Building an AAV vector pipeline

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

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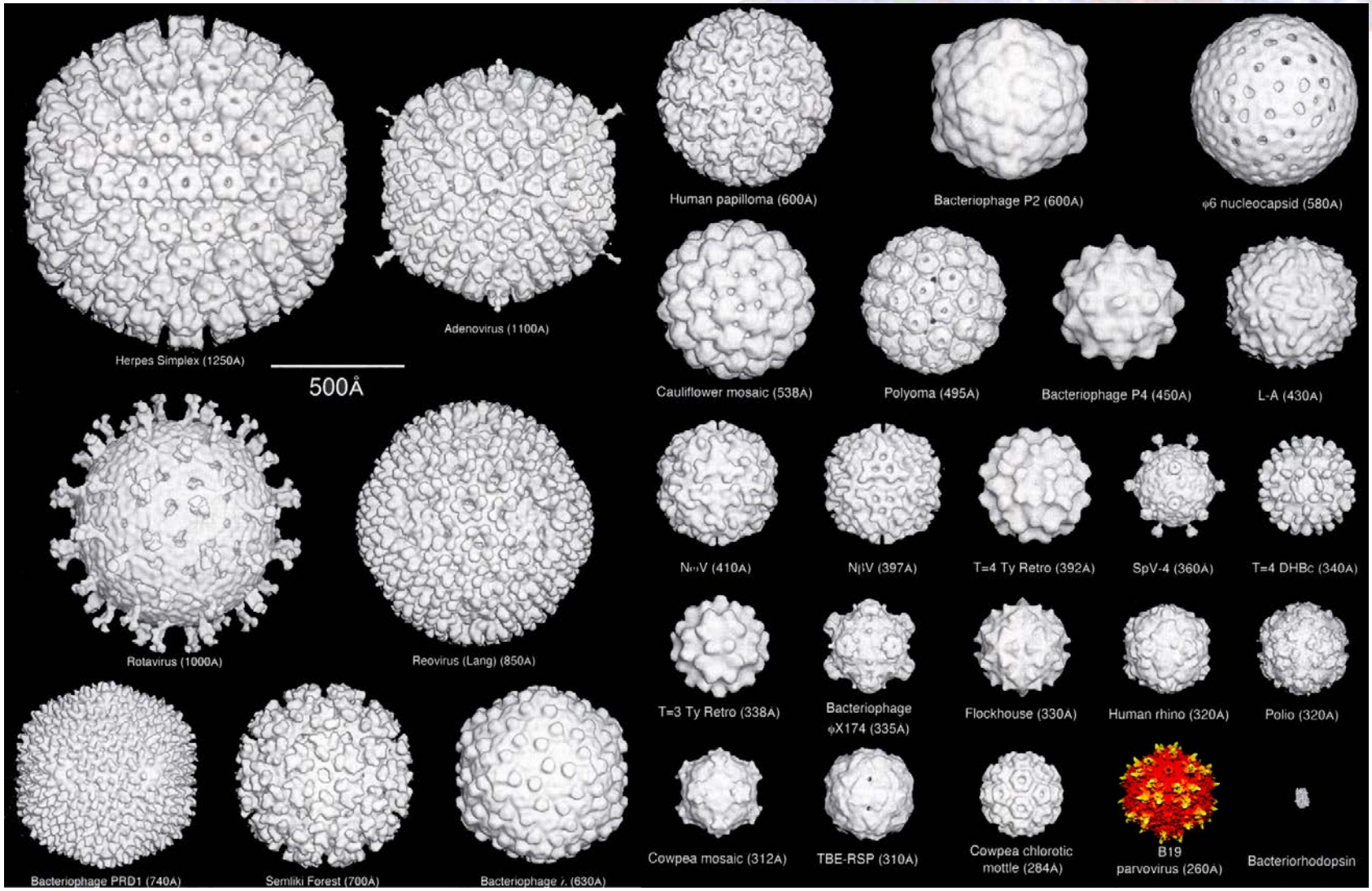
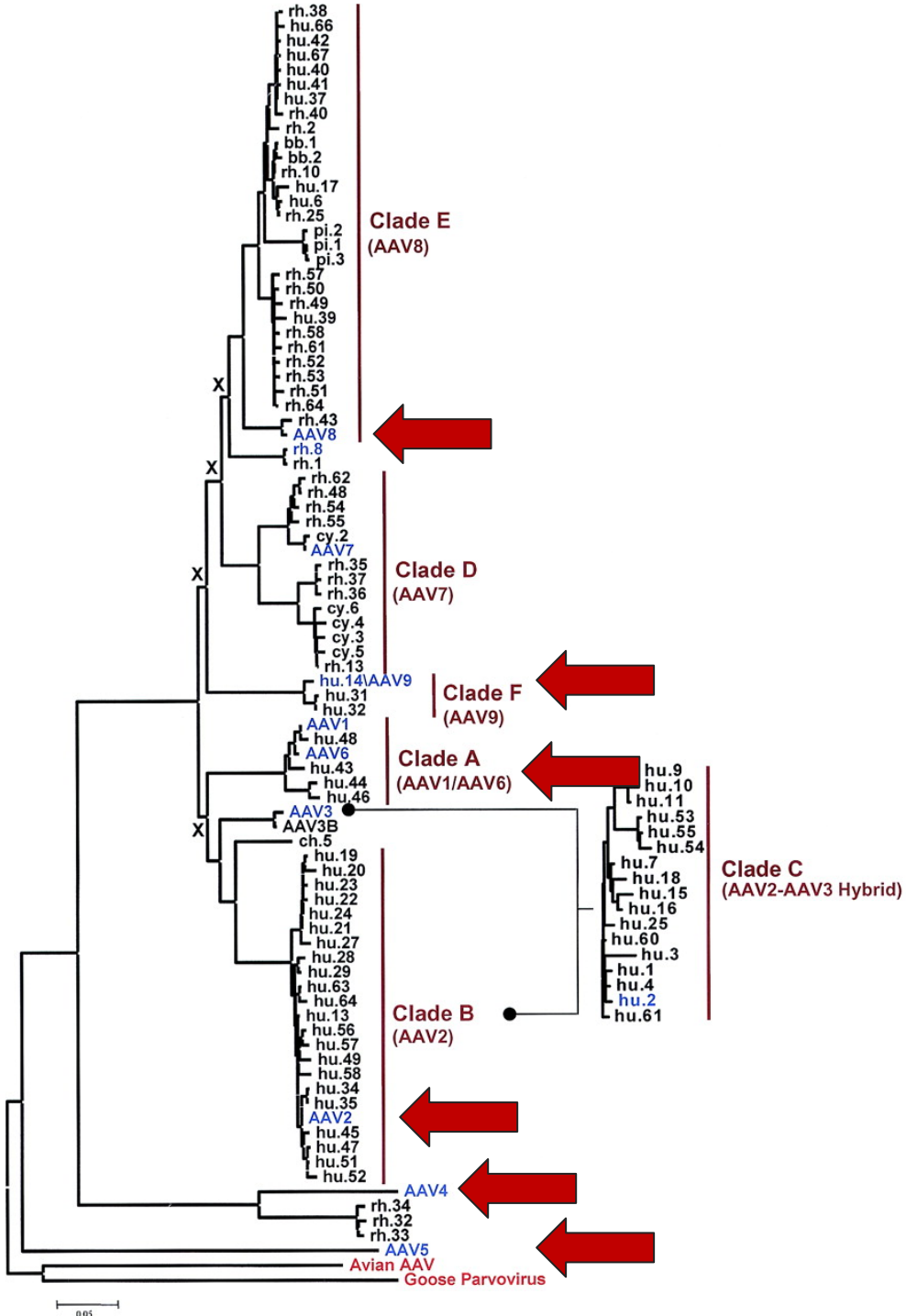
12 Apr 2013
@ NIH Workshop
Gene Therapy: Charting a Future Course

Parvoviridae: Adeno-Associated Virus (AAV)



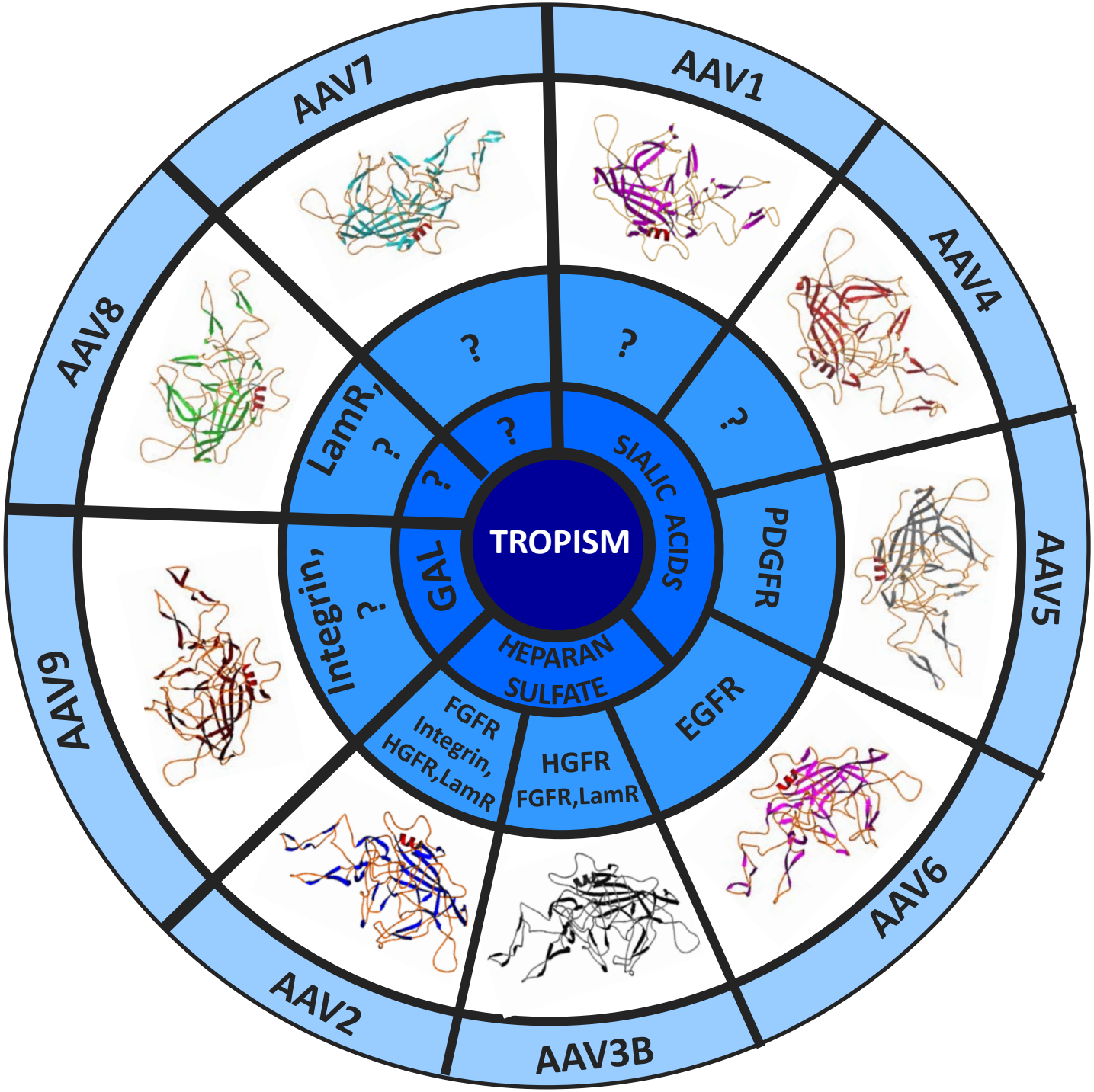
Baker et al., 1999



AAV: Natural Diversity

Neighbor-joining phylogenies of the VP1 protein sequence of natural AAV isolates

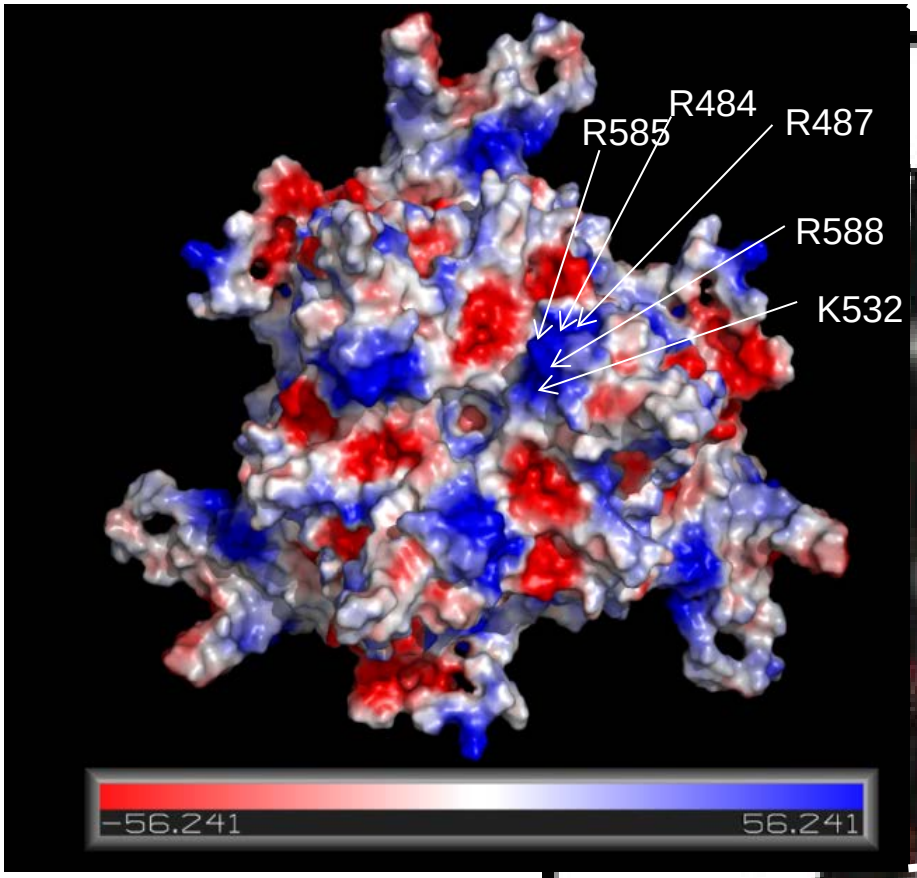
Gao, G. et al. 2004. J. Virol.

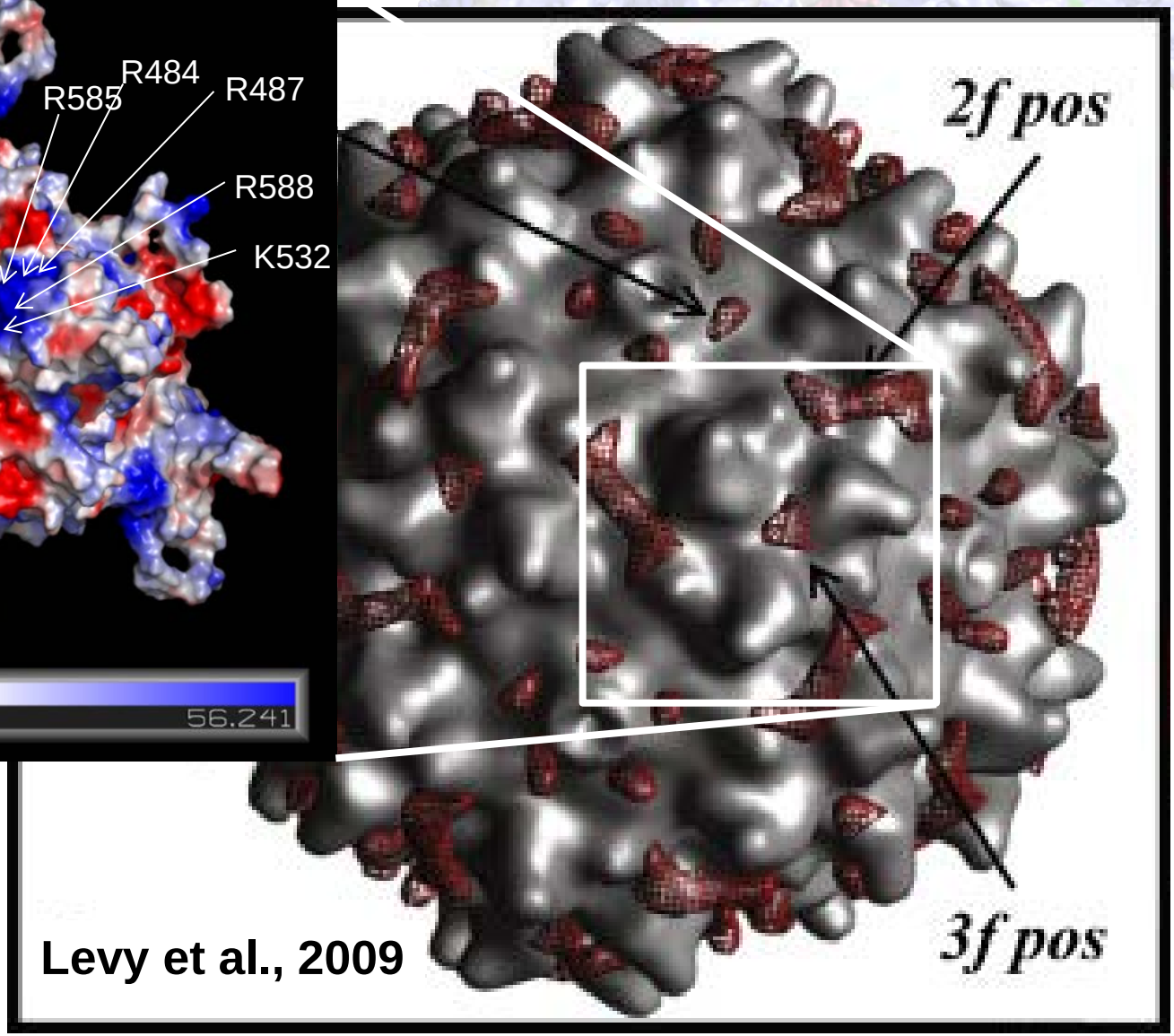

TROPISM
SIALIC ACIDS
PDGFR
EGFR
HGFR
FGFR,LamR
FGFR
Integrin,
HGFR,LamR
HEPARAN
SULFATE
GAL
Integrin,
?
LamR,
?
?
?
?
?
?
AAV1
AAV4
AAV5
AAV6
AAV3B
AAV2
AAV9
AAV8
AAV7

Building an AAV vector pipeline…

Understanding & re-engineering AAV biology to improve recombinant vector safety & efficacy for human gene therapy

Glycan receptor footprints: Heparin & AAV2



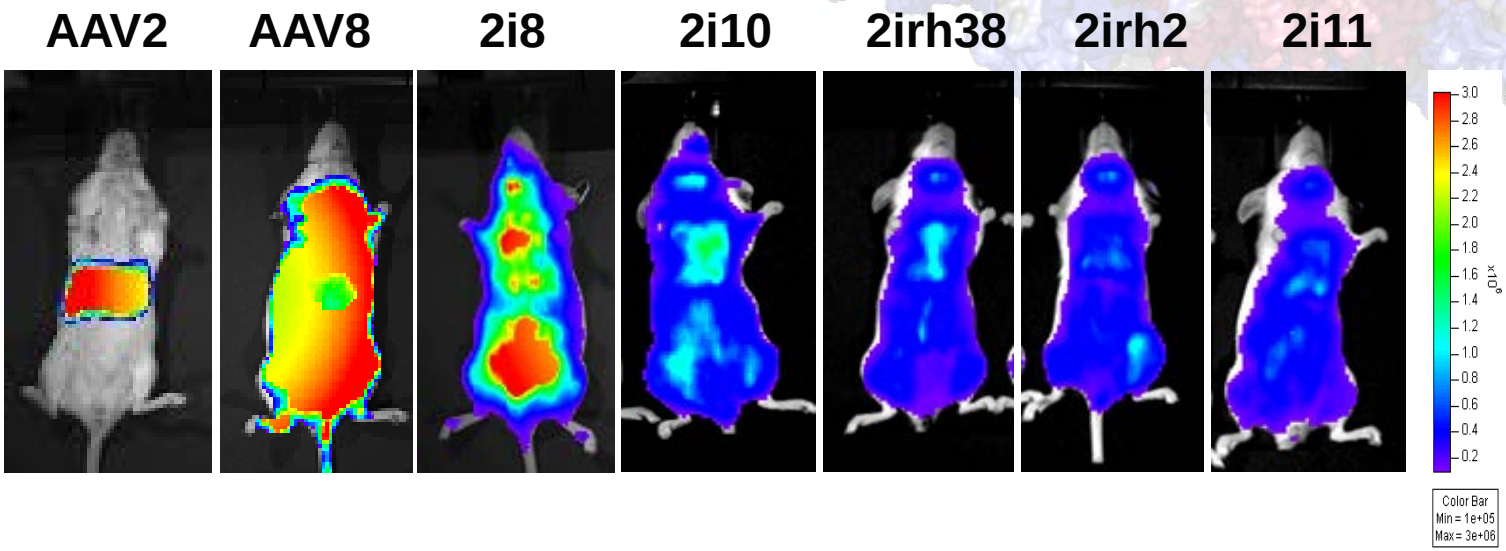


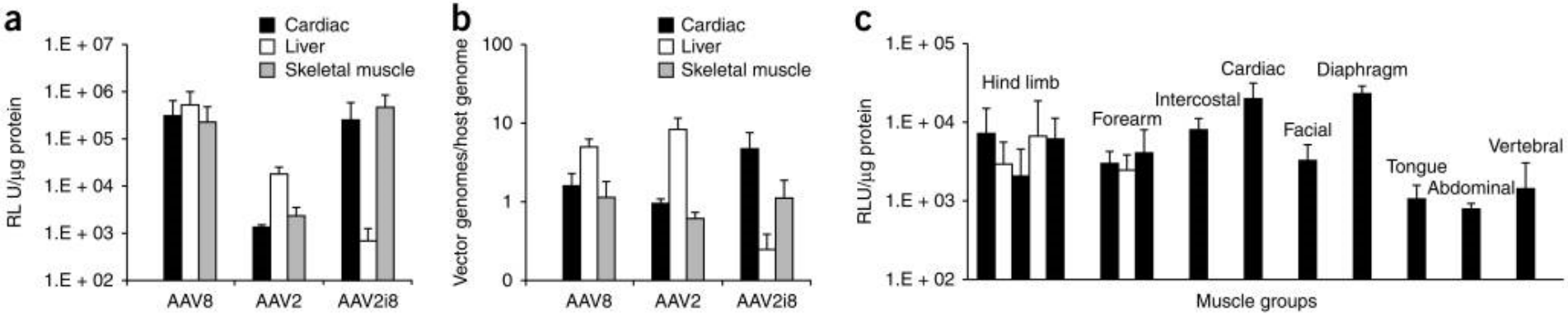
Levy et al., 2009

Opie *et* al., 2005; Kern *et* al., 2003; O'Donnell et al., 2009

Reengineering the AAV2:Heparin Footprint - I

Redirecting viral vectors from liver to heart & muscle

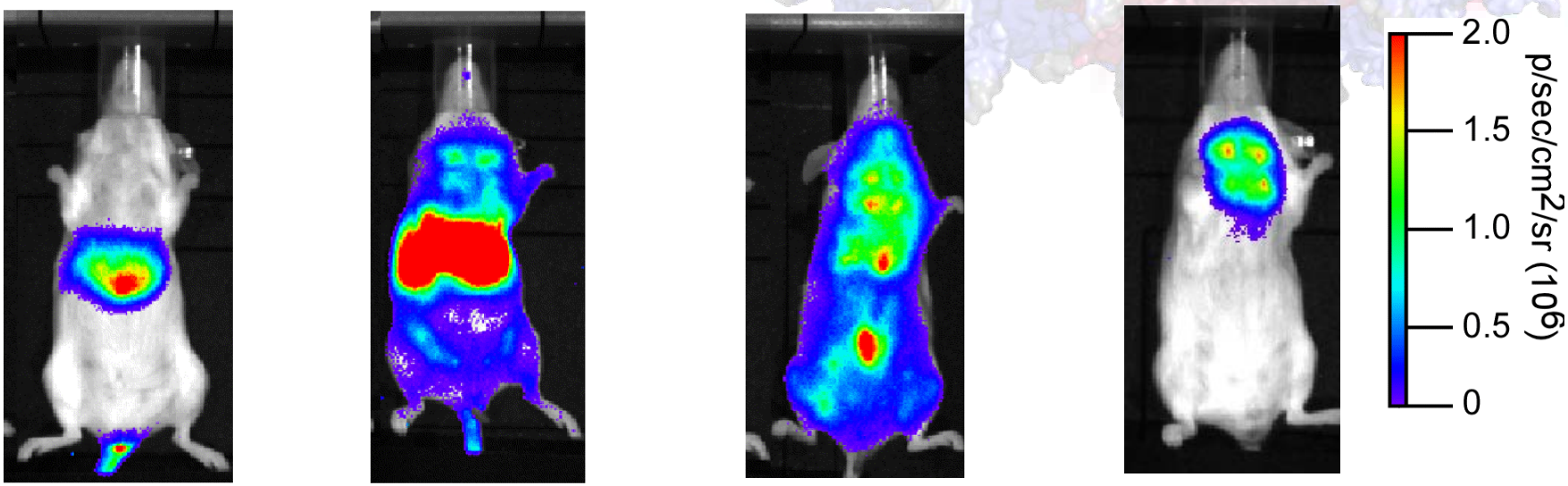




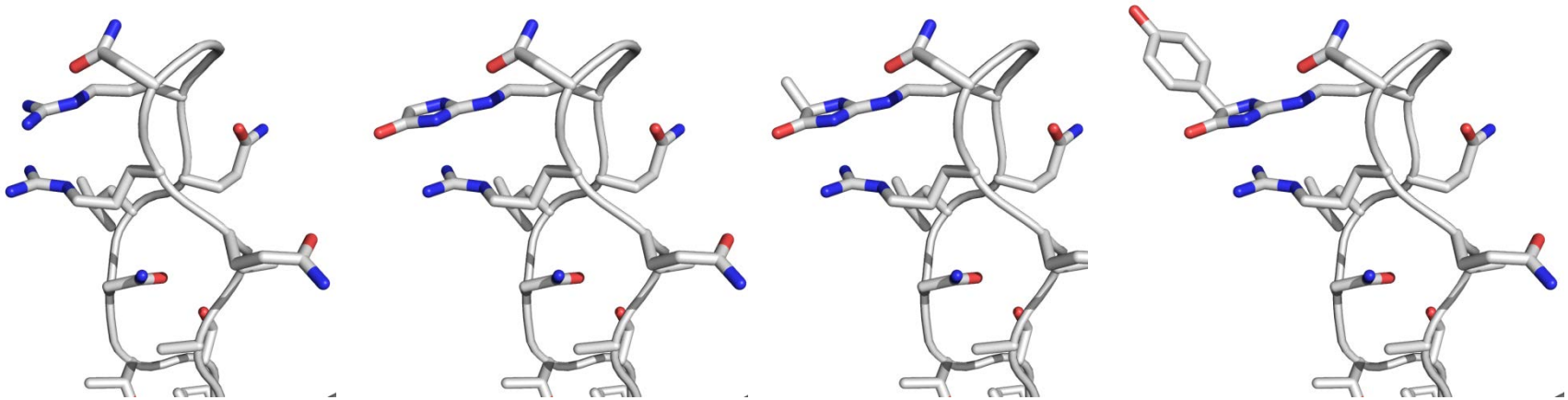
Asokan *et al.*, Nat Biotechnol, 2010

Reengineering the AAV2:Heparin Footprint – II

Fine tuning viral vector tropism

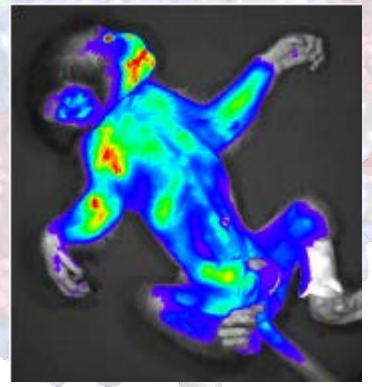


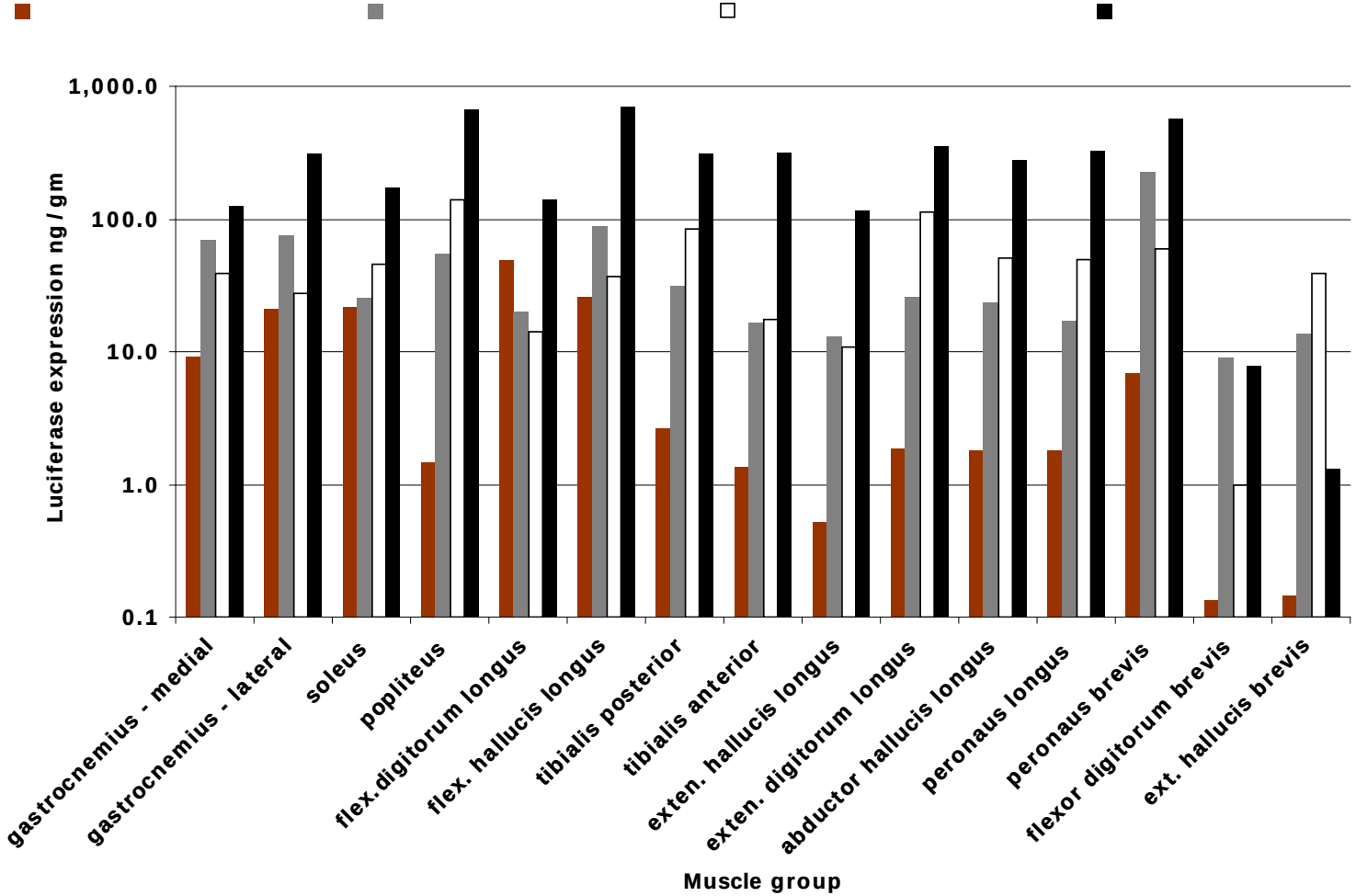
WT **GO** **MGO** **HPGO**



Horowitz et al., Bioconjug Chem, 2010

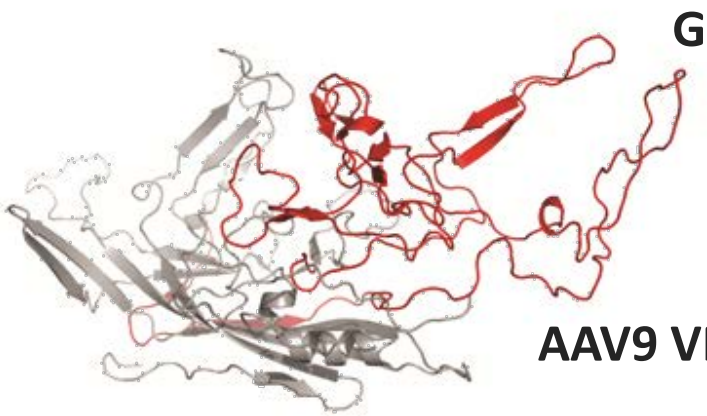
Large Animal Models:
AAV2i8 is muscle-tropic in primates

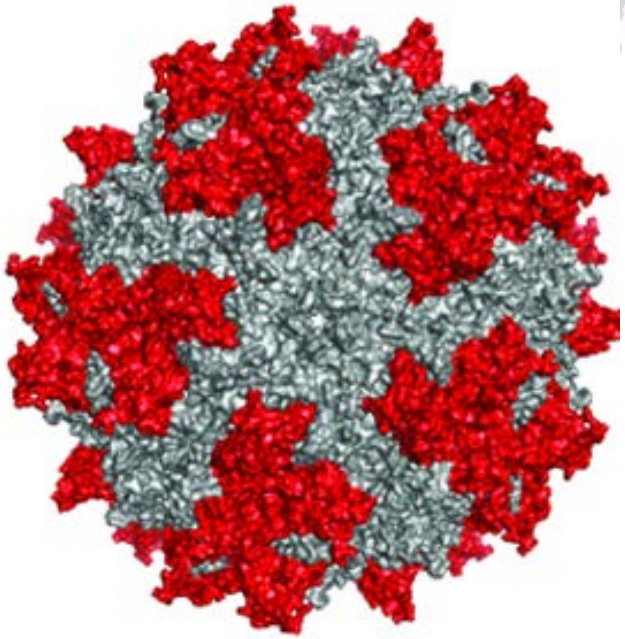


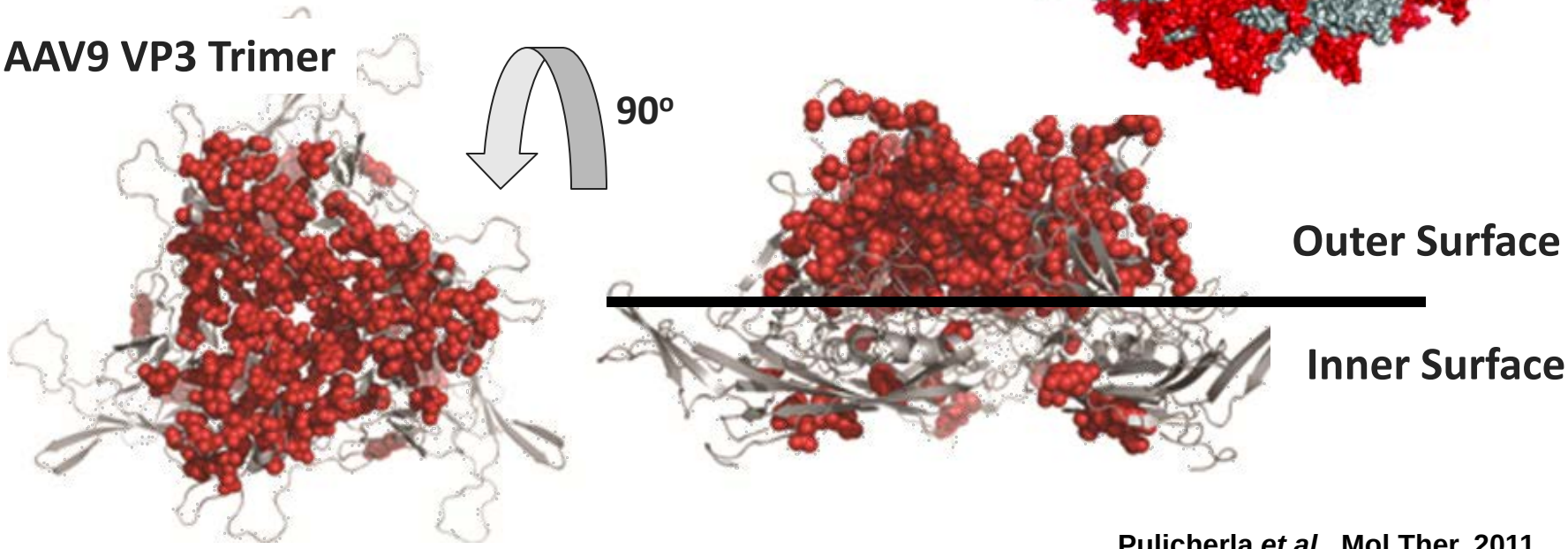


Tarantal, Wolff, Samulski and Asokan, Unpublished

Reengineering unknown AAV receptor footprints



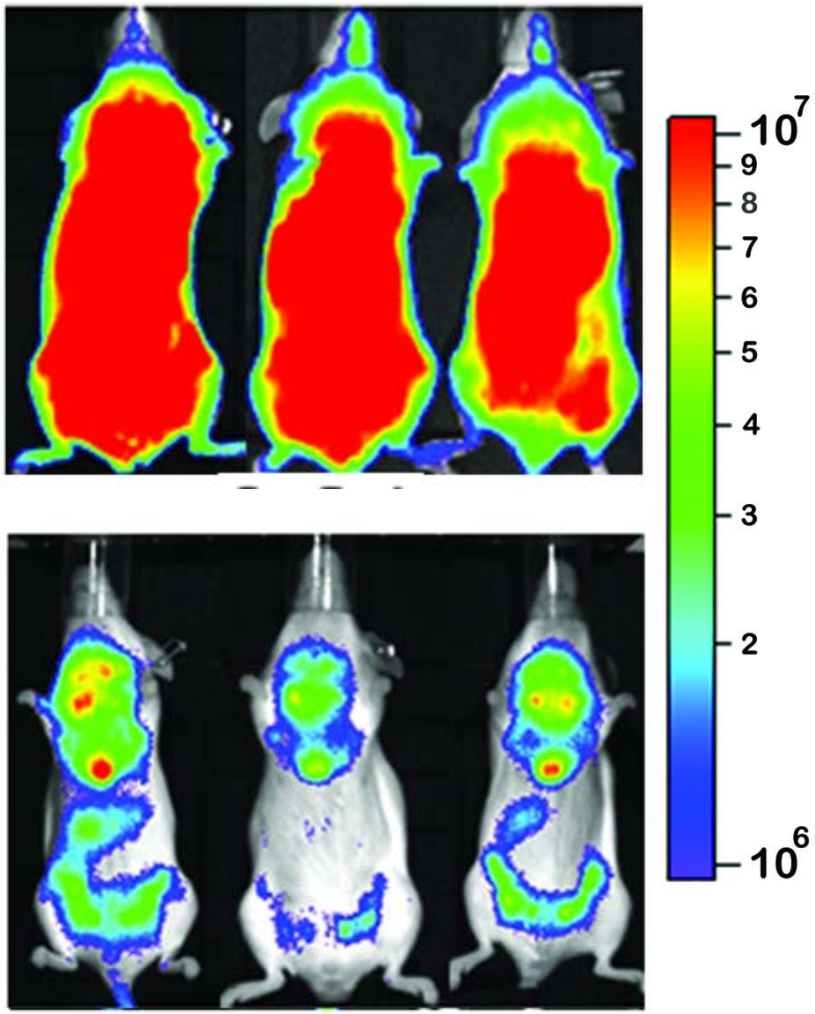


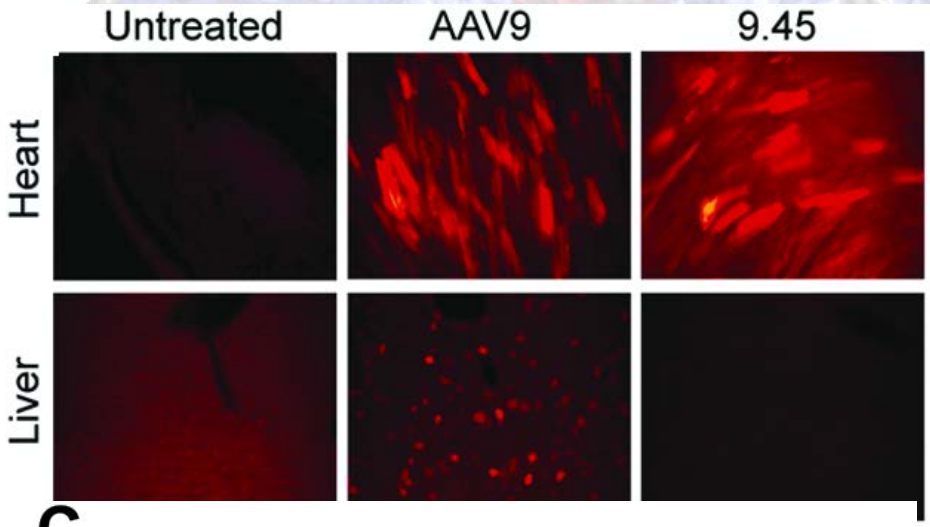


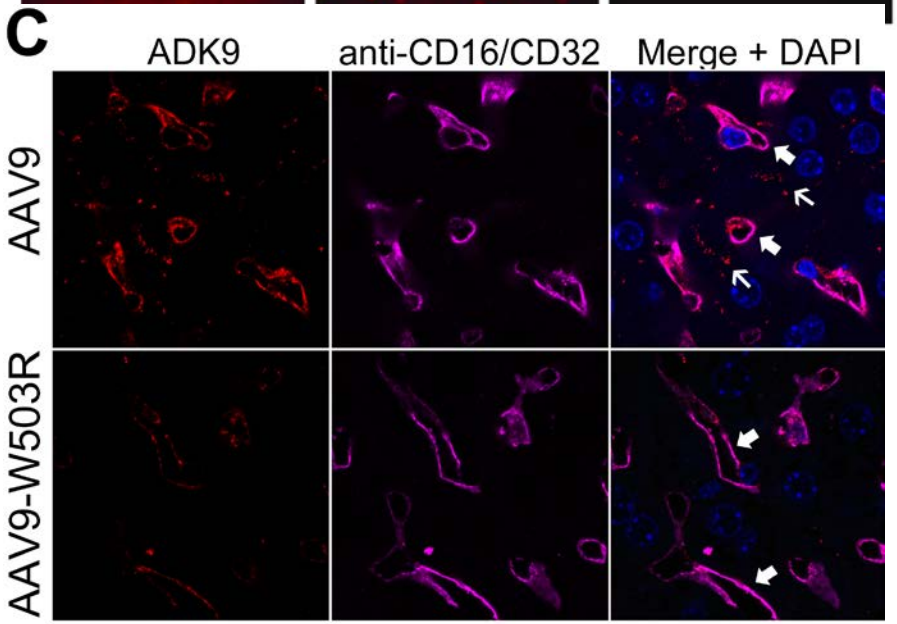
Pulicherla *et al*., Mol Ther, 2011

Reengineering unknown receptor footprints: AAV9

De-targeting viral vectors from the liver

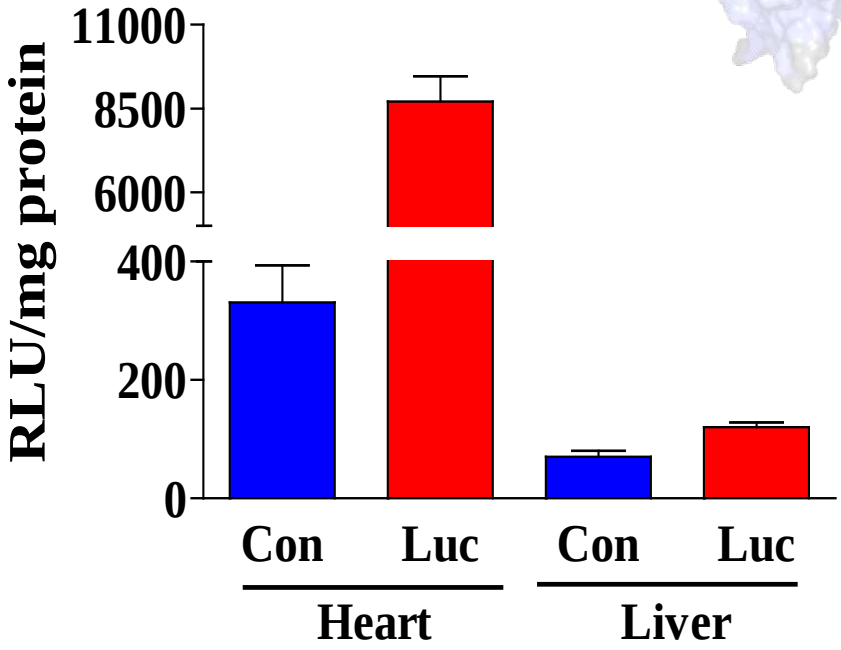






Pulicherla *et al.*, Mol Ther, 2011, Shen et al., 2012

Intracoronary AAV Delivery in Pigs

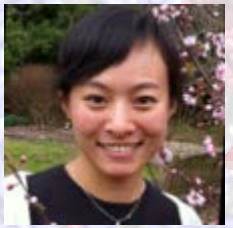


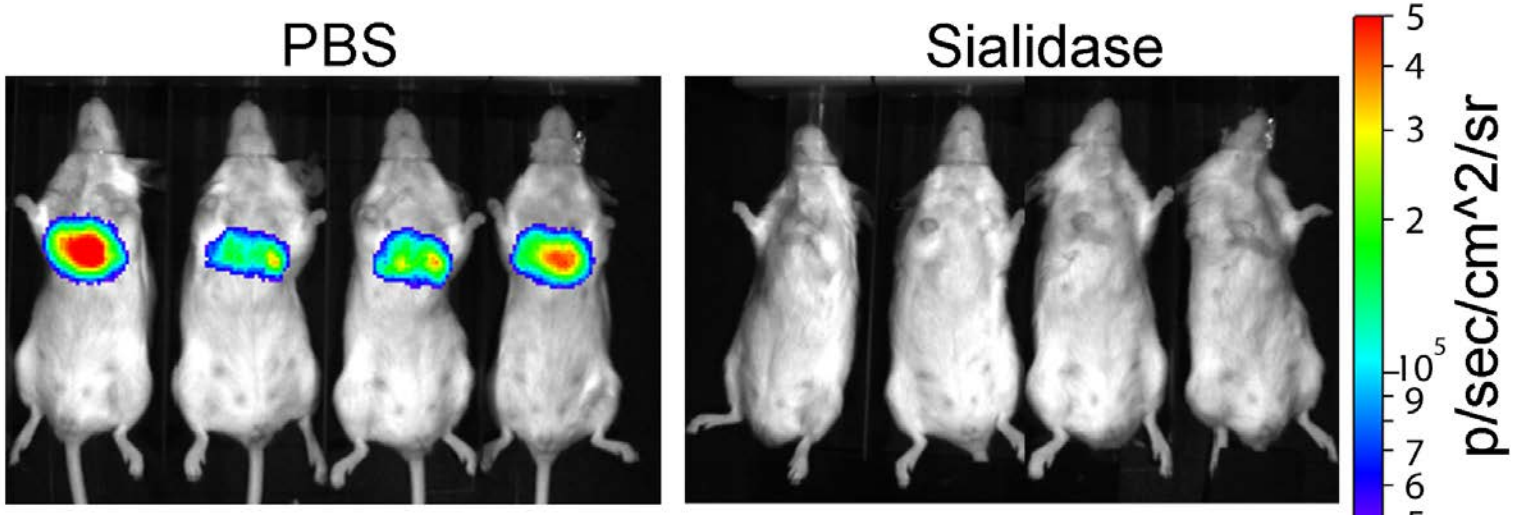
- AAV9.45-luc (10^{14} gc) delivered in LAD during nitroprusside infusion (30 kg pig, killed 6 weeks after intracoronary delivery)
- LV luciferase activity increased 26-fold (mean value of 48 samples)
- In contrast, luciferase activity was increased only 1.6 fold in liver, indicating relative cardiac specificity

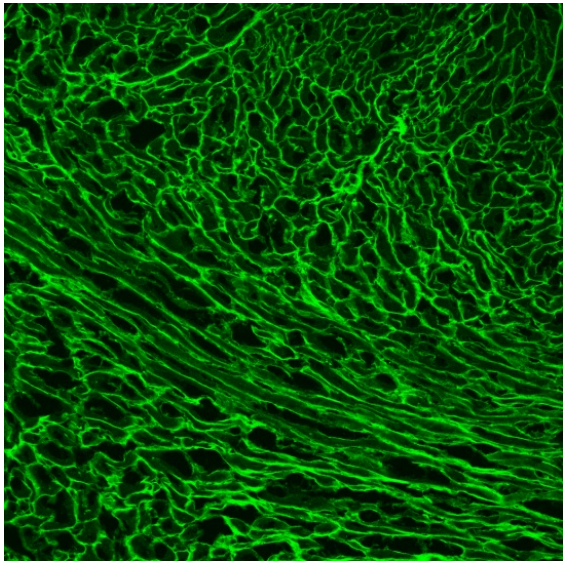
Kirk Hammond (UCSD) and A Asokan, unpublished

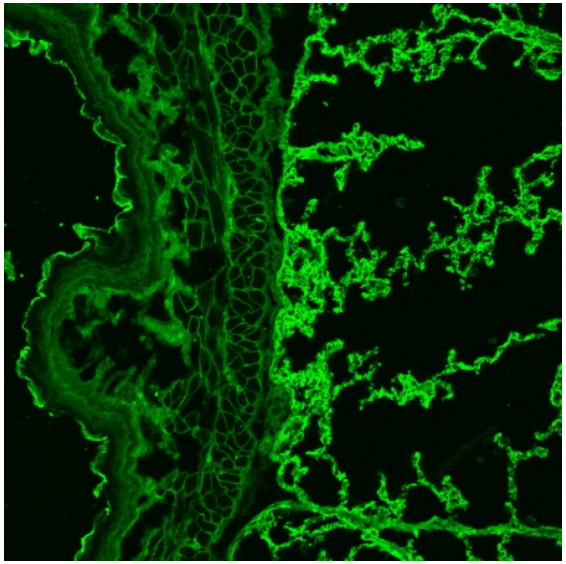
Reengineering virus-glycan interactions: AAV4

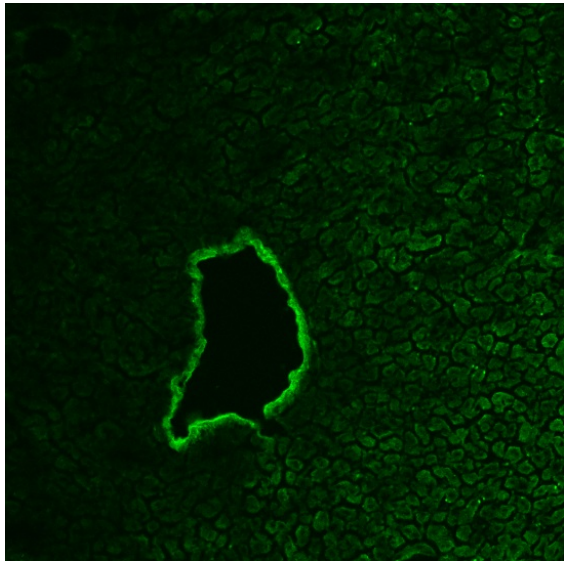
Dissecting cardiopulmonary tropism





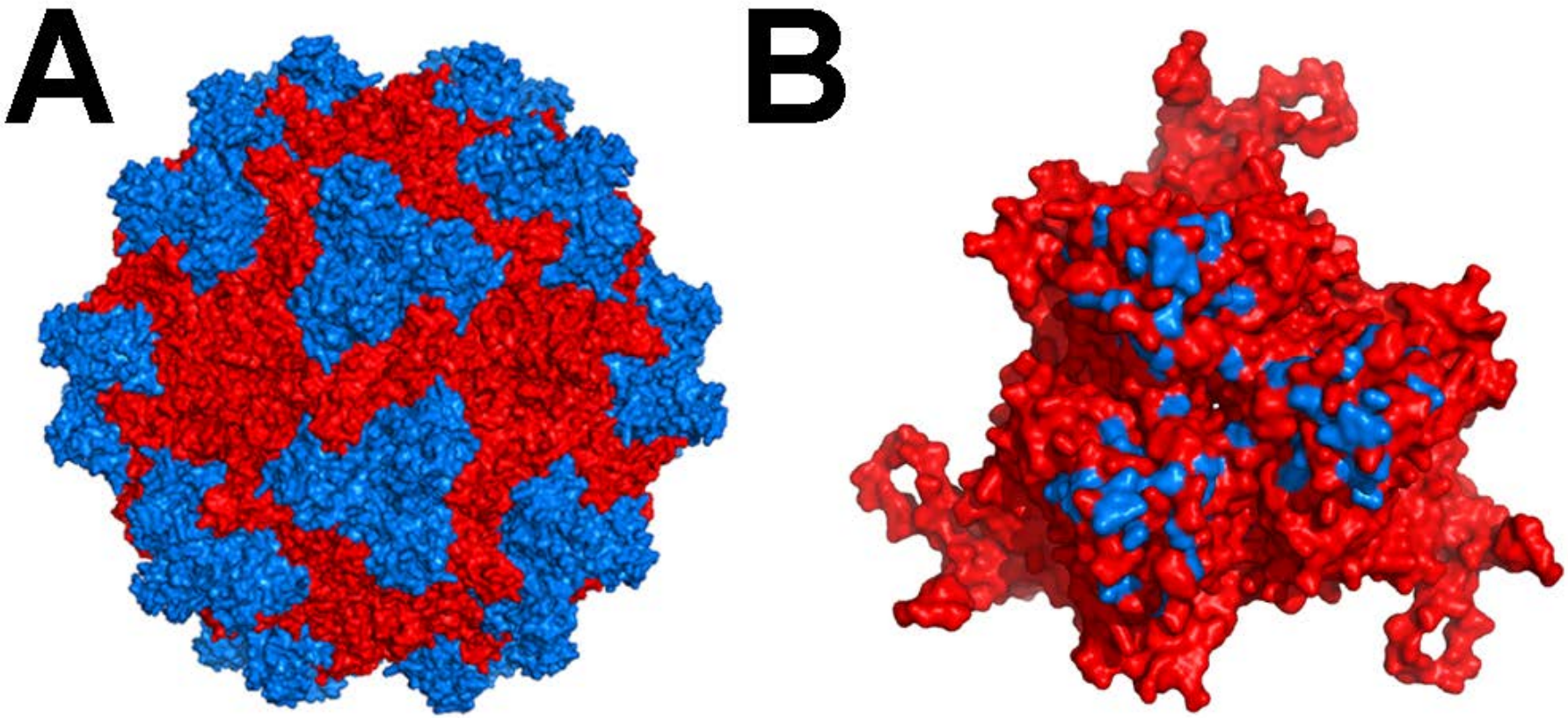






Shen & Asokan, unpublished

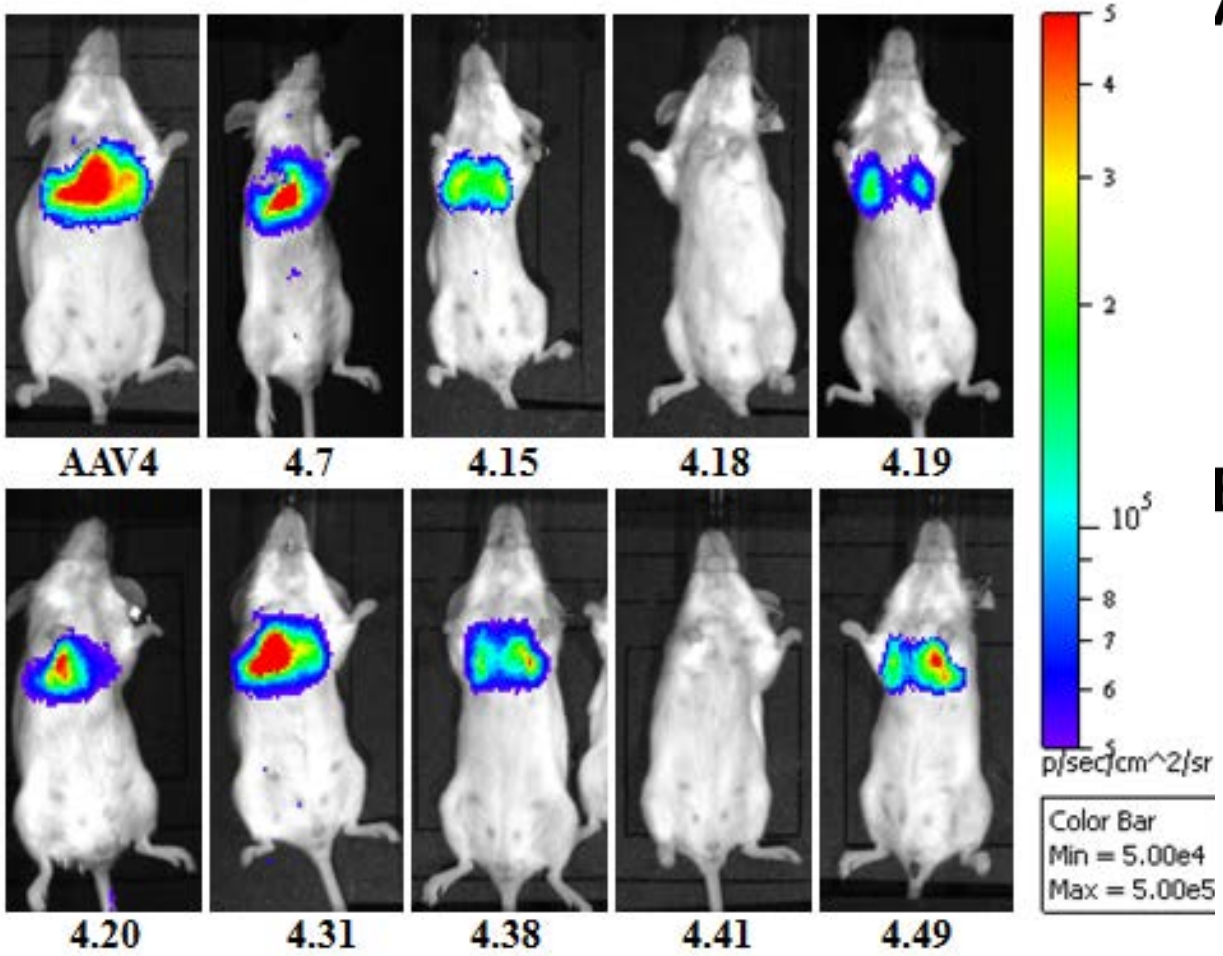
Reengineering AAV4:Mucin interactions

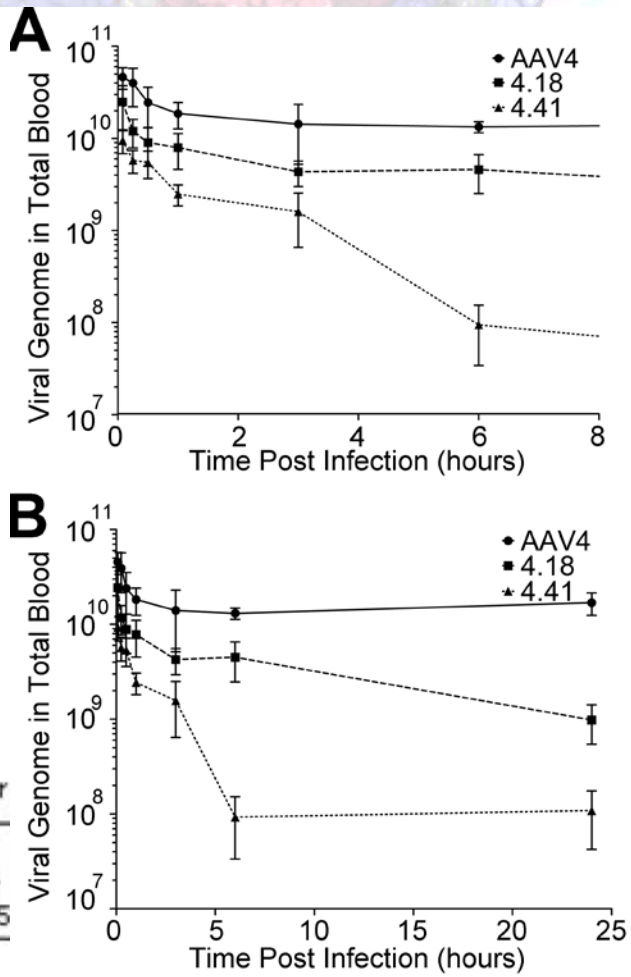


Shen & Asokan, unpublished

Reengineering AAV4:Mucin interactions

Dissecting cardiopulmonary tropism



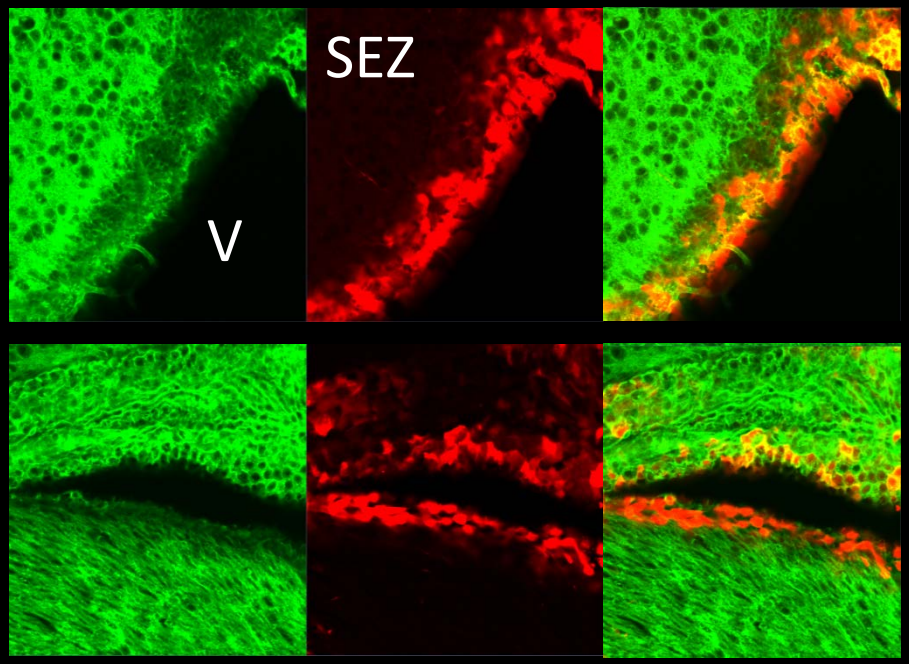


Shen & Asokan, unpublished

Reengineering AAV4:Mucin interactions

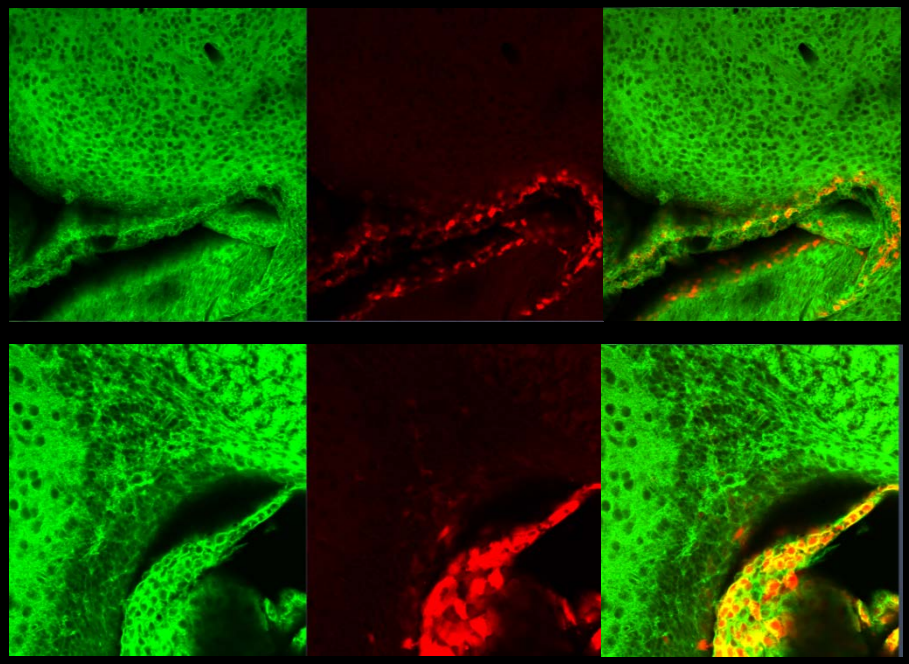
Dissecting CNS Tropism

AAV4



Mucin tdTomato Overlay

AAV4.18 Mutant

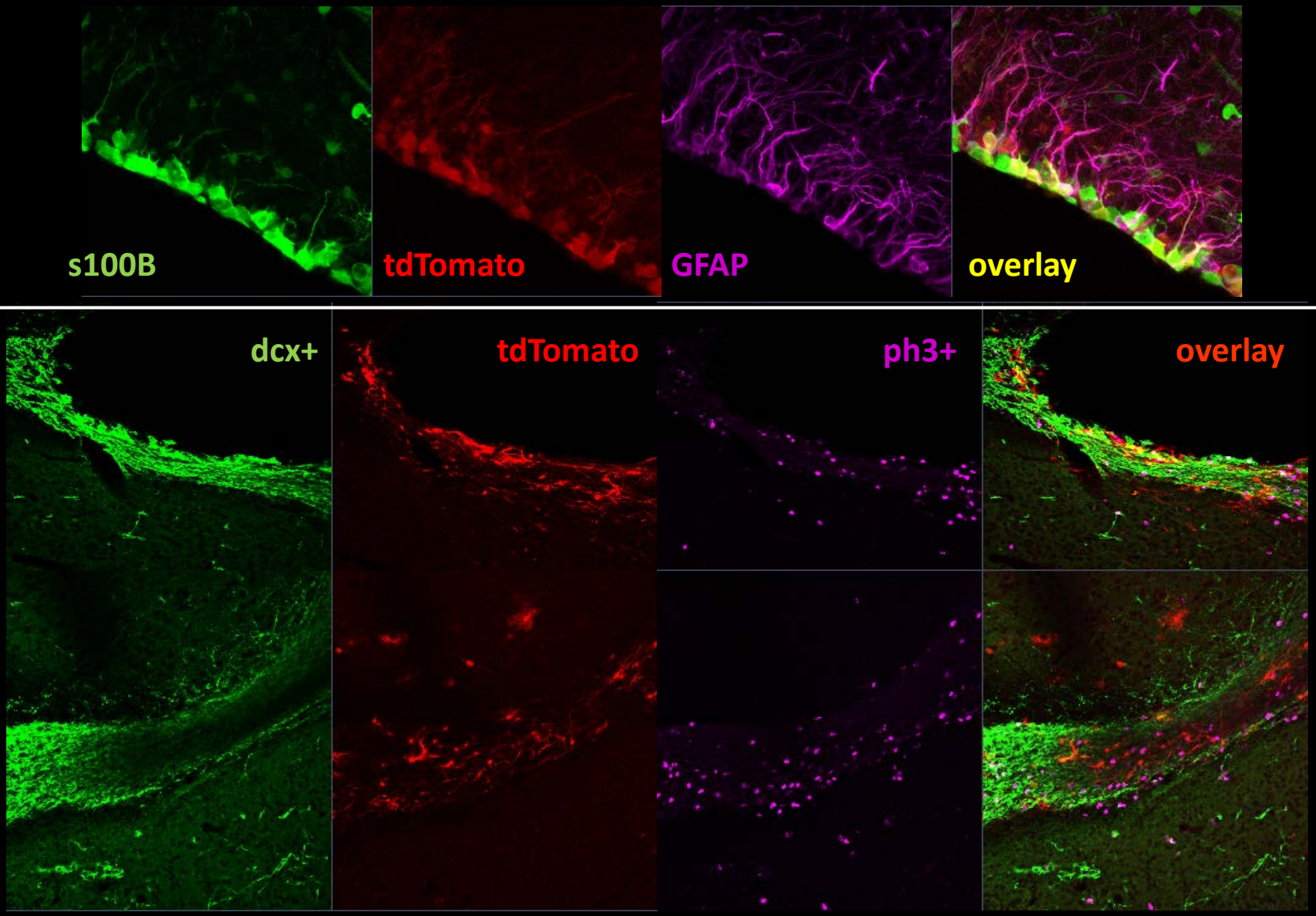


Mucin tdTomato Overlay

Giridhar & Asokan, unpublished

Reengineering AAV4:Mucin interactions

Expanding the CNS Gene Transfer Toolkit



Giridhar & Asokan, unpublished

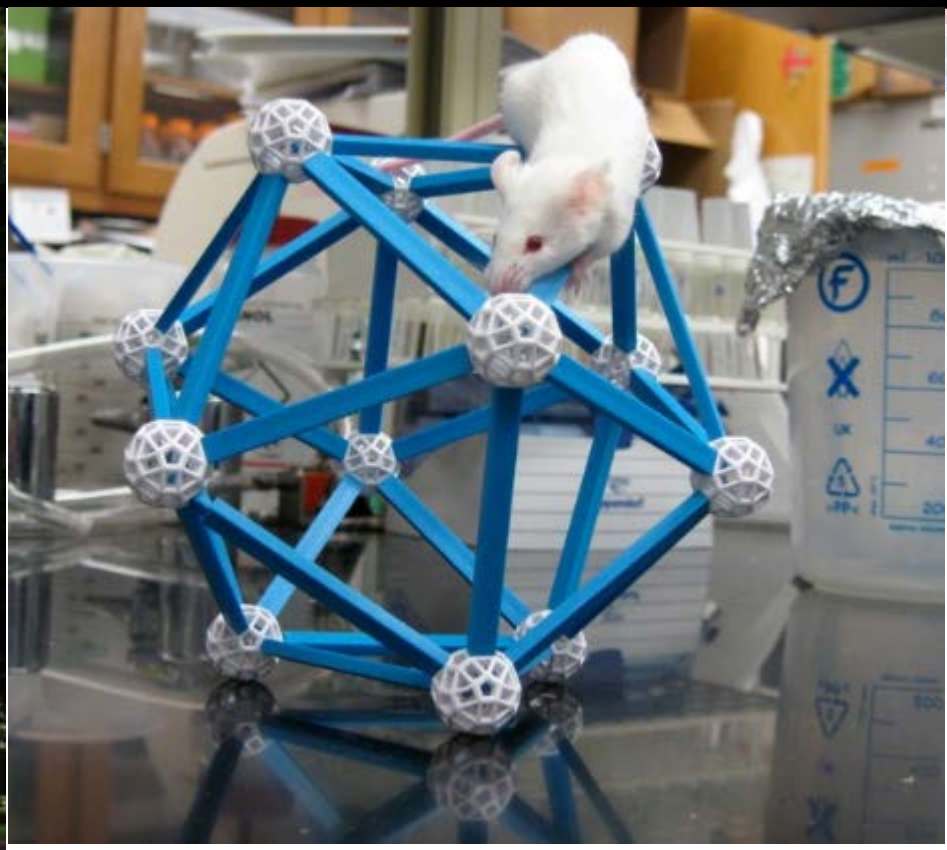
Building an AAV vector pipeline…

- Safety
  - Re-engineer AAV strains to decrease off-target transduction
- Safety & Efficacy
  - Identify & re-engineer AAV strains to increase transduction efficiency & decrease effective vector dose
- Efficacy
  - Identify & re-engineer strains to escape pre-existing immunity

ASOKANLAB

SYNTHETIC VIROLOGY AND GENE THERAPY

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL



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