Novel Capsid LSV1 Has a Unique 3D Structure at the Loop Substitution Area - Confers Superior Retinal Transduction from Intravitreal Injection

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ASGCT May 19th 2022

Disclosure

- I am an employee of Adverum Biotechnologies and hold shares in the company.

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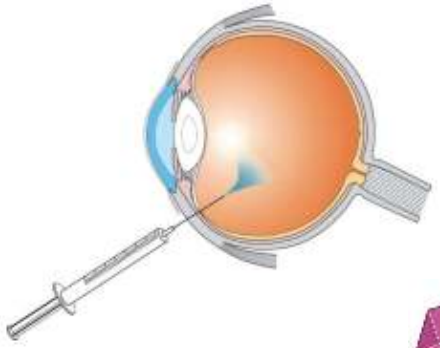
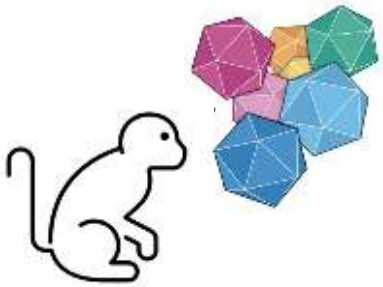
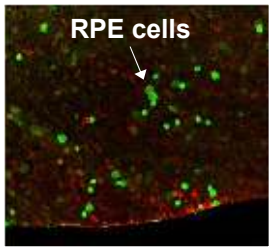
Summary



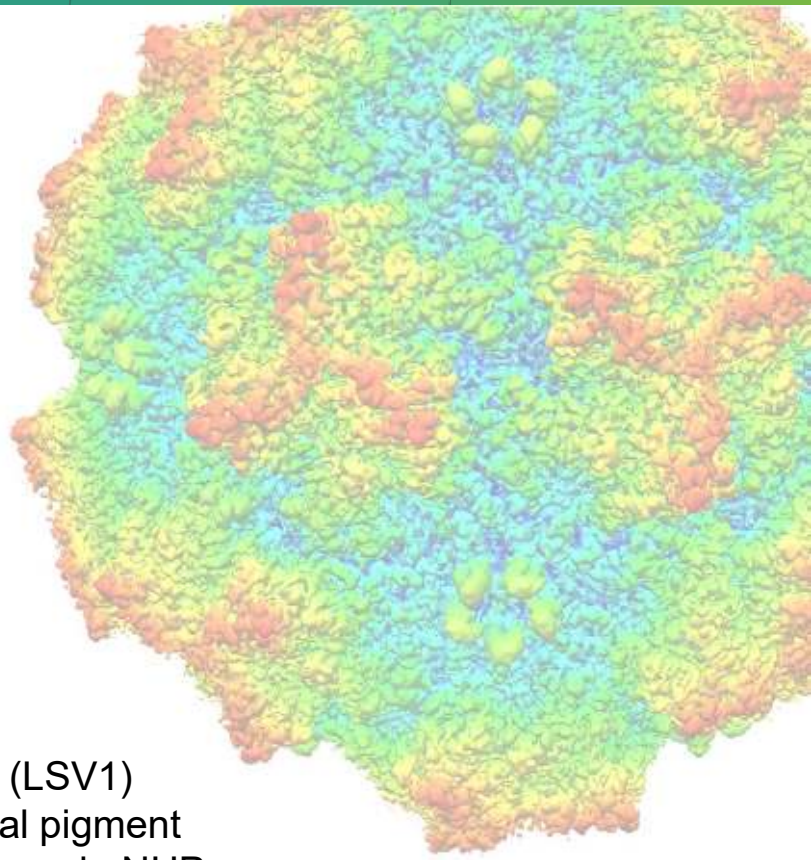
Goal: develop AAVs that can transduce the retina following intravitreal (IVT) administration to provide functional cures for ocular disorders



Method: generate AAV library and select variants that transduce non-human primate (NHP) retina from IVT injections



Outcome: loop swap variant 1 (LSV1) transduces the retina and retinal pigment epithelium (RPE) from the vitreous in NHP and has a novel AAV structure



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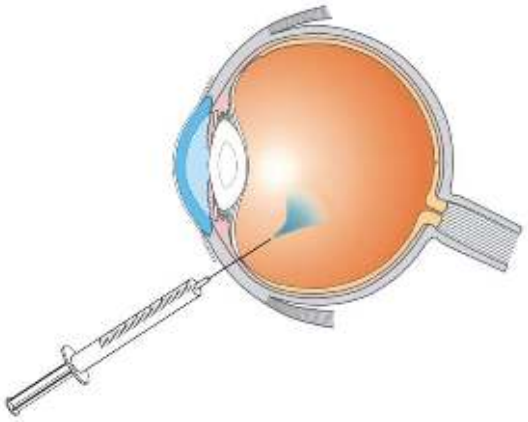
Intravitreal Injections (IVT) Have the Potential to Non-Invasively Transduce the Entire Retina

Goal:

- Provide potential curative therapies to ocular disorders by delivering therapeutic DNA to the retina via AAV
- IVT delivery enables pan-retinal transduction
  - Common, noninvasive

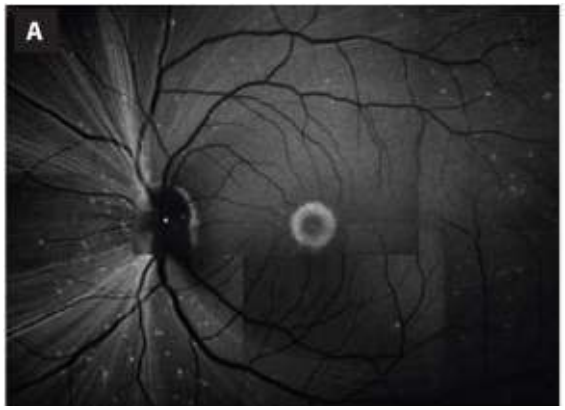


Honda et al., Int J Nanomedicine 2013

Problem:

- Naturally occurring AAV serotypes poorly transduce the retina from the vitreous
  - Expression mostly limited to the fovea

3 weeks post IVT
AAV2(4YF)-CMV-GFP in NHP
Little to no GFP expression

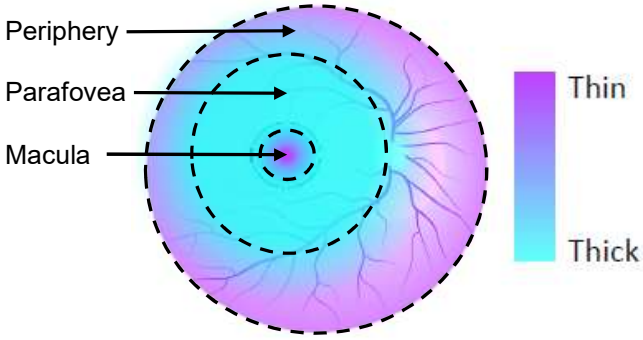


Dalkara et al., Sci Trans Med 2013

Cause:

- The Inner Limiting Membrane (ILM) impedes AAV from entering the retina
  - Largely composed of heparin sulfate proteoglycans (HSPGs)

Primate ILM Thickness



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Diana Cepeda

Development of New AAV Variants That can Effectively Transduce the Retina From IVT in Non-Human Primates

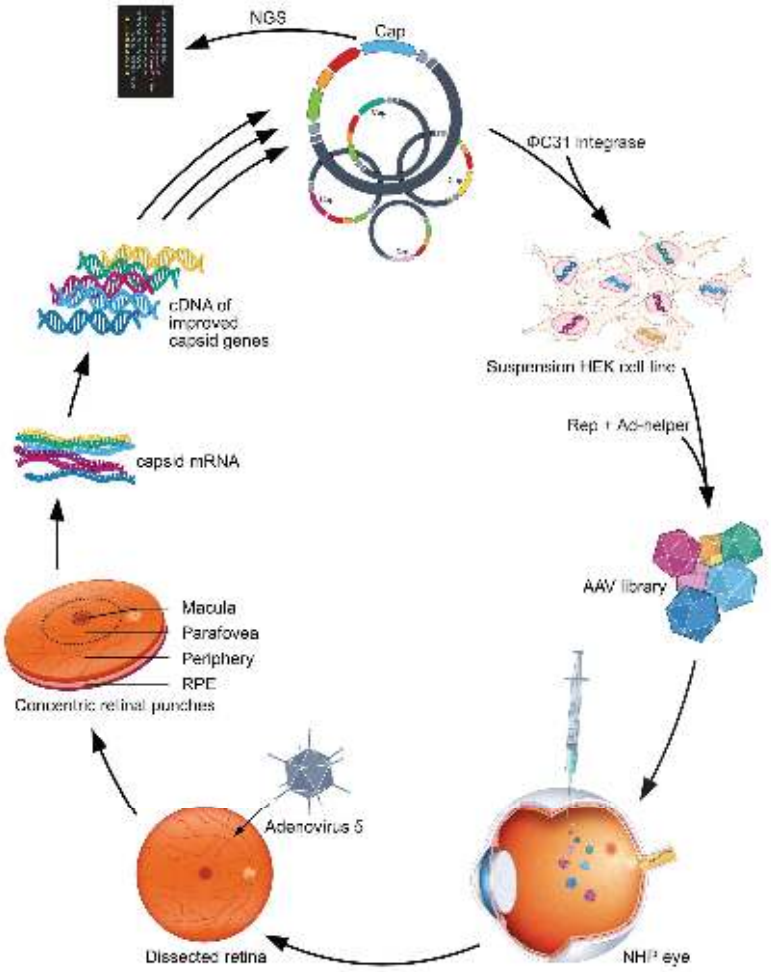

Illustration: Gardenia Gonzalez Gil

Iterative selection of AAV from hard-to-reach areas of NHP retina

1. Plasmid AAV capsid libraries (~10^6 unique variants)
2. Stable cell AAV capsid libraries
   - Controls for cross packaging, preservable
3. AAV library
4. AAV library intravitreally injected into NHP eye
5. NHP eye enucleated, dissected, and infected with Ad5 to boost AAV mRNA production
6. Concentric punches taken from different parts of the retina and RPE
   - Parafovea and RPE sections primarily used to select for AAV that effectively transduce the ILM
7. mRNA isolated from retinal punches
8. cDNA library from AAV mRNA

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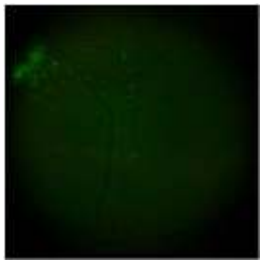
AAV2.5T Was Used as the Backbone for the Libraries

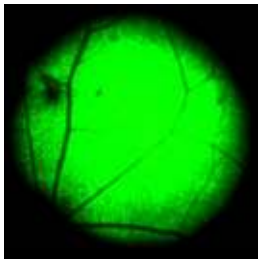
- AAV2.5T (Excoffon et al, 2009)
  - VP1 unique region of AAV2
  - VP2 & VP3 of AAV5
  - One point mutation: A582T

- AAV2.5T effectively transduces retinal cells from subretinal injections but not IVT

IVT



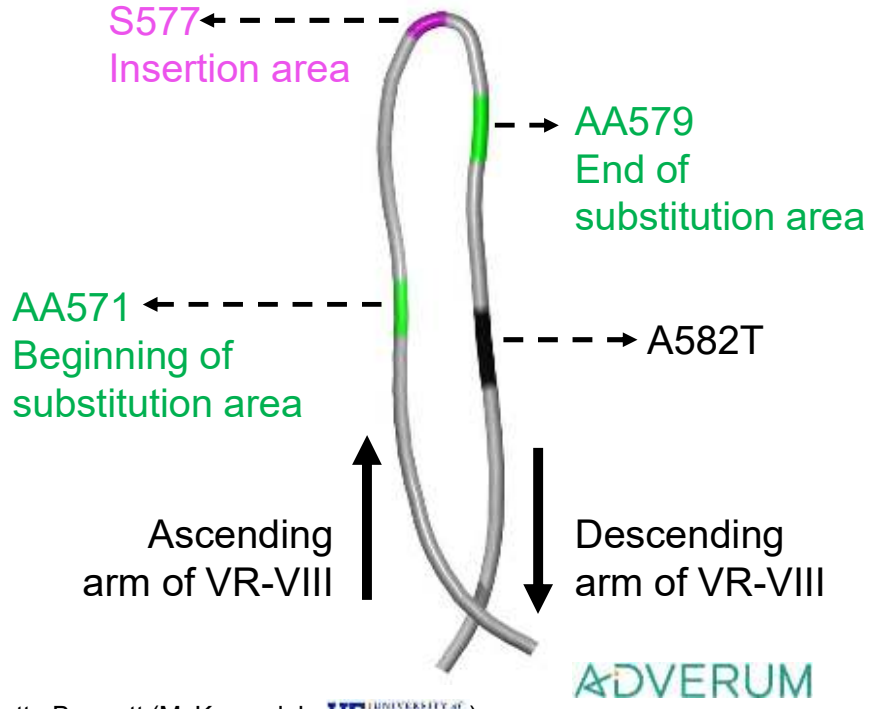
Subretinal



AAV2.5T-CMV-GFP
Mongolian gerbil
fluorescent fundus
8 weeks post injection

➢Ability to transduce, but impeded by ILM

- Two libraries: **Loop Swap Library (LSL)** and **Loop Insertion Library (LIL)**
  - LSL: 9 amino acids ***substituted*** from 571-579
  - LIL: 10 amino acids ***inserted*** after S577



Antonette Bennett (McKenna lab, UF UNIVERSITY of FLORIDA)

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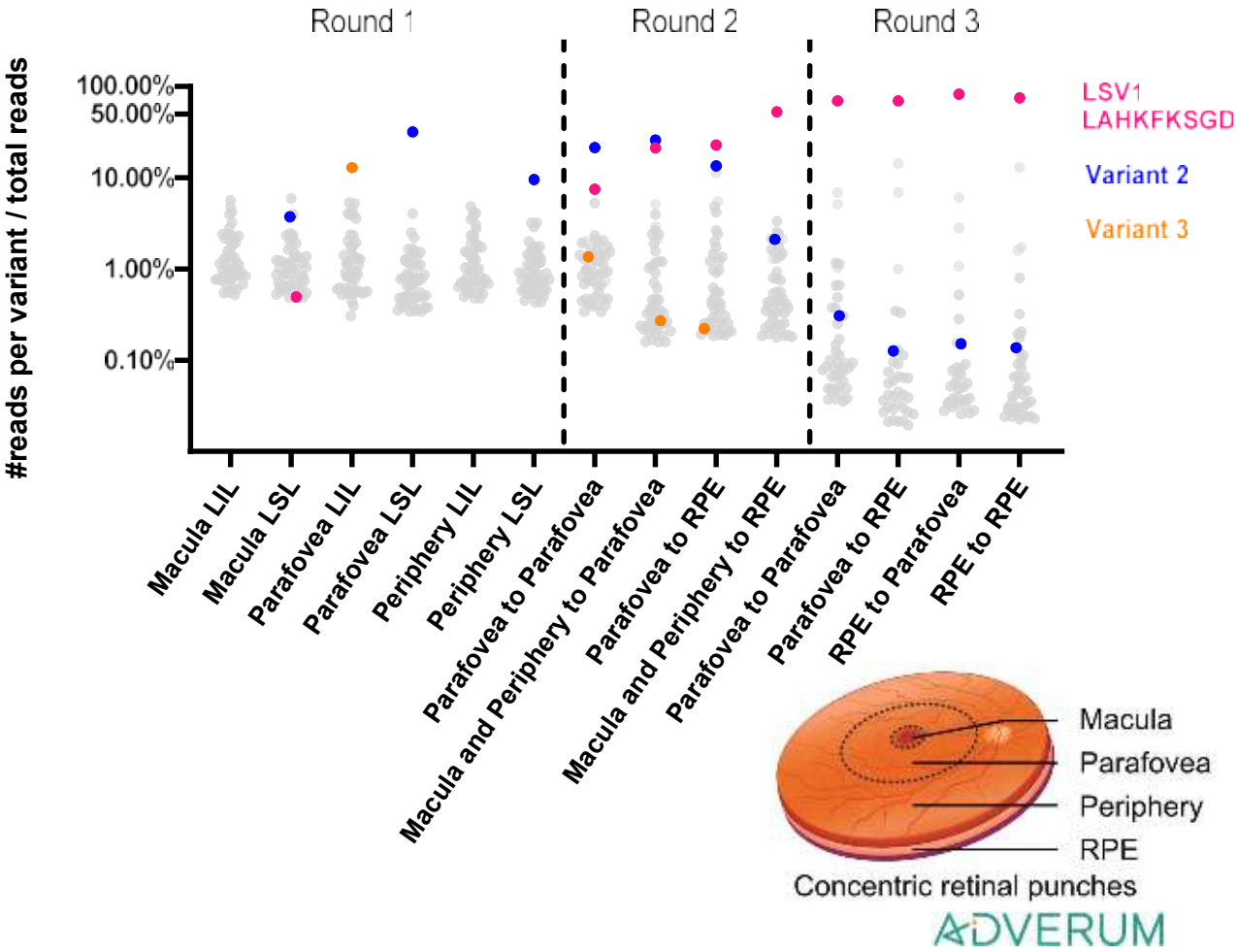
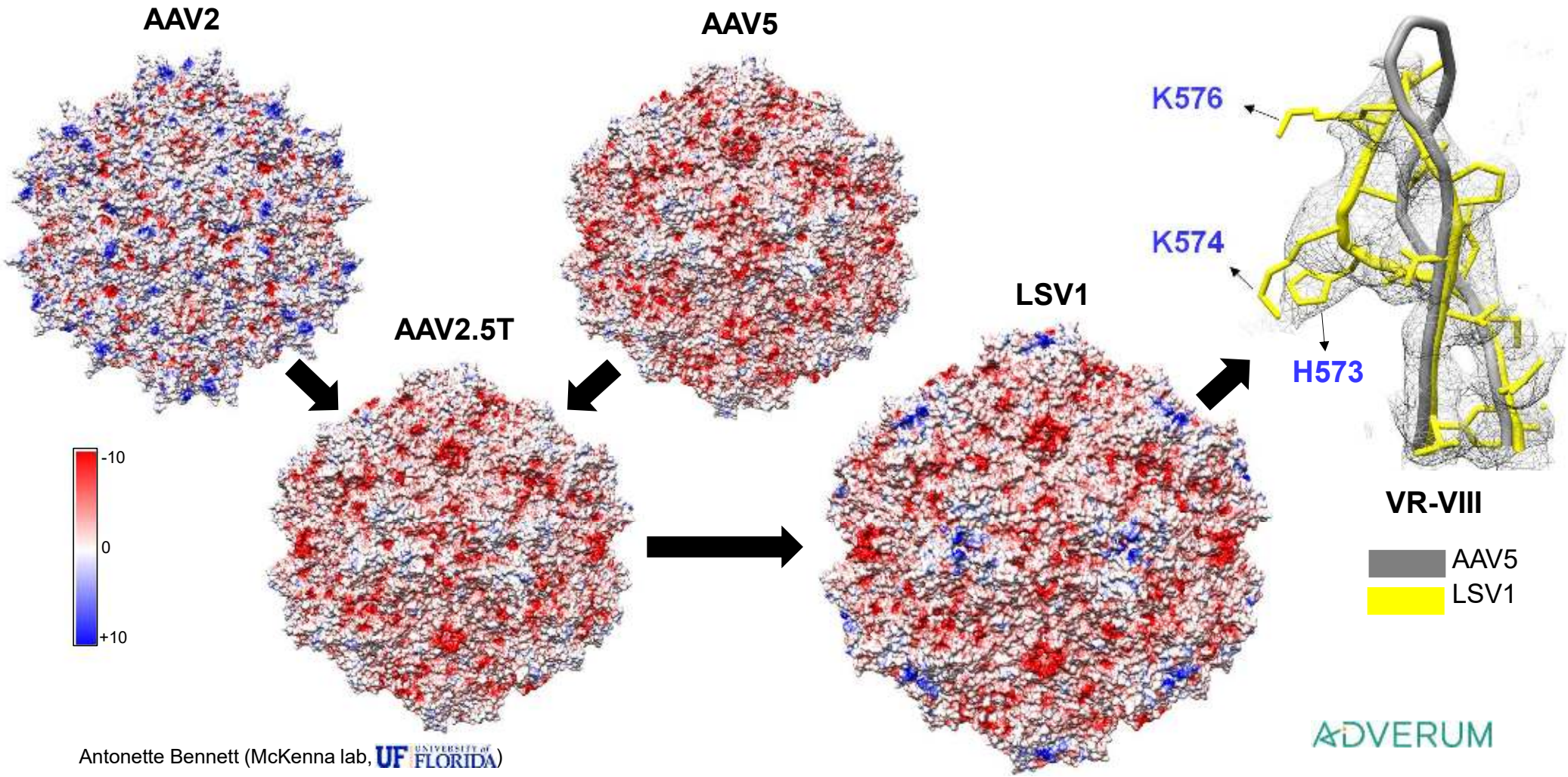
LSV1 LAHKFKSGD Loop Is Strongly Enriched After Three Rounds of Selection in NHP

All libraries were sequenced by NGS to determine prevalence of each unique sequence

- Round 1: All retinal areas selected to generate next round's AAV library
- Rounds 2&3: Only parafovea and RPE selected to generate next round's AAV library
- After 3 rounds of selection, LAHKFKSGD (Loop Swap Variant 1, LSV1) was highly enriched (>90% of libraries)



LSV1 CryoEM Structure Shows Positive Charge Imparted by Loop Substitution



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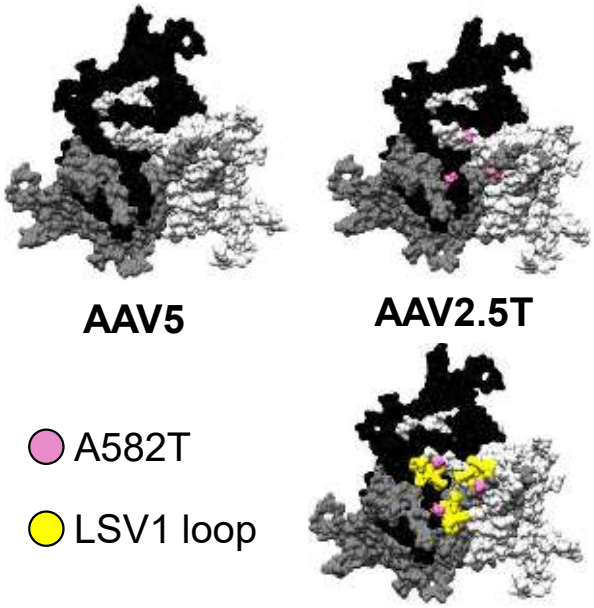
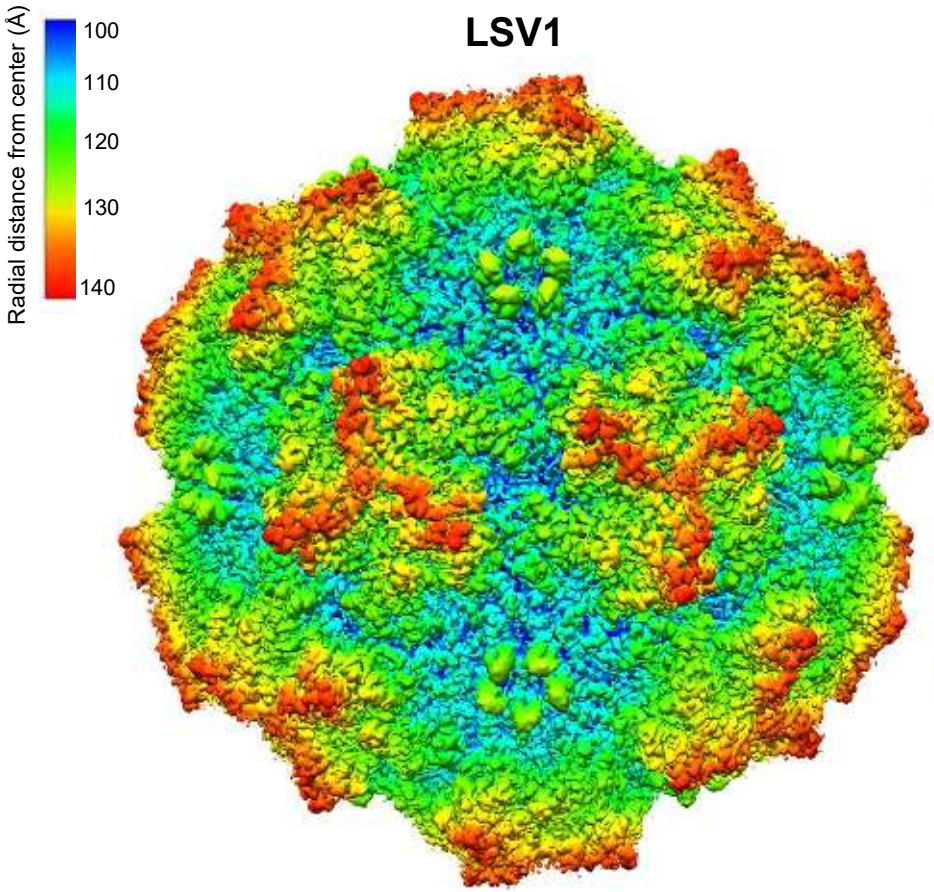
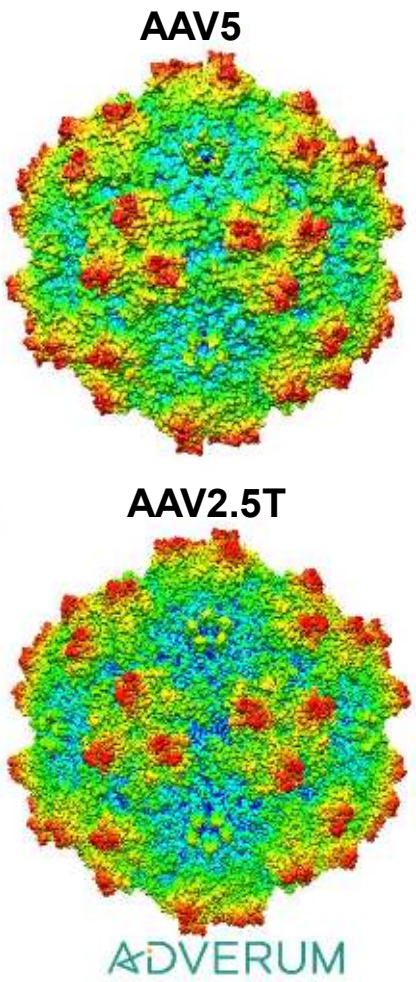
CryoEM Structure of LSV1 Reveals Unique 3-Fold Axis

LSV1 loop in 3-fold axis area causes extra density to fill in area between protrusions

Trimer







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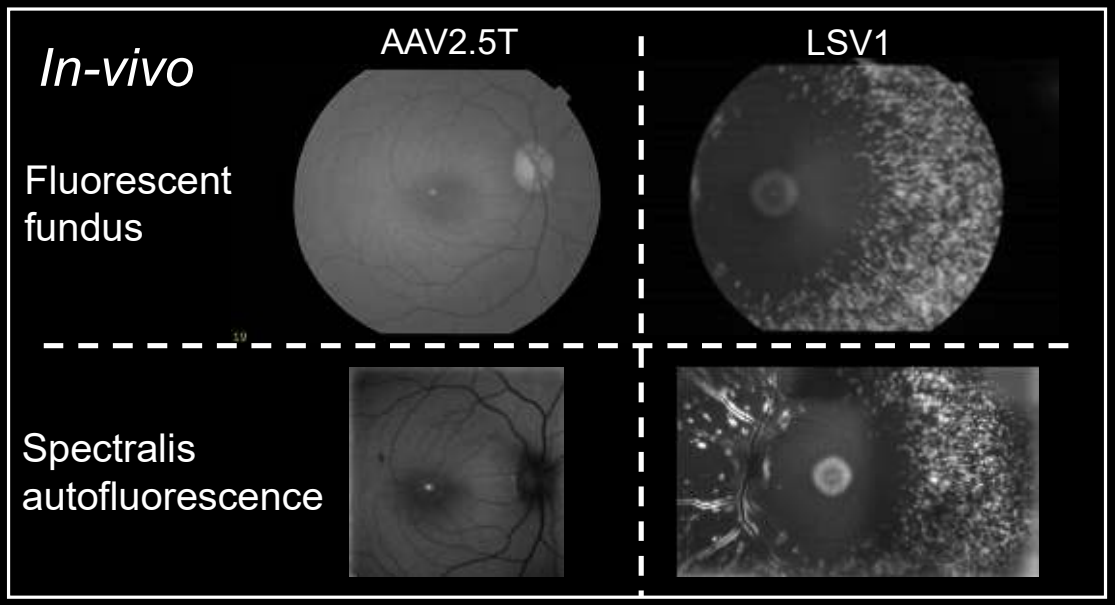
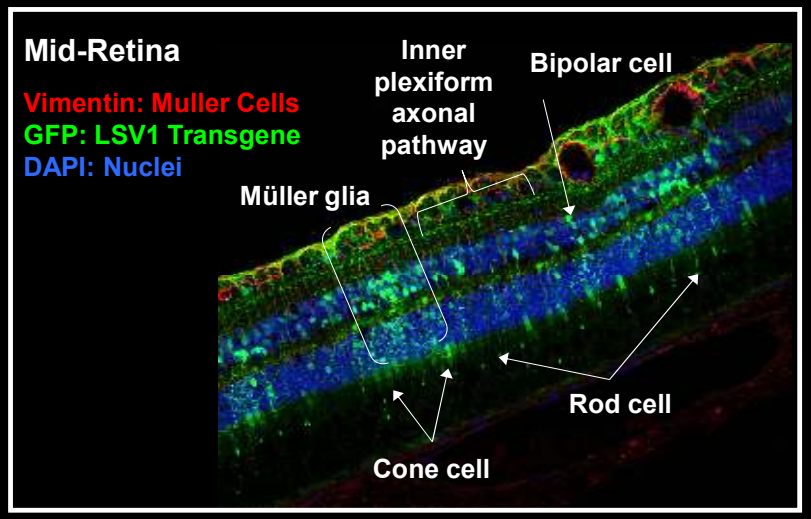
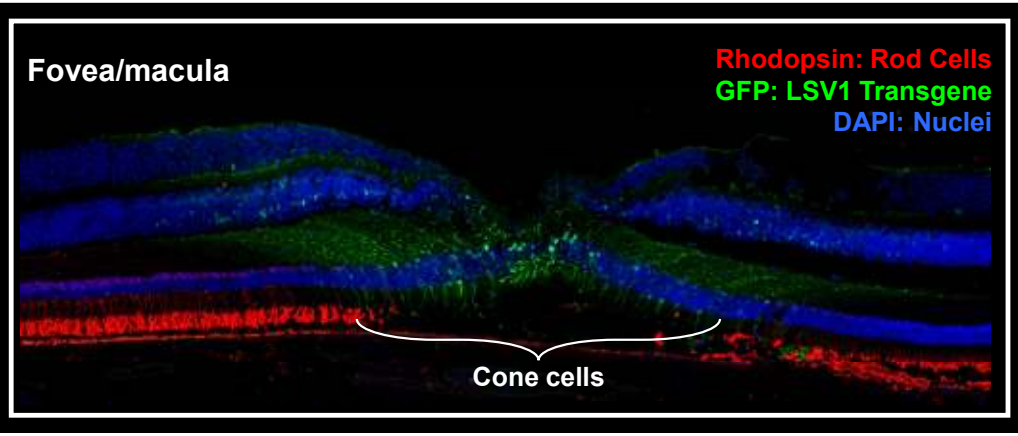
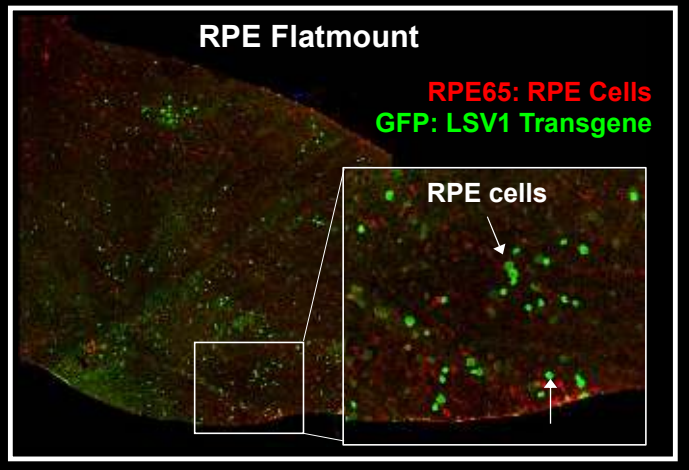
LSV1-CMV-GFP Displays Broad Cellular Tropism and Deep Retinal Tissue Penetration by IVT in NHP





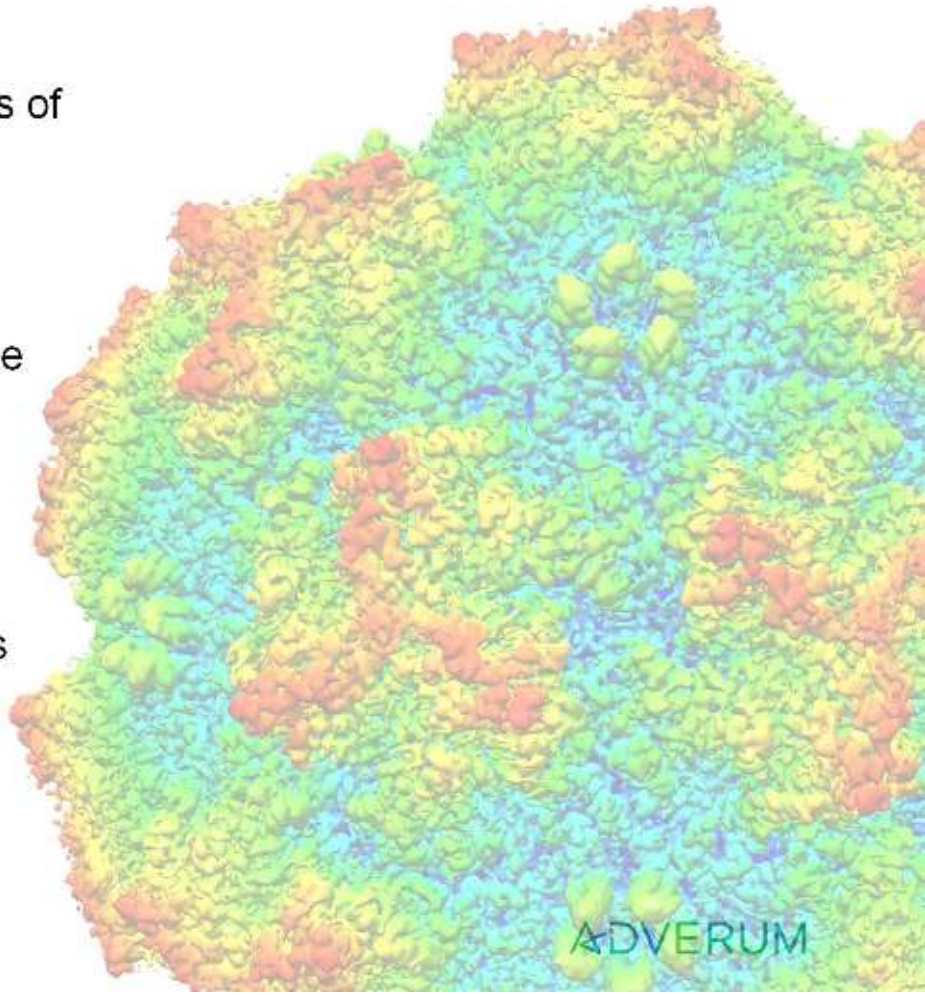




Diana Cepeda
Julio Nieves

Conclusions

- Identified LSV1 from a library of over one million variants of AAV2.5T
  - LSV1 was enriched after three iterative rounds of selection following IVT administration in NHP retina
- LSV1 loop imparts new biochemical characteristics to the AAV2.5T parental backbone
  - Heparin affinity, ability to cross the ILM
- LSV1 has a unique 3D structure
- LSV1 efficiently transduces NHP retina from the vitreous
  - High expression in the fovea and periphery
  - RPE expression



Acknowledgments

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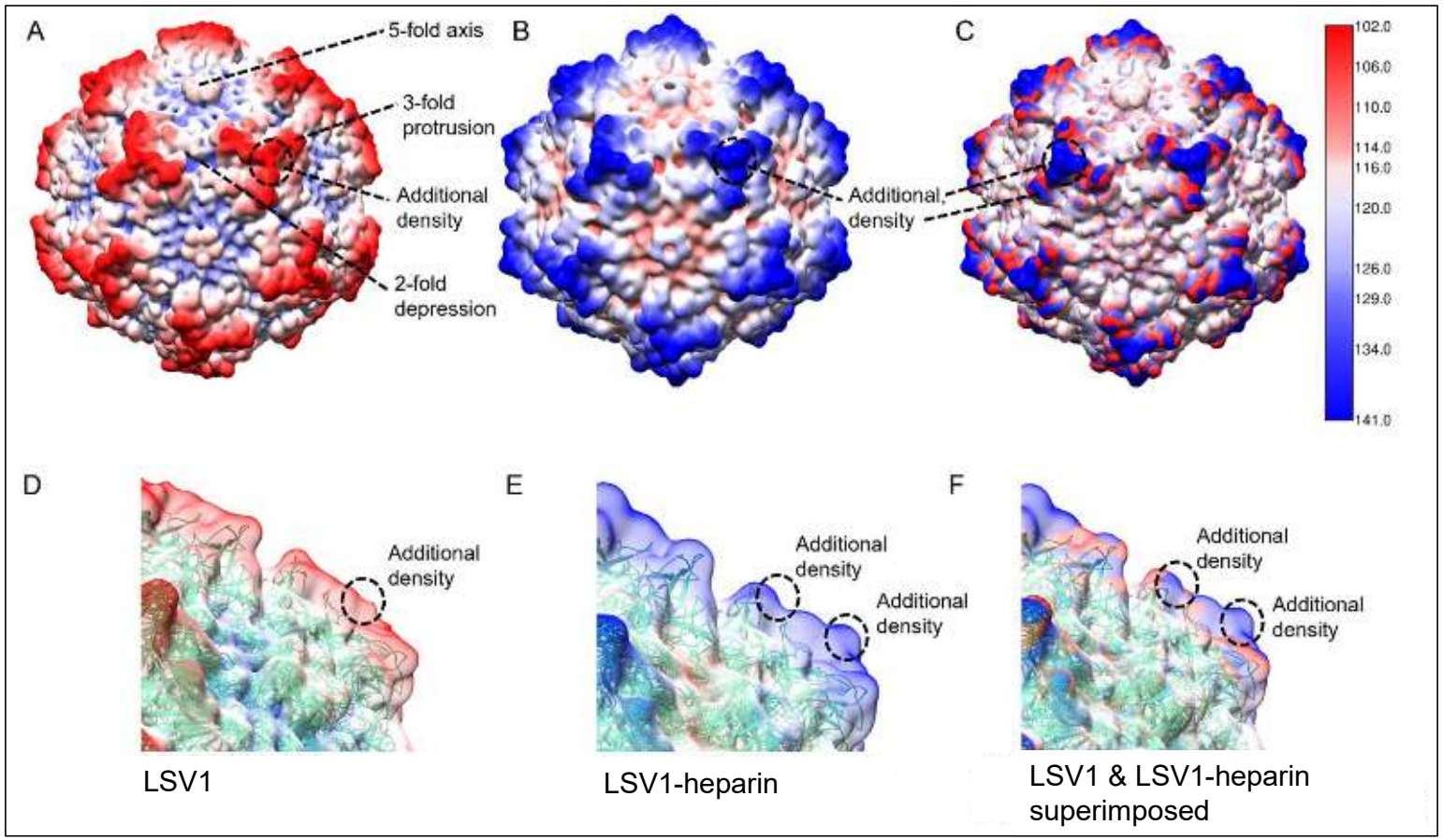
- Brahim Belbellaa
- Julio Nieves
- Alex Tai
- Brigit Riley
- Diana Cepeda
- Annahita Keravala
- Rangoli Aeran
- Hao To
- Tawny Neal Qureshi
- Awilda Rosario
- Edward Yeh
- Alexa Levinson
- Christopher Chavez
- George Todorov
- Claire Gelfman
- Mehdi Gasmi

UF UNIVERSITY of FLORIDA

- Antonette Bennett
- Robert McKenna
- Mavis Agbandje-McKenna

Thank you!

Bonus – Heparin Is Coordinated at the 3-Fold Axis Where the Additional Density Resides



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