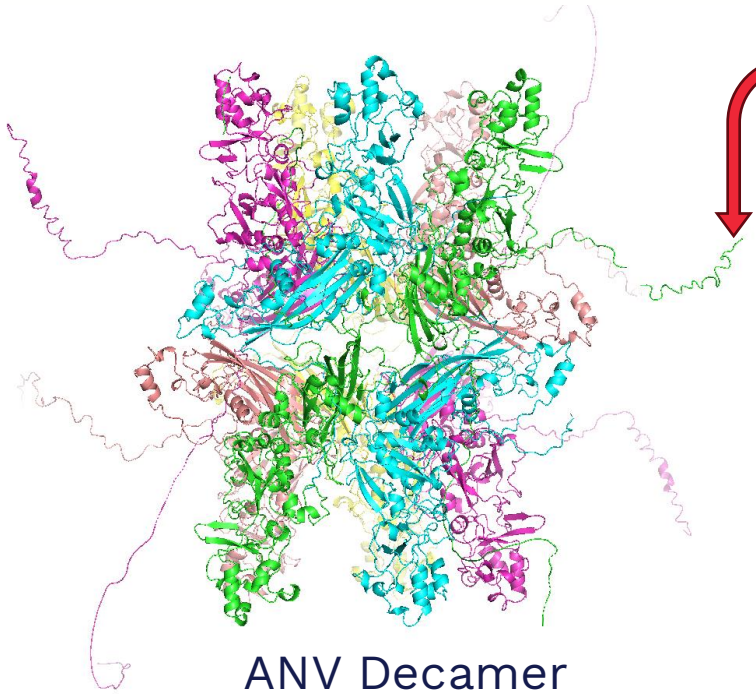
Extensive physical and functional characterization of “naked payload”



# The challenges of ORF1

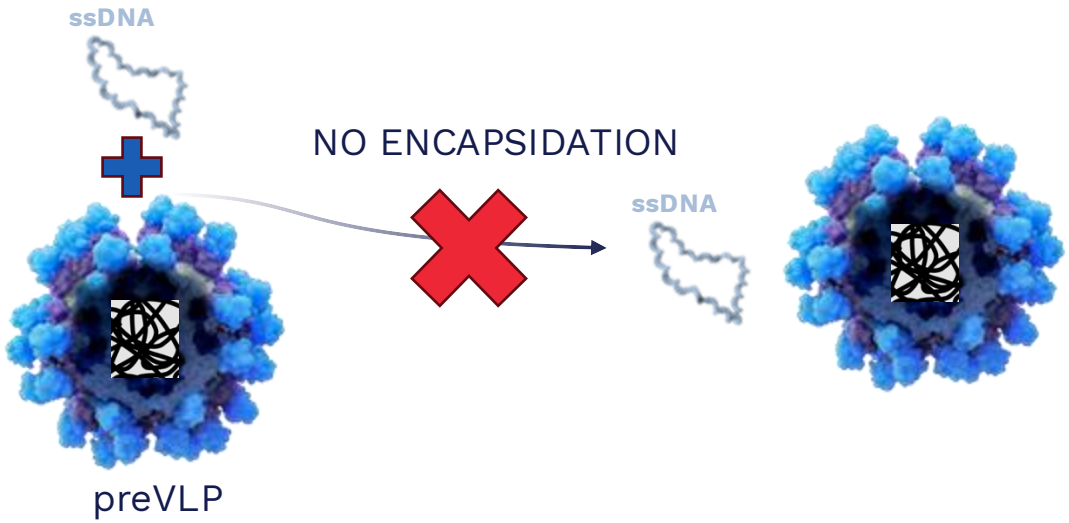


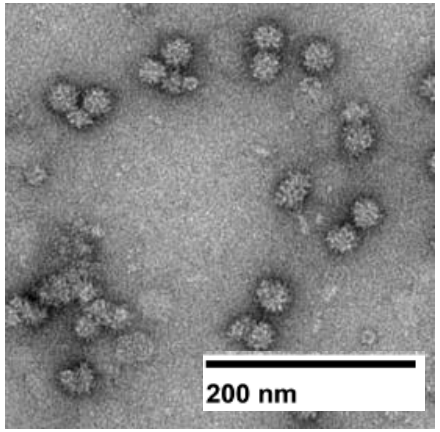
ANV Decamer
(2 capsomeres)

**ARM (Arginine Rich Motif) domain**

- Highly positively charged at physiologic pH
- Important for binding payload
- **Also binds host cell nucleic acid**



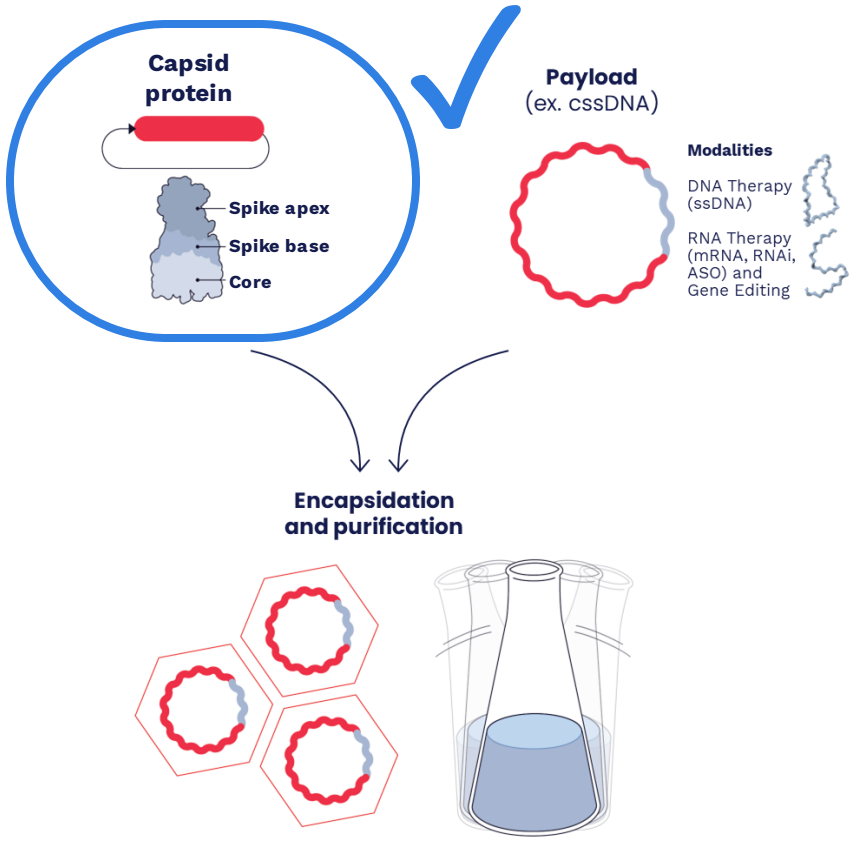

preVLP



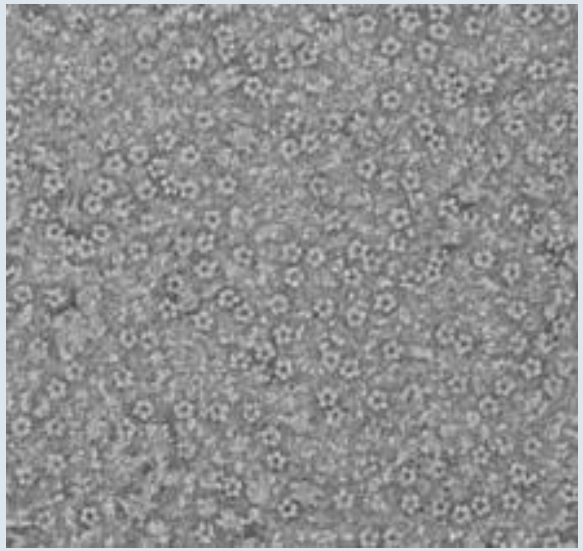

**preVLPs**

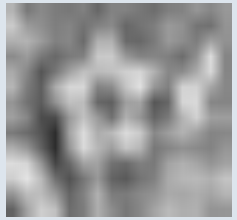
# Generating capsid sub-units

## AnelloBricks®: *in vitro* assembly

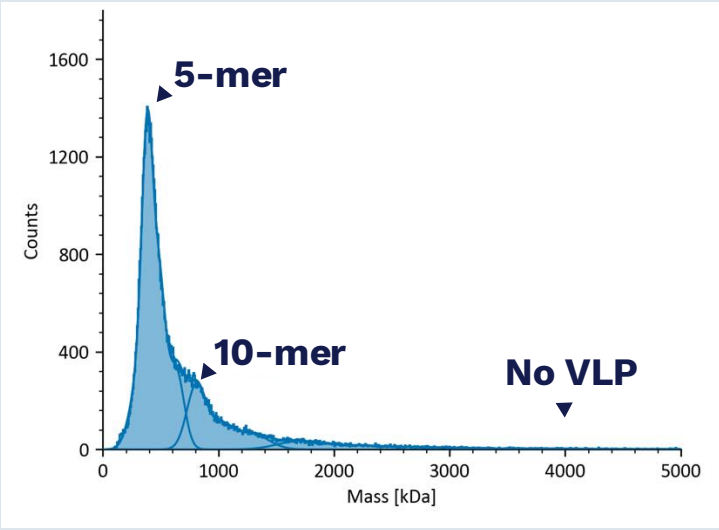



## Protein engineering to prevent VLP assembly



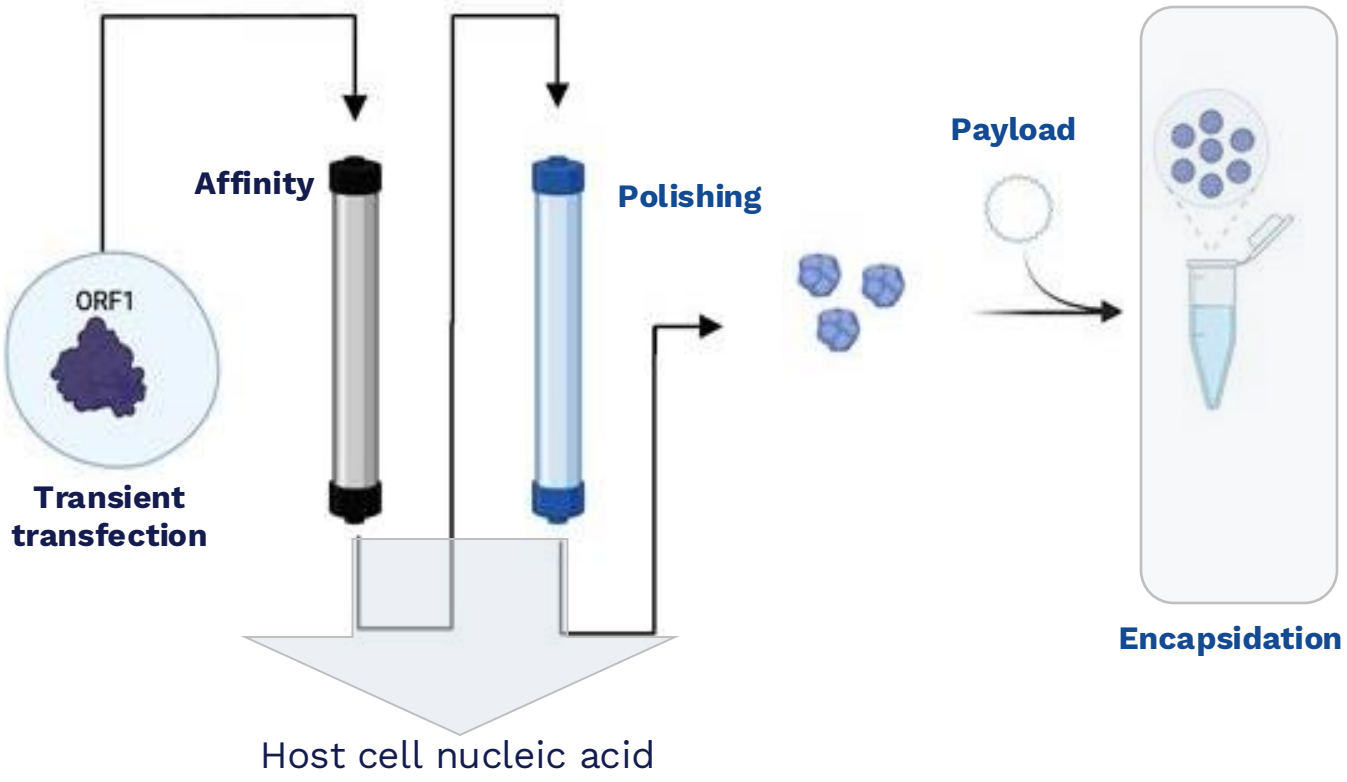
Pentamer structures prevent premature VLP formation



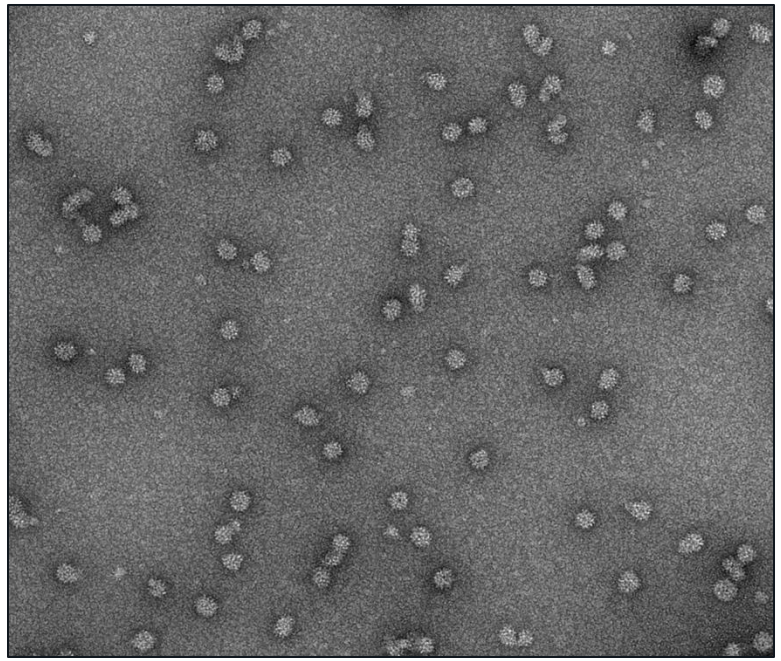

MASS PHOTOMETRY

# AnelloBricks® production process

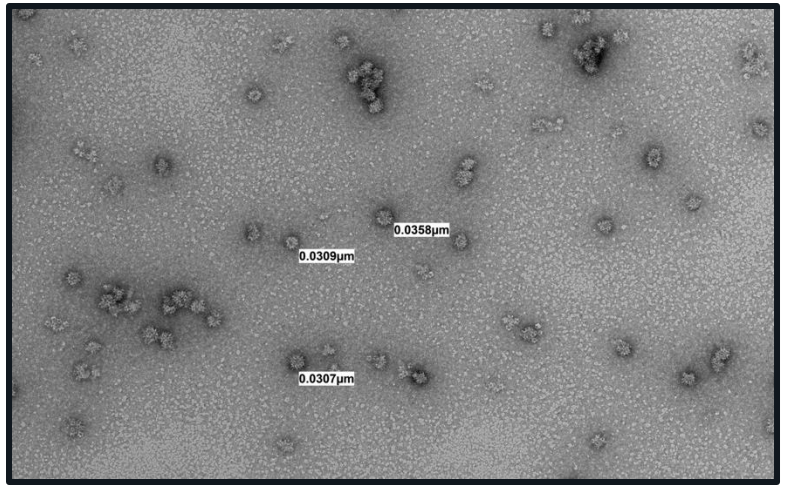
Encapsidation achieved with a variety of nucleic acid payloads



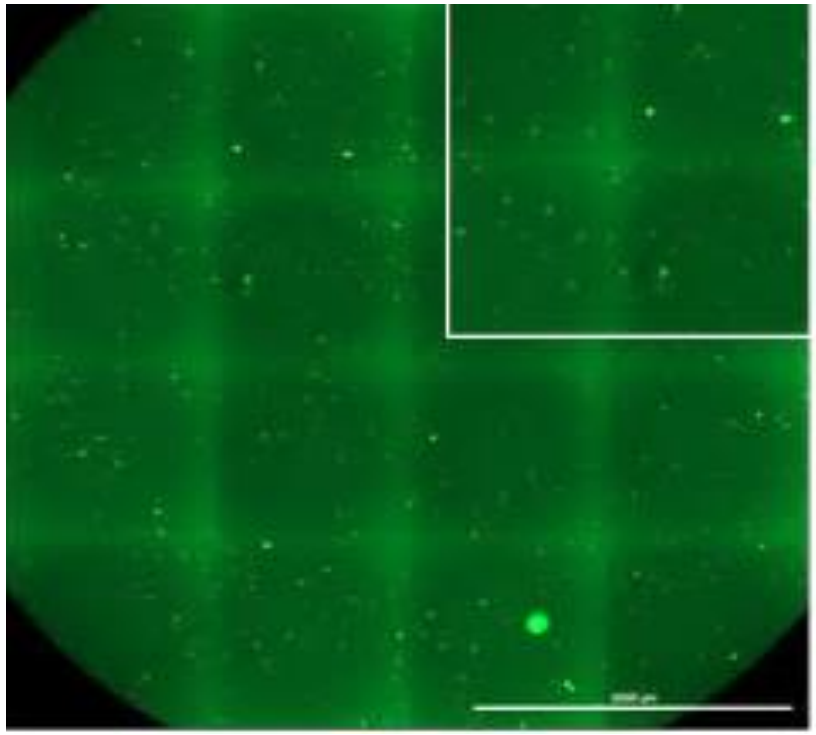

**cssDNA**



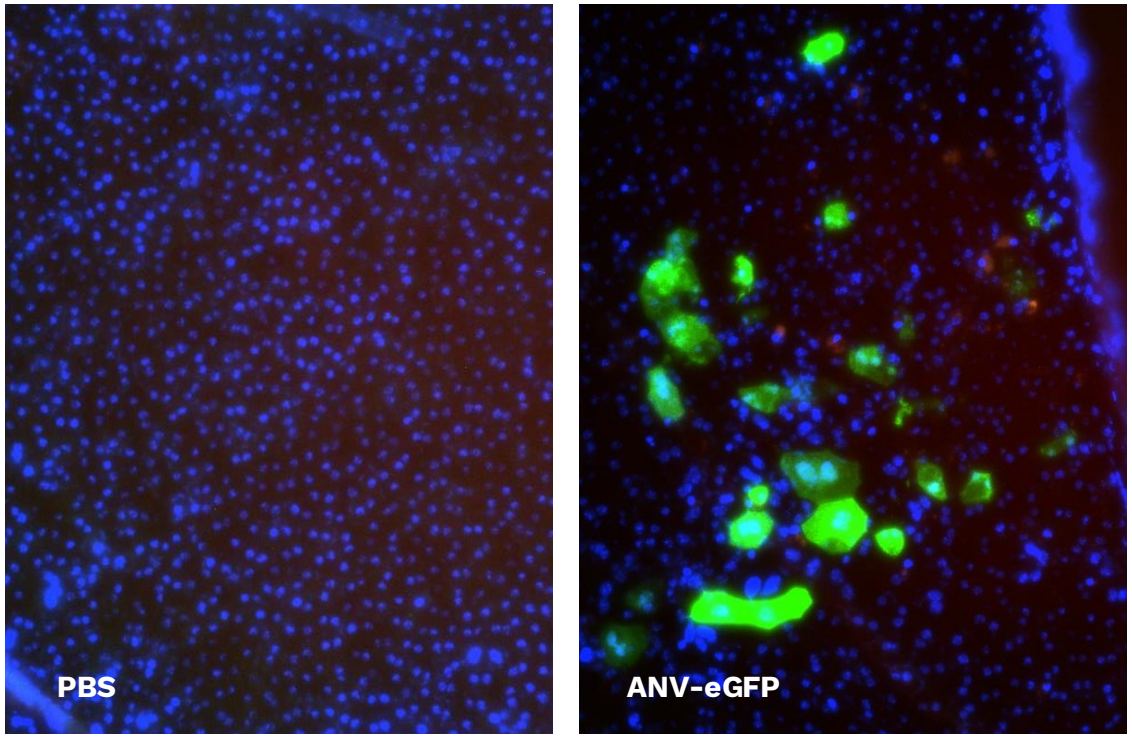
**circRNA**



# Successful *in vitro* and *in vivo* Transduction – eGFP vector



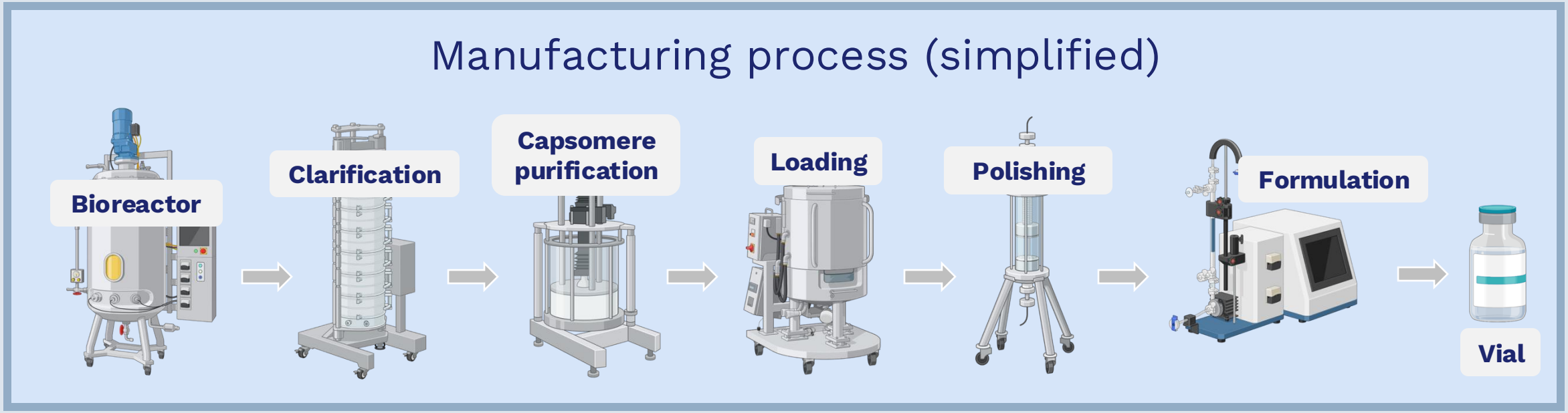
293TT cells




Mouse sub-retinal injection

# AnelloBricks® Manufacturing Process Architecture

*Complexity reduced to the level of recombinant protein production*




Since 1g ORF1 = ~1e17 capsids, a single large-scale campaign (500-2kL) can produce a tremendous quantity of AnelloVector®, even at low purification yields

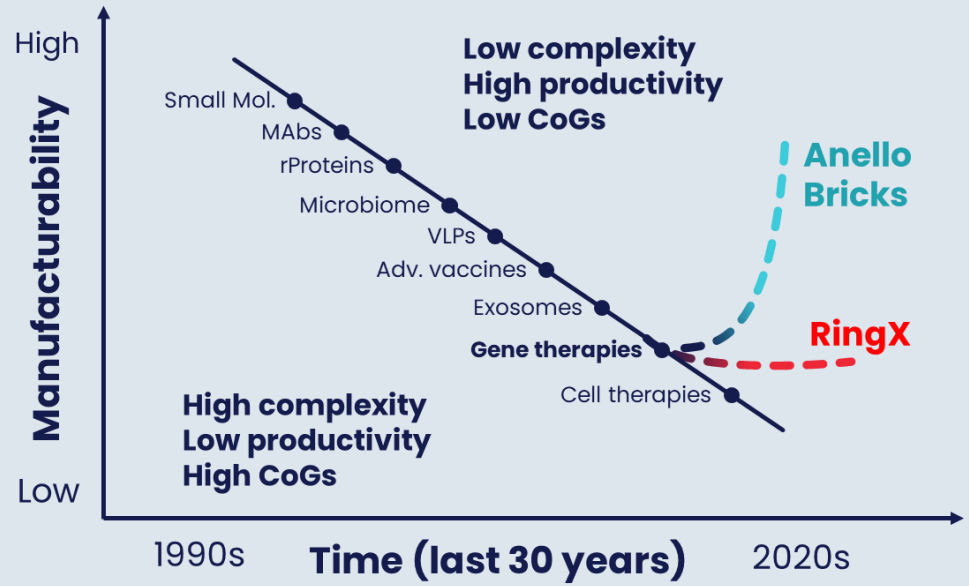
# AnelloBricks® provides a path to dramatic cost reductions

Increased manufacturability could redefine what is possible with genetic medicines

**Estimated Cost Per Dose of Genetic Medicines Produced with AnelloBricks**

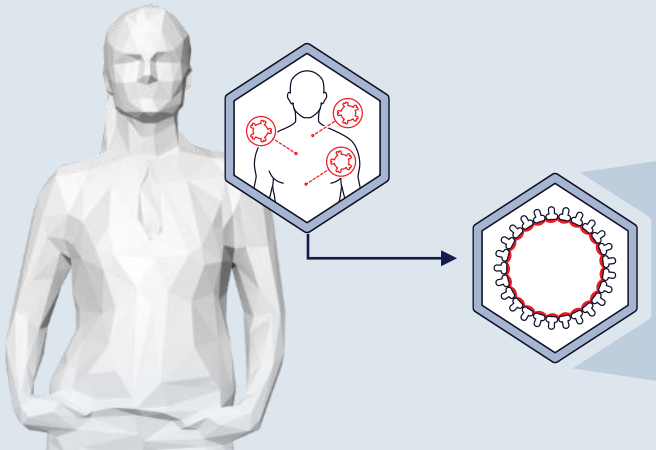
| Application | Dose | Cost |
|---|---|---|
| Eye | 1e11 | <$1.00 |
| Systemic (Low Dose) | 1e15 | $3,125 |
| Systemic (High Dose) | 1e16 | $31,250 |

- Assumes 2% total process yield
- **20% total process yield could lead to an additional order of magnitude reduction in cost**



# Ring's Platform

## End-to-end drug discovery to commercial drug platform unlocked by AnelloBricks®



### AnelloScope

Anellovirus discovery engine to mine sequences from human tissues



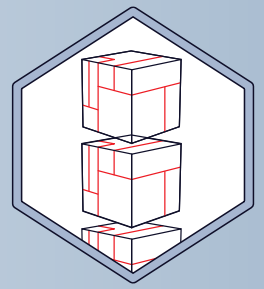
### AnelloVector®

Powerful anellovirus-based platforms delivering payloads

**Genetic modalities**

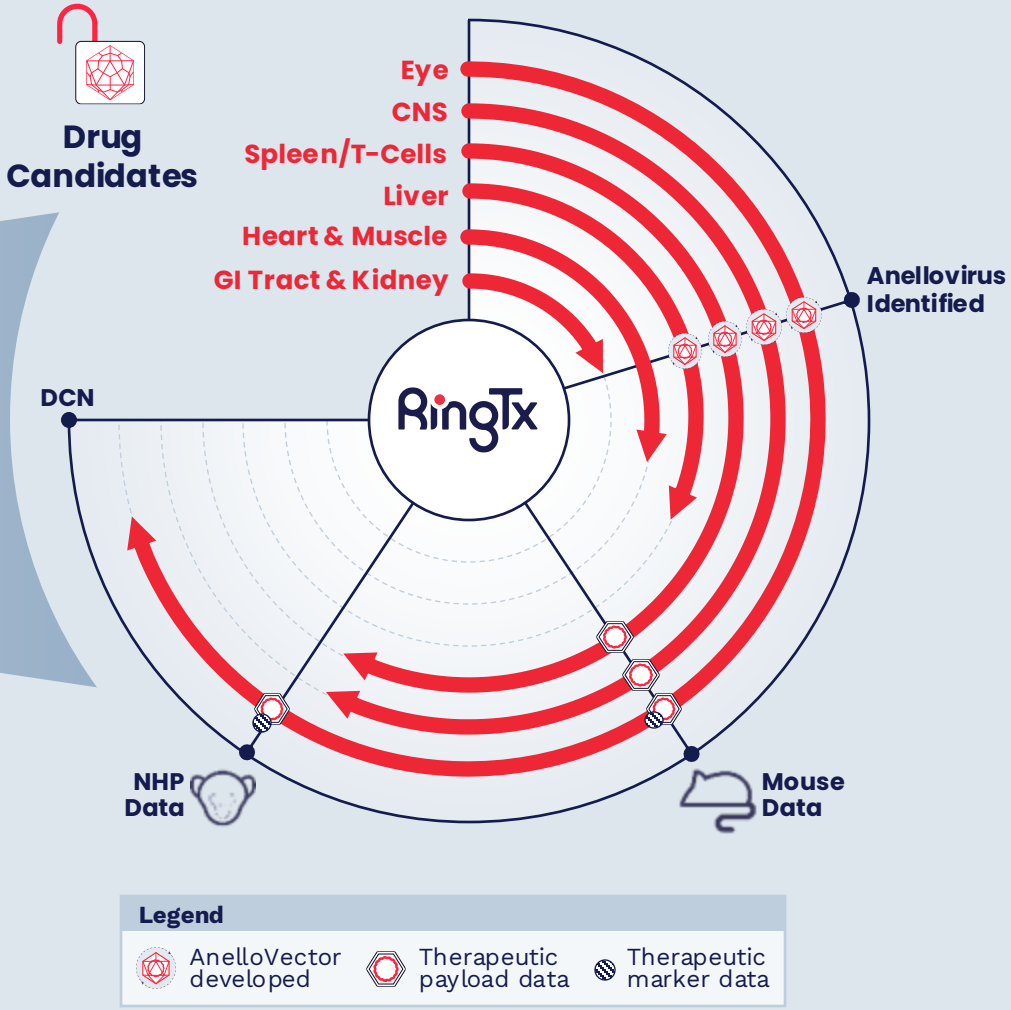
**DNA Therapy**
(ssDNA)

**RNA Therapy**
(mRNA, RNAi, ASO) and Gene Editing



### AnelloBricks®

Highly scalable, modular-based manufacturing strategy leveraging in-house expertise and external partners





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# Acknowledgements


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  Riana Pozsgai
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  Maciej Nogalski

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  Kurt Swanson
  Shu-hao Liou
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  Hillary Rodgers
  Saadman Islam
  Jared Pitts
  Cesar Arze
  Harish Swaminathan
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  Yong Chang
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