

Bioprocess Tools & Designs for the Purification of Viral Vectors for Gene Therapy

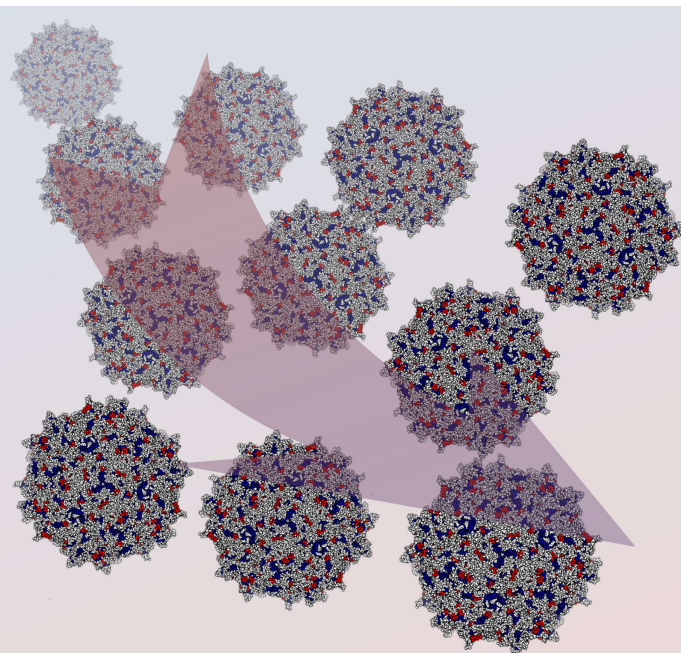
Stefano Menegatti & Group

Chemical and Biomolecular Engineering

Biomanufacturing Training and Education Center (BTEC)

NC State University

**Bioprocess International
September 21-23, 2021**



Intro

LigaGuard

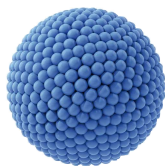
AAVidity

Results

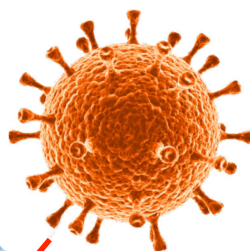
Summary

Gene Therapy Products (GTPs) and Vectors

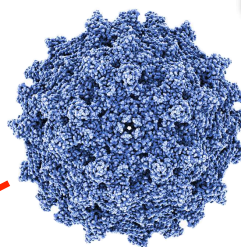
Synthetic



Lentivirus



AAV

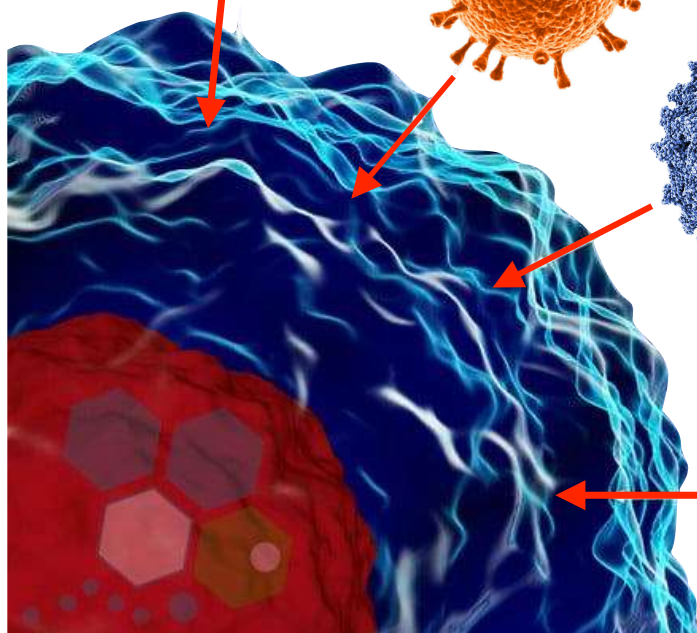


2 AAV products approved
~150 clinical trials

Growing GTP market
in vivo - ex vivo - in vitro

Critical quality attributes
Titer, Full vs. Empty,
Transduction activity ...

Expression in HEK293 cells
Bind-and-Elute purification
Batch manufacturing process



Intro

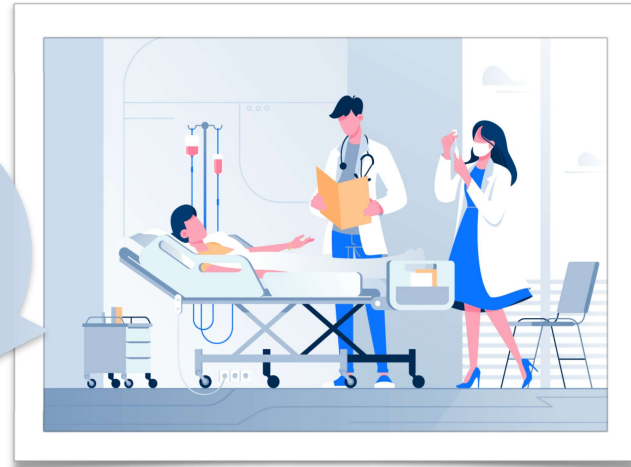
LigaGuard

AAVidity

Results

Summary

From Bench to Bedside ...



Intro

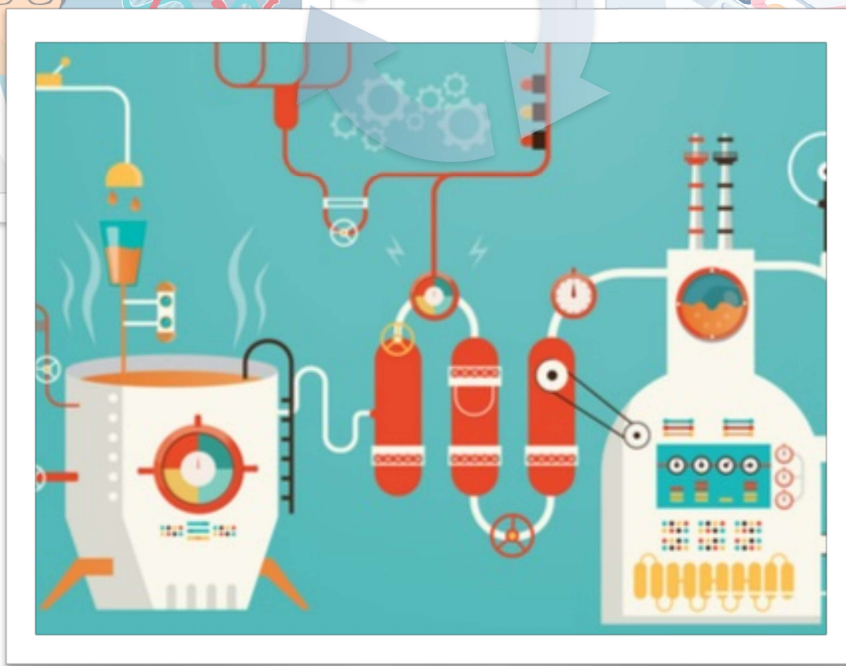
LigaGuard

AAVidity

Results

Summary

... There's Bioprocess in between!



Intro

LigaGuard

AAVidity

Results

Summary

... There's Bioprocess in between!

**mAb-inspired bioprocess technologies
and process designs**

Need for a GTP-dedicated toolbox:

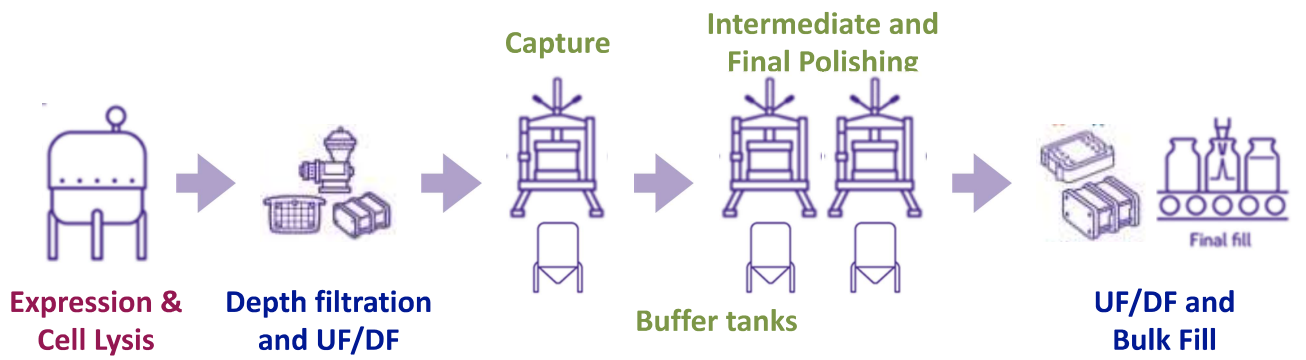
Purification technologies

Integrate Us ↔ Ds

Process modalities

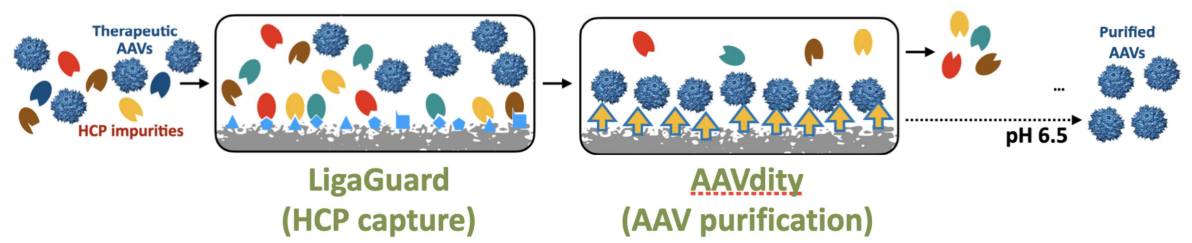
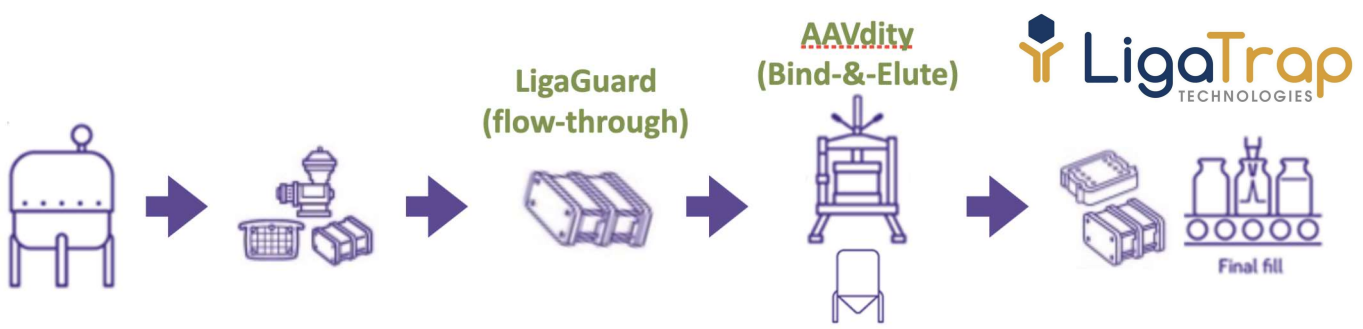
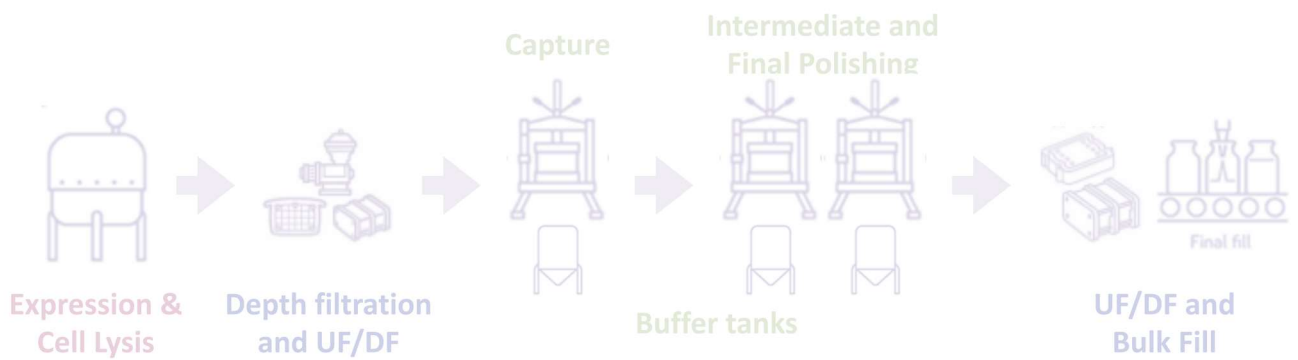


Current AAV Purification Process



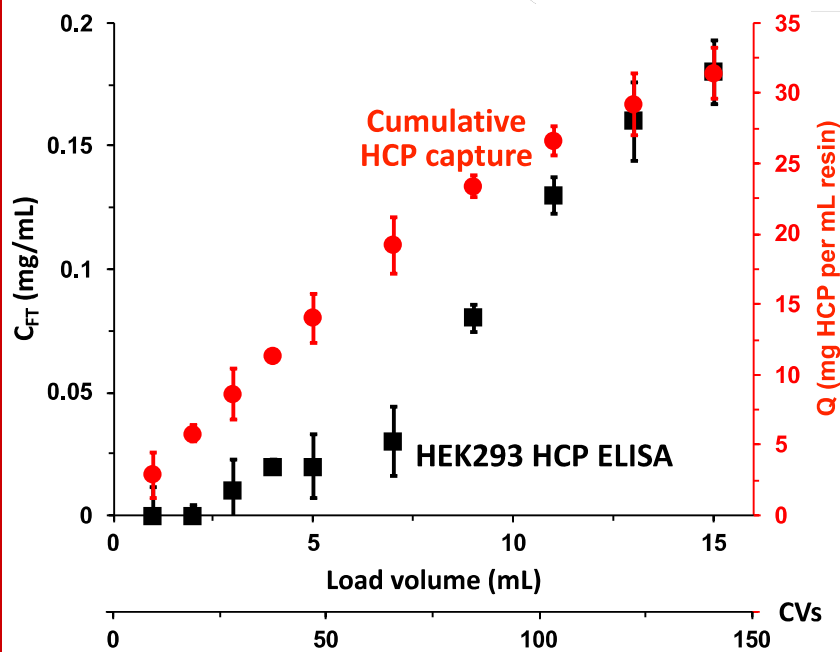
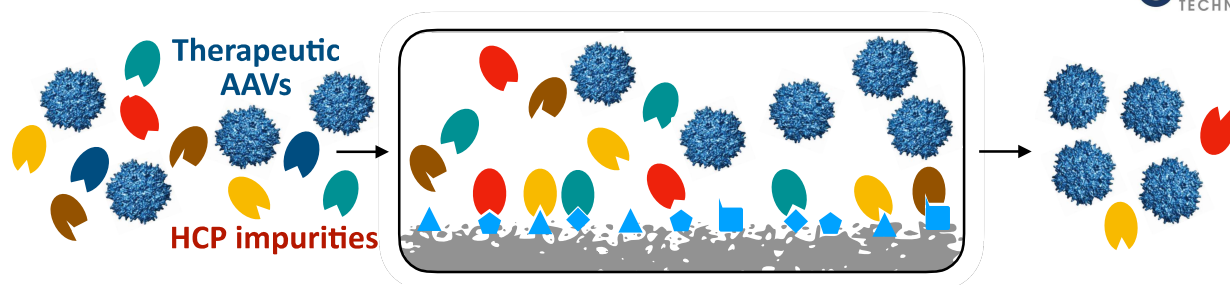
Intro LigaGuard AAVdity Results Summary

Current AAV Purification Process



Intro	LigaGuard	AAVidity	Results	Summary
-------	------------------	----------	---------	---------

LigaGuard™ adsorbent



Load	0.75 mg/mL
DBC _{10%} (RT: 0.5 min)	23.3 mg/mL
DBC _{10%} (RT: 1 min)	24.1 mg/mL
DBC _{10%} (RT: 2 min)	29.5 mg/mL
DBC _{10%} (RT: 5 min)	31.2 mg/mL

Intro

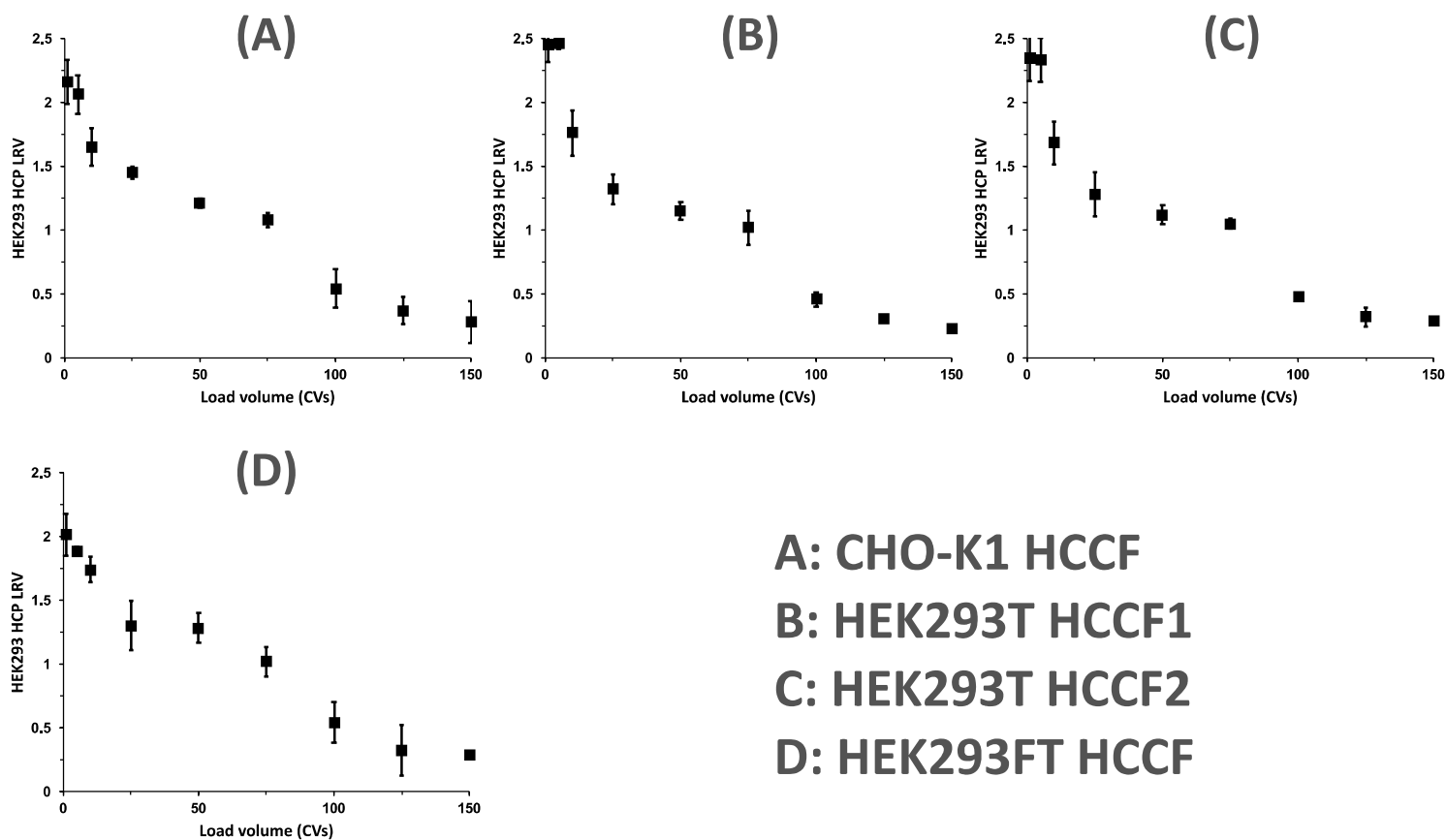
LigaGuard

AAVidity

Results

Summary

HCP capture from industrial HCCFs *ELISA-based Clearance (LRV)*



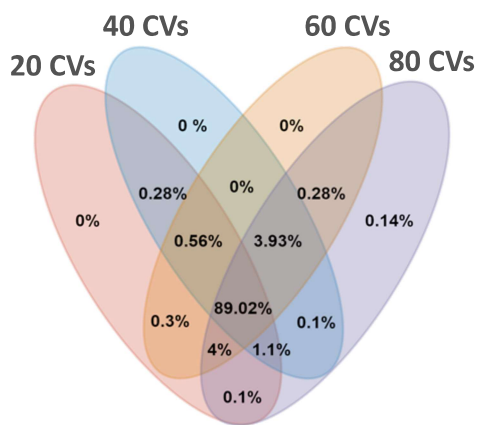


Proteomics-based Clearance

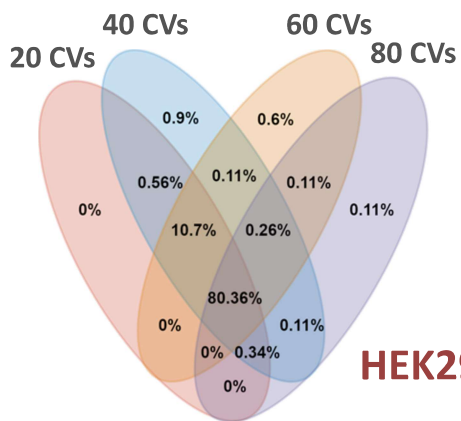
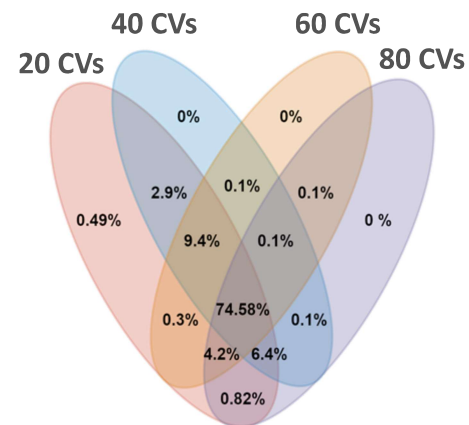
CHO-K1 HCCF



HEK293T HCCF1



HEK293T HCCF2



HEK293FT HCCF

% values calculated as

$$X\% = \frac{\text{Number of HCPs in FT\#}}{\text{Number of HCPs in Feed}}$$

Intro

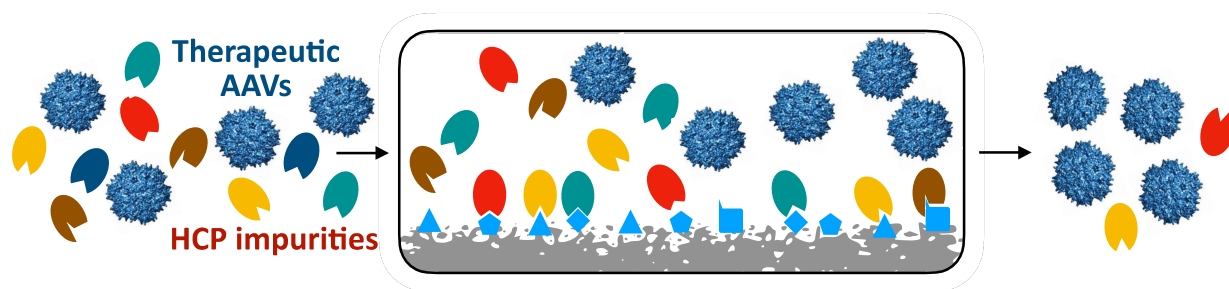
LigaGuard

AAVidity

Results

Summary

LigaGuard™: AAV purification in Flow-Through Mode



AAV particle recovery: >97%

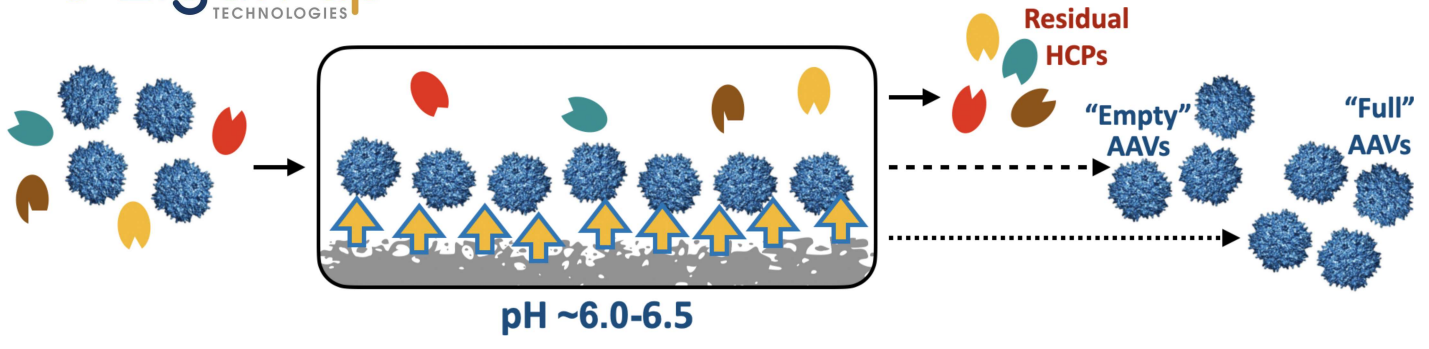
Genome recovery: >92% (unpackaged)

DNA LRV > 2.0

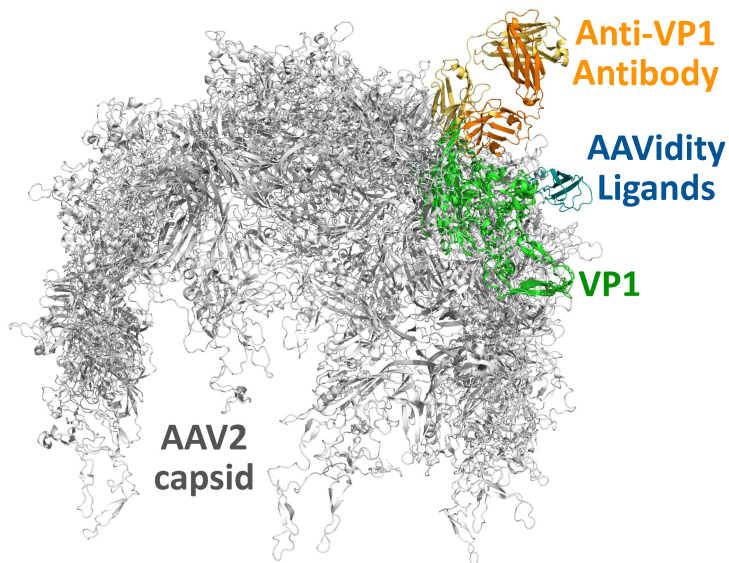
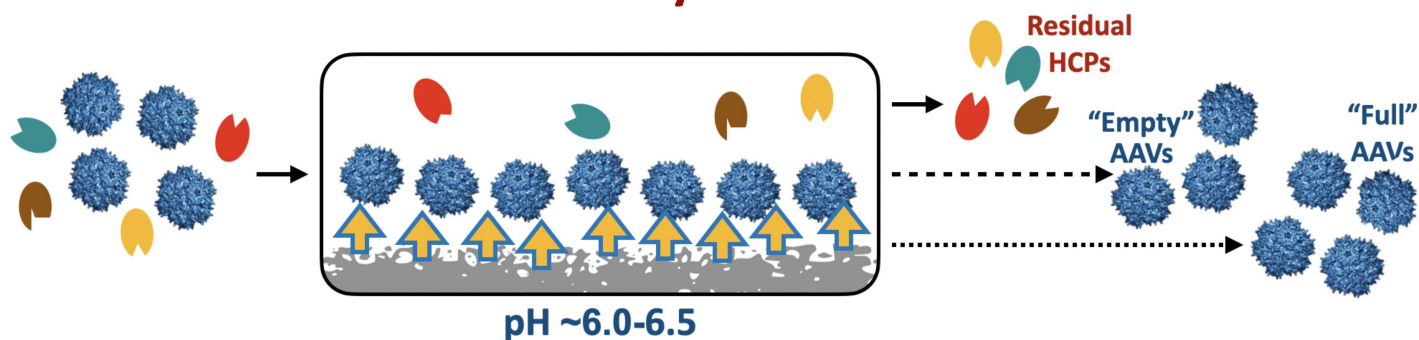
HCP LRV > 1.0



AAVidity adsorbent



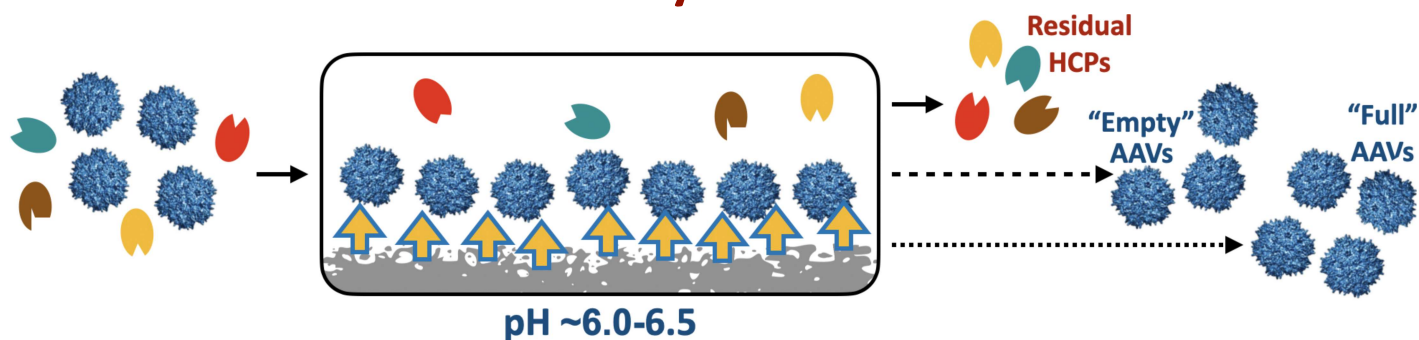
AAVidity adsorbent



AAVidity ligands: small peptide ligands (< 10 aa) designed to:

- Target the VP1 domain of the AAV capsids for universal AAV binding;
- Bind with mild affinity ($K_D \sim 10^{-5}$ M) for mild elution conditions.

AAVidity adsorbent



Nota Bene:
AAVidity is a “polyclonal” peptide ligand ensemble that ensures **interaction with multiple AAV serotypes**

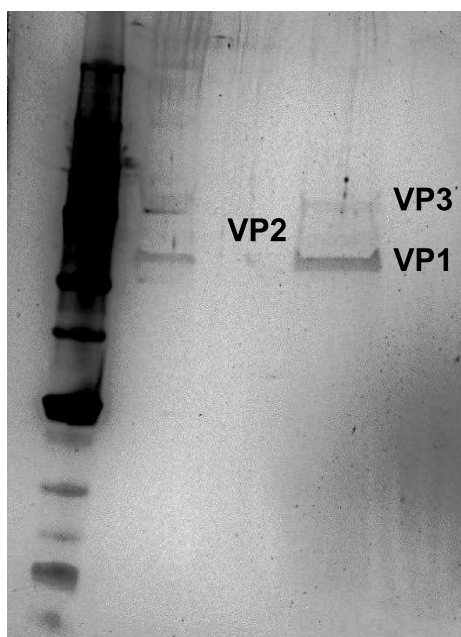
peptide
AAV
ing;
- Bind with mild affinity ($K_D \sim 10^{-5}$ M)
for mild elution conditions.

AAV2 capsid

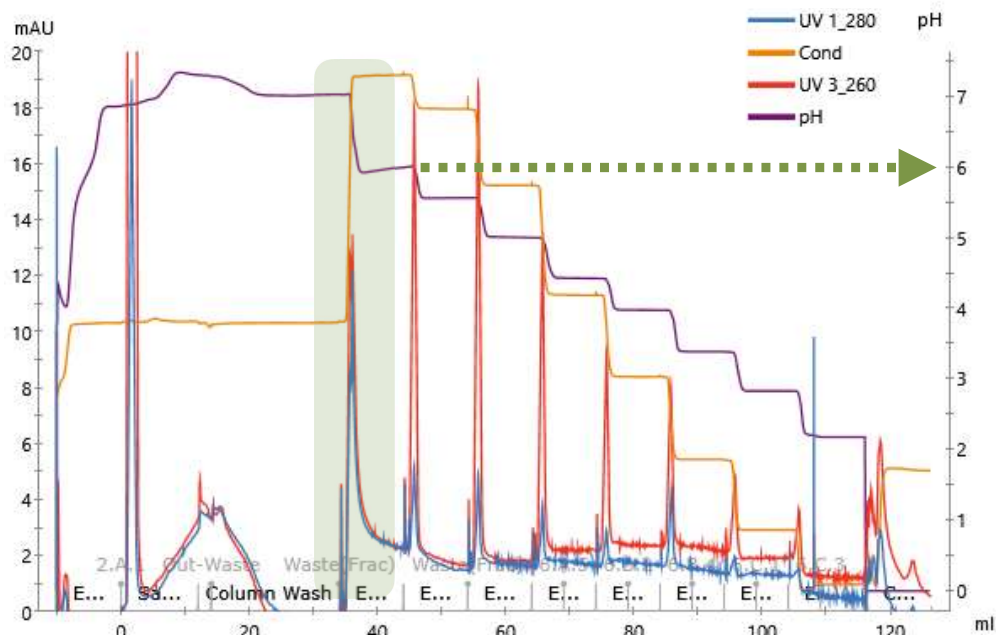


Multi Step pH Gradient – AAVidity Resin upflow elution (pure AAV2 injection)

Pure AAV2 feed



VP1 – 87 kDa
VP2 – 72 kDa
VP3 – 62 kDa



ELISA-measured yield

pH 6.5: 78% pH 5.5: 9%
pH 6.0: 12% pH ≤ 5: 2%

Intro

LigaGuard

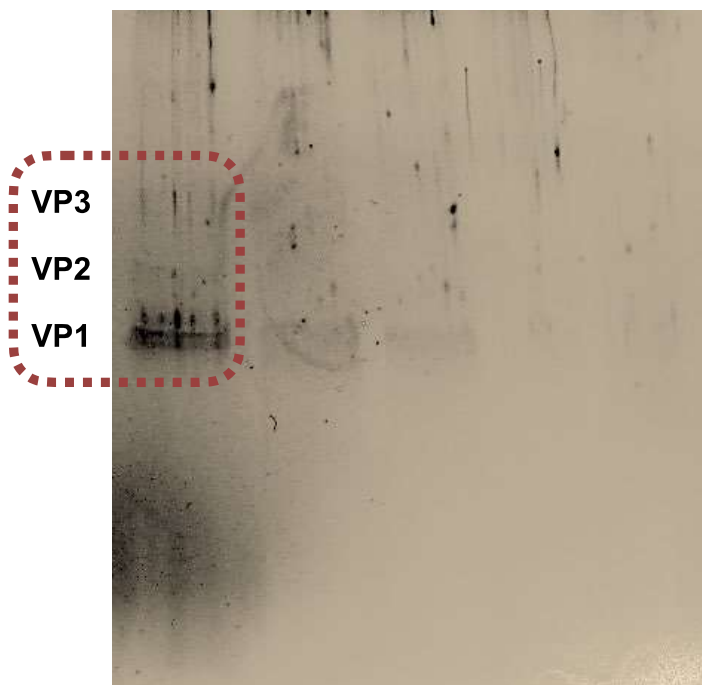
AAVidity

Results

Summary

Multi Step pH Gradient – AAVidity Resin upflow elution (pure AAV2 injection)

1 2 3 4 5



Lane 1 – Elution @ pH 6.3

Lane 2 – Elution @ pH 6.0

Lane 3 – Elution @ pH 5.5

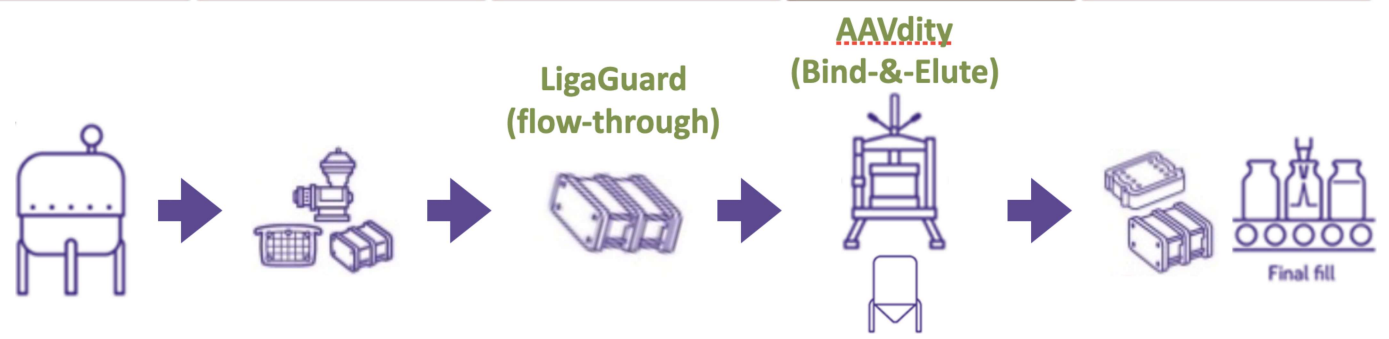
Lane 4 – Elution @ pH 5.0

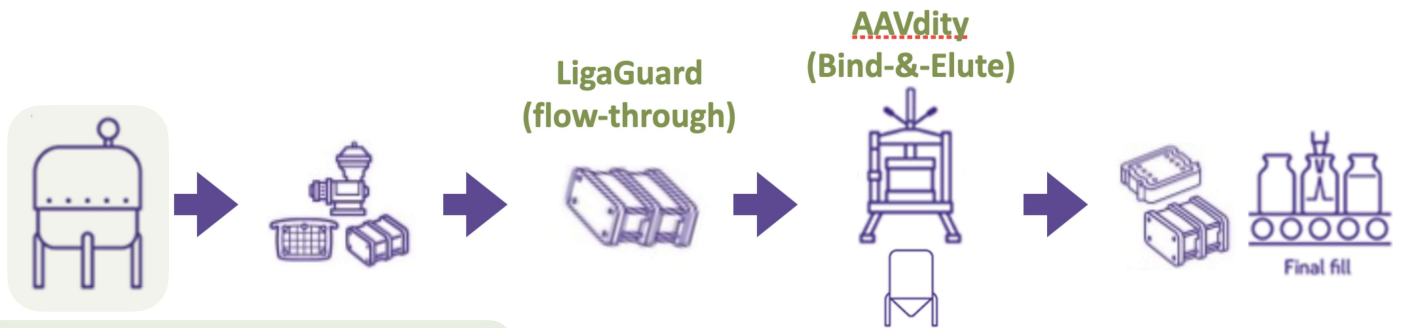
Lane 5 – Elution @ pH 4.5

VP1 – 87 kDa

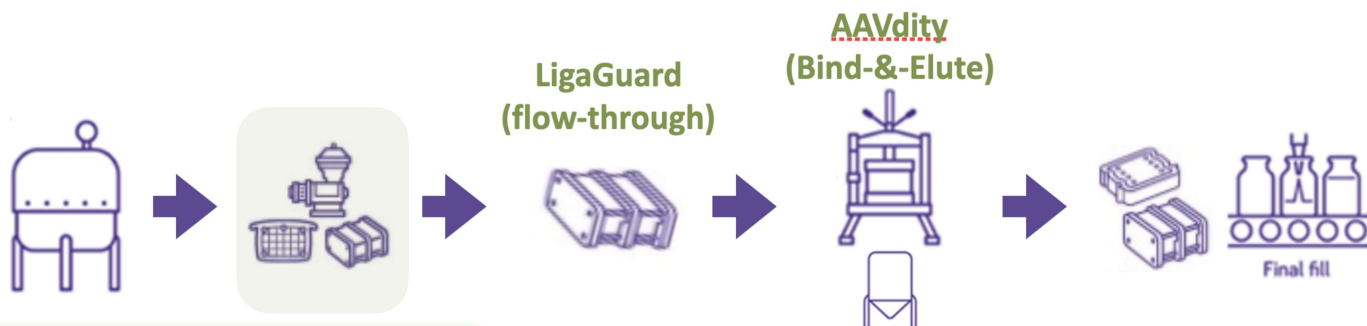
VP2 – 72 kDa

VP3 – 62 kDa





Cells: HEK293 (2.5×10^6 cells/mL)
SFM4Transfx-293 serum-free in shaker flasks; T: 28 °C; pH: 6.4
Product: AAV2 at $\sim 8.3 \times 10^9$ vg/mL



Cells: HEK293 (2.5×10^6 cells/mL)
 SFM4Transfx-293 serum-free in shaker flasks; T: 28 °C; pH: 6.4
Product: AAV2 at $\sim 8.3 \times 10^9$ vg/mL

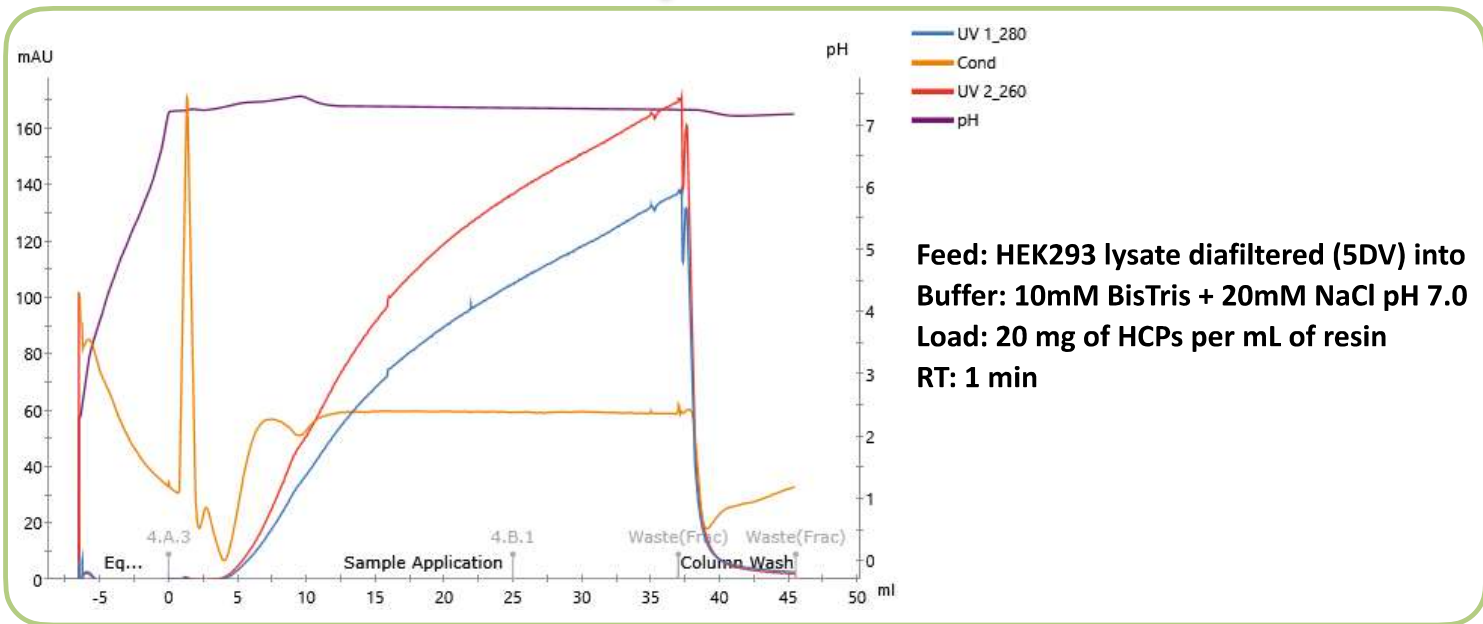
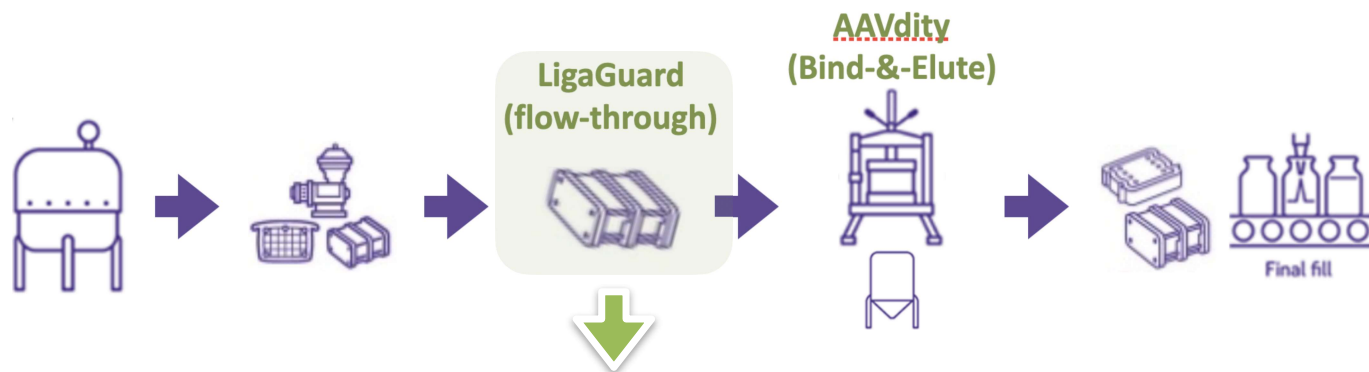
Cell lysis (0.5% Triton X-100 and 5 μ g/ml of RNase A)
 Centrifugation: 3,700 \times g for 30 minutes.
 Filtration: 0.45 μ m Sartoscale filter
 TFF: 5 DVs on a PES membrane (MWCO 100 kDa)
 Buffer: 10 mM Bis-Tris + 20 mM NaCl, pH 7.0
AAV2 at 2.49×10^{11} vp/mL; HCPs: 0.748 mg/mL



Cells: HEK293 (2.5×10^6 cells/mL)
SFM4Transfx-293 serum-free in shaker flasks; T: 28 °C; pH: 6.4
Product: AAV2 at $\sim 8.3 \times 10^9$ vg/mL

Cell lysis (0.5% Triton X-100 and 5µg/ml of RNase A)
Centrifugation: 3,700×g for 30 minutes.
Filtration: 0.45 µm Sartoscale filter
TFF: 5 DVs on a PES membrane (MWCO 100 kDa)
Buffer: 10 mM Bis-Tris + 20 mM NaCl, pH 7.0
AAV2 at 2.49×10^{11} vg/mL; HCPs: 0.748 mg/mL

Load: 30 CVs; RT: 1 min
Product: AAV2 at 1.12×10^{10} vg/mL
HCPs: 0.049 mg/mL (~ 1.18 LRV)





**LigaGuard
(flow-through)**



**AAVdity
(Bind-&-Elute)**



Final fill

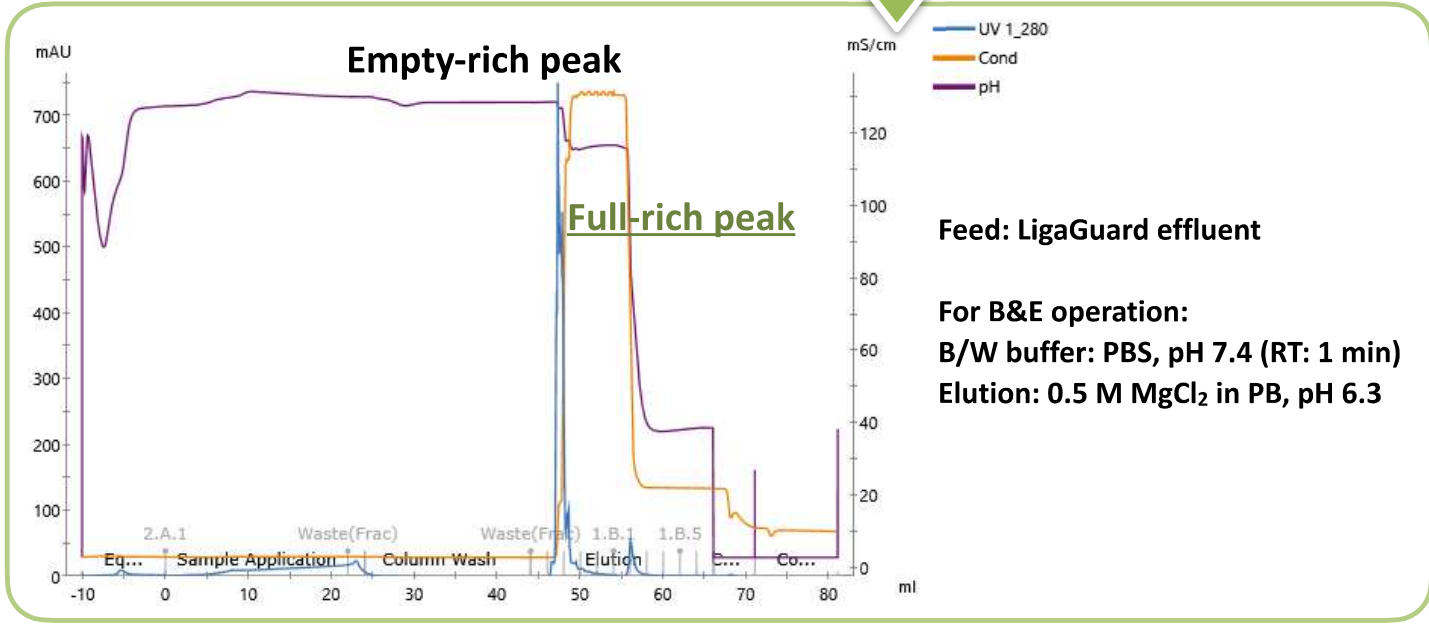
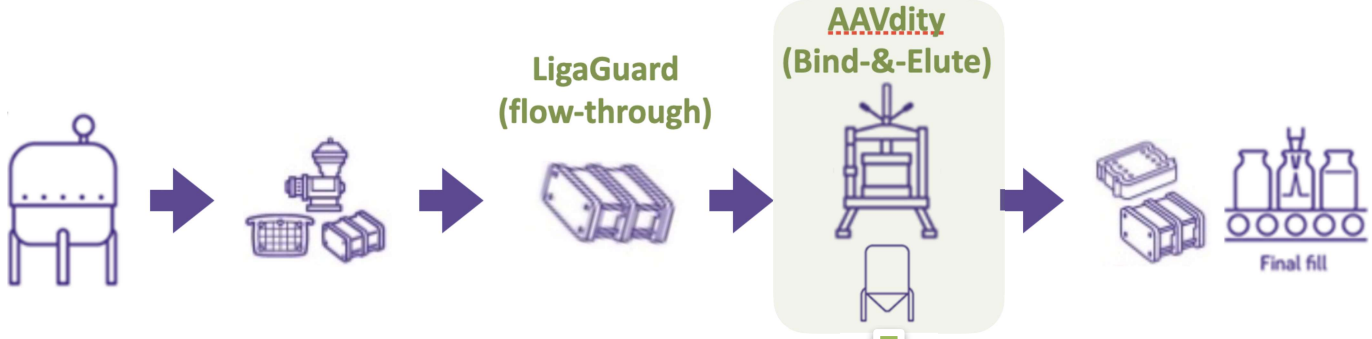
Cells: HEK293 (2.5×10^6 cells/mL)
SFM4Transfx-293 serum-free in shaker flasks; T: 28 °C; pH: 6.4
Product: AAV2 at $\sim 8.3 \times 10^9$ vg/mL

Buffer: 0.5M MgCl₂ in PB, pH 6.3
RT: 1 min
Product: AAV2 at 0.89×10^{13} vg/mL
HCPs: 0.003 mg/mL (~ 2.4 LRV)
Reg: 0.2M Citrate in pH 2.5

Cell lysis (0.5% Triton X-100 and 5µg/ml of RNase A)
Centrifugation: 3,700×g for 30 minutes.
Filtration: 0.45 µm Sartoscale filter
TFF: 5 DVs on a PES membrane (MWCO 100 kDa)
Buffer: 10 mM Bis-Tris + 20 mM NaCl, pH 7.0
AAV2 at 2.49×10^{11} vg/mL; HCPs: 0.748 mg/mL

Load: 30 CVs; RT: 1 min
Product: AAV2 at 1.12×10^{10} vg/mL
HCPs: 0.049 mg/mL (~ 1.18 LRV)

Intro LigaGuard AAVidity **Results** Summary



Intro

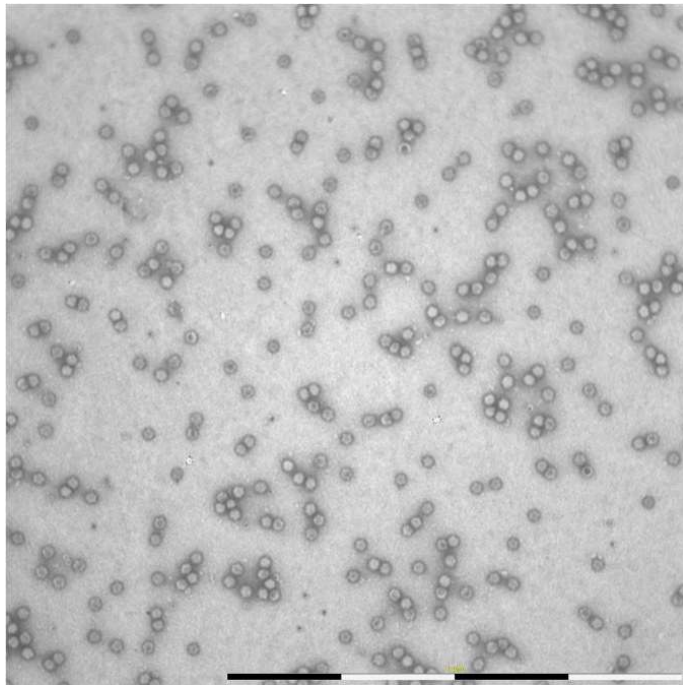
LigaGuard

AAVidity

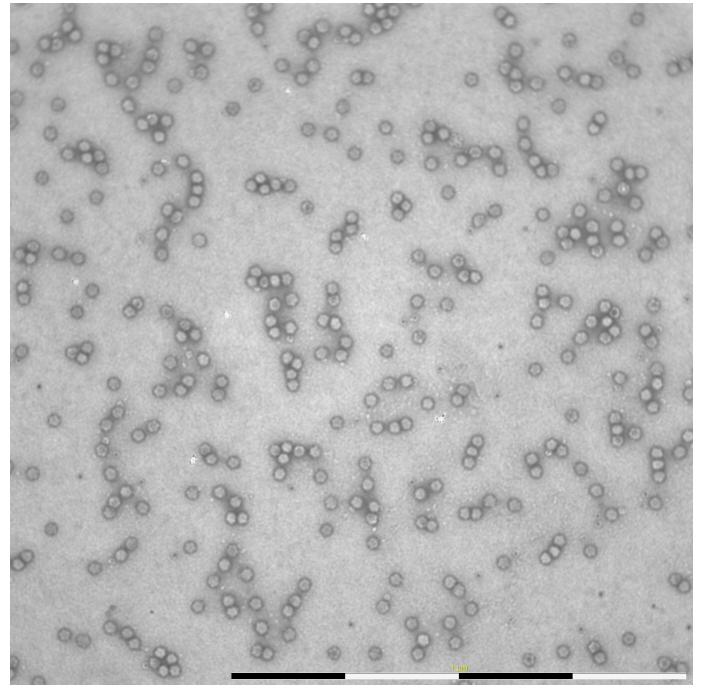
Results

Summary

Full:Empty AAVs



Empty-rich peak



Full-rich peak

Intro

LigaGuard

AAVidity

Results

Summary

Transduction Activity of (affinity eluted) AAVs

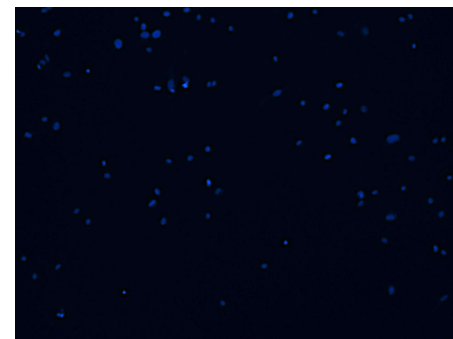
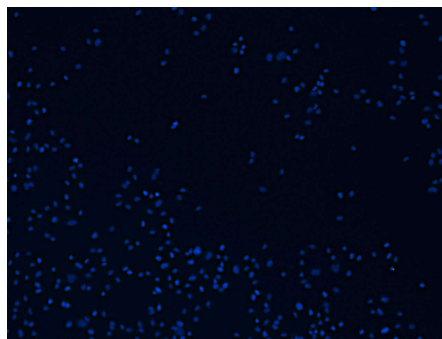
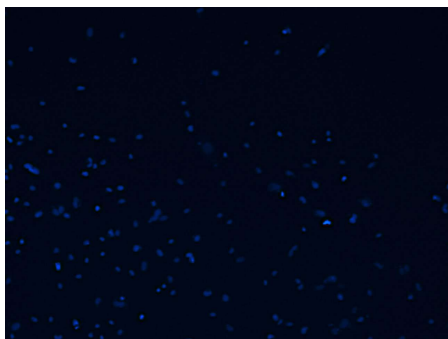
HuH7 cells at 10^5 vg/cell

LigaTrap™
LigaGuard™ + AAVidity

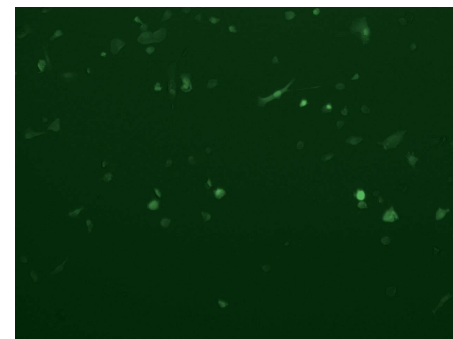
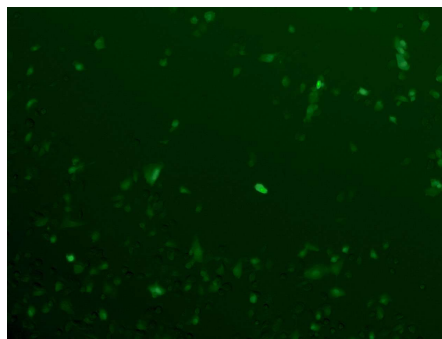
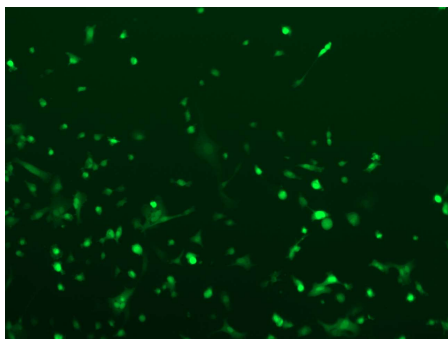
POROS™
CaptureSelect™ AAVX

Cytiva
HiTrap Capto AVB

DAPI



EGFP





**LigaGuard
(flow-through)**



**AAVdity
(Bind-&-Elute)**



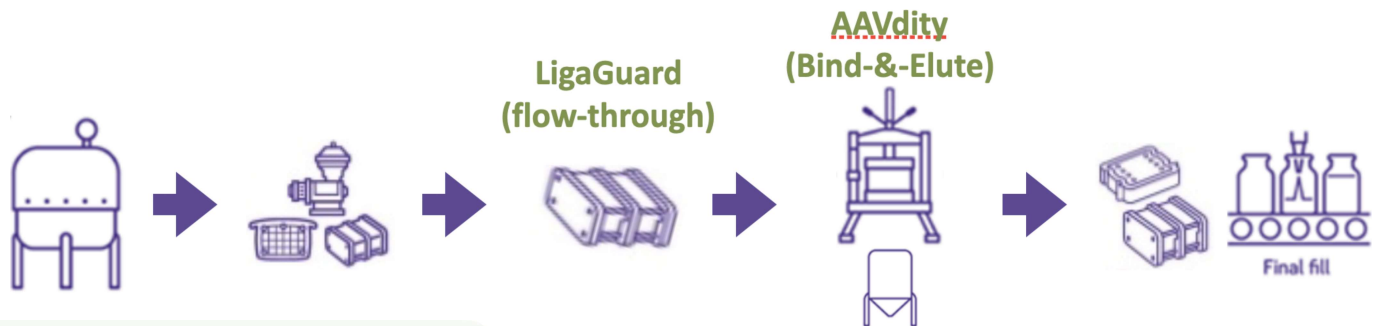
Cells: HEK293 (2.5×10^6 cells/mL)
SFM4Transfx-293 serum-free in shaker flasks; T: 28 °C; pH: 6.4
Product: AAV2 at $\sim 8.3 \times 10^9$ vg/mL

Buffer: 0.5M $MgCl_2$ in PB, pH 6.3
RT: 1 min
Product: AAV2 at 0.89×10^{13} vg/mL
HCPs: 0.003 mg/mL (~ 2.4 LRV)
Reg: 0.2M Citrate in pH 2.5

Cell lysis (0.5% Triton X-100 and 5 μ g/ml of RNase A)
Centrifugation: 3,700 \times g for 30 minutes.
Filtration: 0.45 μ m Sartoscale filter
TFF: 5 DVs on a PES membrane (MWCO 100 kDa)
Buffer: 10 mM Bis-Tris + 20 mM NaCl, pH 7.0
AAV2 at 2.49×10^{11} vg/mL; HCPs: 0.748 mg/mL

CIMmultus QA monolith (1 mL)
Load: 2×10^{12} vg/mL_R; RT: 1 min
Elution: 53 mM NaCl, pH 8.95
Product: AAV2 at 3.18×10^{12} vg/mL
F:E = 4.9:95.1
HCPs < 1 μ g/mL (~ 2.9 LRV)

Load: 30 CVs; RT: 1 min
Product: AAV2 at 1.12×10^{10} vg/mL
HCPs: 0.049 mg/mL (~ 1.18 LRV)



Cells: HEK293 (2.5 10⁶ cells/mL)
 SFM4Transf...
 shaker f...
 Product

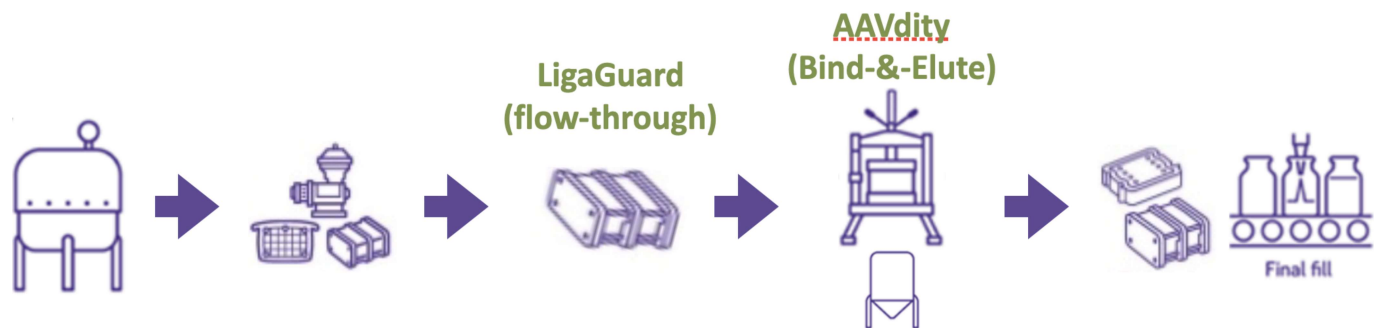
Feedstock

AAV2 titer: 8.3 10⁹ vg/mL
 Full:Empty ~ 81.8:18.2
 HCP titer: 0.748 mg/mL

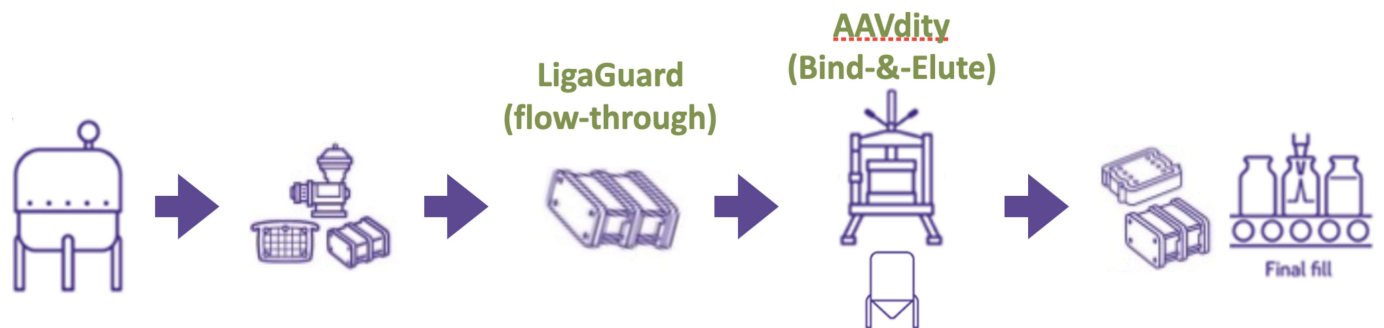
Final product

AAV2 titer: 3.18 10¹² vg/mL; Full:Empty ~ 95.1:4.9;
 Genome recovery ~ 91%; Transduction eff ~ 92%;
 HCP titer < 1 µg/mL (~2.9 LRV)

(1 mL)
 1 min
 8.95
 10¹² vg/mL
 V)



	POROS™ CaptureSelect™ AAVX	Cytiva HiTrap Capto AVB	LigaGuard + AAVidity Process
Final AAV titer (full AAV2 recovery)	9.8 10 ¹¹ vg/mL (84.1%)	1.15 10 ¹² vg/mL (83.7%)	3.18 10 ¹² vg/mL (76.8%)
Full / Empty ratio	95.5 : 4.5	82. : 4.5	95.1 : 4.9
Transduction activity (inc: 10⁵ VG/cell, 24 hr)	4.8 10 ⁴ pg _{eGF} mL ⁻¹ 7.2 10 ² VG cell ⁻¹	1.05 10 ⁵ pg _{eGF} mL ⁻¹ 8.2 10 ² VG cell ⁻¹	5.1 10 ⁵ pg _{eGF} mL ⁻¹ 4.1 10 ³ VG cell ⁻¹
HEK293T HCP removal (LRV)	~ 2.98	~ 3.08	~ 2.90
Productivity	9.8 10 ¹¹ vg/hr	8.1 10 ¹¹ vg/hr	1.9 10 ¹² vg/hr



	AAV2 ✓		AAV5 ✓		AAV9 ✓	
	POROS	LigaTrap	Cytiva	LigaTrap	Cytiva	LigaTrap
AAV titer (vg mL ⁻¹)	9.8 10 ¹¹	3.18 10 ¹²	1.0 10 ¹²	2.88 10 ¹²	1.1 10 ¹²	2.05 10 ¹²
F:E	95.5:4.5	95.1:4.9	83.8:16.2	91.7:8.3	94.8:5.2	95.3:4.7
HCP LRV	~ 2.98	~ 2.90	~ 3.11	~ 2.87	~ 3.05	~ 2.87
eGFP⁺ cells (%)	61.5 ± 5.4	72.1 ± 2.4	57.1 ± 3.3	66.1 ± 4.0	84.1 ± 2.7	86.1 ± 1.4

Intro

LigaGuard

AAVidity

Results

Summary

AAV Purification: LigaGuard™ + AAVidity

- Rapid and efficient process
- “Universal” AAV recovery
- Gentle purification: high product activity
- Excellent clearance of HEK293 HCP and DNA

Future work: silica resins and 3D printed monoliths

- Low cost of good: single-use adsorbents
- High (affinity) binding capacity
- Adjustable surface back-charge
- Optimize gradient elution: purification + E/F

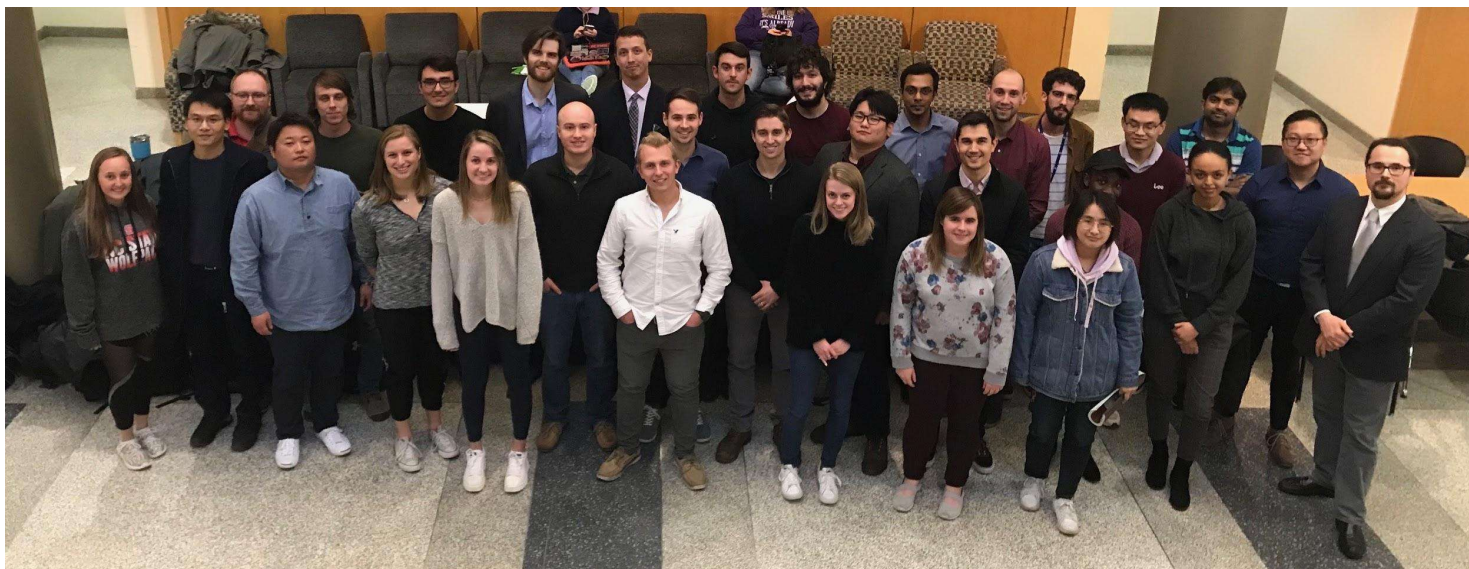
Intro

LigaGuard

AAVidity

Results

Summary



KENAN INSTITUTE
ENGINEERING, TECHNOLOGY & SCIENCE
NC STATE UNIVERSITY



**THE NONWOVENS
INSTITUTE**

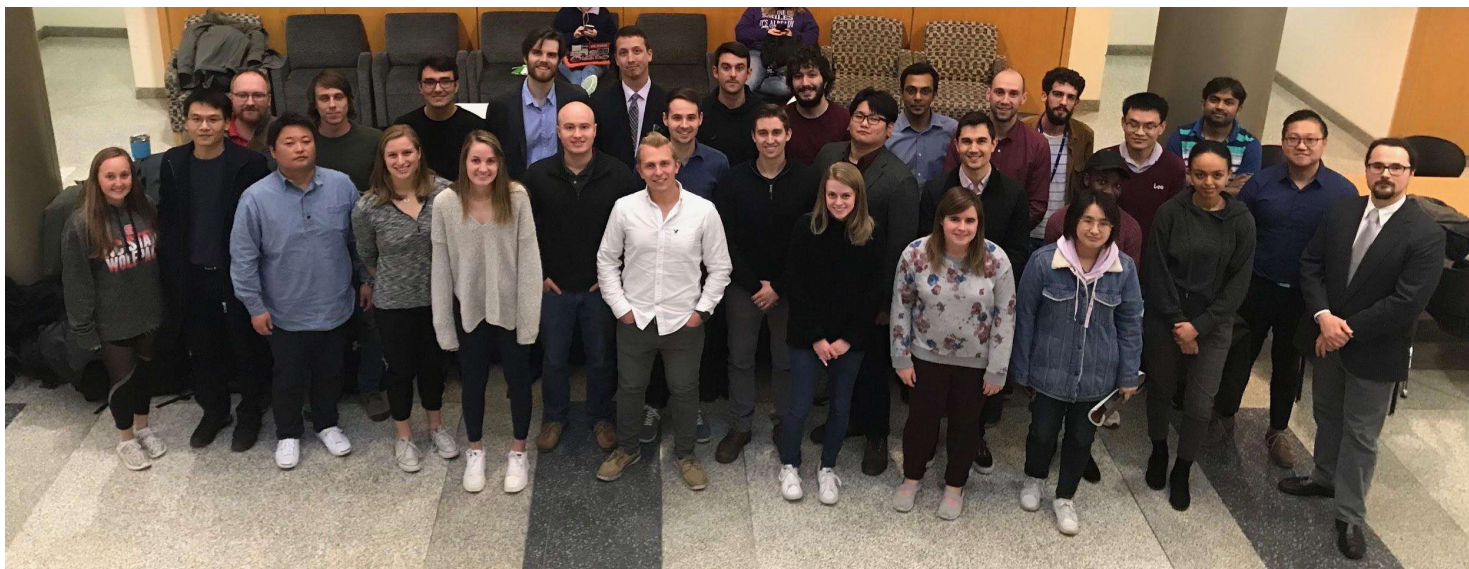
Intro

LigaGuard

AAVidity

Results

Summary



NOVO
nordisk
fonden



MERCK



KBI
BIOPHARMA

Genentech



LigaTrap
TECHNOLOGIES

CSL Behring
Biotherapies for Life™

EASTMAN

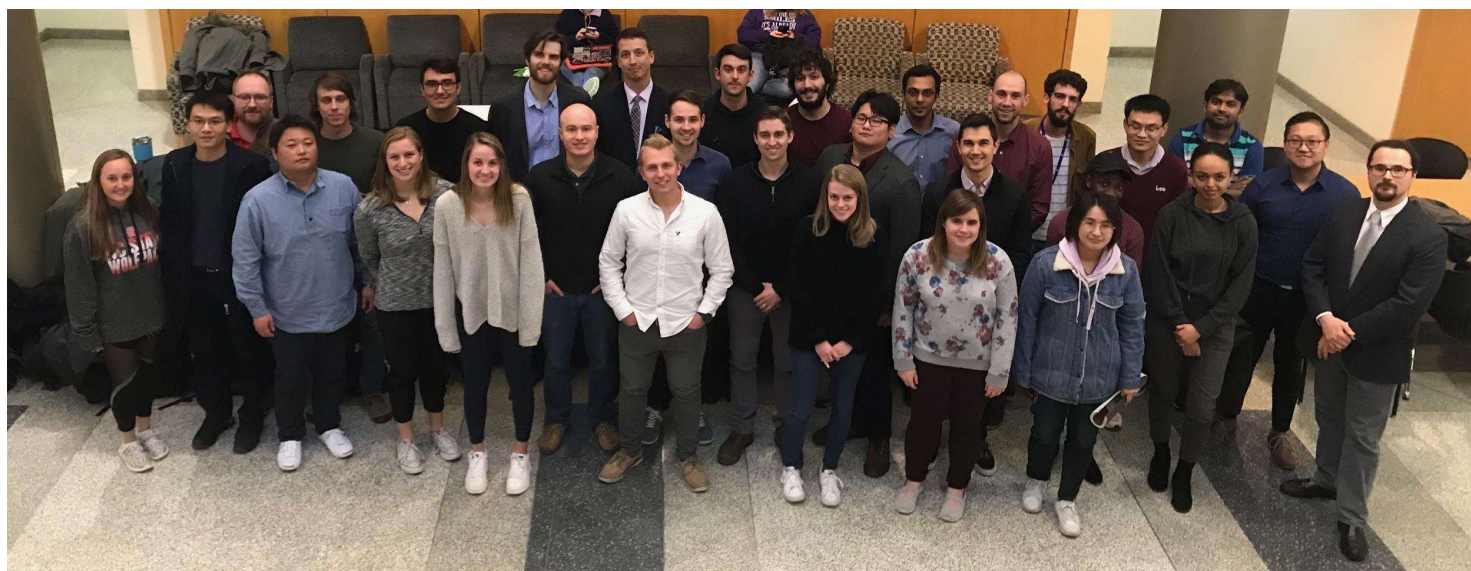
Intro

LigaGuard

AAVidity

Results

Summary



<https://www.cbe.ncsu.edu/person/smenega/>
<https://biopep.wordpress.ncsu.edu>

Come visit us!

Intro

LigaGuard

AAVidity

Results

Summary

Selection of the ligands

