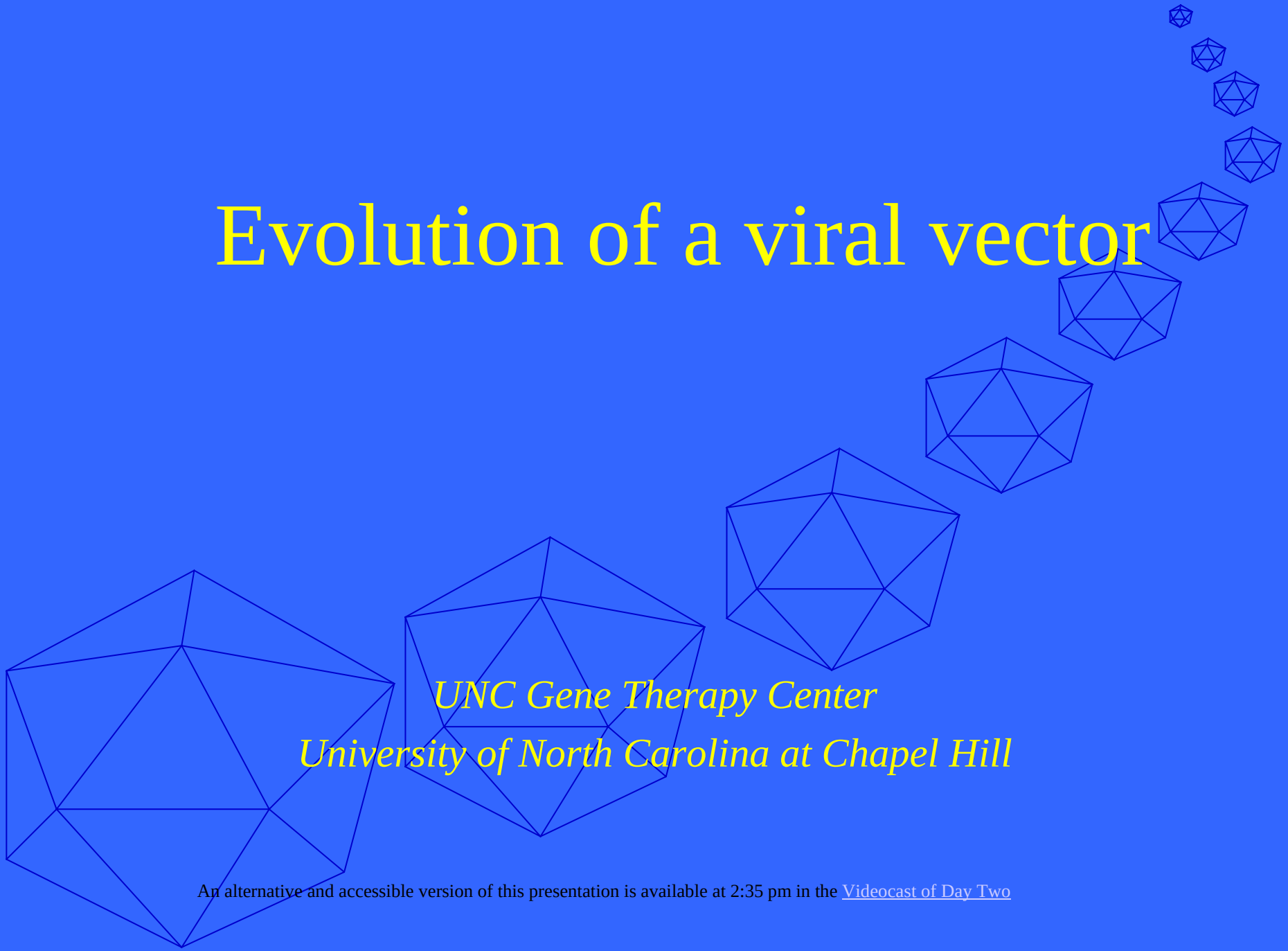
Evolution of a viral vector

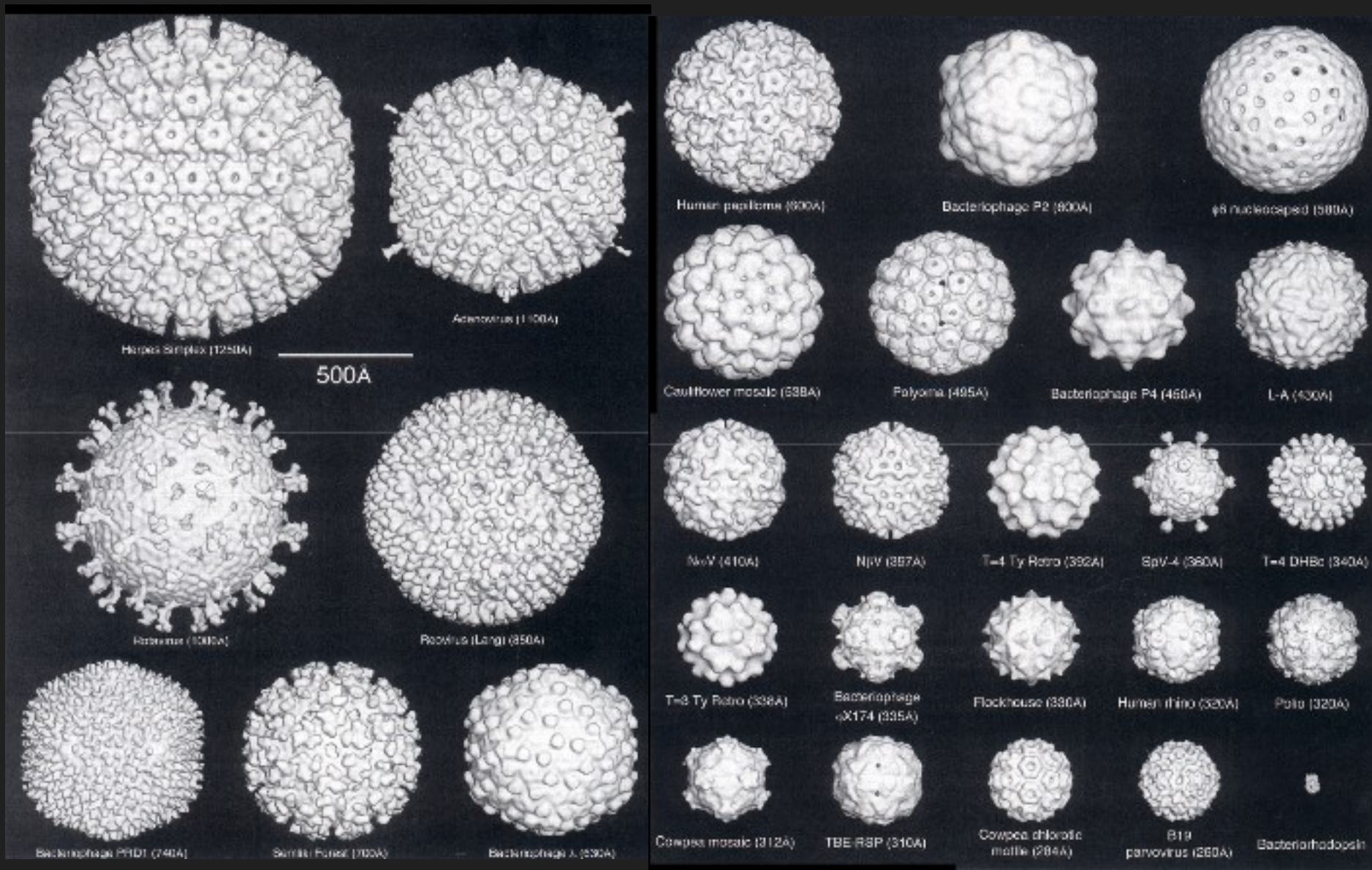


UNC Gene Therapy Center

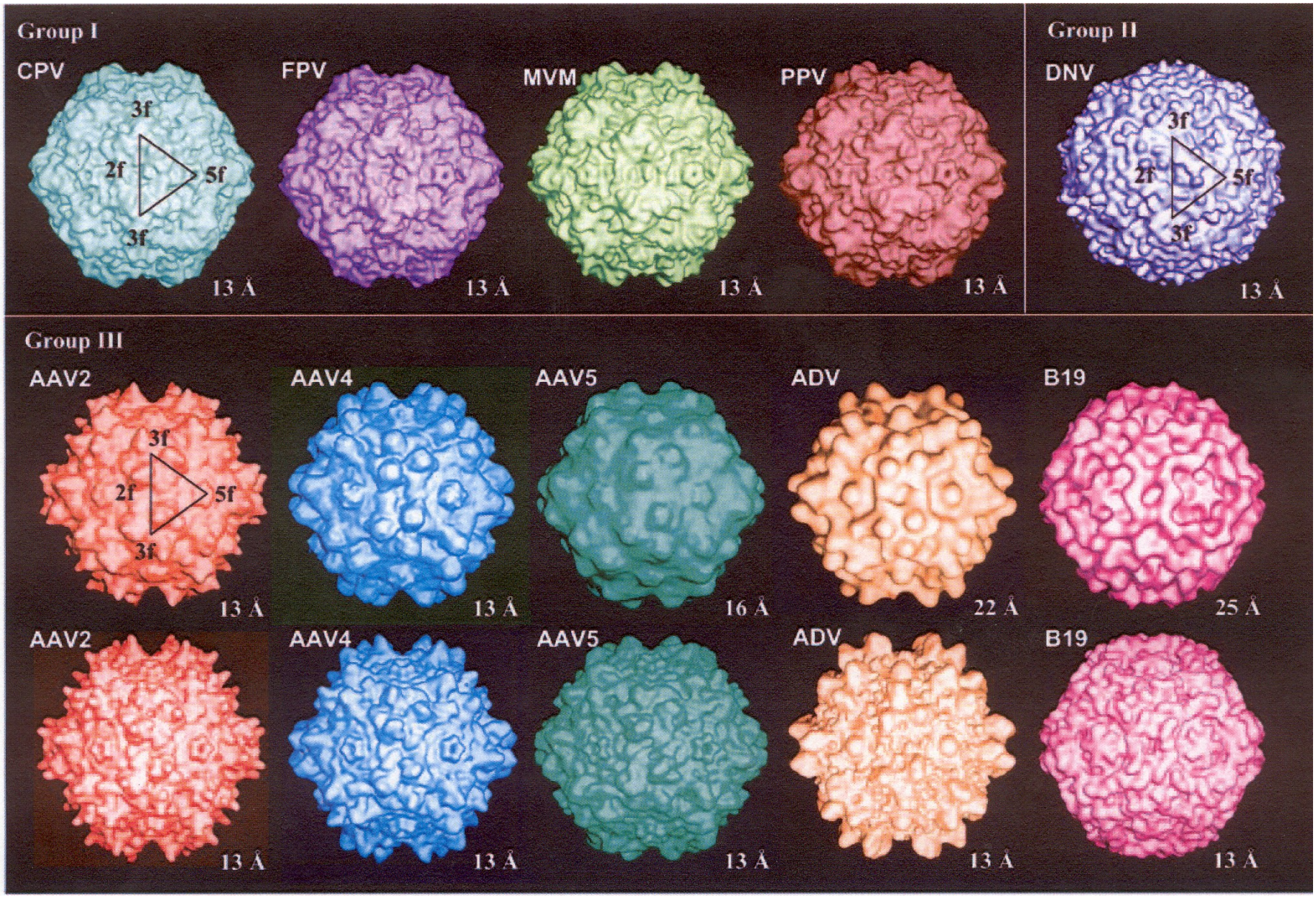
University of North Carolina at Chapel Hill

An alternative and accessible version of this presentation is available at 2:35 pm in the Videocast of Day Two

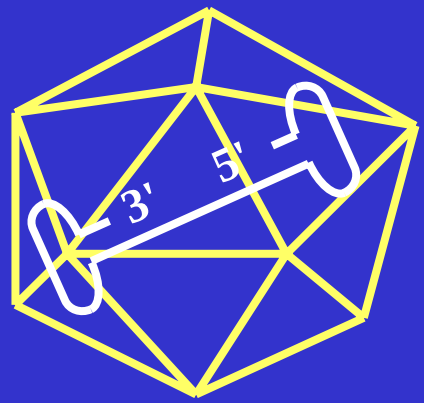
I do not have significant financial interests related to this conference.



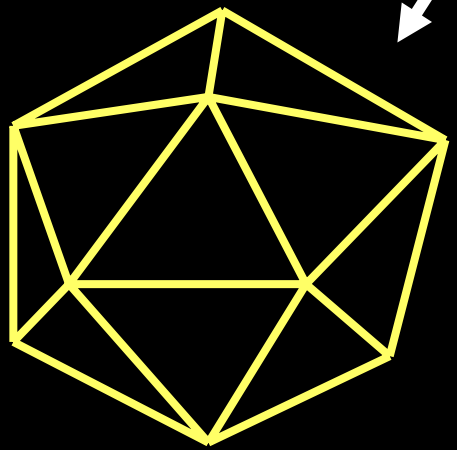
Baker et al., 1999. Microbiol. Mol. Biol. Rev. 63:862-922.


Group I
CPV
3f
2f
5f
3f
13 Å
FPV
13 Å
MVM
13 Å
PPV
13 Å
Group II
DNV
3f
2f
5f
3f
13 Å
Group III
AAV2
3f
2f
5f
3f
13 Å
AAV4
13 Å
AAV5
16 Å
ADV
22 Å
B19
25 Å
AAV2
13 Å
AAV4
13 Å
AAV5
13 Å
ADV
13 Å
B19
13 Å

AAV as a Gene Delivery Tool



Capsid

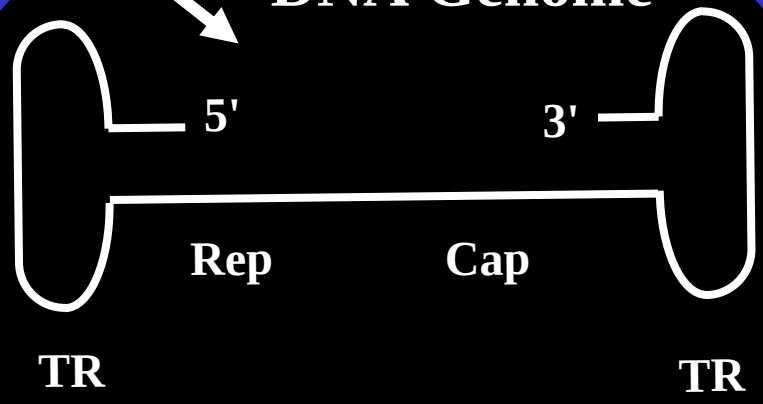


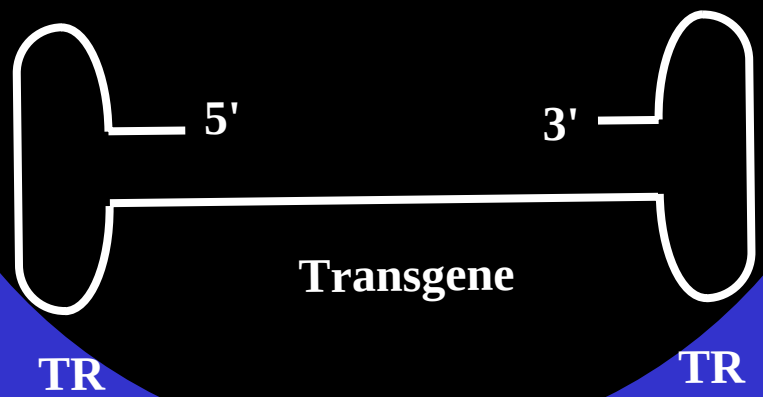
wtAAV

Recombinant

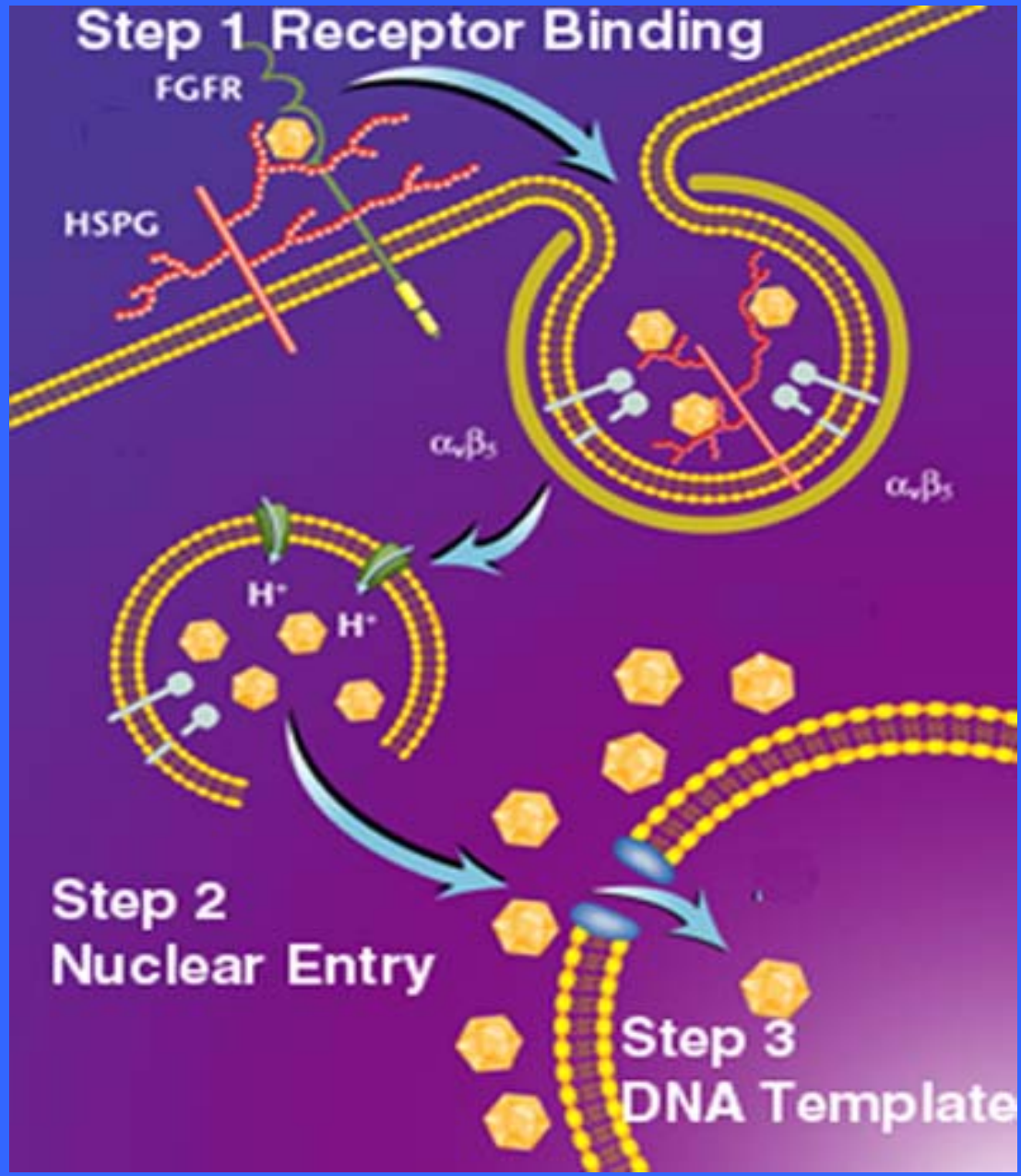
(rAAV)

Single-stranded DNA Genome



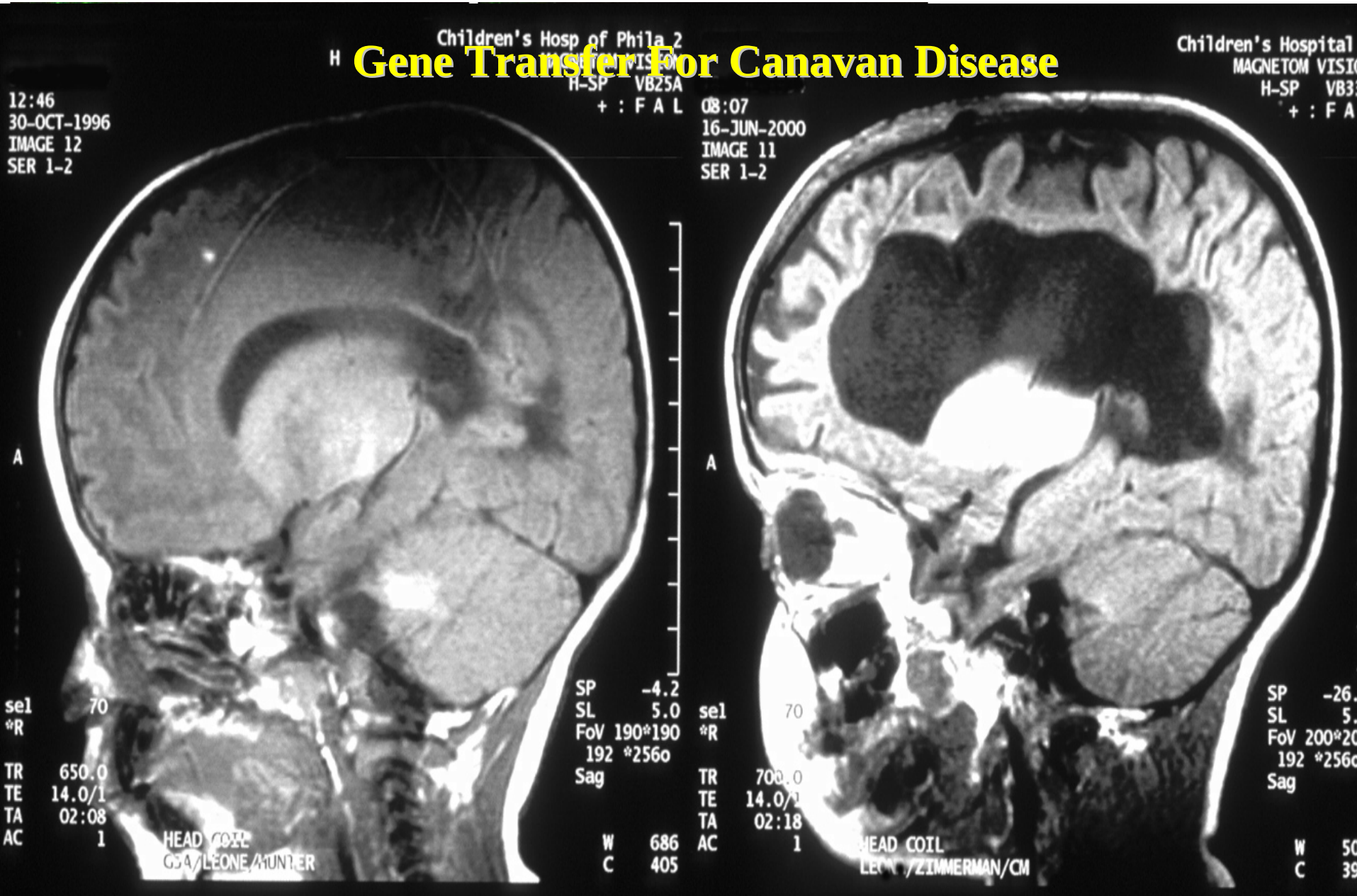


AAV Entry


Step 1 Receptor Binding
FGFR
HSPG
$\alpha_v\beta_5$
$\alpha_v\beta_5$
H+
H+
Step 2
Nuclear Entry
Step 3
DNA Template

Vector Transduction In Vivo

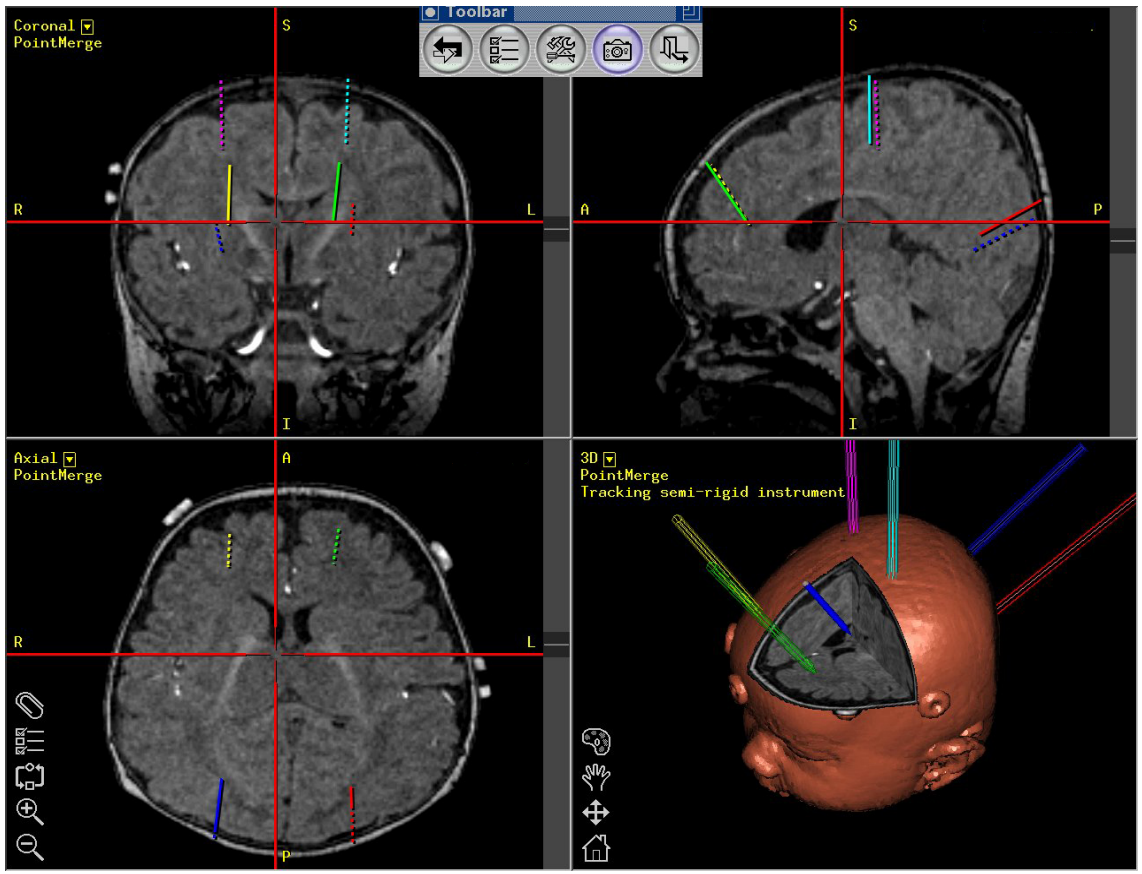
Gene Transfer For Canavan Disease



Immune responses to AAV in a phase I study for Canavan disease.

McPhee SW, Janson CG, Li C, Samulski RJ, Camp AS Francis J, Shera D, Lioutermann L, Feely M, Greese A, Leone P.

Journal of Gene Medicine 2006 May :8(5):577-88.



Clinical trials in neurological disorders using AAV vectors: promises and challenges.

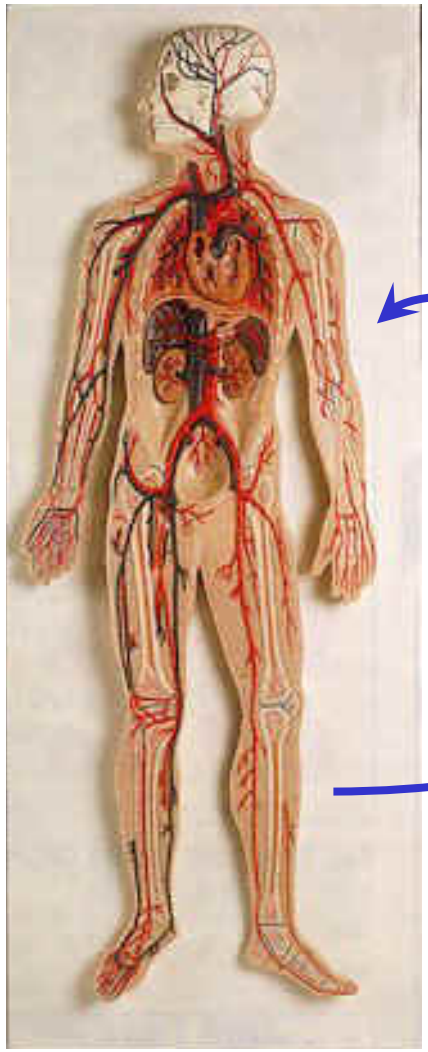
Mandel RJ,
Burger C.
University of Florida College of Medicine, Department of Neuroscience, PO Box 100244, Gainesville, FL 32610, USA. rmandel@ufl.edu

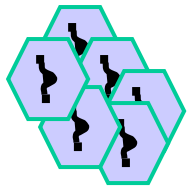
Currently, there are **five phase I clinical trials** of recombinant adeno-associated viral vectors for the treatment of neurological disorders. Two Parkinson's disease (PD), the third trial is aimed at treating Canavan's disease, a pediatric leukodystrophy, the fourth trial targets Alzheimer's disease (AD), and the fifth will attempt to target the lysosomal storage disorder, Batten's disease.
Other gene therapy treatment strategies for PD and other disorders, such as amyotrophic lateral sclerosis, are also on the horizon.

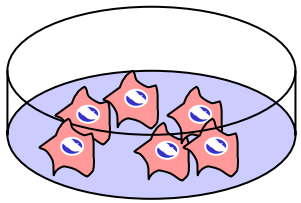
Ex vivo

Virus+gene

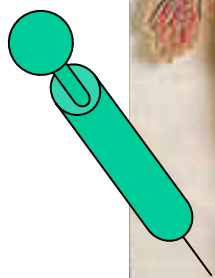
In vivo

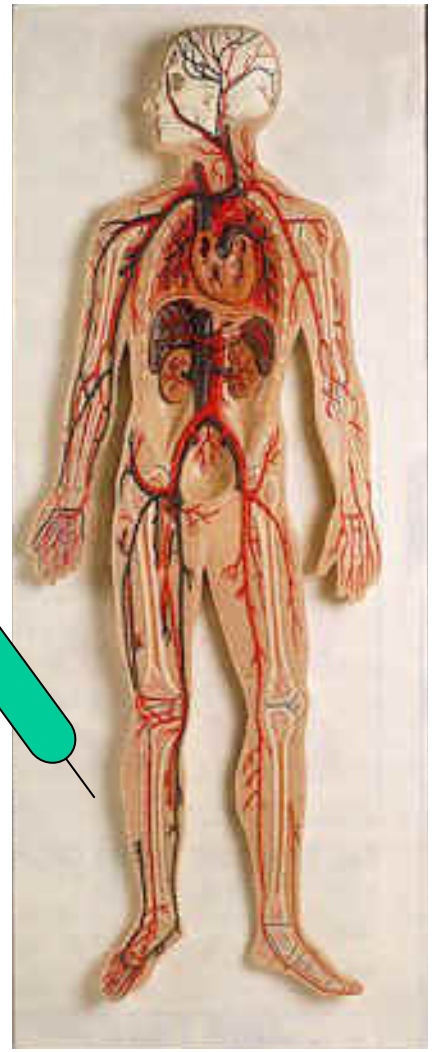






Patient's
Stem cells






Vector Development

To Increase Vector gene expression

- **Optimizing Expression Cassette**
- **Increase AAV Transduction Efficiency by Using rAAV Serotype Vectors**

AAV1: W Xiao, et al. J. of Virology, 1999, p3994

AAV3: E Rutledge, et al. J. of Virology, 1998, p309
A Handa, et al. J. of general Virology, 2000, p2077

AAV4: J Chiorini, et al. J. of Virology, 1997, p6823
B Davidson,et al. PNAS, 2000

AAV5: J Chiorini, et al. J. of Virology, 1999, p1309
J Zabner, et al. J of Virology, 2000, p3852

AAV7,8 Gao, et al. PNAS, 2002

JOURNAL OF VIROLOGY, Jan. 2002, p. 000–000
0022-538X/02/$04.00+0 DOI: 10.1128/JVI.76.2.000–000.2002

Vol. 76, No. 2

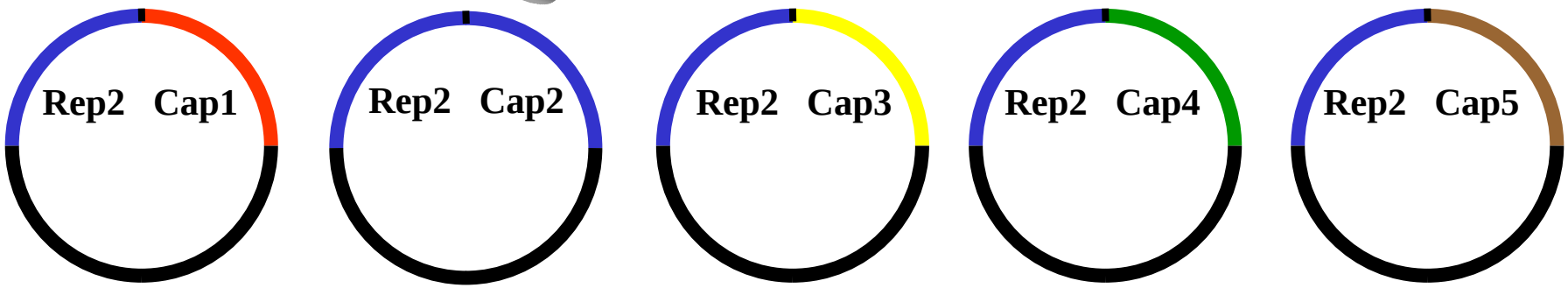
Cross-Packaging of a Single Adeno-Associated Virus (AAV) Type 2 Vector Genome into Multiple AAV Serotypes Enables Transduction with Broad Specificity

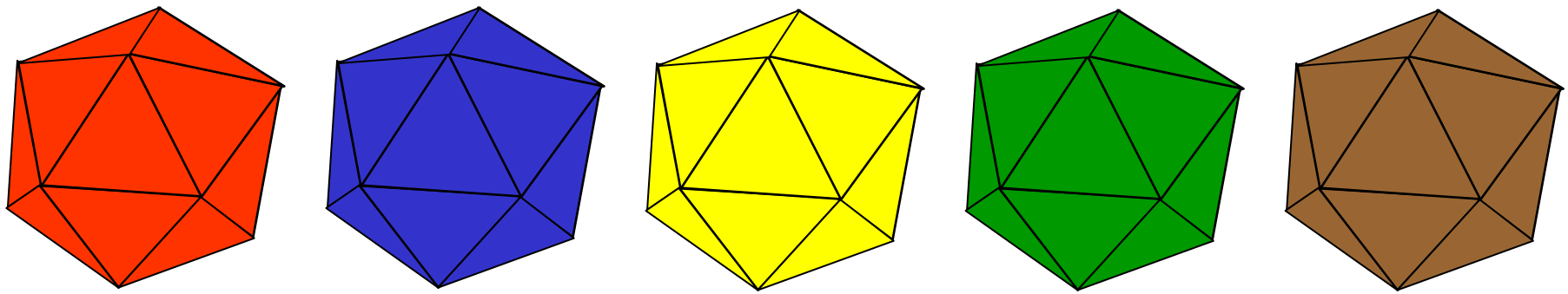
Joseph E. Rabinowitz,[1] Fabienne Rolling,[2] Chengwen Li,[1] Hervè Conrath,[2] Weidong Xiao,[3] Xiao Xiao,[4] and R. Jude Samulski[1,5]*

Gene Therapy Center[1] and Department of Pharmacology,[5] University of North Carolina, Chapel Hill, North Carolina; Laboratoire de Thérapie Génique, CHU Hotel-DIEU, 44035 Nantes Cedex 01, France[2]; Division of Hematology, Department of Pediatrics, University of Pennsylvania School of Medicine and Children's Hospital of Philadelphia, Philadelphia, Pennsylvania[3]; and Department of Microbiology, University of Pittsburgh, Pittsburgh, Pennsylvania[4]

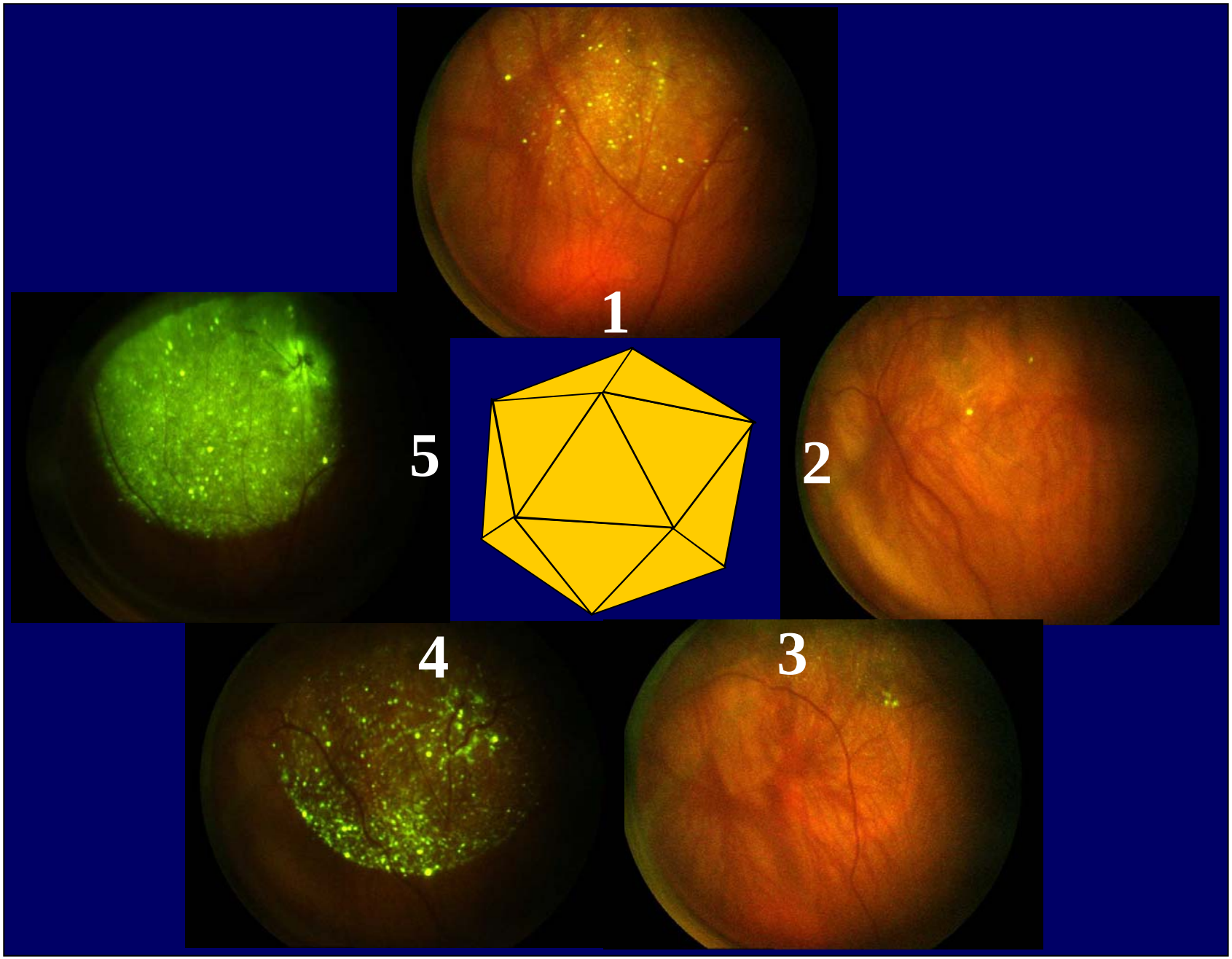
Received 7 August 2001/Accepted 9 October 2001

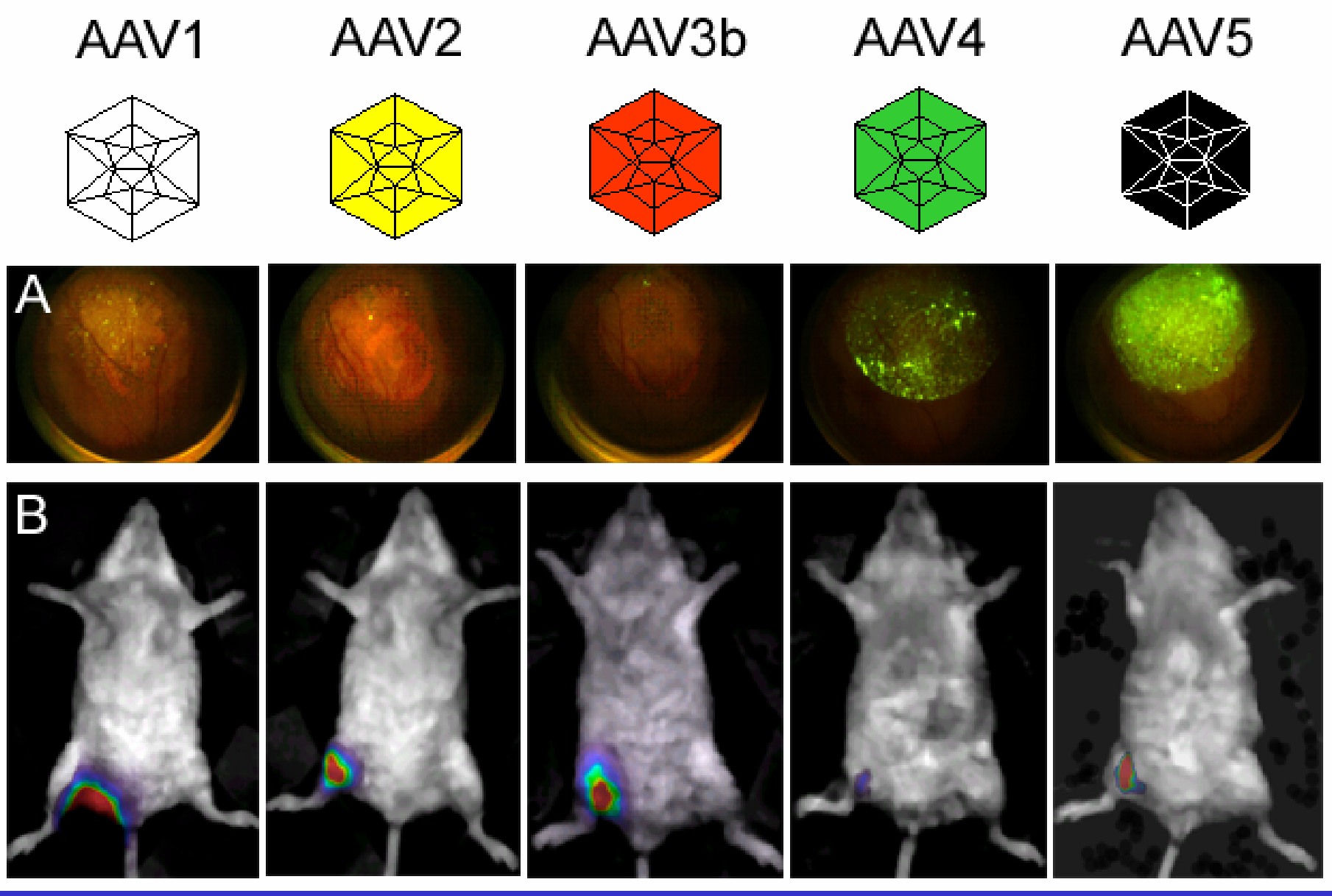
Hybrid Vectors



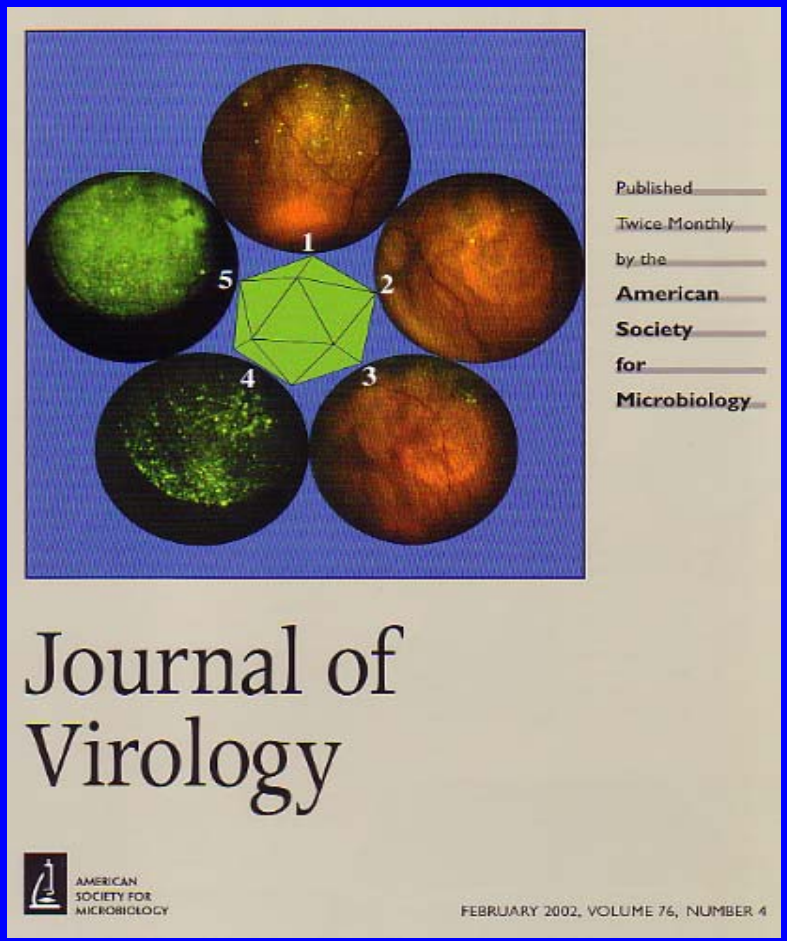


rAAV1 rAAV2 rAAV3 rAAV4 rAAV5


1
2
3
4
5


AAV1
AAV2
AAV3b
AAV4
AAV5
A
B

Tissue Tropism



Rabinowitz, et. al. 2002

New AAV Serotypes

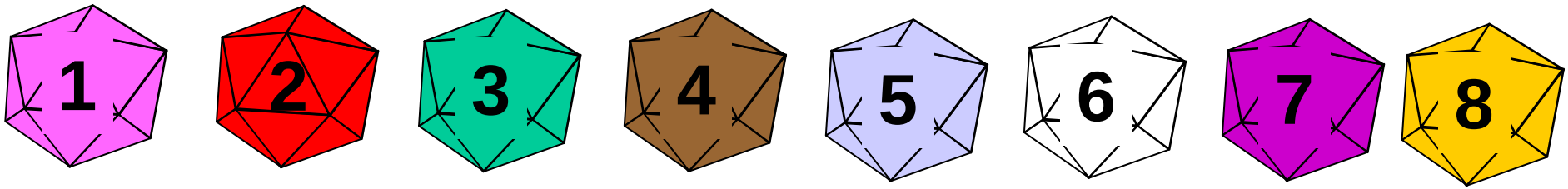
Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy

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

Institute for Human Gene Therapy and Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104; and Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104

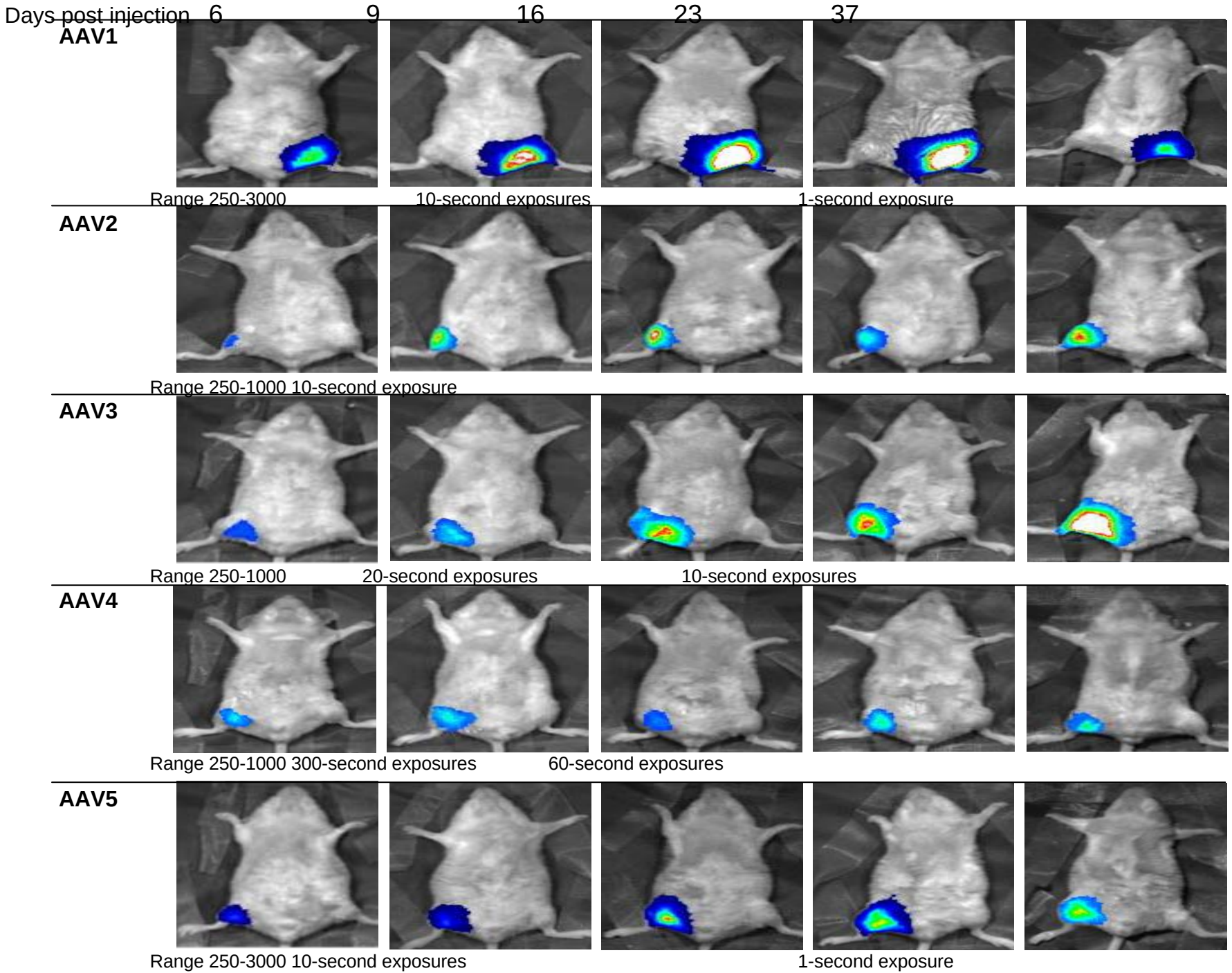
Communicated by Thomas E. Shenk, Princeton University, Princeton, NJ, July 10, 2002 (received for review March 21, 2002)

AAV SEROTYPES



1	2	3	4	5	6	7	8
Muscle	Liver	Sucks	Retina	Muscle Liver Retina	like AAV1	Muscle	Liver

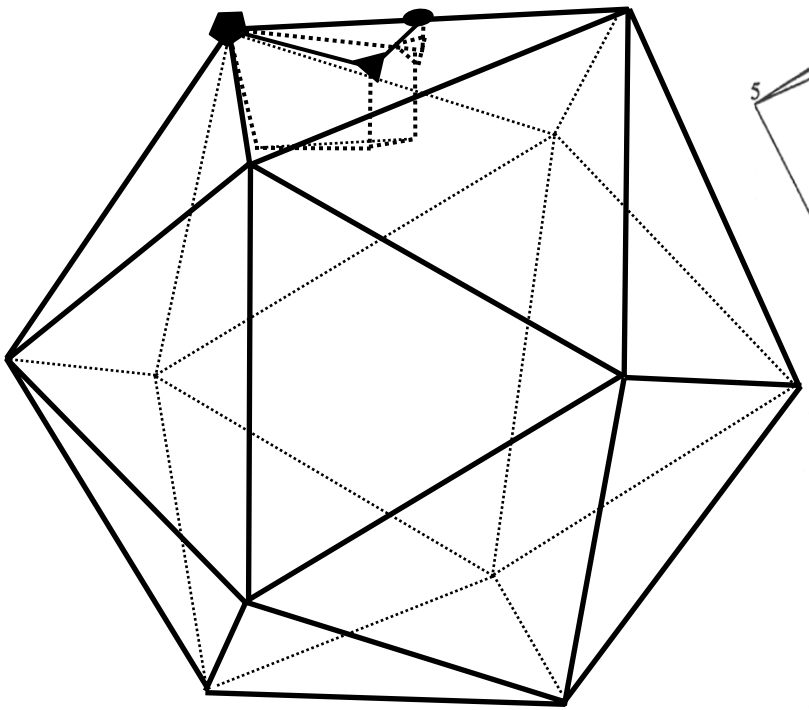
Over 100 new isolates of AAV


Days post injection 6 9 16 23 37
AAV1
Range 250-3000 10-second exposures 1-second exposure
AAV2
Range 250-1000 10-second exposure
AAV3
Range 250-1000 20-second exposures 10-second exposures
AAV4
Range 250-1000 300-second exposures 60-second exposures
AAV5
Range 250-3000 10-second exposures 1-second exposure

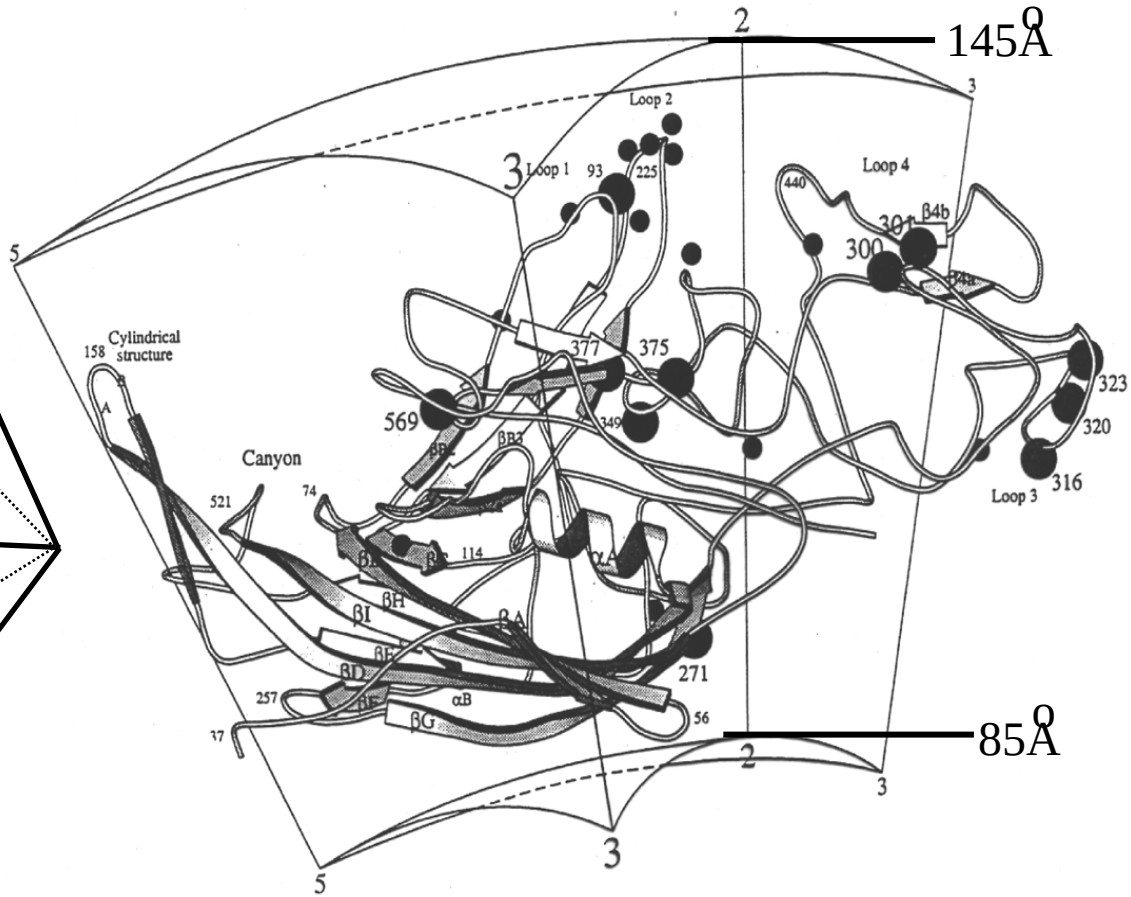
Jude

Field of Gene Therapy

Parvovirus Capsid Structure

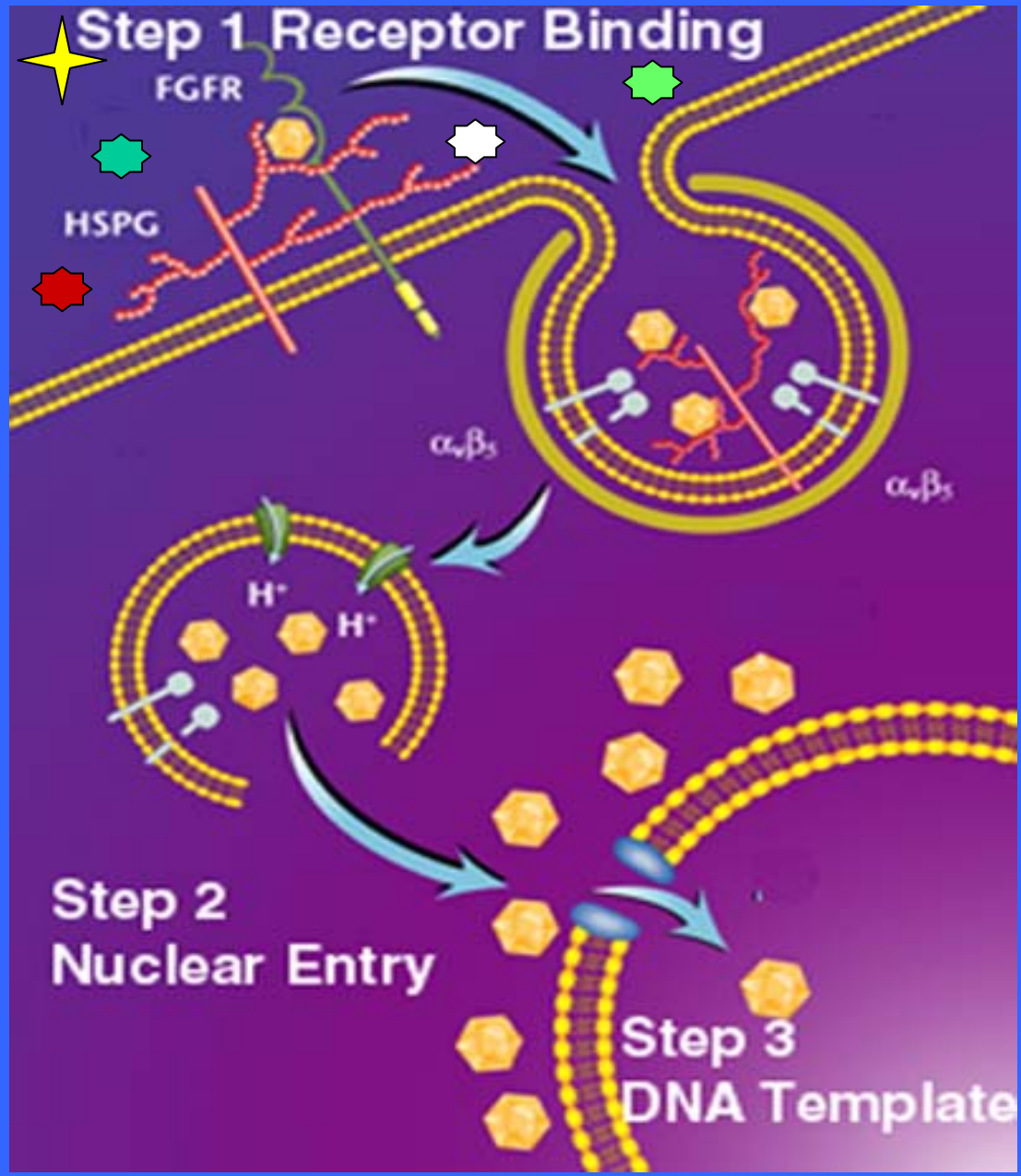


T= 1 symmetry, 60 subunits

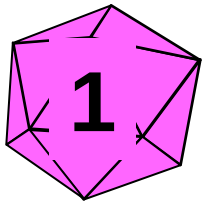


From Rossman and Chapman

AAV Entry


Step 1 Receptor Binding
FGFR
HSPG
$\alpha_v\beta_5$
$\alpha_v\beta_5$
H+
H+
Step 2
Nuclear Entry
Step 3
DNA Template

AAV SEROTYPES



Muscle

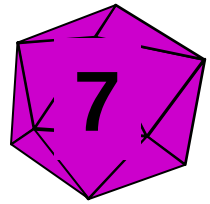
Liver

Sucks

Retina

Muscle
Liver
Retina

like AAV1



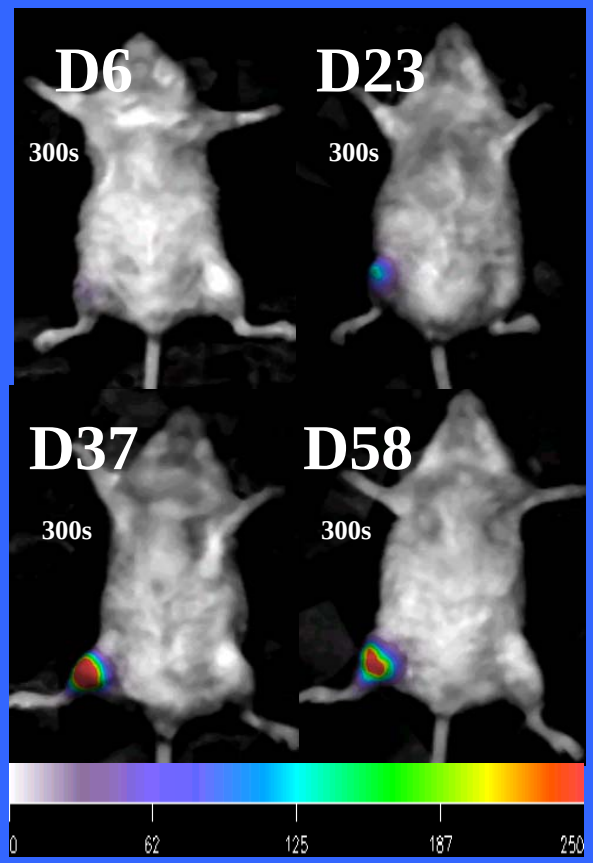
Muscle

Liver

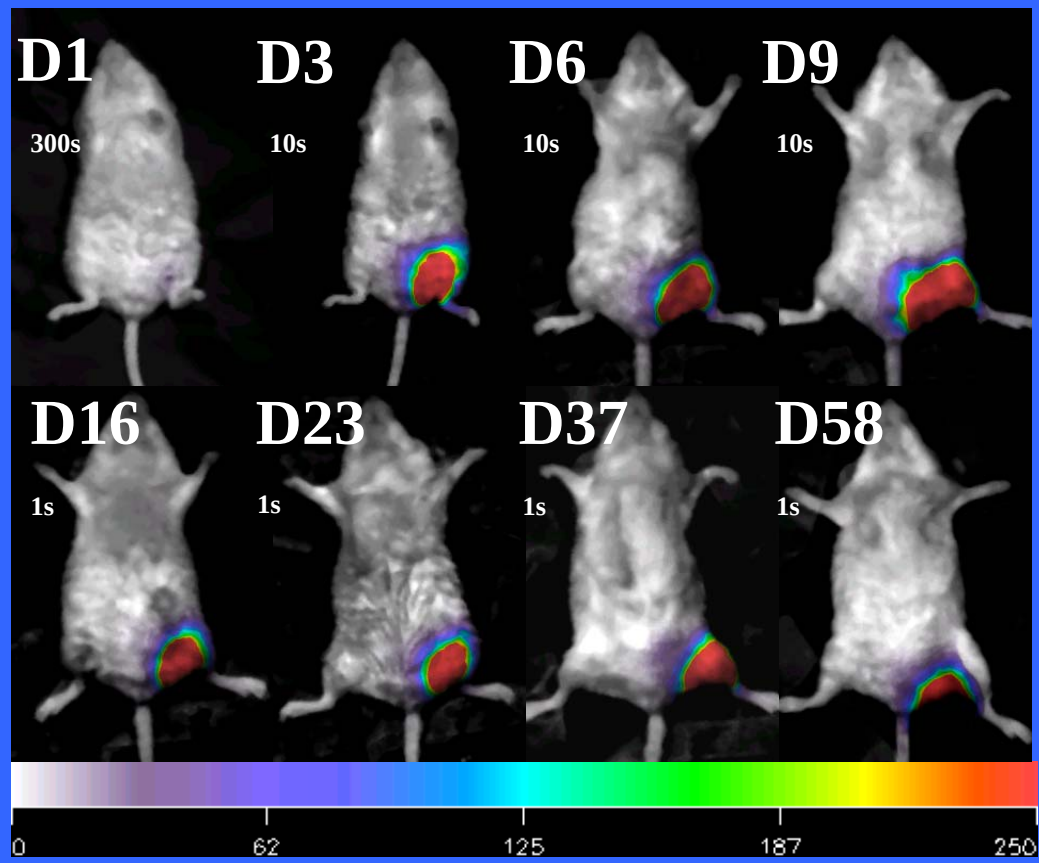
CHIMERIC VECTORS

Proof of principle: to identify amino acids of AAV1 and/or AAV7 that are responsible for muscle tropism, and to engineer these amino acids into AAV2 (non-muscle tropic) background.

Kenitics of AAV serotypes *in vivo*

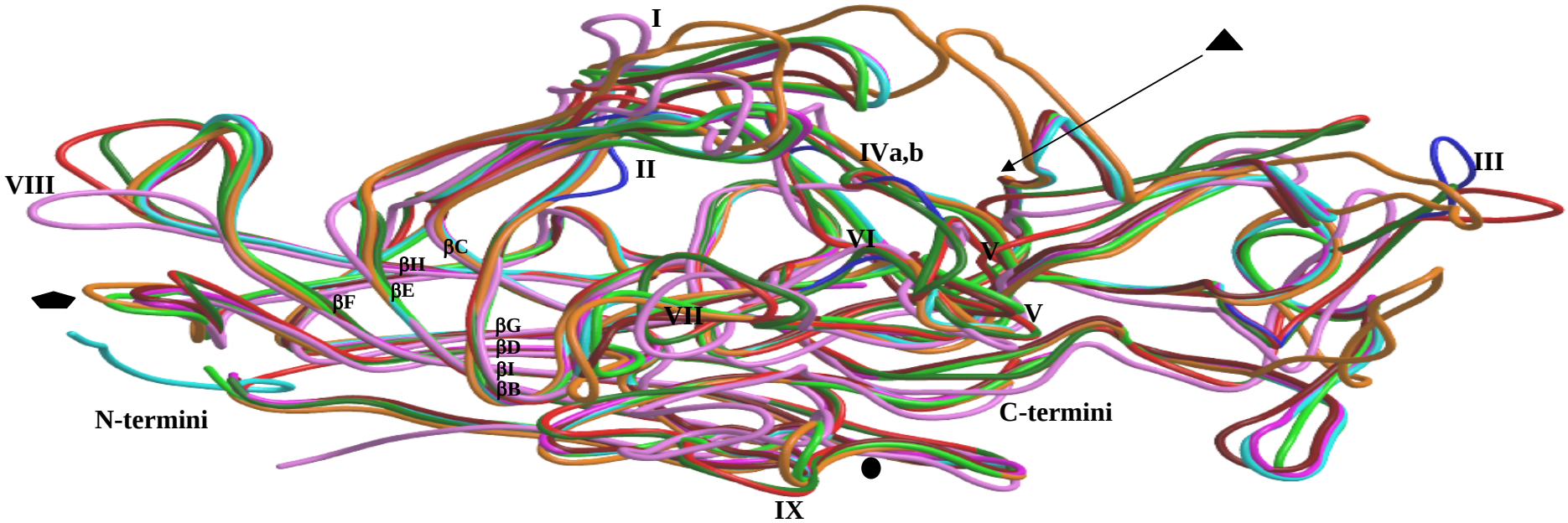


rAAV2, Muscular Injection



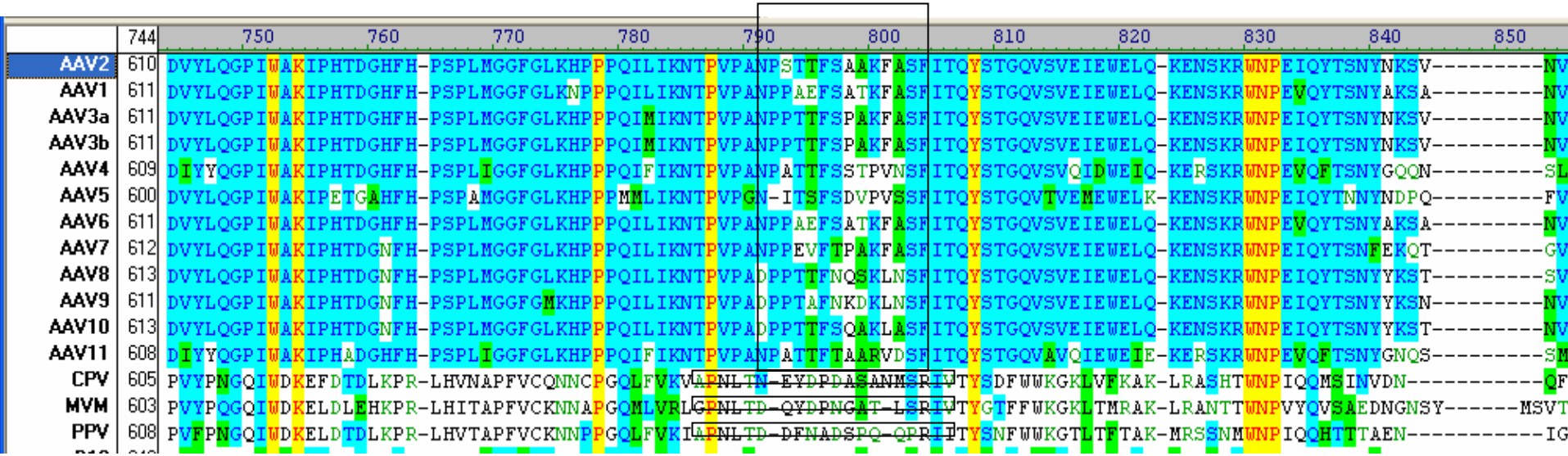
rAAV1, Muscular Injection

Ref: Images from Dr. Joe Rabinowitz

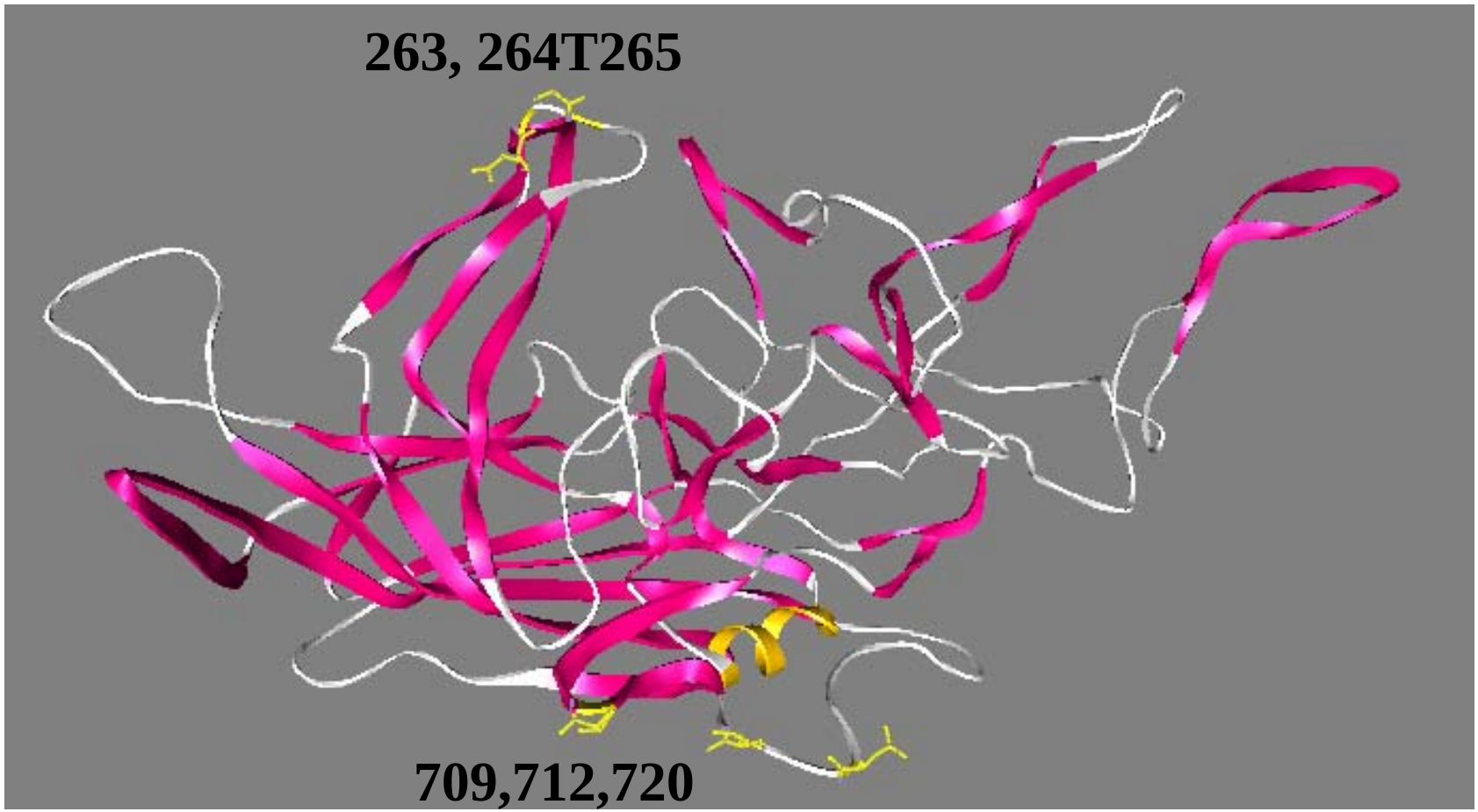


JVI01692-04-Fig6

Figure 6. Superimposition of coil representations of the VP3 monomers of AAV2 (atomic coordinates) (in red), the pseudo-atomic models of AAV4 (in blue) and AAV5 (in dark green) as in figure 5, the VP2/VP3 monomers of the atomic coordinates of B19 (in pink) (37), CPV (in cyan) (67), FPV (in magenta) (51), MVM (in green) (4), PPV (in brown) (53) and the VP2 pseudo-atomic coordinates of ADV (in orange) (44). Variable surface loop regions labeled **I**-**IX** are as in Figures 3 and 5. The N- and C-termini of the VPs are indicated. The approximate icosahedral 2-, 3- and 5-fold axes are indicated by the filled oval, triangle and pentagon, respectively.

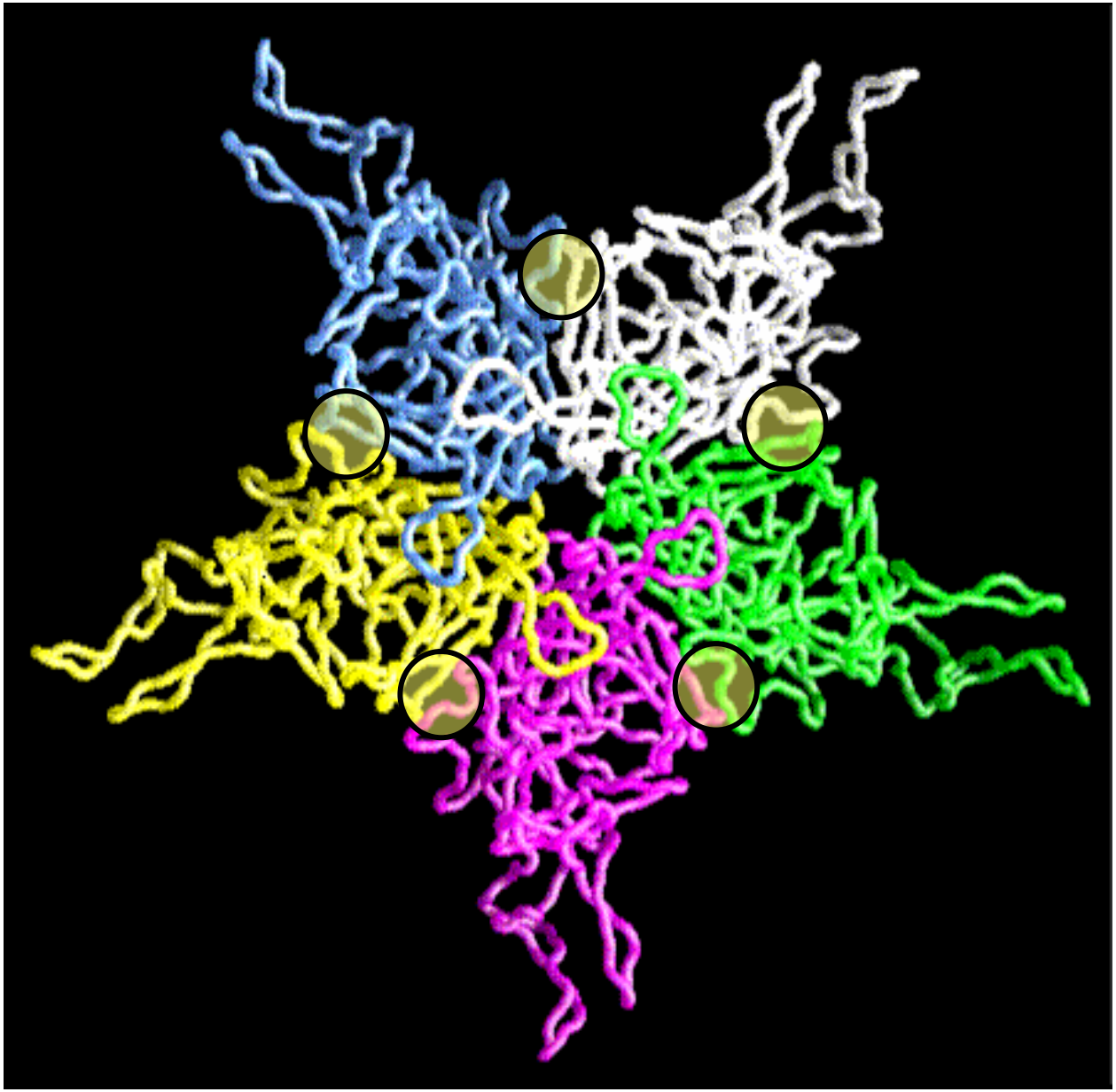

744
750
760
770
780
790
800
810
820
830
840
850
AAV2 610 DVYLQGPIWAKIPHTDGHFH-PSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQ-KENSKRWNPEIQYTSNYNKSV---------NV
AAV1 611 DVYLQGPIWAKIPHTDGHFH-PSPLMGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQ-KENSKRWNPEVQYTSNYAKSA---------NV
AAV3a 611 DVYLQGPIWAKIPHTDGHFH-PSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQ-KENSKRWNPEIQYTSNYNKSV---------NV
AAV3b 611 DVYLQGPIWAKIPHTDGHFH-PSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQ-KENSKRWNPEIQYTSNYNKSV---------NV
AAV4 609 DIYYQGPIWAKIPHTDGHFH-PSPLIGGFGLKHPPPQIFIKNTPVPANPATTFSSTPVNSFITQYSTGQVSVQIDWEIQ-KERSKRWNPEVQFTSNYGQQN---------SL
AAV5 600 DVYLQGPIWAKIPETGAHFH-PSPAMGGFGLKHPPPMMLIKNTPVPGN-ITSFSDVPVSSFITQYSTGQVTVEMEWELK-KENSKRWNPEIQYTNNYNDPQ---------FV
AAV6 611 DVYLQGPIWAKIPHTDGHFH-PSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQ-KENSKRWNPEVQYTSNYAKSA---------NV
AAV7 612 DVYLQGPIWAKIPHTDGNFH-PSPLMGGFGLKHPPPQILIKNTPVPANPPEVFTPAKFASFITQYSTGQVSVEIEWELQ-KENSKRWNPEIQYTSNFEKQT---------GV
AAV8 613 DVYLQGPIWAKIPHTDGNFH-PSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQSKLNSFITQYSTGQVSVEIEWELQ-KENSKRWNPEIQYTSNYYKST---------SV
AAV9 611 DVYLQGPIWAKIPHTDGNFH-PSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQ-KENSKRWNPEIQYTSNYYKSN---------NV
AAV10 613 DVYLQGPIWAKIPHTDGNFH-PSPLMGGFGLKHPPPQILIKNTPVPADPPTTFSQAKLASFITQYSTGQVSVEIEWELQ-KENSKRWNPEIQYTSNYYKST---------NV
AAV11 608 DIYYQGPIWAKIPHADGHFH-PSPLIGGFGLKHPPPQIFIKNTPVPANPATTFTAARVDSFITQYSTGQVAVQIEWEIE-KERSKRWNPEVQFTSNYGNQS---------SM
CPV 605 PVYPNGQIWDKEFDTDLKPR-LHVNAPFVCQNNCPGQLFVKVAPNLTN-EYDPDASANMSRIVTYSDFWWKGKLVFKAK-LRASHTWNPIQQMSINVDN----------QF
MVM 603 PVYPQGQIWDKELDLEHKPR-LHITAPFVCKNNAPGQMLVRLGPNLTD-QYDPNGATLSRIVTYGTFFWKGKLTMRAK-LRANTTWNPVYQVSAEDNGNSY------MSVT
PPV 608 PVFPNGQIWDKELDTDLKPR-LHVTAPFVCKNNPPGQLFVKIAPNLTD-DFNADSPQ-QPRIITYSNFWWKGTLTFTAK-MRSSNMWNPIQQHTTTAEN----------IG

Rational Mutagenesis of AAV2

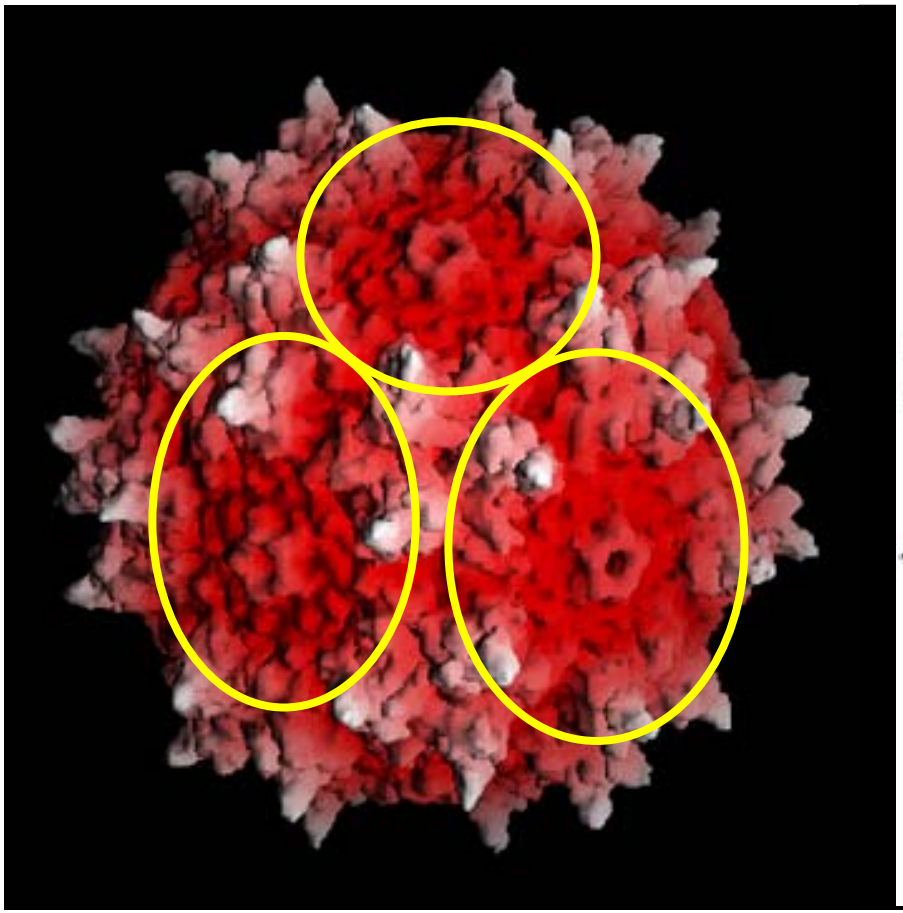


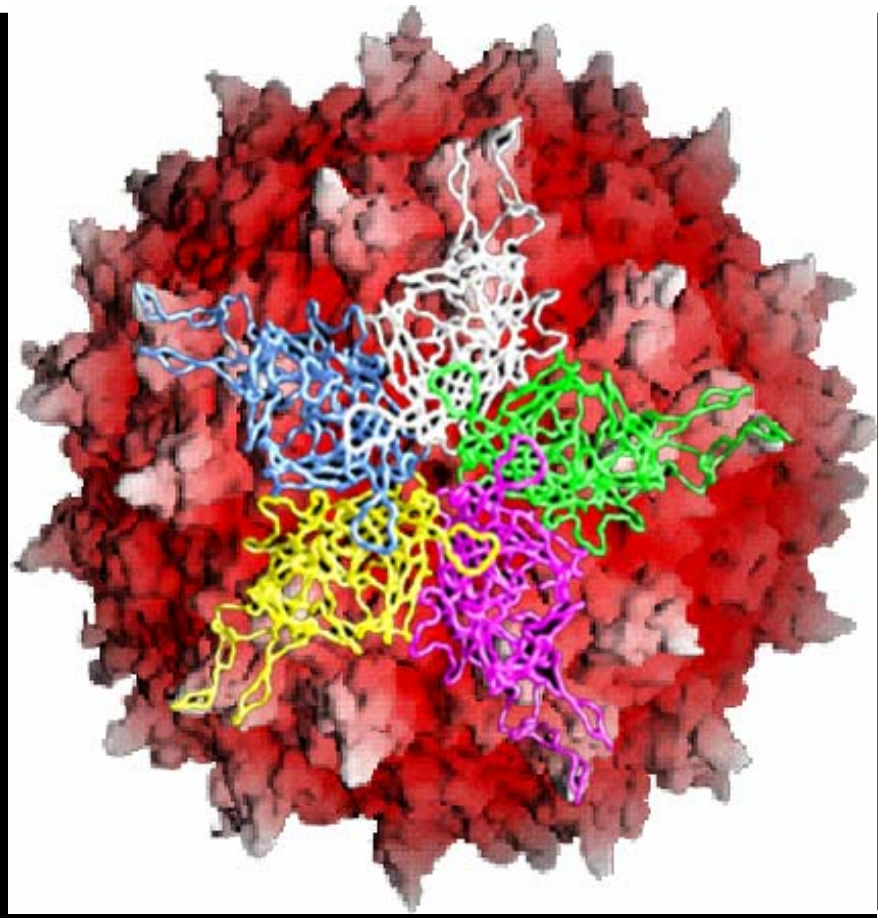
Dawn Bowles

Position on Pentamer

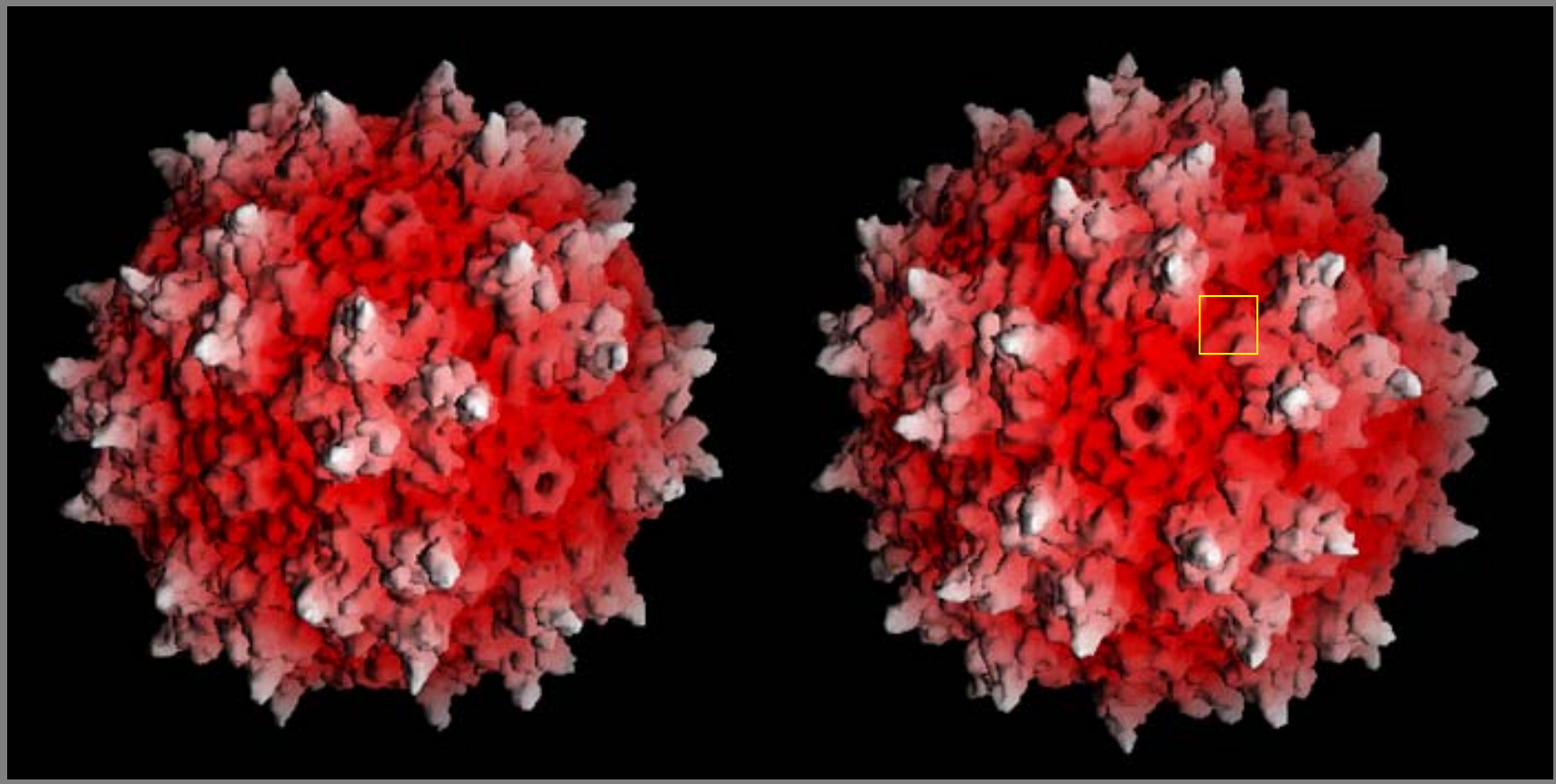


Relationship between Pentamer and Topology Map



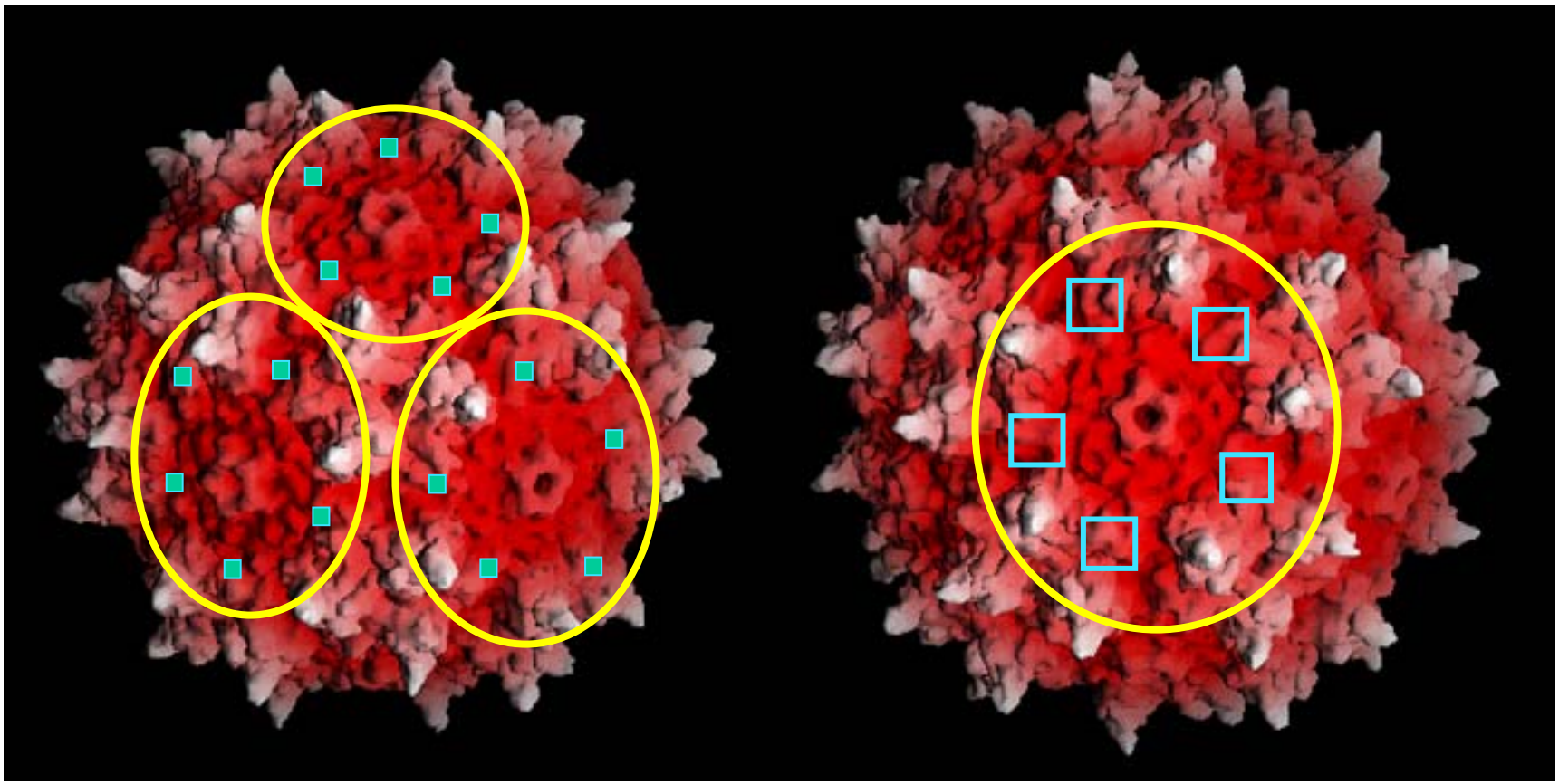


AAV2 Surface Topology



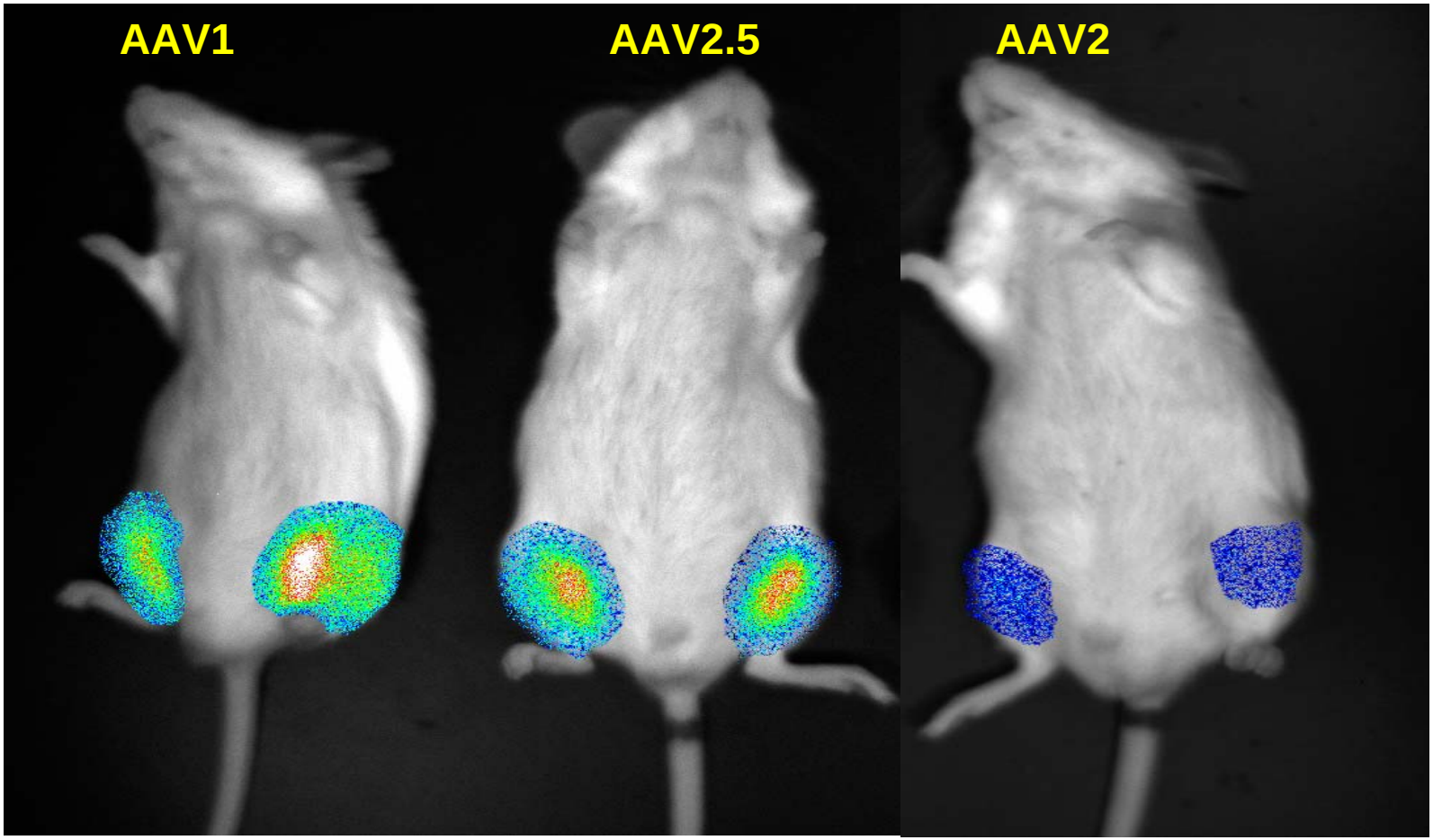
Surface topology rendering of AAV2 drawn down the threefold (left) and fivefold (right) axes. Dark to light coloring represents increasing distance from center of virus.

AAV2 Surface Topology



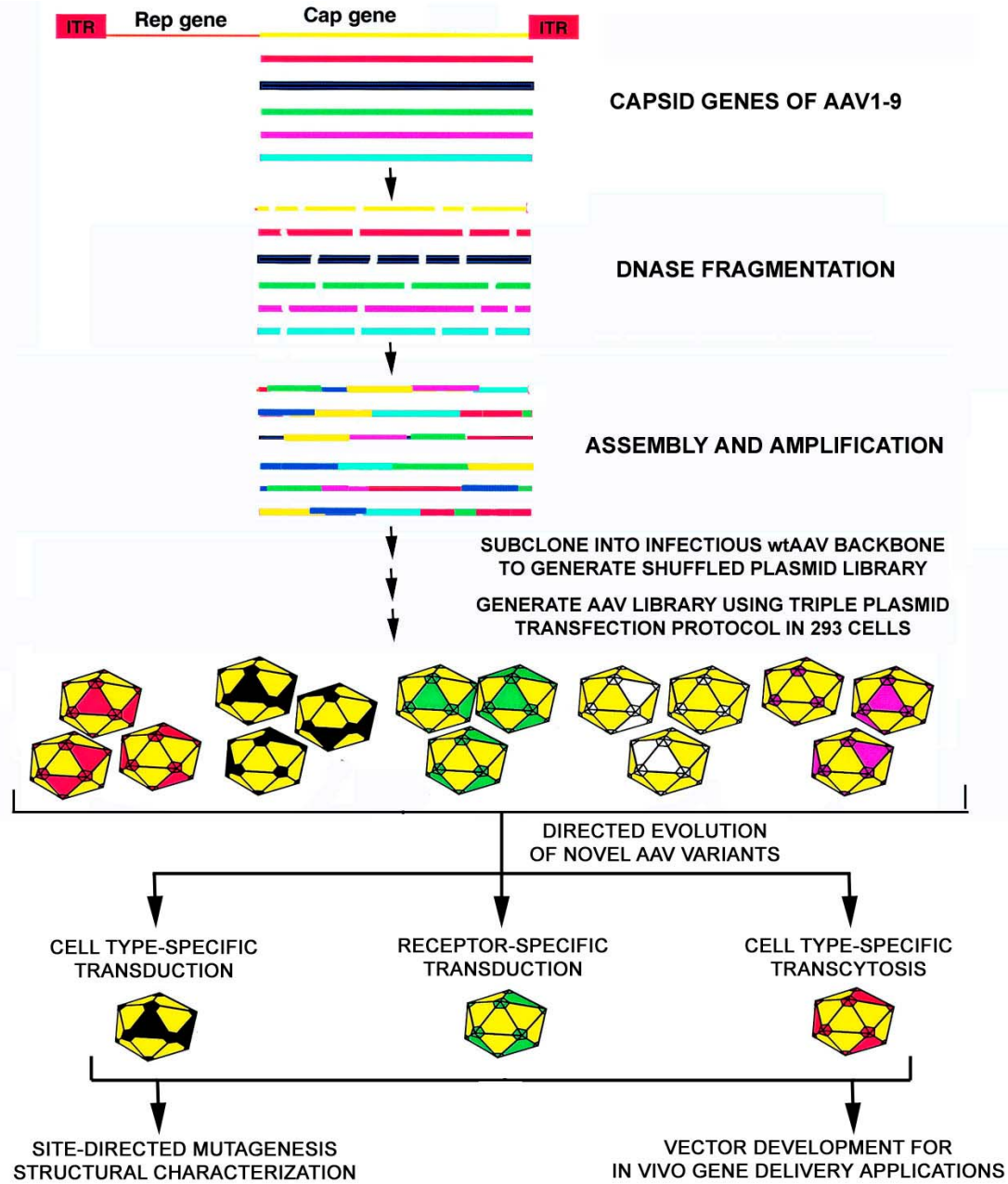
Surface topology rendering of AAV2 drawn down the threefold (left) and fivefold (right) axes. Dark to light coloring represents increasing distance from center of virus.

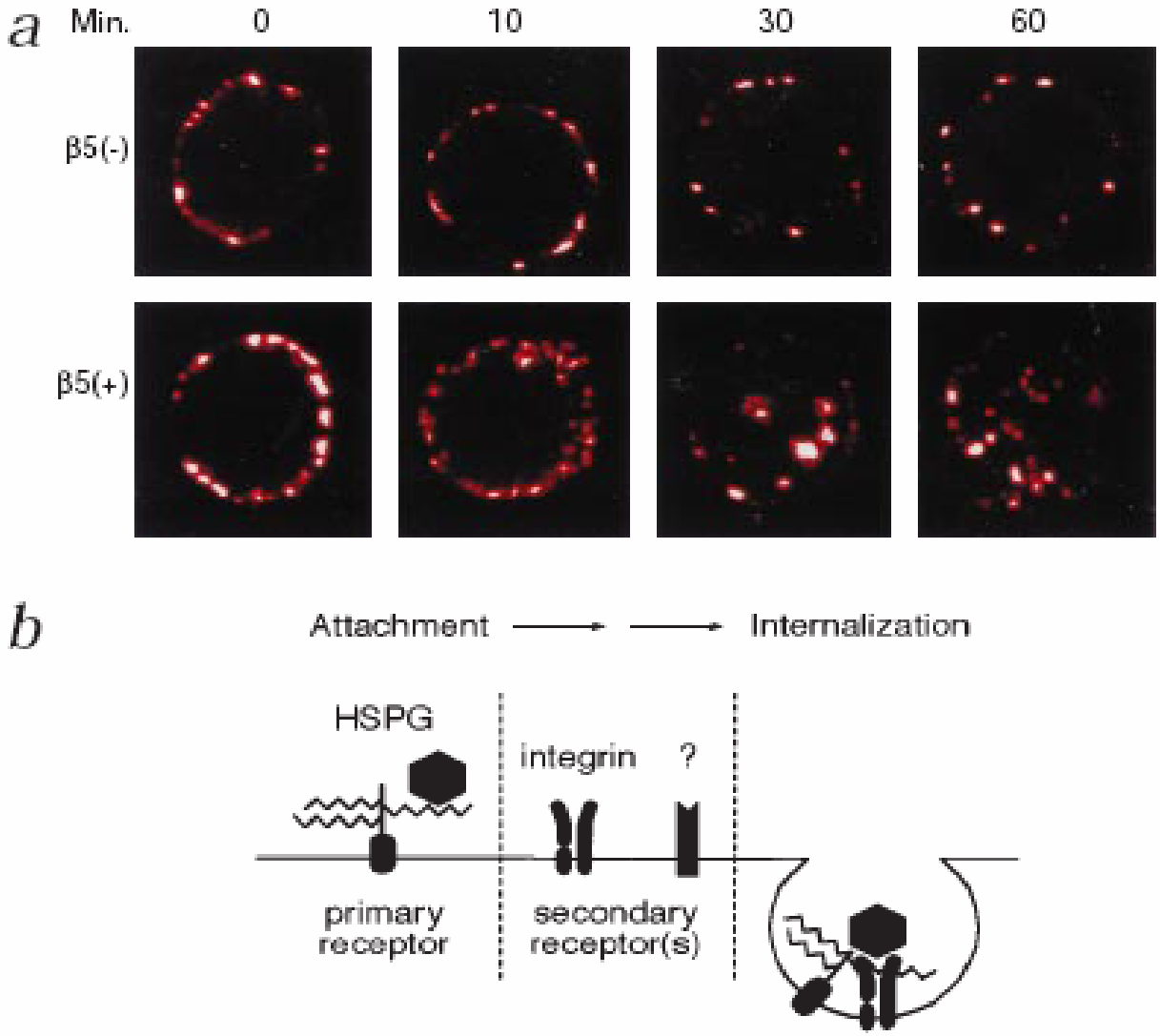
Imaging of Luciferase Transgene in BALB/C Mice
Day 4 post injection

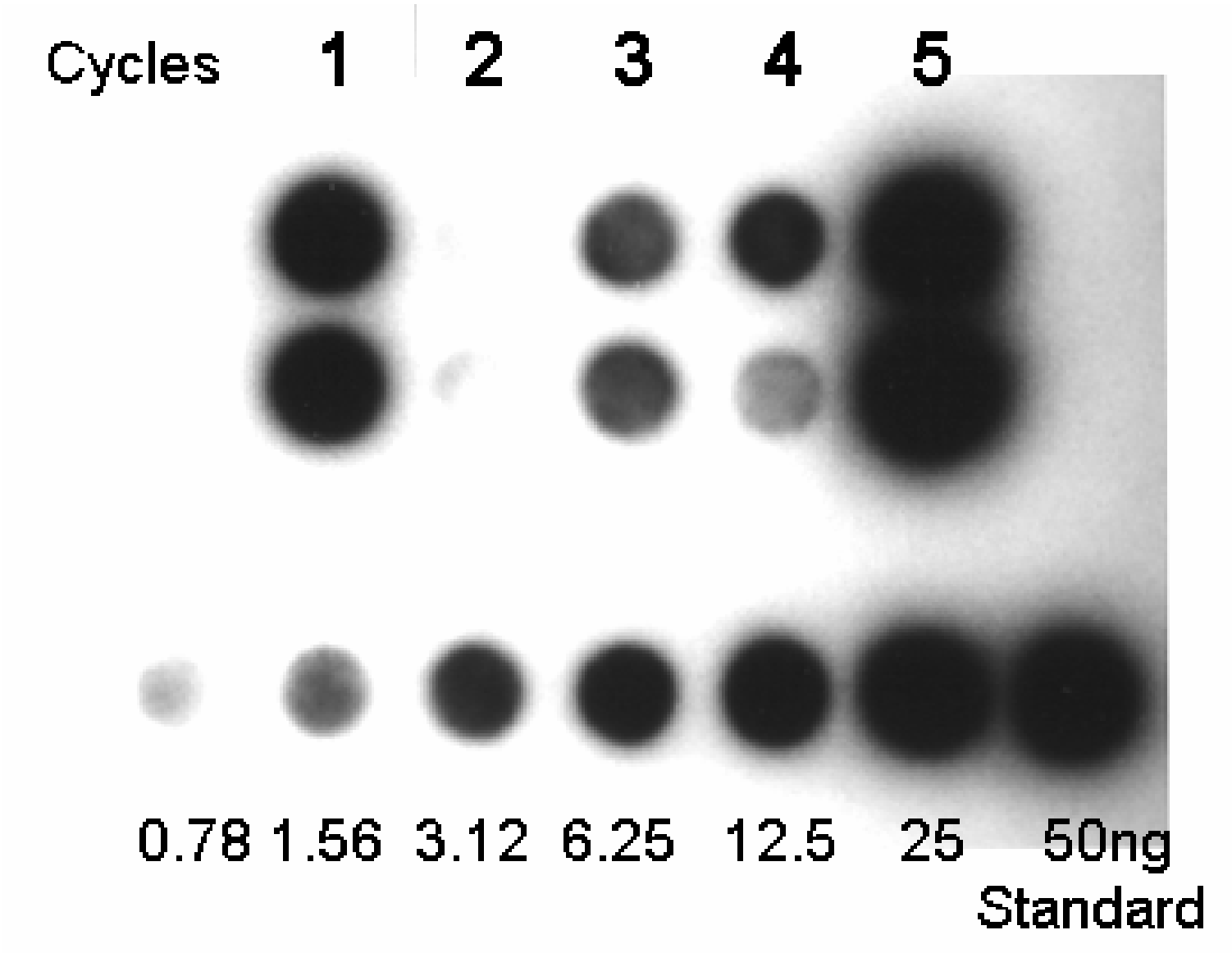


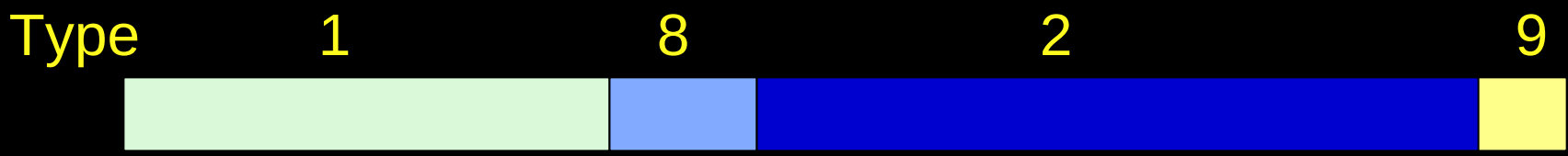
$1x10^{10}$ particles injected into each gastrocnemius

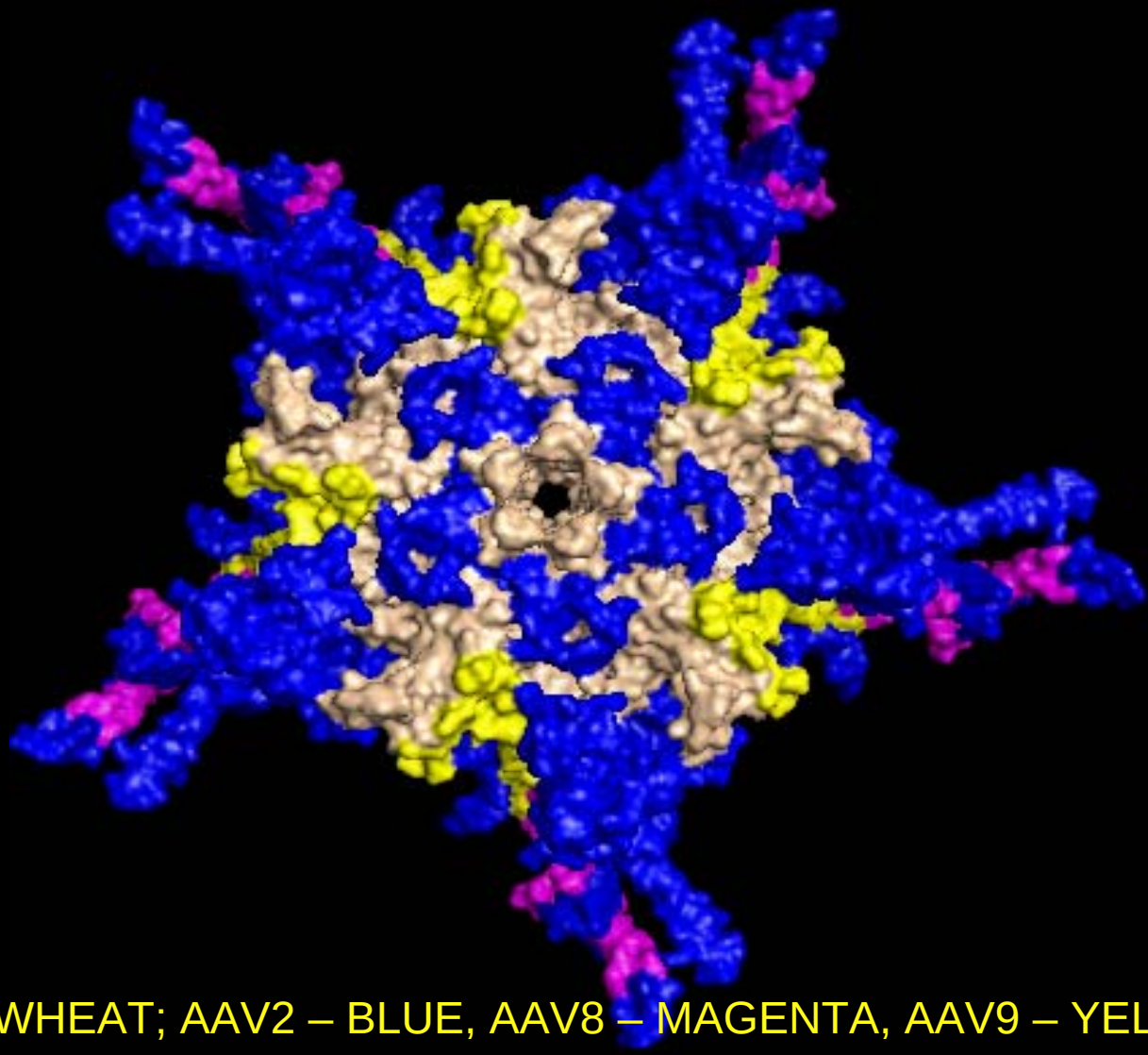

What a difference a little DNA makes


ITR
Rep gene
Cap gene
ITR
CAPSID GENES OF AAV1-9
DNASE FRAGMENTATION
ASSEMBLY AND AMPLIFICATION
SUBCLONE INTO INFECTIOUS wtAAV BACKBONE
TO GENERATE SHUFFLED PLASMID LIBRARY
GENERATE AAV LIBRARY USING TRIPLE PLASMID
TRANSFECTION PROTOCOL IN 293 CELLS
DIRECTED EVOLUTION
OF NOVEL AAV VARIANTS
CELL TYPE-SPECIFIC
TRANSDUCTION
RECEPTOR-SPECIFIC
TRANSDUCTION
CELL TYPE-SPECIFIC
TRANSCYTOSIS
SITE-DIRECTED MUTAGENESIS
STRUCTURAL CHARACTERIZATION
VECTOR DEVELOPMENT FOR
IN VIVO GENE DELIVERY APPLICATIONS

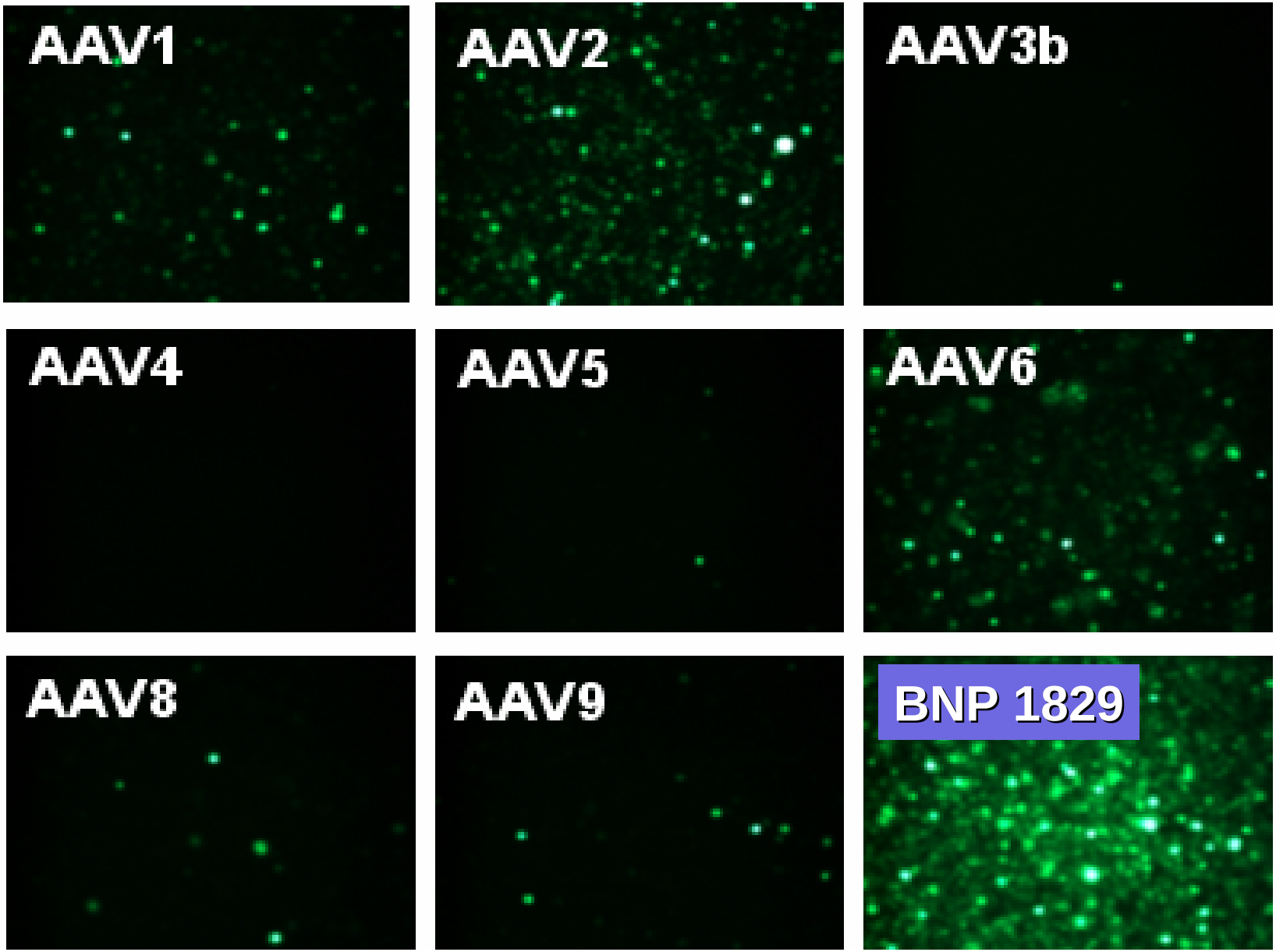

a
Min.
0
10
30
60
β5(-)
β5(+)
b
Attachment
Internalization
HSPG
integrin
?
primary receptor
secondary receptor(s)


Cycles
1
2
3
4
5
0.78
1.56
3.12
6.25
12.5
25
50ng
Standard

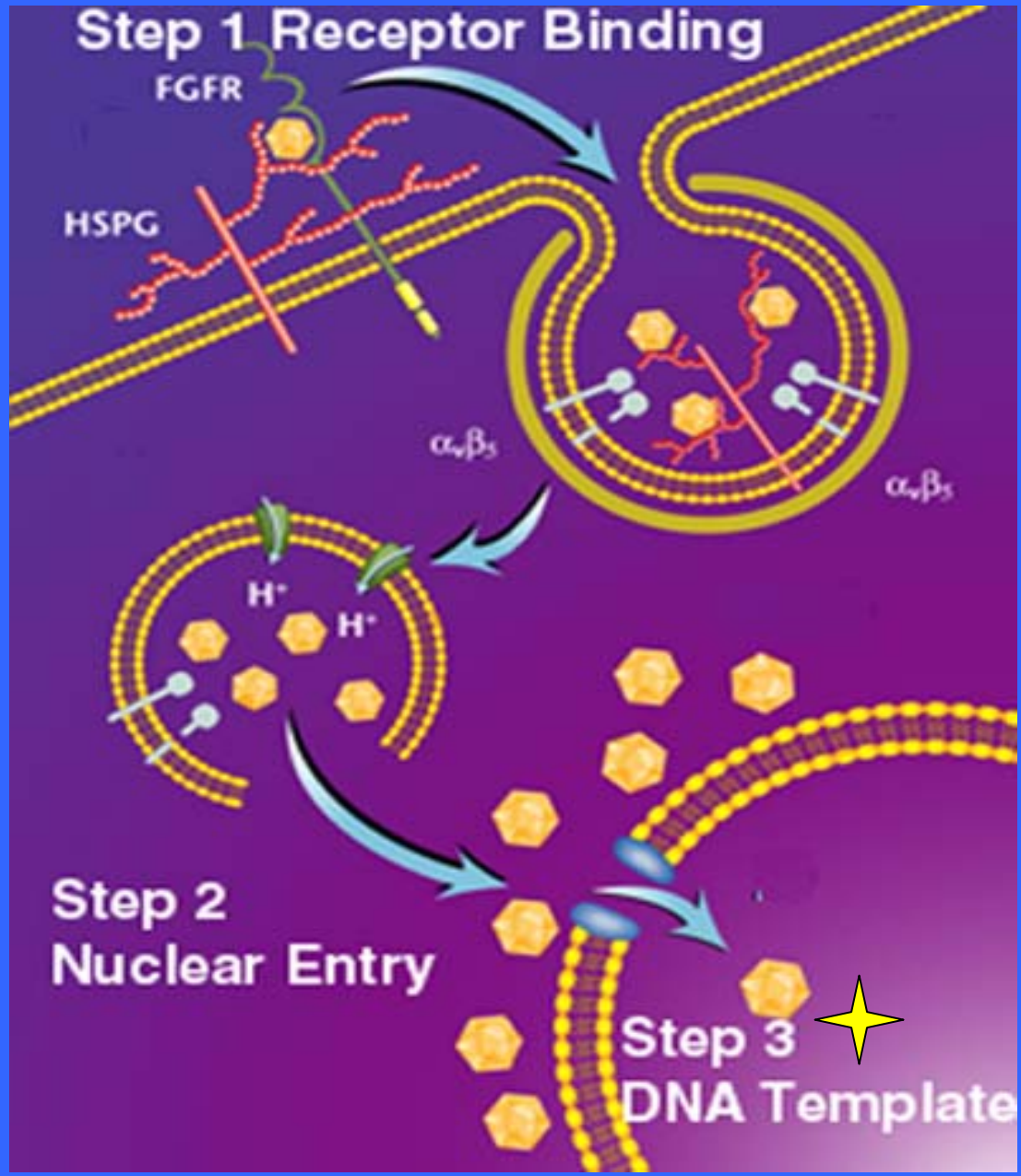




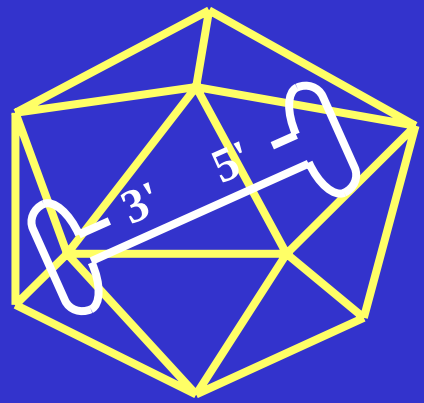
AAV1 – WHEAT; AAV2 – BLUE, AAV8 – MAGENTA, AAV9 – YELLOW
REGIONS ARE MODELED ON AAV2 CRYSTAL STRUCTURE


AAV1
AAV2
AAV3b
AAV4
AAV5
AAV6
AAV8
AAV9
BNP 1829

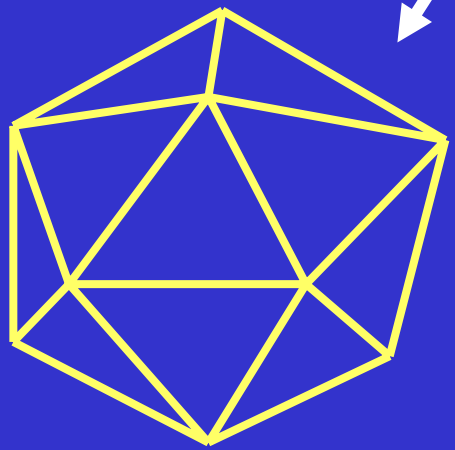
AAV Entry


Step 1 Receptor Binding
FGFR
HSPG
$\alpha_v\beta_5$
$\alpha_v\beta_5$
H+
H+
Step 2
Nuclear Entry
Step 3
DNA Template

AAV as a Gene Delivery Vector

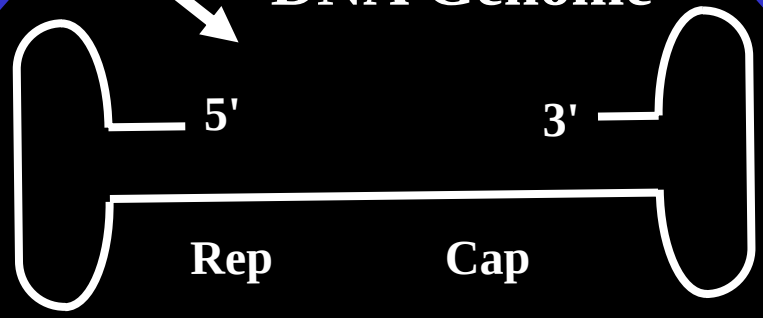


Capsid



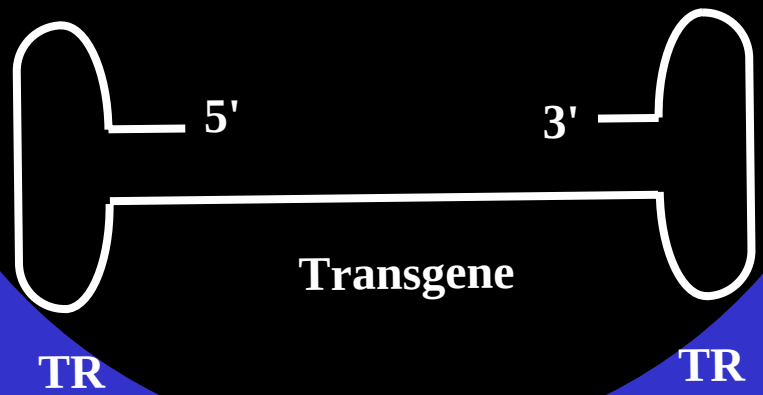
wtAAV

Single-stranded
DNA Genome

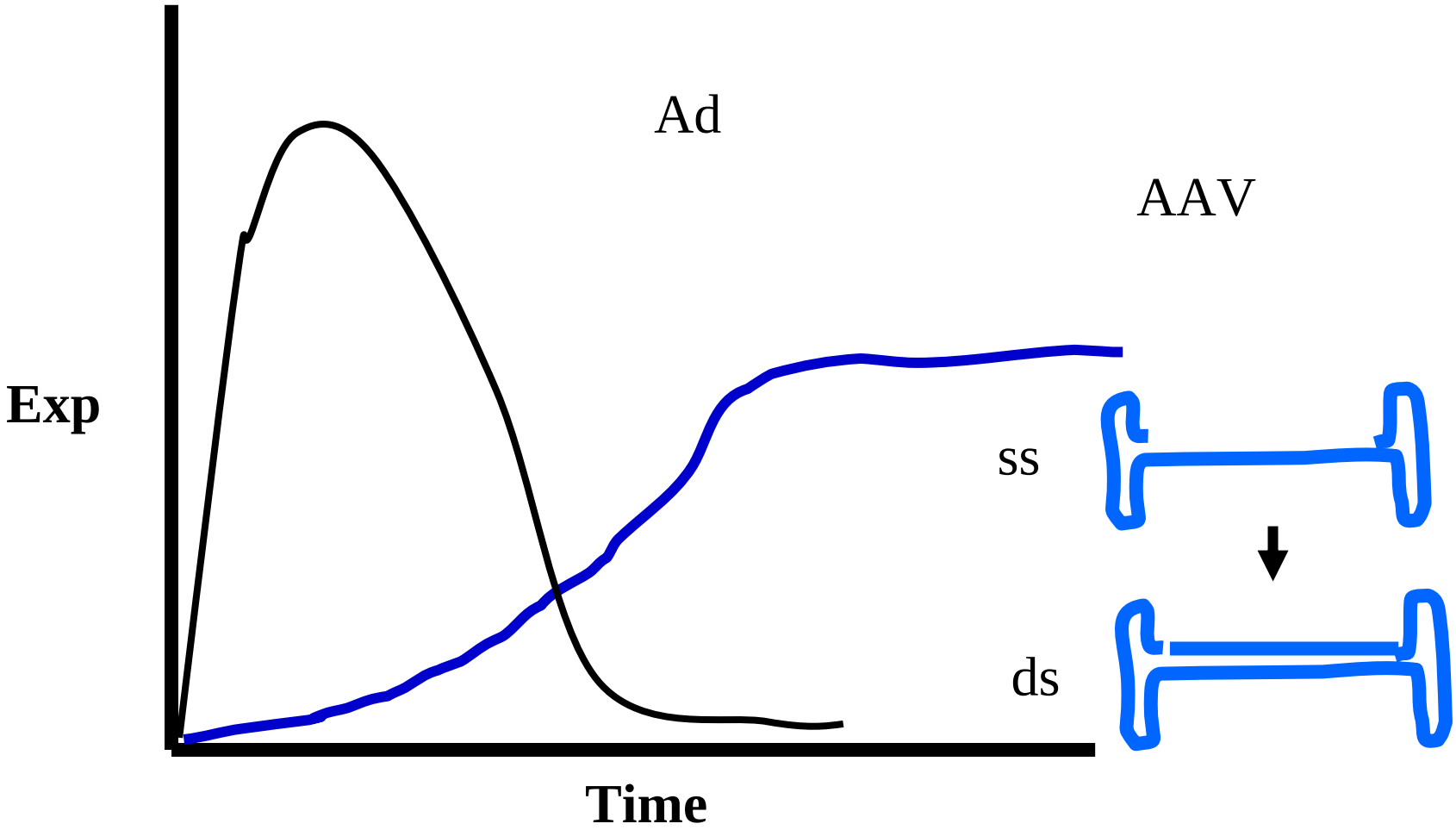


Recombinant

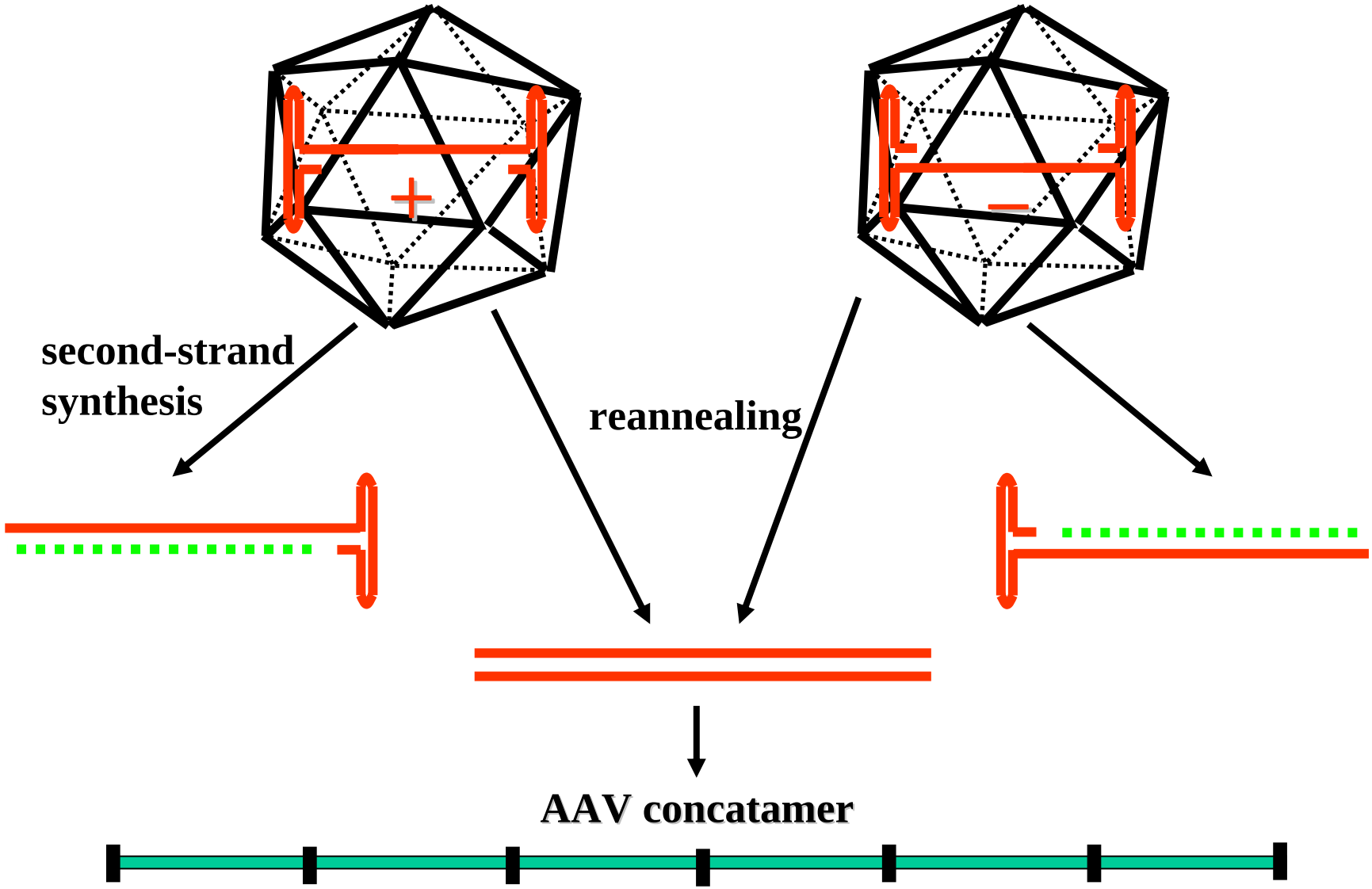
(rAAV)



Understanding AAV Gene Expression



AAV Mediated Gene Expression



Gene Therapy (2001) 8, 1248–1254

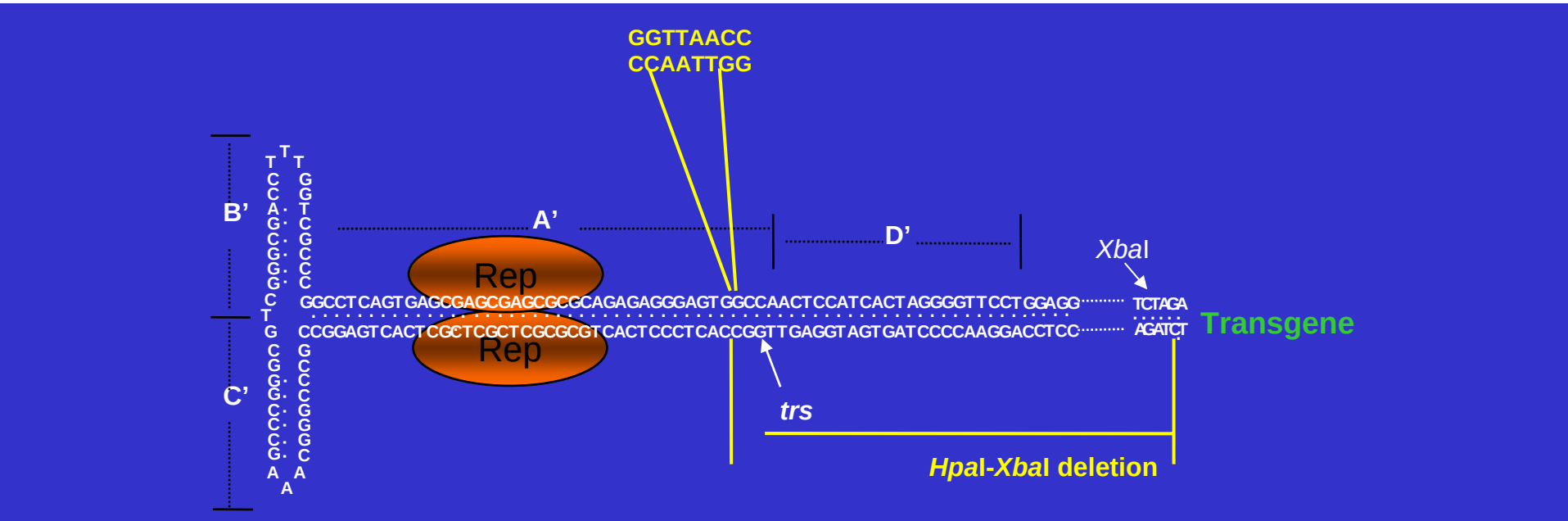
www.nature.com/gt

RESEARCH ARTICLE

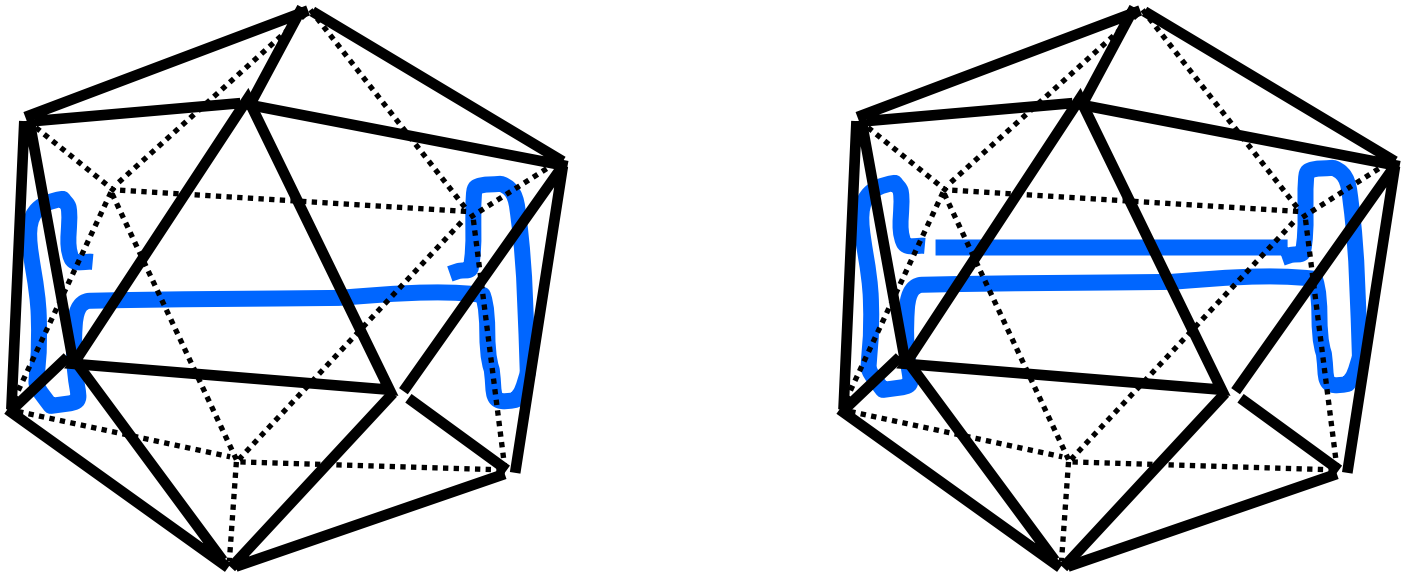
Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis

DM McCarty[1,2], PE Monahan[1,3] and RJ Samulski[1,4]

[1]UNC Gene Therapy Center, [2]School of Pharmacy, [3]Department of Pediatrics, [4]Department of Pharmacology University of North Carolina at Chapel Hill, NC, USA

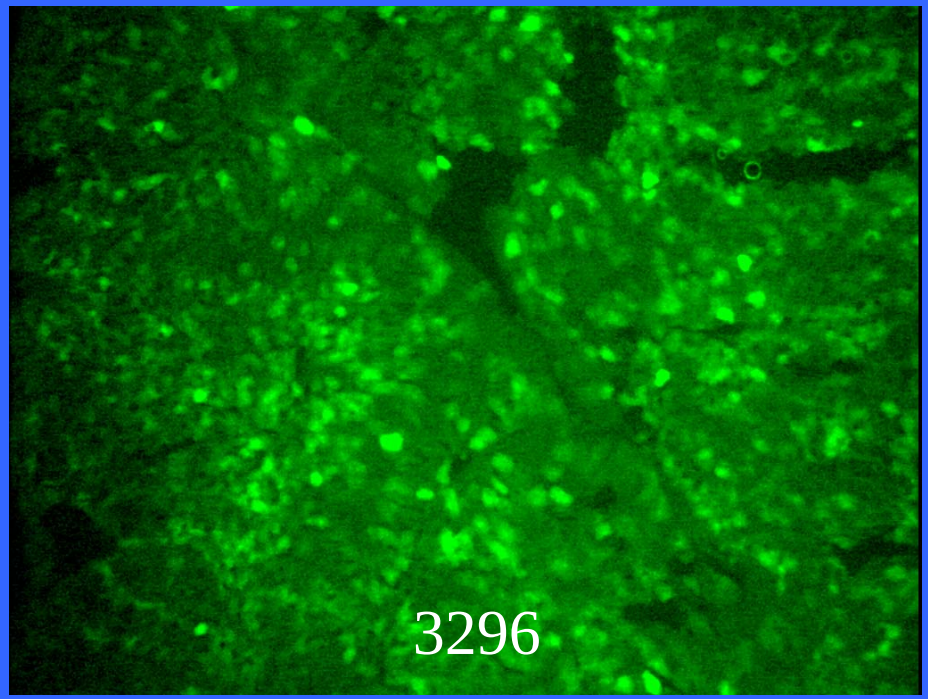


AAV Duplex Vectors

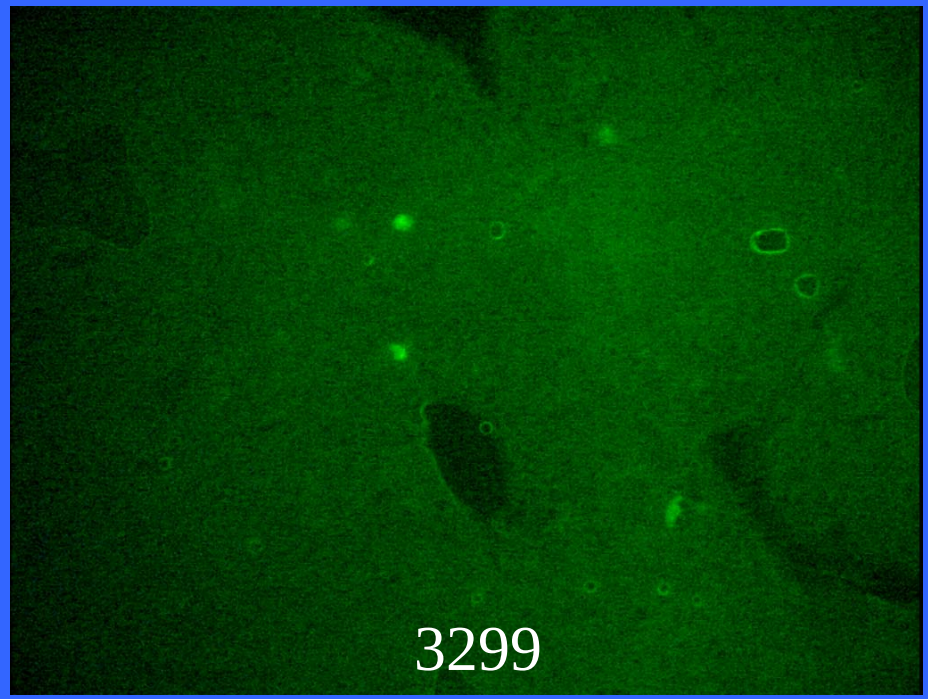


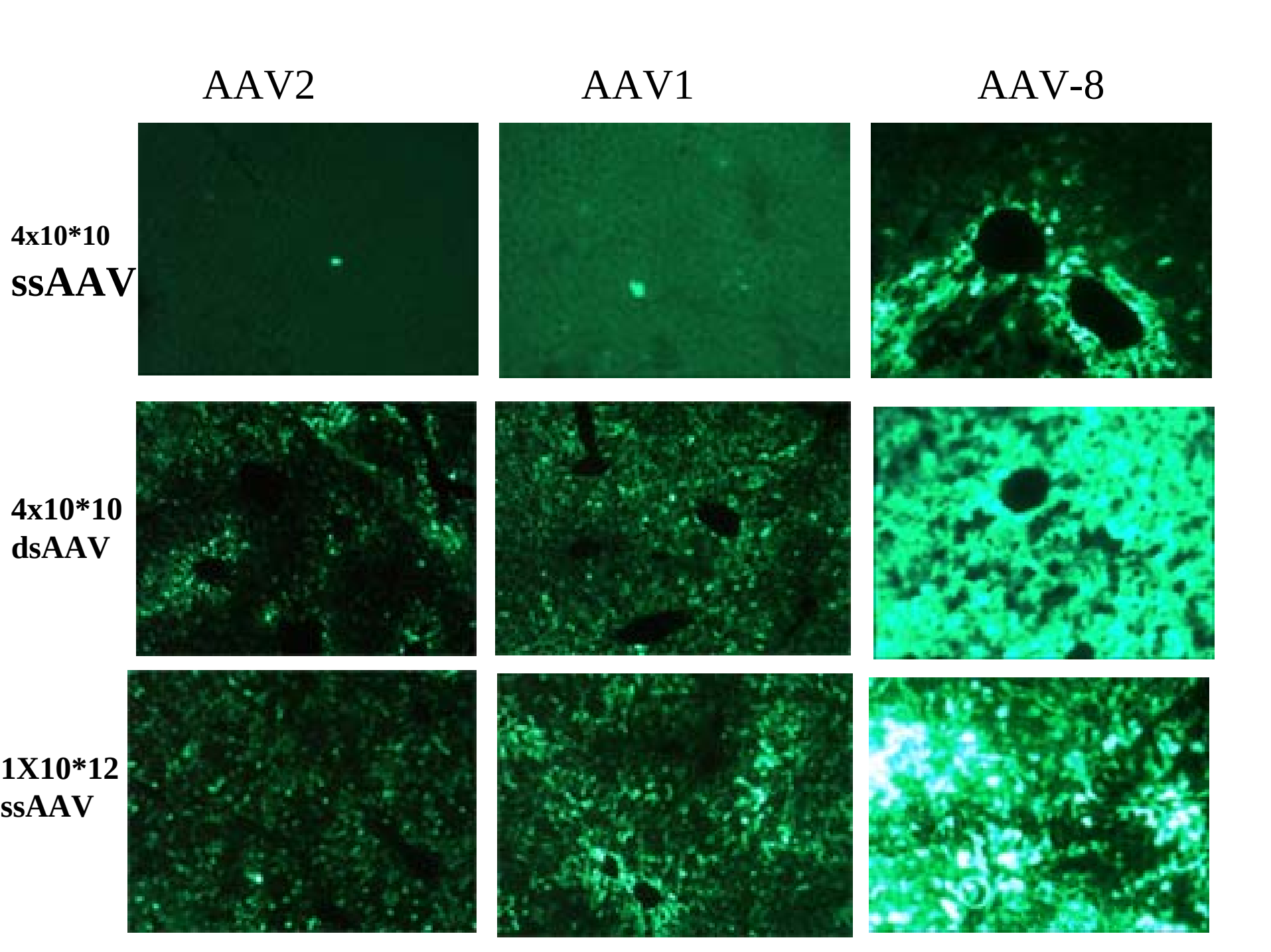
ss-DNA ds-DNA

Dimer Vs. Monomer
AAV-2 LSP-GFP.
6 weeks PI with 5 x 10^{10} Particles

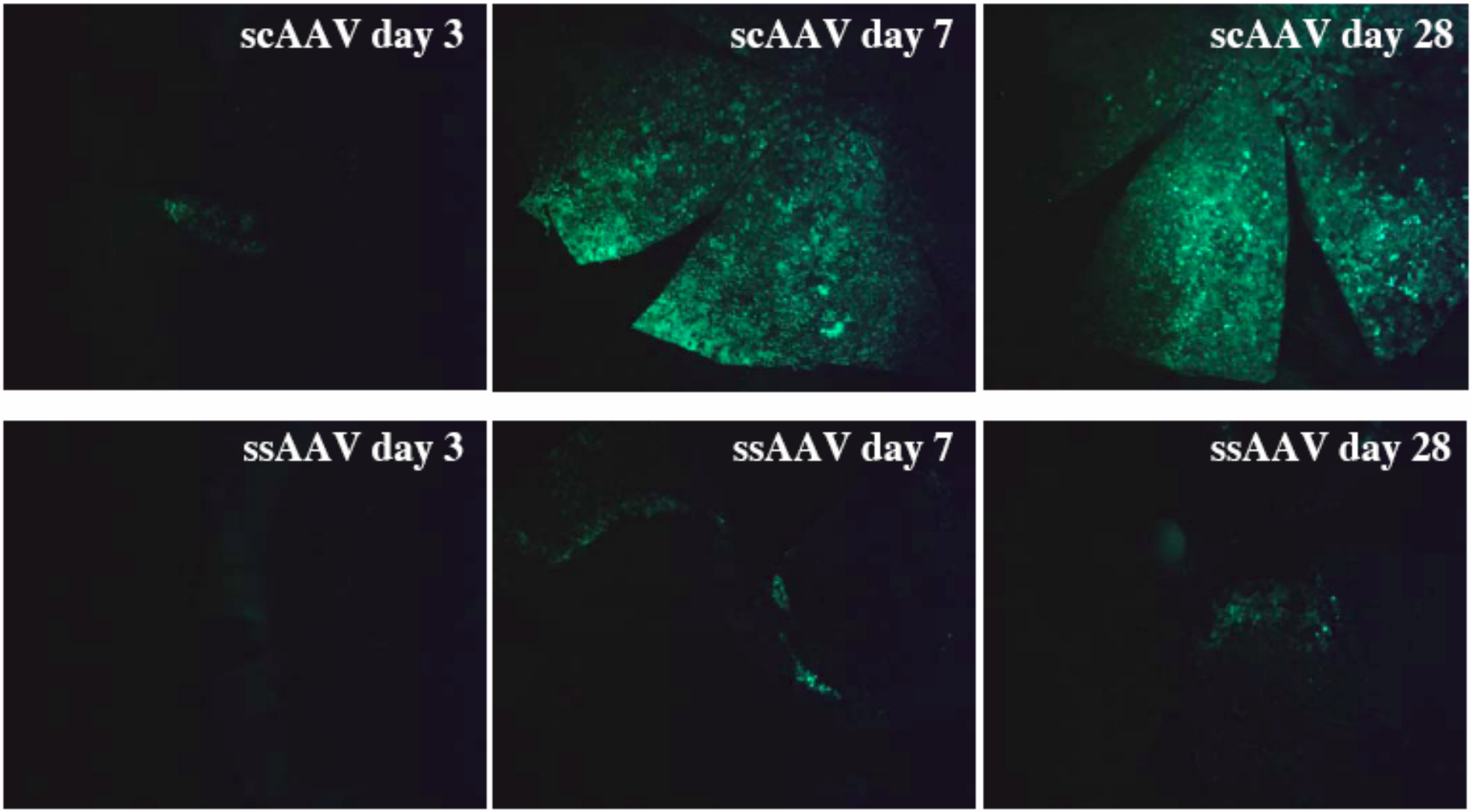


5 x 10^{10}

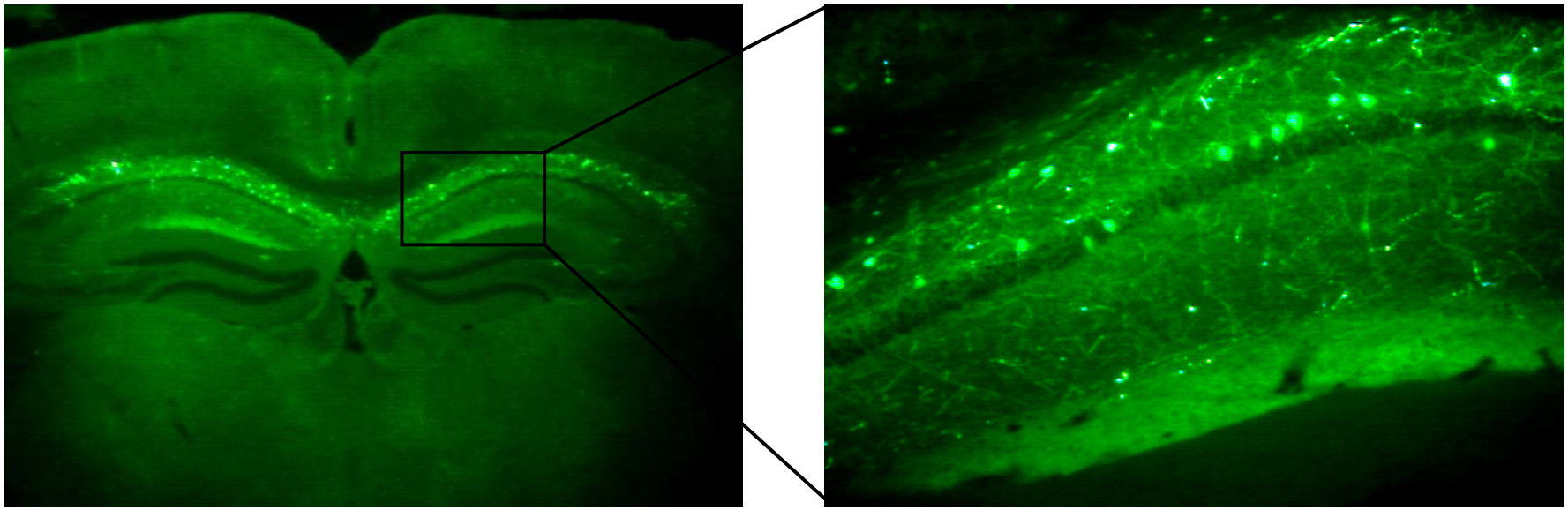



AAV2
AAV1
AAV-8
4x10*10
ssAAV
4x10*10
dsAAV
1X10*12
ssAAV

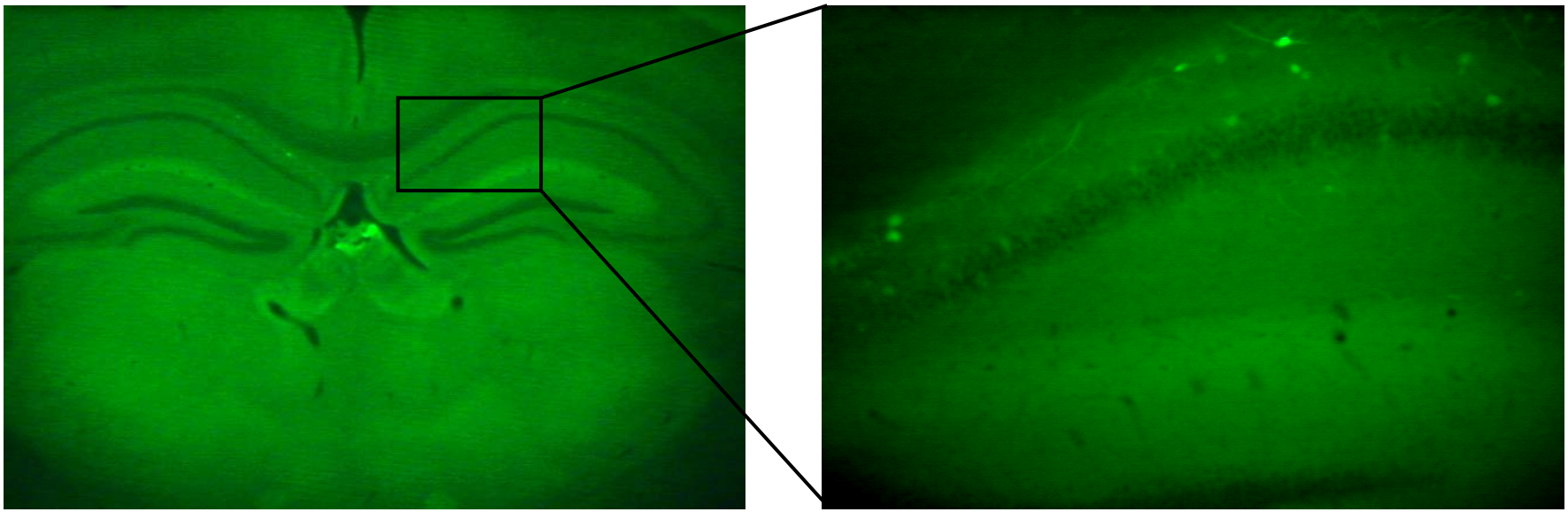
Ocular Gene Transfer with Self Complementary AAV Vectors



ds



ss

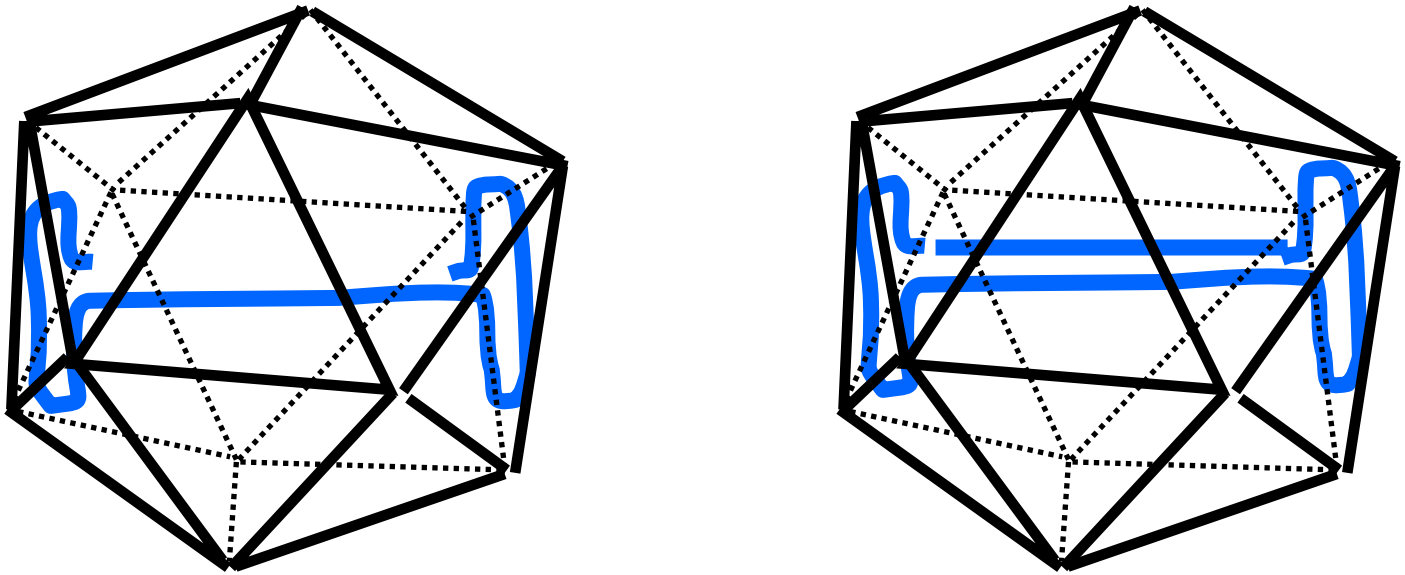


Double-Stranded AAV Papers

List of papers (1-20)

1. **McCarty, D. M., P. E. Monahan, and R. J. Samulski.** 2001. Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. Gene Ther **8:**1248-54.
2. **McCarty, D. M., H. Fu, P. E. Monahan, C. E. Toulson, P. Naik, and R. J. Samulski.** 2003. Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo. Gene Ther **10:**2112-8.
3. **Fu, H., J. Muenzer, R. J. Samulski, G. Breese, J. Sifford, X. Zeng, and D. M. McCarty.** 2003. Self-complementary adeno-associated virus serotype 2 vector: global distribution and broad dispersion of AAV-mediated transgene expression in mouse brain. Mol Ther **8:**911-7.
4. **Wang, Z., H. I. Ma, J. Li, L. Sun, J. Zhang, and X. Xiao.** 2003. Rapid and highly efficient transduction by double-stranded adeno-associated virus vectors in vitro and in vivo. Gene Ther **10:**2105-11.
5. **Ding, W., Z. Yan, R. Zak, M. Saavedra, D. M. Rodman, and J. F. Engelhardt.** 2003. Second-strand genome conversion of adeno-associated virus type 2 (AAV-2) and AAV-5 is not rate limiting following apical infection of polarized human airway epithelia. J Virol **77:**7361-6.
6. **Nakai, H., S. Fuess, T. A. Storm, L. A. Meuse, and M. A. Kay**. 2003. Free DNA ends are essential for concatemerization of synthetic double-stranded adeno-associated virus vector genomes transfected into mouse hepatocytes in vivo. Mol Ther **7:**112-21.
7. **Zhong, L., L. Chen, Y. Li, K. Qing, K. A. Weigel-Kelley, R. J. Chan, M. C. Yoder, and A. Srivastava.** 2004. Self-complementary adeno-associated virus 2 (AAV)-T cell protein tyrosine phosphatase vectors as helper viruses to improve transduction efficiency of conventional single-stranded AAV vectors in vitro and in vivo. Mol Ther **10:**950-7.
8. **Zhong, S., S. Sun, and B. B. Teng.** 2004. The recombinant adeno-associated virus vector (rAAV2)-mediated apolipoprotein B mRNA-specific hammerhead ribozyme: a self-complementary AAV2 vector improves the gene expression. Genet Vaccines Ther **2:**5.
9. **Choi, V. W., D. M. McCarty, and R. J. Samulski.** 2005. AAV hybrid serotypes: improved vectors for gene delivery. Curr Gene Ther **5:**299-310.
10. **Choi, V. W., R. J. Samulski, and D. M. McCarty.** 2005. Effects of adeno-associated virus DNA hairpin structure on recombination. J Virol **79:**6801-7.
11. **Hacker, U. T., L. Wingenfeld, D. M. Kofler, N. K. Schuhmann, S. Lutz, T. Herold, S. B. King, F. M. Gerner, L. Perabo, J. Rabinowitz, D. M. McCarty, R. J. Samulski, M. Hallek, and H. Buning.** 2005. Adeno-associated virus serotypes 1 to 5 mediated tumor cell directed gene transfer and improvement of transduction efficiency. J Gene Med **7:**1429-38.
12. **Nathwani, A. C., J. T. Gray, C. Y. Ng, J. Zhou, Y. Spence, S. N. Waddington, E. G. Tuddenham, G. Kemball-Cook, J. McIntosh, M. Boon-Spijker, K. Mertens, and A. M. Davidoff.** 2005. Self complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. Blood.
13. **Rehman, K. K., Z. Wang, R. Bottino, A. N. Balamurugan, M. Trucco, J. Li, X. Xiao, and P. D. Robbins.** 2005. Efficient gene delivery to human and rodent islets with double-stranded (ds) AAV-based vectors. Gene Ther **12:**1313-23.
14. **Ren, C., S. Kumar, D. R. Shaw, and S. Ponnazhagan.** 2005. Genomic stability of self-complementary adeno-associated virus 2 during early stages of transduction in mouse muscle in vivo. Hum Gene Ther **16:**1047-57.
15. **Thomas, C. E., T. A. Storm, Z. Huang, and M. A. Kay**. 2004. Rapid uncoating of vector genomes is the key to efficient liver transduction with pseudotyped adeno-associated virus vectors. J Virol **78:**3110-22.
16. **Wei, X., C. Zhao, J. Jiang, J. Li, X. Xiao, and D. W. Wang.** 2005. Adrenomedullin gene delivery alleviates hypertension and its secondary injuries of cardiovascular system. Hum Gene Ther **16:**372-80.
17. **Xu, D., D. McCarty, A. Fernandes, M. Fisher, R. J. Samulski, and R. L. Juliano.** 2005. Delivery of MDR1 small interfering RNA by self-complementary recombinant adeno-associated virus vector. Mol Ther **11:**523-30.
18. **Zhang, N., N. Clement, D. Chen, S. Fu, H. Zhang, P. Rebollo, R. M. Linden, and J. S. Bromberg.** 2005. Transduction of pancreatic islets with pseudotyped adeno-associated virus: effect of viral capsid and genome conversion. Transplantation **80:**683-90.
19. **Zhu, T., L. Zhou, S. Mori, Z. Wang, C. F. McTiernan, C. Qiao, C. Chen, D. W. Wang, J. Li, and X. Xiao.** 2005. Sustained whole-body functional rescue in congestive heart failure and muscular dystrophy hamsters by systemic gene transfer. Circulation **112:**2650-9.
20. **Aldrich, W. A., C. Ren, A. F. White, S. Z. Zhou, S. Kumar, C. B. Jenkins, D. R. Shaw, T. V. Strong, P. L. Triozzi, and S. Ponnazhagan.** 2006. Enhanced transduction of mouse bone marrow-derived dendritic cells by repetitive infection with self-complementary adeno-associated virus 6 combined with immunostimulatory ligands. Gene Ther **13:**29-39.

AAV Duplex Vectors

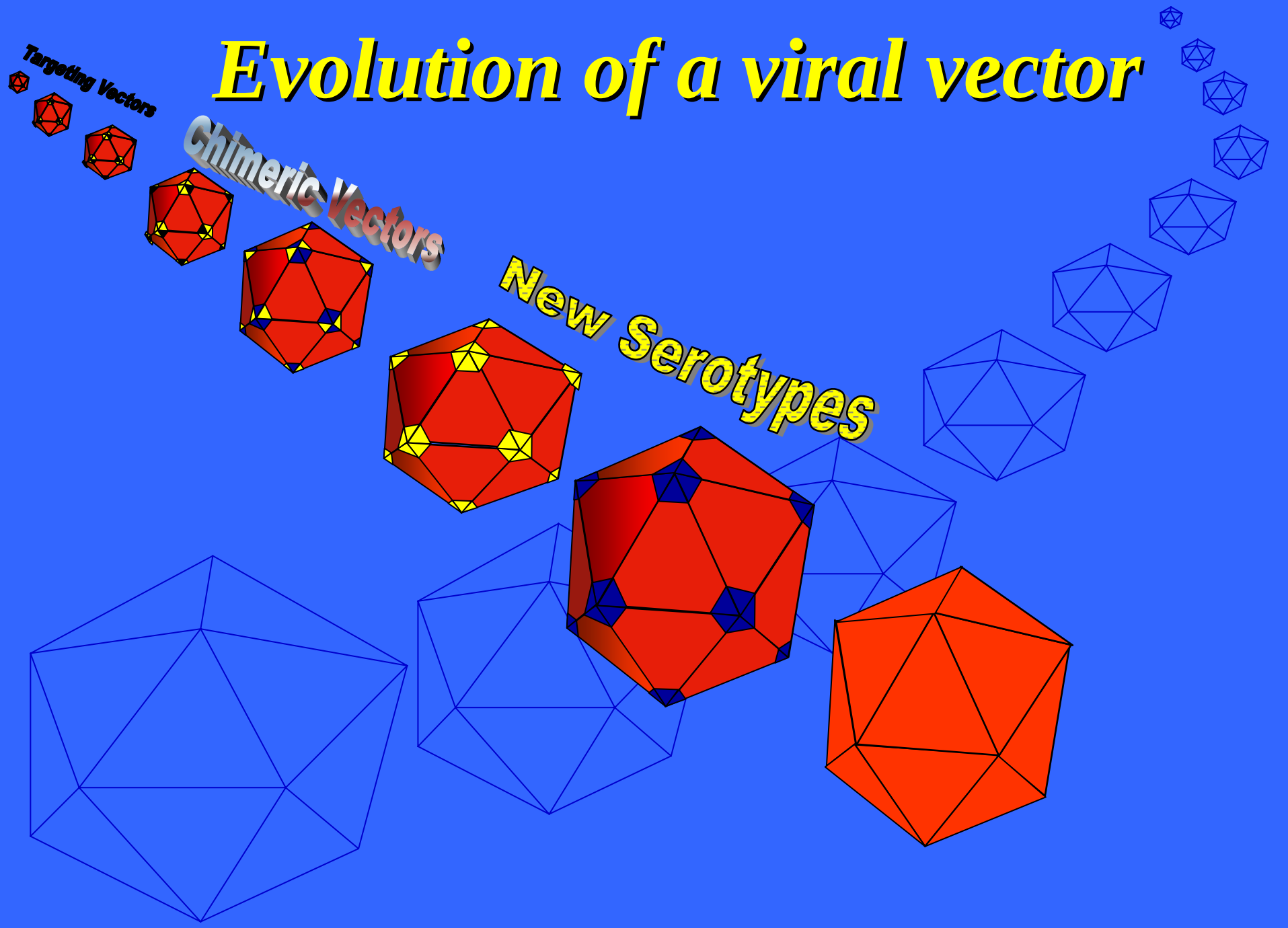


ss-DNA ds-DNA

AAV Double Stranded Vectors

1) Early Transgene Onset
2) More Efficient in all Tissue (20-1000 fold)
3) Compatible with all AAV
4) Reduce overall dose (50-100 fold)
5) Reduce production demand

Evolution of a viral vector



Clinical trials in neurological disorders using AAV vectors: promises and challenges.

Mandel RJ,
Burger C.
University of Florida College of Medicine, Department of Neuroscience, PO Box 100244, Gainesville, FL 32610, USA. rmandel@ufl.edu

Currently, there are five phase I clinical trials of recombinant adeno-associated viral vectors for the treatment of neurological disorders. Two Parkinson's disease (PD), the third trial is aimed at treating Canavan's disease, a pediatric leukodystrophy, the fourth trial targets Alzheimer's disease (AD), and the fifth will attempt to target the lysosomal storage disorder, Batten's disease.
Other gene therapy treatment strategies for PD and other disorders, such as amyotrophic lateral sclerosis, are also on the horizon.

Attenuation of seizures and neuronal death by AAV vector galanin expression and secretion

Haberman, R, Samulaksi RJ, McCown, T.
Gene Therapy Center and Department of Psychiatry,

University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599, USA

Nature Medicine Aug 2003

Adeno-associated Virus-Mediated Expression and Constitutive Secretion of Galanin Suppresses Limbic Seizure Activity in Vivo

Thomas J. McCown*
Gene Therapy Center and Department of Psychiatry, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599, USA

Molecular Therapy May 2006

Acknowledgements

R. J. Samulski Laboratory

Rebecca Haberman
Joe Rabinowitz
Doug McCarty
Paul Monohan
Dawn Bowles
Chengwen Li
Wupin Li
Zhijian Wu

Vector Core
Xiaohuai Zhou
Jeff Beechman
Angelique Camp
Lori Nisi
Victoria, Xing Hau,

Thomas McCown UNC
Gene Redmond Yale
Paola Leone Robert Wood Johnson