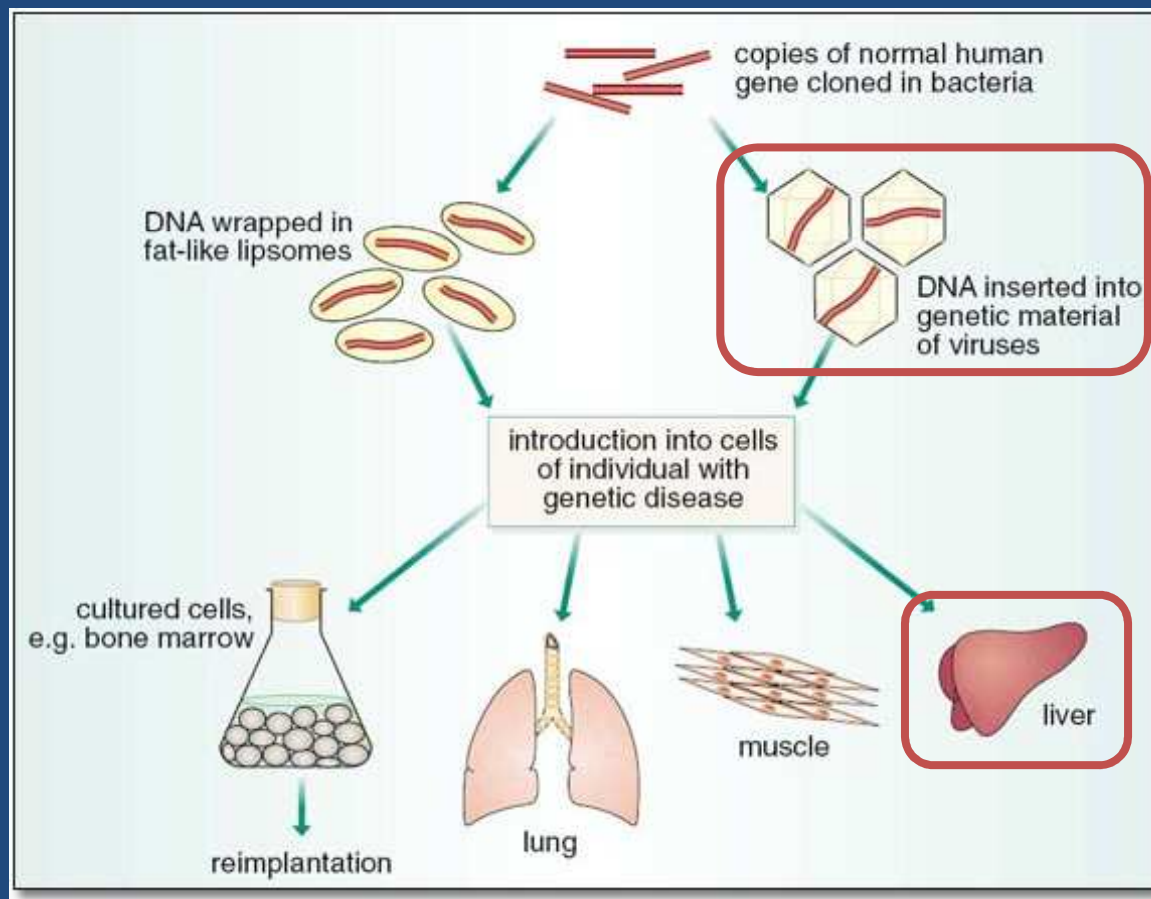


# Principles of human gene therapy

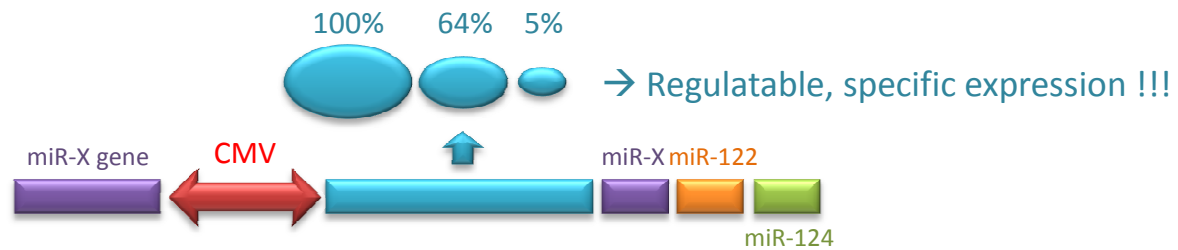
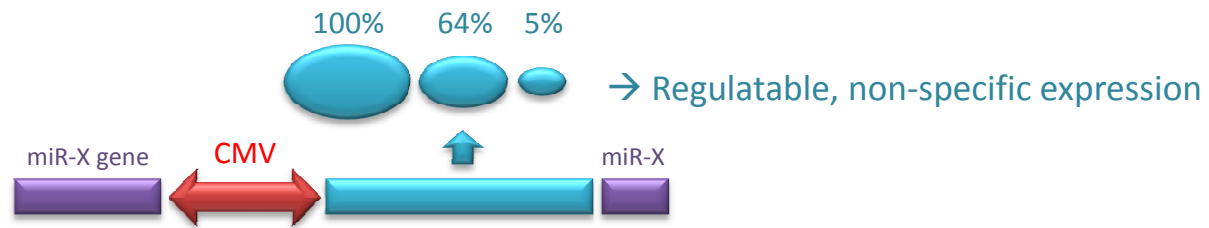
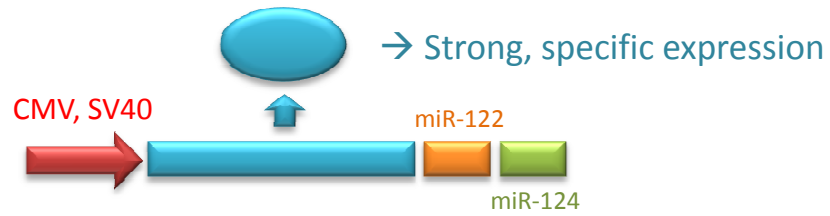
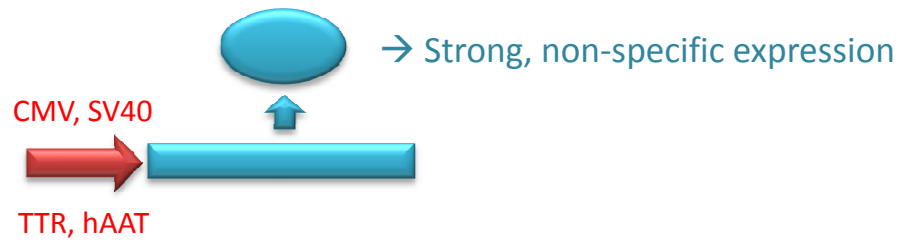
"The insertion of normal or genetically altered genes into cells through the use of recombinant DNA technology - usually done to replace or repair defective genes, or to suppress abnormally expressed or disease-associated genes, as part of the treatment of genetic disorders."

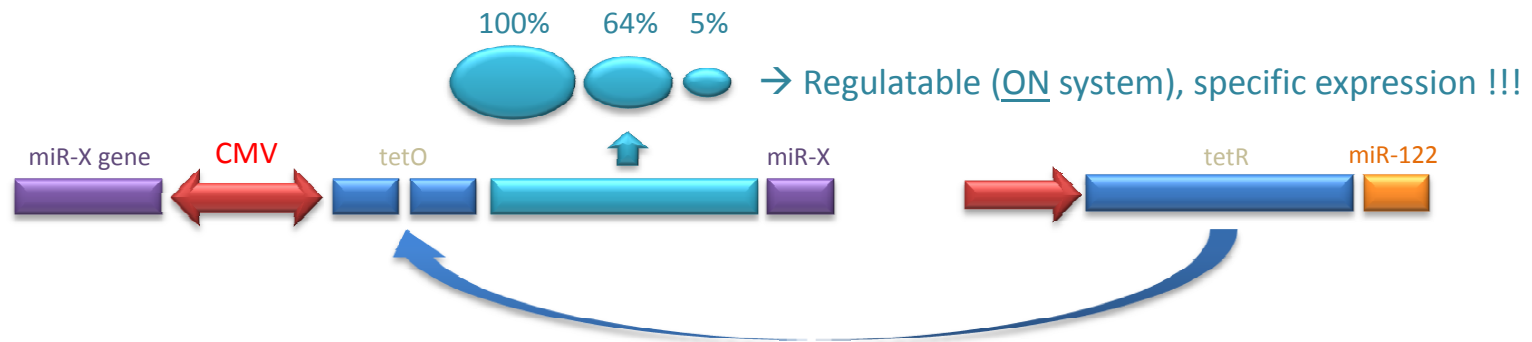
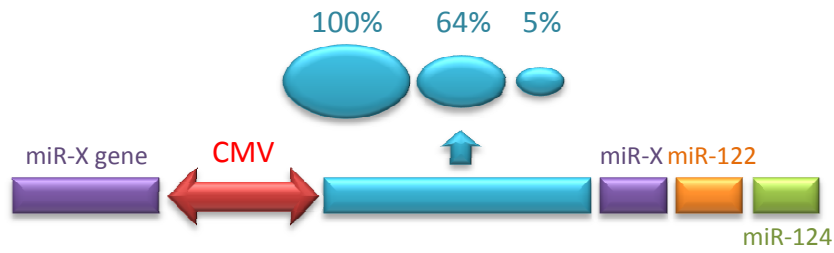


1) Gene

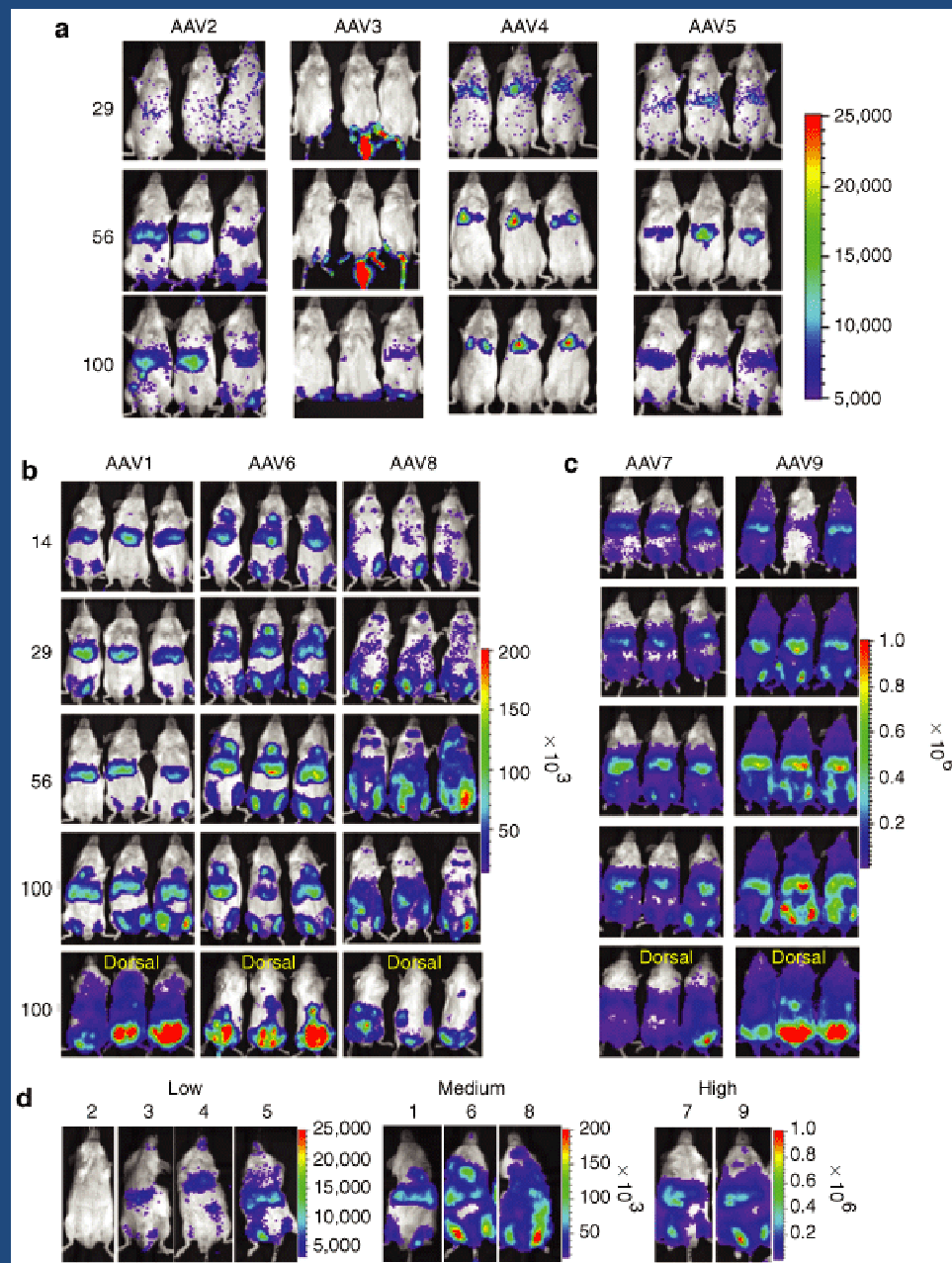
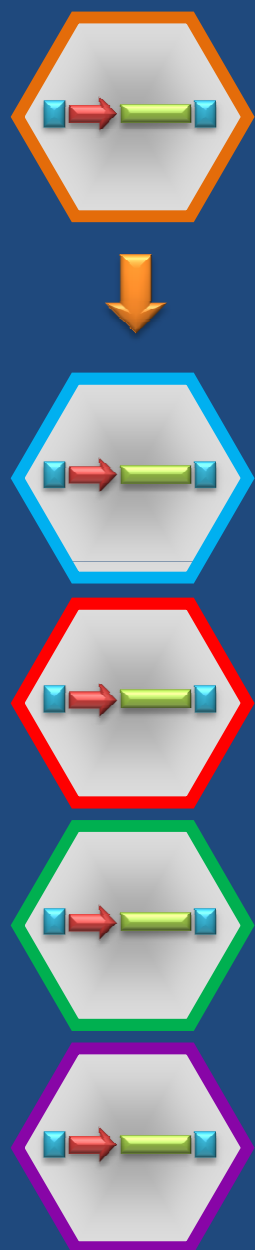
2) Vector

3) Target

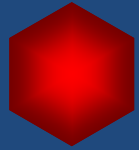




# AAV serotypes differ in tropism

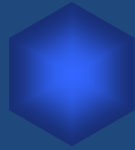


# Towards “personalized” AAV/RNAi-based gene therapy



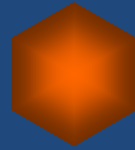
AAV-2

+ good *in vitro*  
- poor *in vivo*  
- high immunity

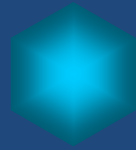


AAV-8

+ great *in vivo*  
+ rare antibodies  
- broad tropism  
- poor *in vitro*

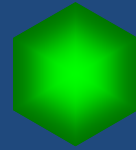


AAV-9

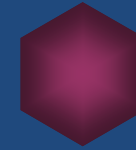


AAV-4

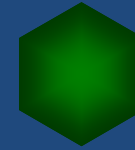
+ very distinct  
+ rare antibodies  
+ unique tropism  
- poor *in vitro*



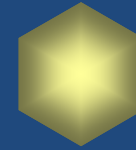
AAV-5



AAAV



BAAV



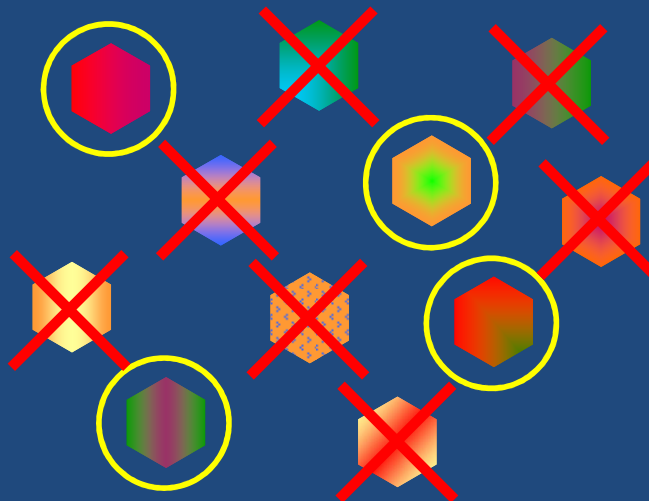
CAAV

+ very distinct  
+ non-primate  
- tropism unclear  
- poor *in vitro*

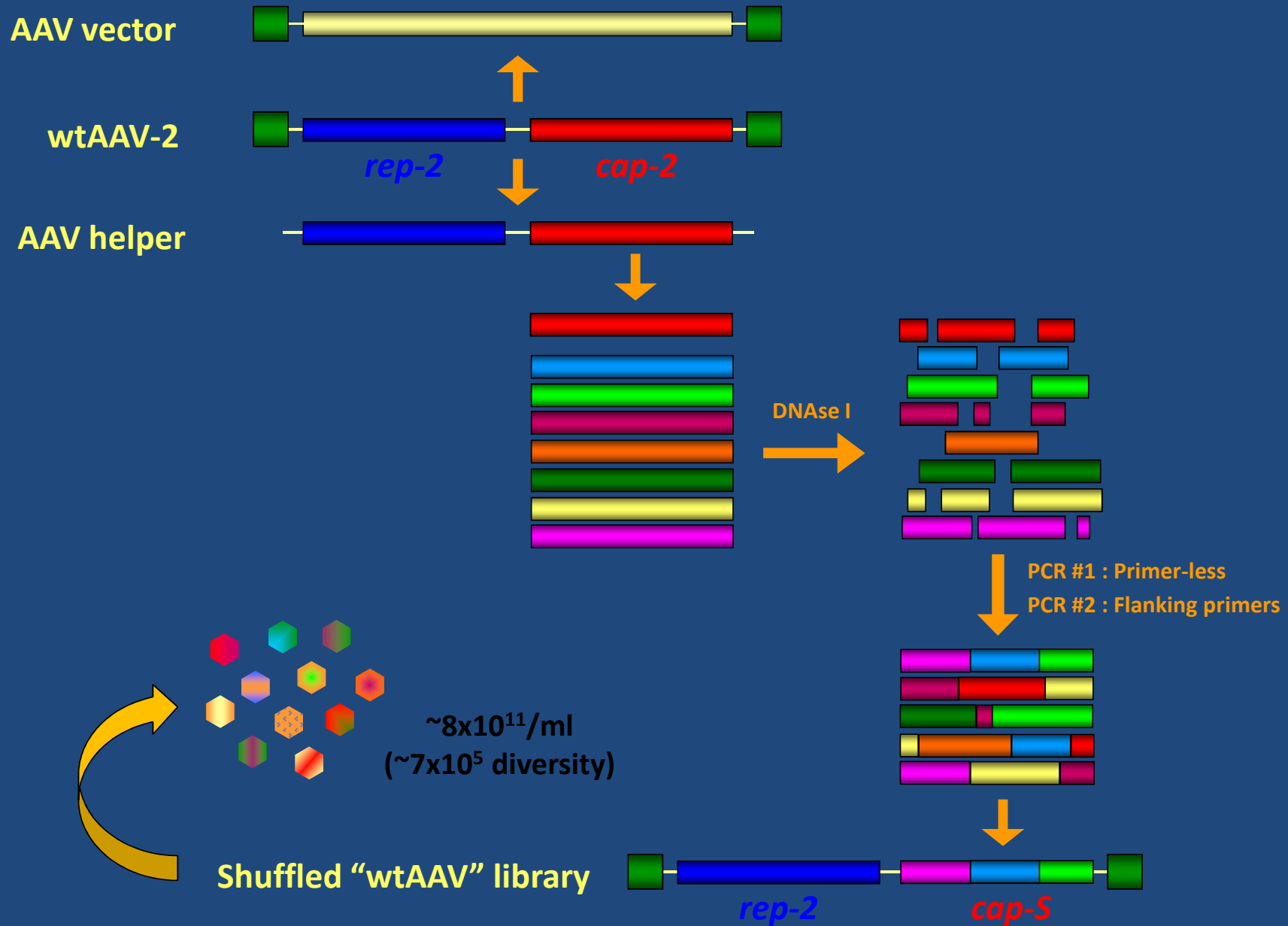
1) Shuffle

2) Select

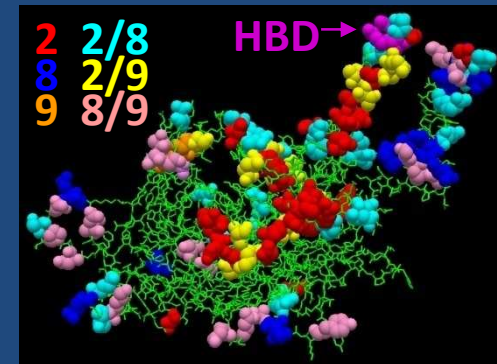
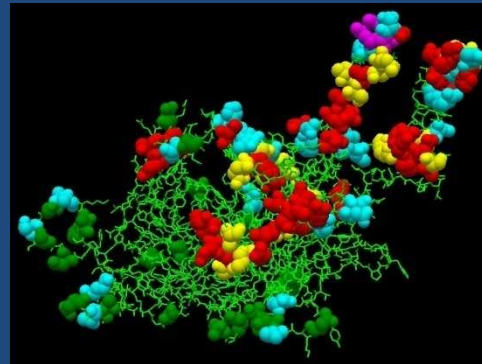
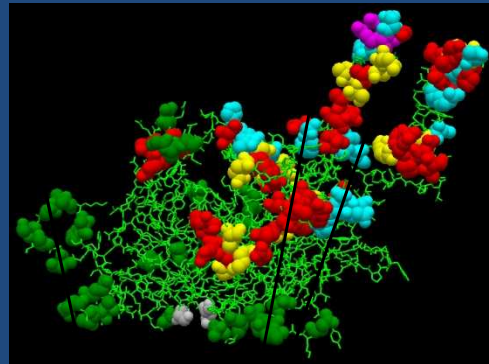
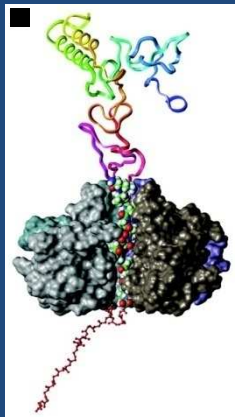
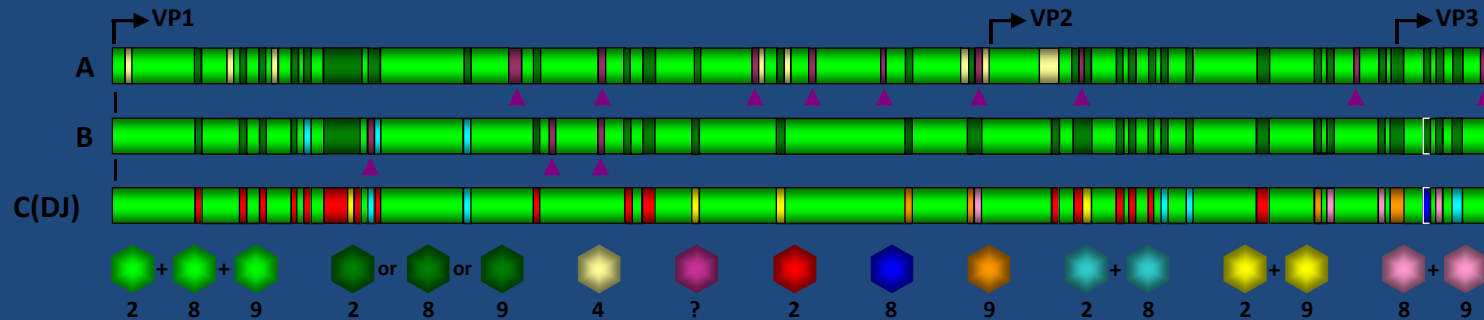
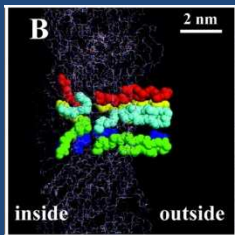
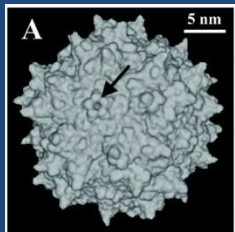
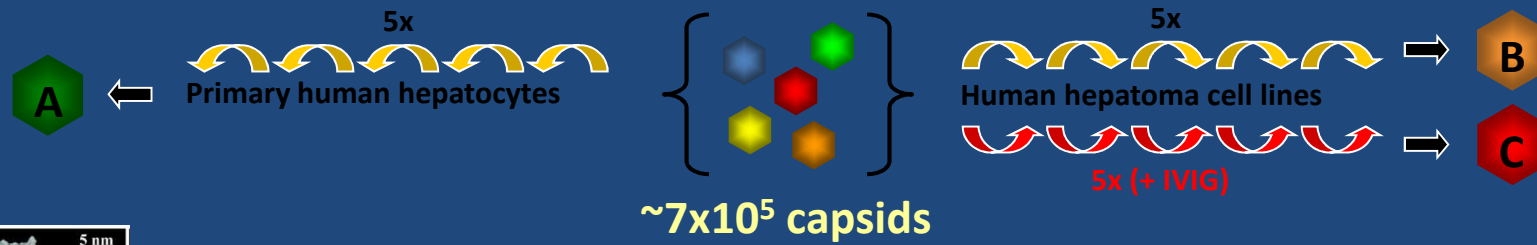
3) Study



# AAV capsid libraries: DNA Family Shuffling approach

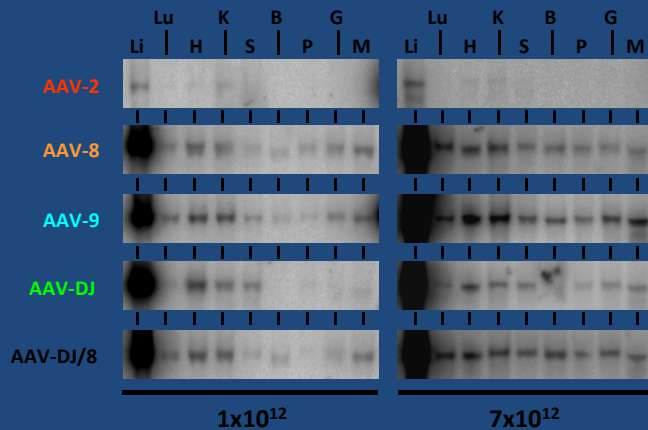
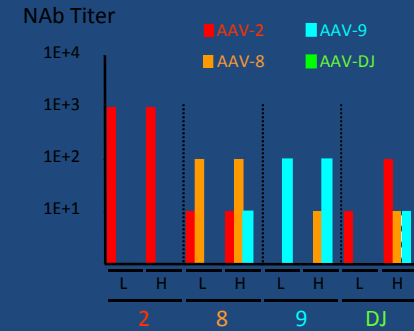
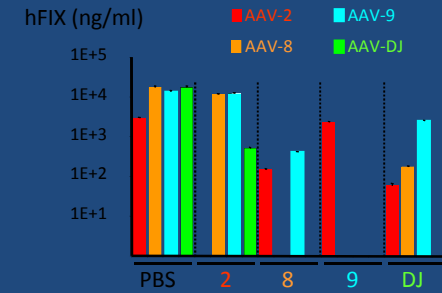
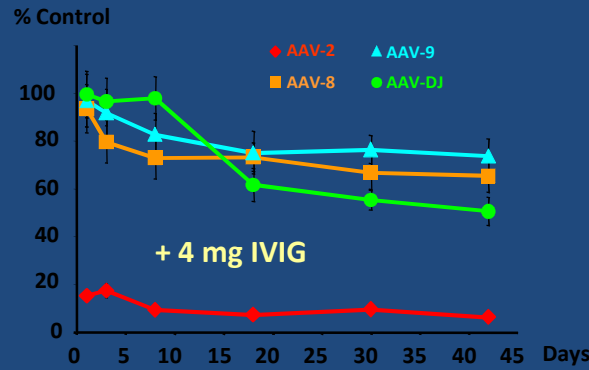
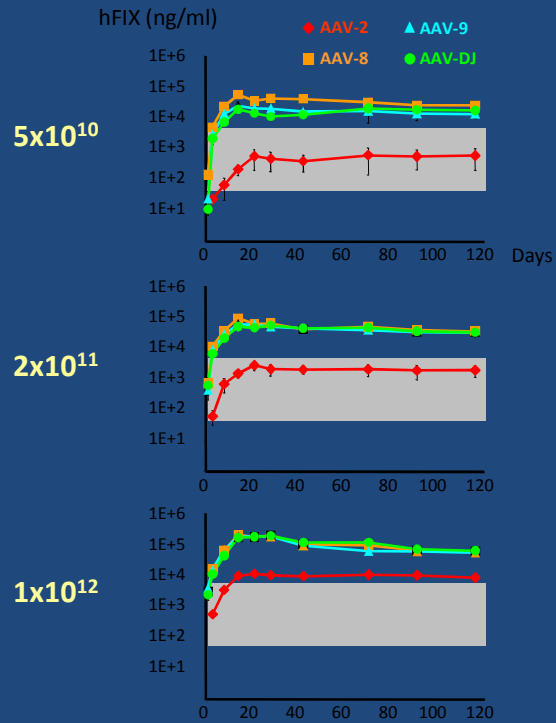


# In vitro evolution of chimeric liver-specific AAV capsids



From: Bleker *et al.*, *J. Virol.* 2005; Kronenberg *et al.*, *J. Virol.* 2005

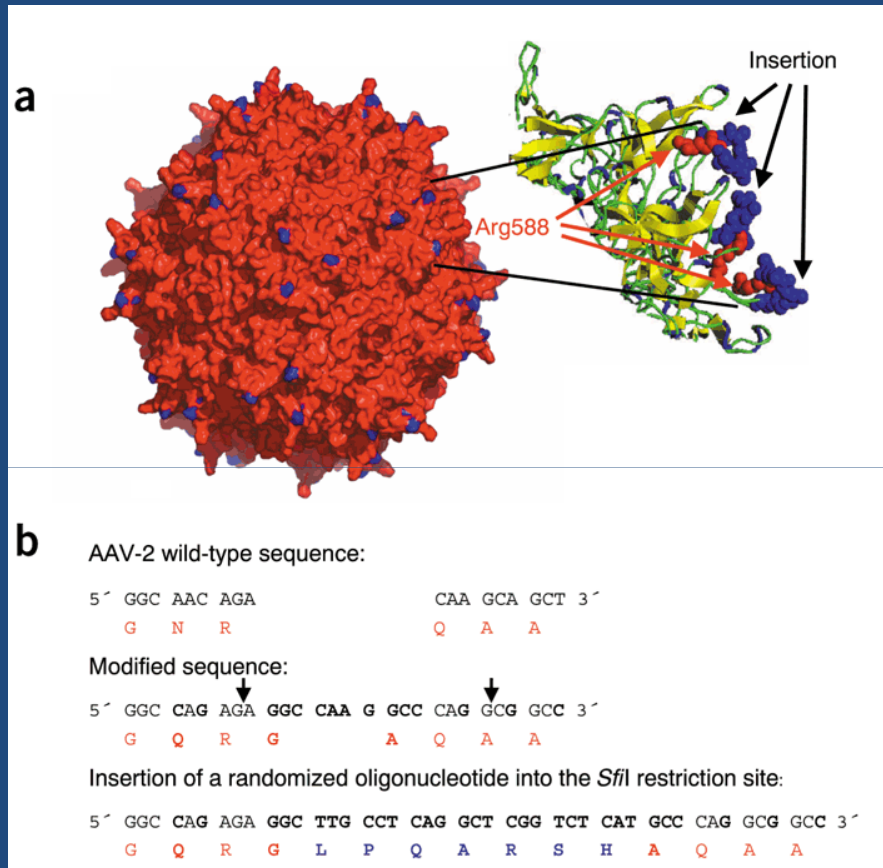
# Superior *in vivo* liver transduction with AAV-DJ



		Lung	Heart	Kidney	Spleen	Brain	Pancreas	Gut	Muscle
AAV-2	1e12	nd	0.7 ± 0.1	0.8 ± 0.1	0.2 ± 0.0	nd	nd	nd	nd
	7e12	nd	1.5 ± .03	2.0 ± 0.3	1.0 ± 0.2	nd	nd	nd	nd
AAV-8	1e12	0.5 ± 0.0	1.2 ± 0.2	0.9 ± 0.2	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.7 ± 0.1
	7e12	2.5 ± 0.3	2.5 ± 0.2	2.6 ± 0.3	1.5 ± 0.2	1.5 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.9 ± 0.2
AAV-9	1e12	0.7 ± 0.1	1.3 ± 0.2	1.1 ± 0.2	0.4 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.8 ± 0.1
	7e12	2.6 ± 0.3	3.6 ± 0.4	3.8 ± 0.4	1.5 ± 0.2	1.8 ± 0.2	1.3 ± 0.2	1.9 ± 0.2	3.0 ± 0.3
AAV-DJ	1e12	0.2 ± 0.0	1.3 ± 0.2	0.8 ± 0.2	0.5 ± 0.1	nd	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
	7e12	0.6 ± 0.1	2.3 ± 0.2	2.1 ± 0.2	1.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.1
AAV-DJ/8	1e12	0.6 ± 0.0	1.3 ± 0.2	0.8 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.7 ± 0.1
	7e12	2.6 ± 0.3	2.5 ± 0.3	2.3 ± 0.3	1.6 ± 0.3	1.8 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	2.0 ± 0.2



# Peptide display as an alternative approach at AAV diversification



## Problems with AAV-2 as parent:

1) Seroprevalence

2) It sucks

3) Multiple (co-)receptors

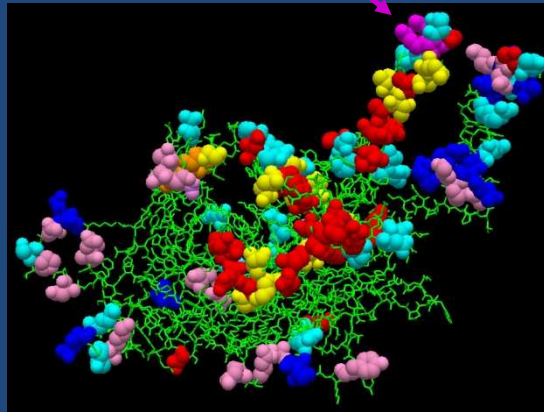
Mueller, O. (...) Kleinschmidt, J.A. *Nat. Biotech.*, 2003

## AAV-DJ as a preferred parent for peptide display libraries

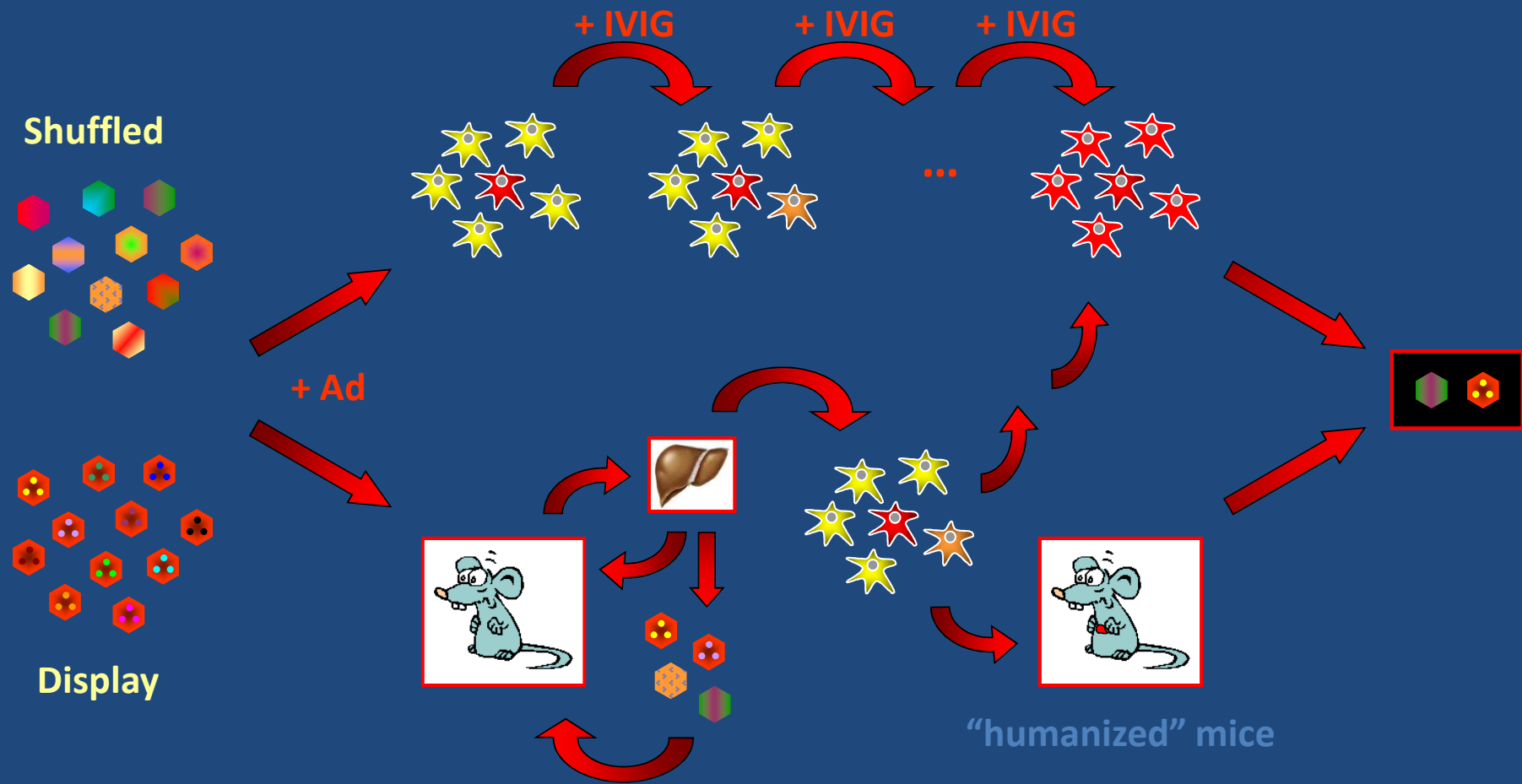
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Q XXXXXXXX  
N L Q R G N R Q A A

Primary receptor binding



# Options for *in vitro* or *in vivo* selection of novel AAV capsids



## Outlook for iGEM

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August: - Design & create miRNA-regulated AAV vector cassettes  
- In parallel: Generation & production of viral capsid libraries  
- Continue work on measurement standard (?)

September: - Selection (5 rounds) of viral libraries → Hepatoma cells, T cells, MEF

October: - Production of rAAV vectors combining best cassettes & capsids  
- Validation & analysis *in vitro* & *in vivo*