Structural basis of AAV affinity chromatography: from design to cryo-EM validation

Nathaniel E Clark[1], Mario Mietzsch[2], Robert McKenna[2], Kelley Kearns[1], Thomas C Scanlon[1] [1]Repligen Corporation, 16 Cavendish Ct Lebanon NH 03766 [2]University of Florida, Gainesville FL 32610

nclark@repligen.com

REPLIGEN

Introduction

To increase patient access to AAV therapies, it is necessary to decrease manufacturing costs. To this end, we developed a suite of alkali-tolerant affinity resins for AAV serotypes 2, 5, 6, 8, and 9. The resulting affinity resins offer best-in-class performance and process economics as they maintain performance through 20+ clean-in-place cycles.

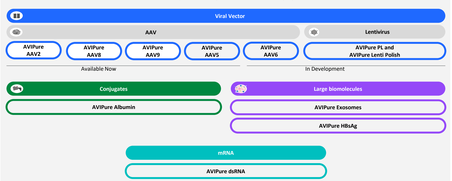
To validate that design and specificity of our AVIPure® AAV-affinity ligands we determined cryo-EM structures of the AAV2, AAV8, and AAV9 ligands in complex with capsids of the appropriate serotype.

The structures map the footprint of AVIPure ligands on the capsids, enabling rational pairing of affinity resin to an engineered capsid. If capsid engineered does not substitute amino acids in AVIPure footprint, successful purification with AVIPure resin is expected.

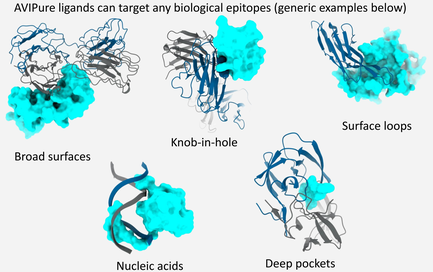
AVIPure scaffolds can bind virtually any biological epitope

AVIPure ligands are: 1) Stable in NaOH 2) Small proteins and peptides 4)Diverse set of >49 scaffolds 3) Manufacturable at scale

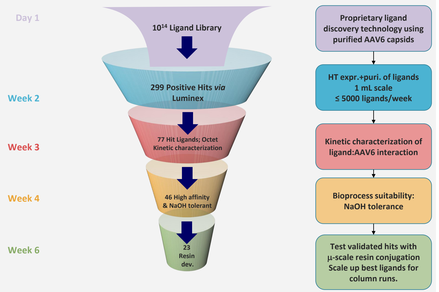
AVIPure® Affinity Resin portfolio and pipeline



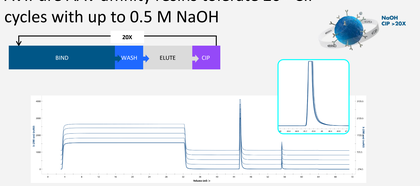
AVIPure ligands can target any biological epitopes (generic examples below)



Creating AAV affinity resins



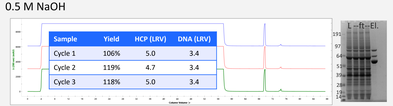
AVIPure AAV affinity resins tolerate 20+ CIP cycles with up to 0.5 M NaOH



Overlay of bind, wash, elute and CIP cycles (5,10,15,20)

AVIPure AAV2 performance data

Consistent performance is maintained though stringent cleaning using 0.5 M NaOH

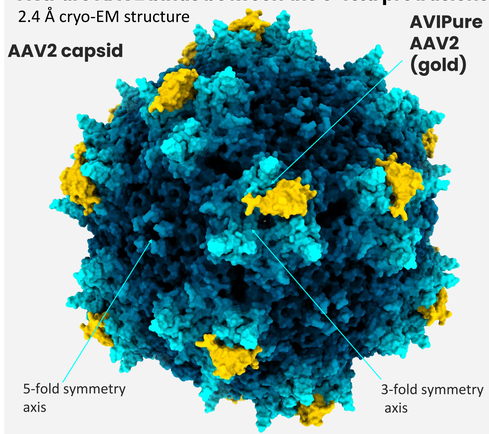


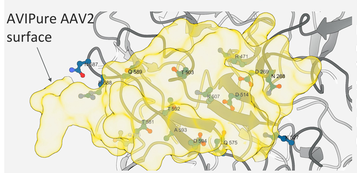
Sample	Yield	HCP (LRV)	DNA (LRV)
Cycle 1	106%	5.0	3.4
Cycle 2	119%	4.7	3.4
Cycle 3	118%	5.0	3.4

0.8 x 10 cm AVIPure® AAV2 OPUS® MiniChrom® column (5 mL CV)

AVIPure AAV2 binds between the 3-fold protrusions

2.4 Å cryo-EM structure

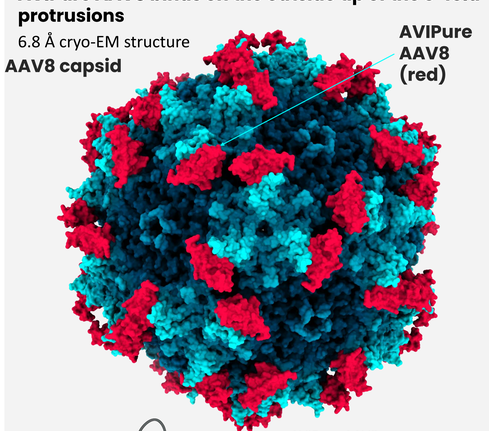


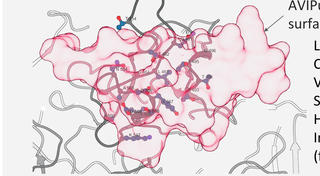


Ligand MW: 7.3 kDa
Contact area: 1,060 Å²
VdW contacts: 128
Salt-bridges: 1
H-bonds: 5
Interf. resi: 17lig, 23 cap.
(from PDBSUM)

AVIPure AAV8 binds on the outside tip of the 3-fold protrusions

6.8 Å cryo-EM structure

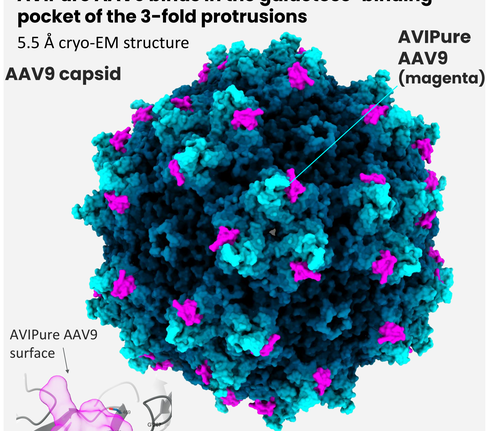




Ligand MW: 6.9 kDa
Contact area: 778 Å²
VdW contacts: 112
Salt-bridges: 2
H-bonds: 5
Interf. resi: 11 lig, 18 capsid
(from PDBSUM)

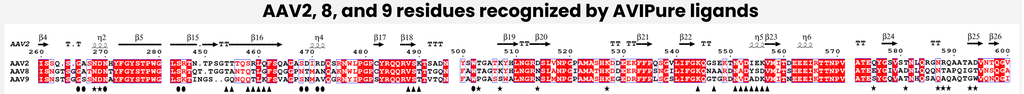
AVIPure AAV9 binds in the galactose-binding pocket of the 3-fold protrusions

5.5 Å cryo-EM structure



Ligand MW: 1.5 kDa Contact area: 614 Å²
VdW contacts: 67 Salt-bridges: 0
H-bonds: 1
Interface residues: 6 lig, 10 capsid
(from PDBSUM)

AAV2, 8, and 9 residues recognized by AVIPure ligands



AVIPure AAV affinity resins are appropriate for capsids that retain these amino acid residues

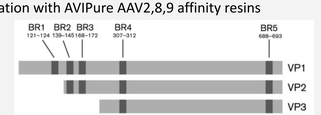
★AAV2 ▲AAV8 ●AAV9

Conclusions

AVIPure ligands are highly specific for AAV serotypes

The ligands bind to different areas of the 3-fold protrusions

Capsid engineering distal to the 3-fold residues should not impair purification with AVIPure AAV2,8,9 affinity resins



Modified from:
Popa-Wagner et a., J. Gen.Vir., 2012 93, 1887-98

Learn more at **repligen.com**