BIA separations

Better purification. Faster.

Ways to improve empty, full and damaged capsids separation

S. Drmota-Prebil, M. Stokelj, S. Peljhan, R. Zigon, M. Leskovec, V. Fujs, B. Goricar, J. Vidic, U. Cernigoj, M. Zidar, U. Sinur, S. Rotar, T. Simcic, P. Gagnon, A. Strancar

October 27, 2020

BIA Separations products and services

Convective Interaction Media (CIM®)
Pre-packed monolithic columns

CIMac™ Analytical and CIMmultus™ Preparative columns

Services, Process development and Technical Support

Development of processes and methods for separation/concentration/purification of large biomolecules.
Custom immobilization, product development,..

Process Analytical Technology (PATfix™)

At-line PAT HPLC suite for **faster process development** and enhanced process control

Integrated Capability from Cell Culture Production through Downstream Processing

Bioprocess scale-up from laboratory to pilot

Managing interface between upstream and downstream

USP control to secure robust DSP

Expert DSP bioprocess knowledge: royalty-free

- pDNA (incl. plasmids larger than 30 kbp) - **pure pDNA, THE key for better transfection and for pure mRNA**
- mcDNA (shorten the pDNA)
- ssRNA and dsRNA, **platform process from E.coli to mRNA**
- Adeno virus
- AAV **(all serotypes, > 20 tested), start with USP development**
- Influenza virus (all serotypes)
- Vaccinia/MVA
- **Exosome**
- Bacteriophage
- IVIG
- IgM and many more

- **> 30 DNA, RNA, virus DSP processes tech transferred to CMOs, sponsors**
- **> 10 AAV DSP processes tech transferred to CMOs, sponsors**

BIA separations

BIA Separations State-of-the-Art production facility >50M USD investment






Expansion to increase production capacity 5x before year-end 2020 and 30x before year-end 2023.

BIA separations

Testimonials

Andy Stober, Senior Vice President of Technical Operations for AveXis:

"We are especially grateful that BIA Separations shared, and operated, with the same sense of urgency we did to help bring gene therapy to the SMA community. BIA's experience with AAV purification and its chromatographic technology were important contributions and we look forward to our continued work together."

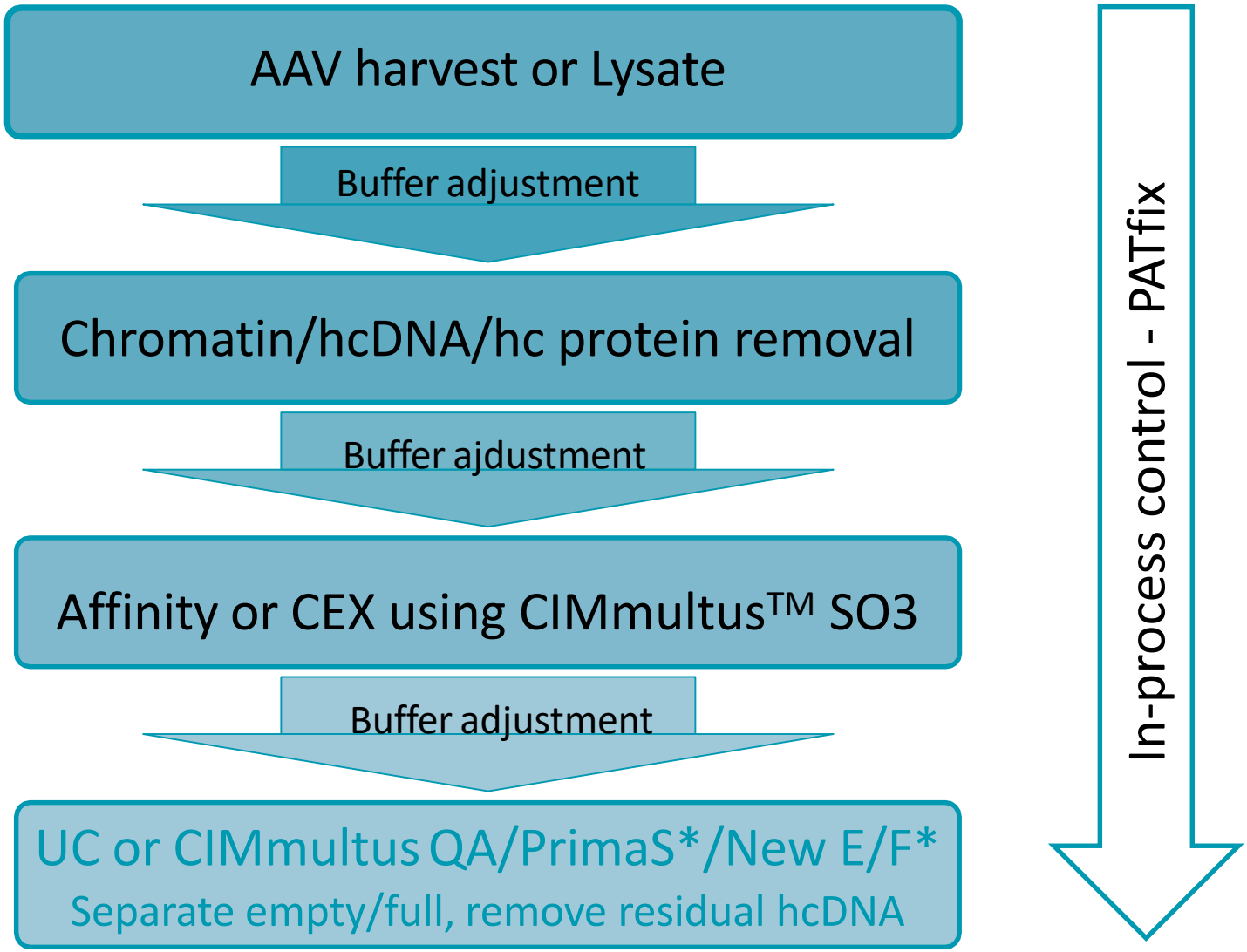
Just 15 months from the lab to manufacturing, not possible without fast analytics.

BIA separations

Ways to improve separation of AAV empty, full and other AAV related particles / capsids

BIA separations

AAV purification process



*available as a prototype

Separation of empty and full capsids using anion exchange chromatography – pertinent IP

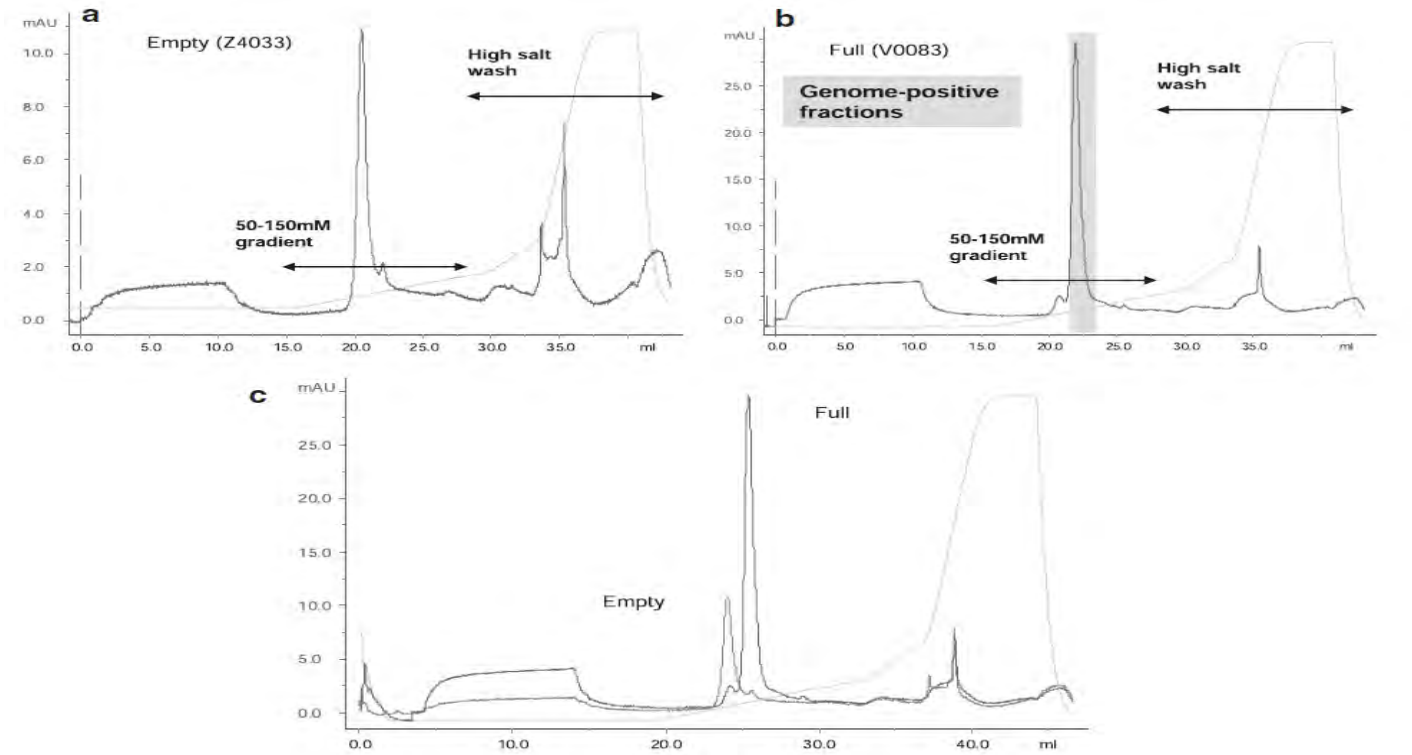


FIG. 3. IEX particle assay. **(a)** Seventy-five microliters of an empty particle AAV8 preparation (lot no. Z4033) was loaded onto a 0.34-ml CIM-QA disk, using FPLC, and eluted with a 50–150 m*M* salt gradient. The y axis shows the absorbance (mAU) at 280 nm and the *x* axis the elution volume (ml). The detected conductivity and absorbance are represented by solid light and dark blue lines, respectively. The vertical dashed pink line represents the point of vector injection. **(b)** A full AAV8 vector preparation (lot no. V0083, 1×10^{12} GC) was run under the same binding/elution conditions as used for the empty particle preparation. Fractions were quantified for vector GC content and those fractions containing >99% of the loaded material are indicated (shaded box). **(c)** An overlay of the elution profiles of the empty and full AAV8 vector preparations is shown.

From Lock et al, Hum. Gene Ther. Met. 23 (2012) 56–64.

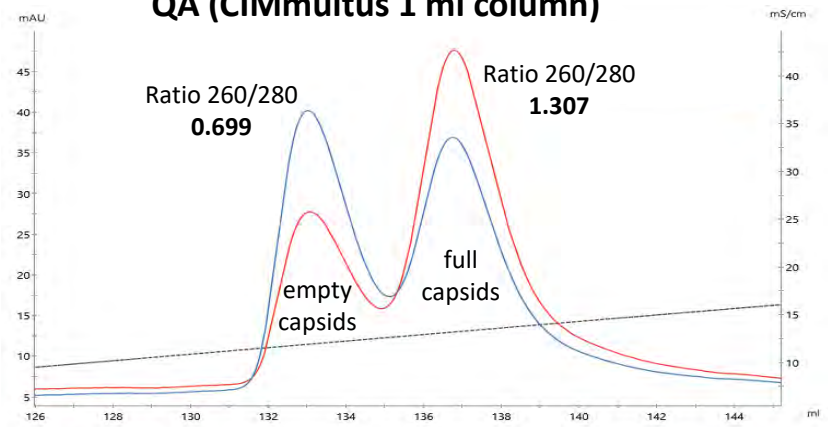
See also: https://patents.google.com/patent/US9198984B2/en
https://patents.google.com/patent/US20160040137A1/en
https://patents.google.com/patent/EP2277996B1/en

BIA separations

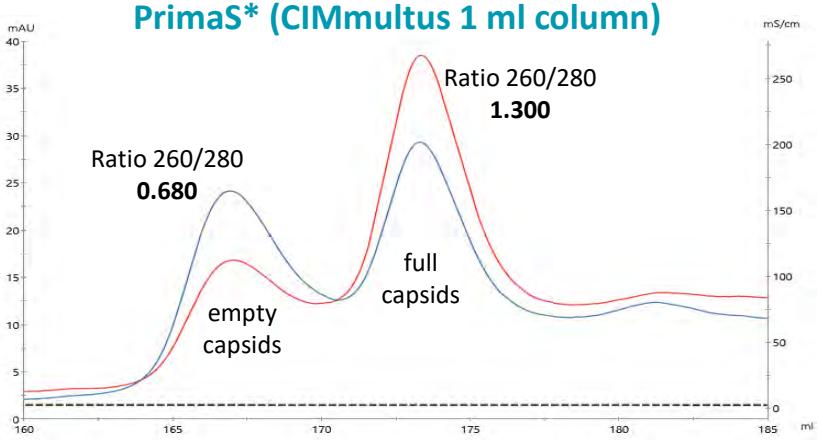
New chromatographic tools to improve separation of empty, full and damaged capsids

Sf9 AAV2/8 pre-purified by cation exchange chromatography

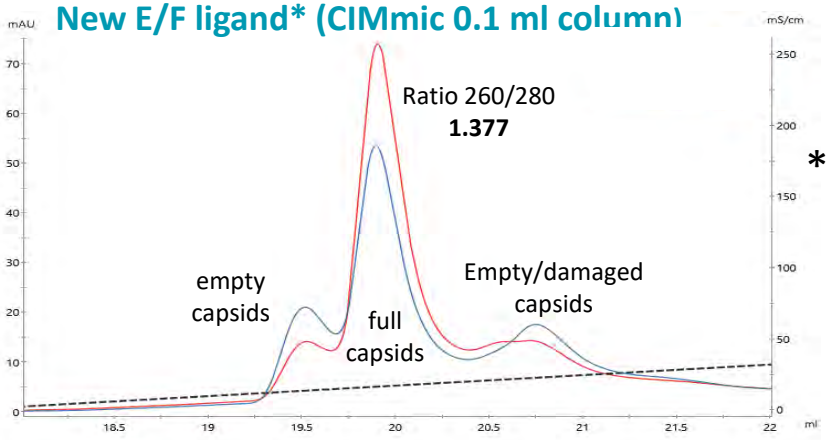
QA (CIMmultus 1 ml column)



PrimaS* (CIMmultus 1 ml column)



New E/F ligand* (CIMmic 0.1 ml column)



*PrimaS and New E/F available only as prototypes

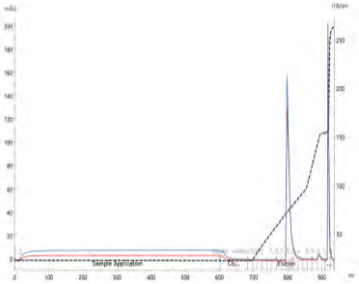
New products separates capsids by a method distinct from Lock's patents

BIA separations

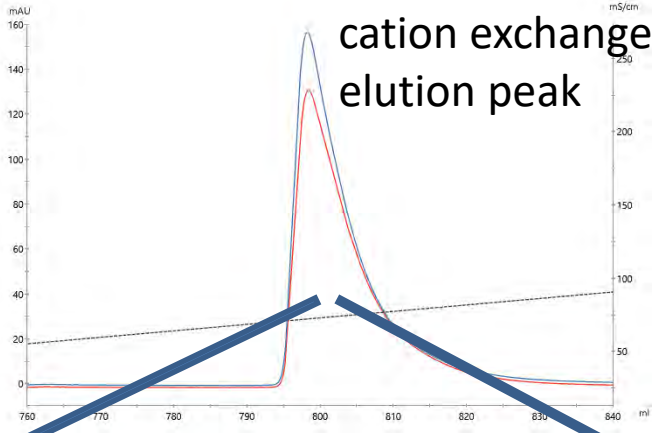
Orthogonal tools to study AAV particles distribution

HPLC using CIMac QA and Ultracentrifuge using same detectors set-up

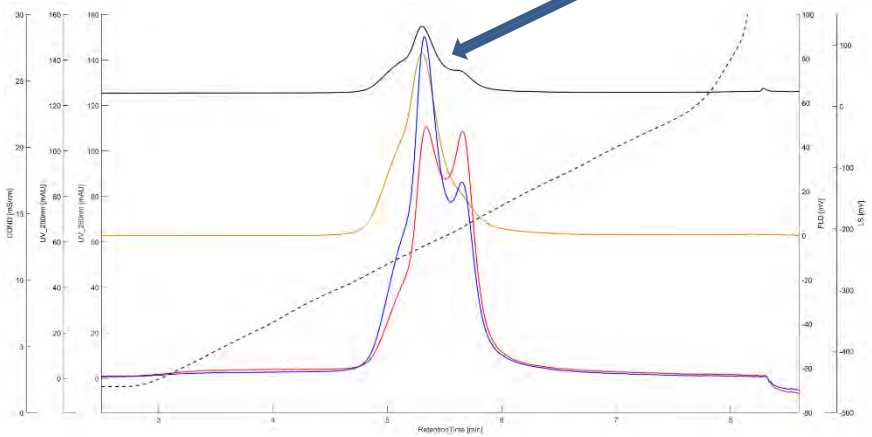
Filtered lysate AAV2/8 from Sf9 ; TFF/Kryptonase™ treatment

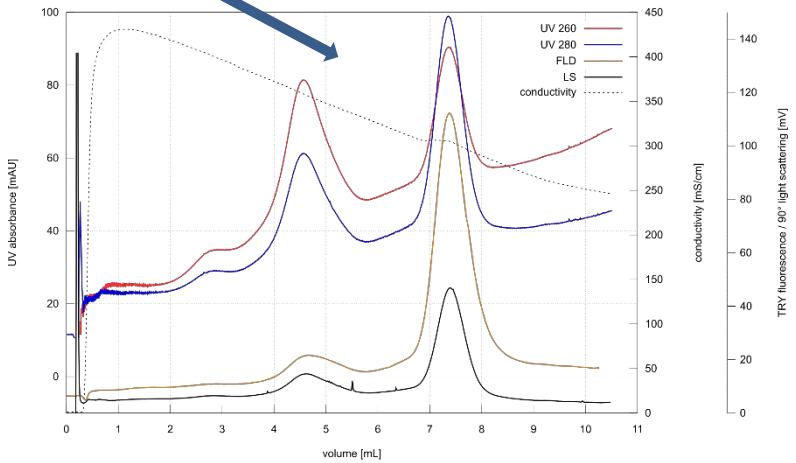


Zoom -in



HPLC detectors:
Red – UV 260 nm
Blue – UV 280 nm
Orange – tryptophan fluorescence
Black - MALS





Chromatogram: HPLC with 3 detectors using QA column – faster gradient

Centrifugram: CsCl ultracentrifuge with 3 detectors – **faster than AUC, less sample needed**

BIA separations

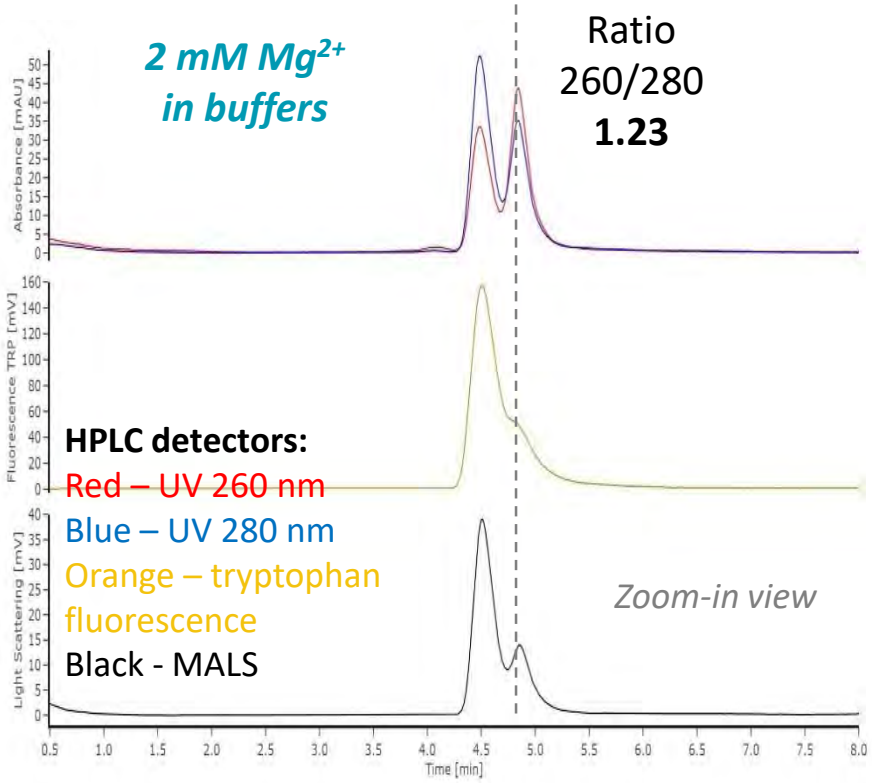
CIM New E/F AAV* capsids separation column based on Mg^{2+} gradient

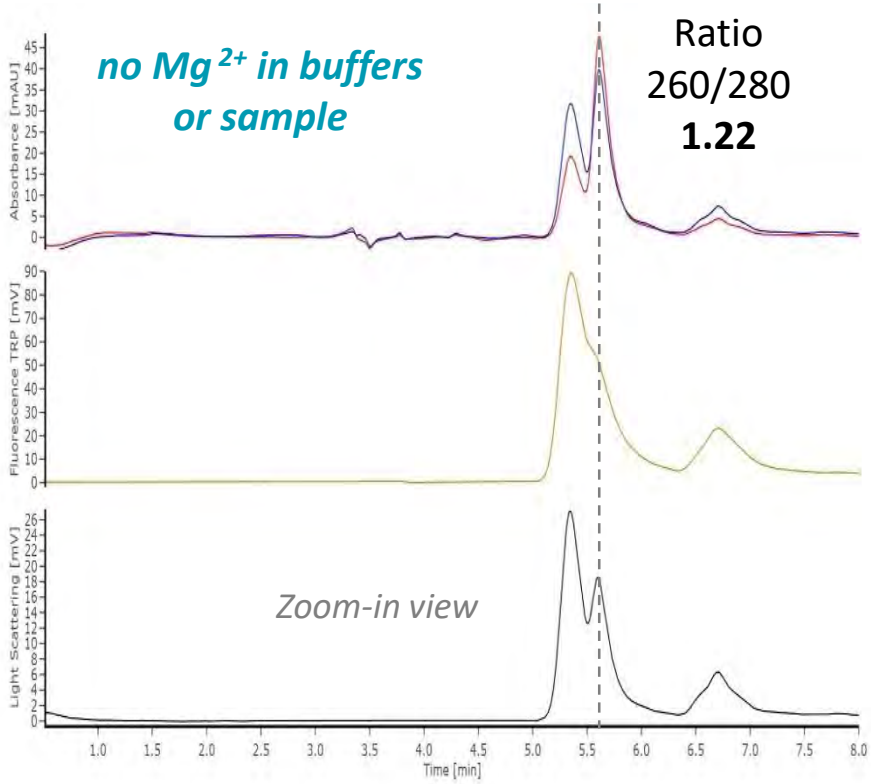
*patent pending

BIA separations

Influence of Mg^{2+} on separation of AAV capsids using CIM QA column

Sample: AAVrh10 pre-purified using CIM SO_3 column





Column: CIMac™ QA (Pores 1.3 µm); Buffer A: 20 mM BTP, 1% Sorbitol, 0.1% Poloxamer, pH 9.5 (+ 2 mM $MgCl_2$ for buffer with Mg^{2+}); Buffer B: Buffer A + 400 mM NaCl (+ 2 mM $MgCl_2$ for buffer with Mg^{2+}); Gradient: 0 % B to 100 % B in 100 CVs; Flow rate: 10 CV/min

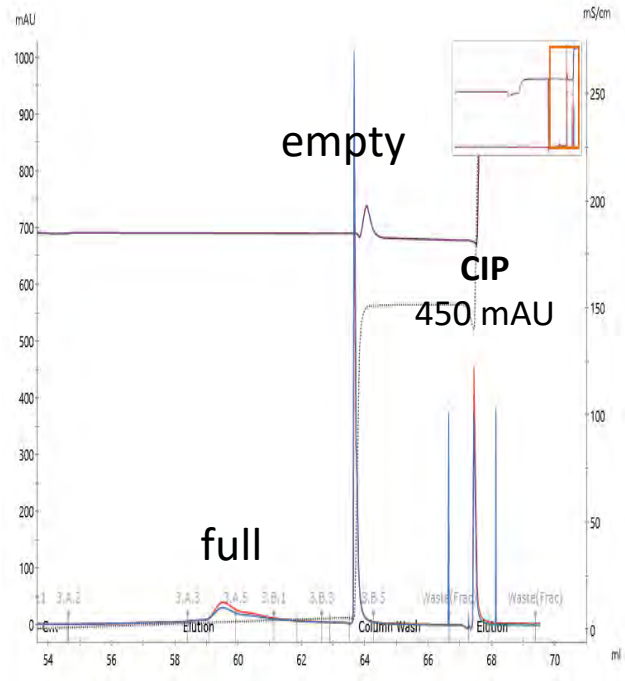
Pay attention on residual Mg and Ca cations in the sample. On the other hand citrate consumes Mg by forming complex with.

BIA separations

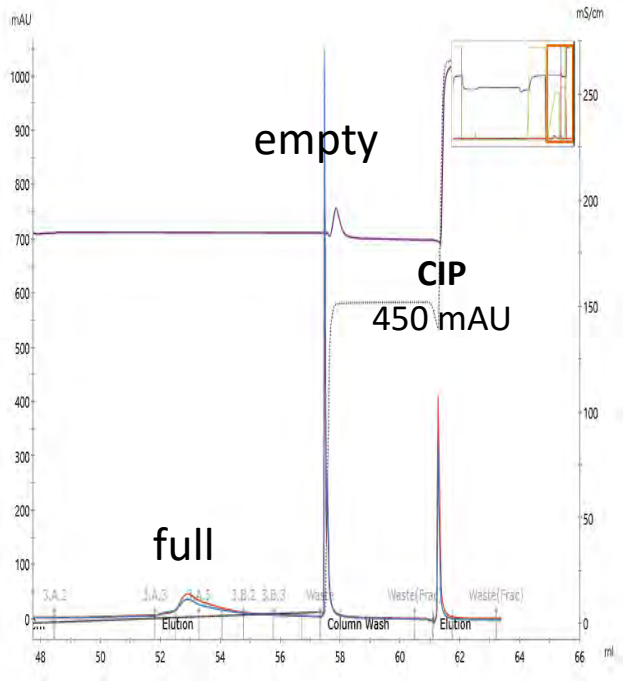
Influence of Mg^{2+} and Fe^{3+} loading of CIM New E/F column* on separation of AAV capsids

Filtered lysate AAV2/8 from Sf9; TFF/Kryptonase treatment, SO3 purif.

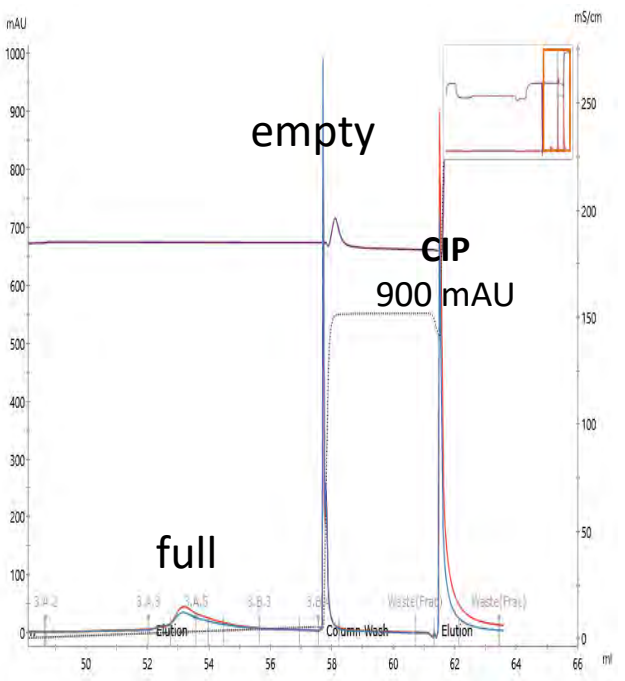
New E/F no metal



New E/F plus Mg



New E/F plus Fe

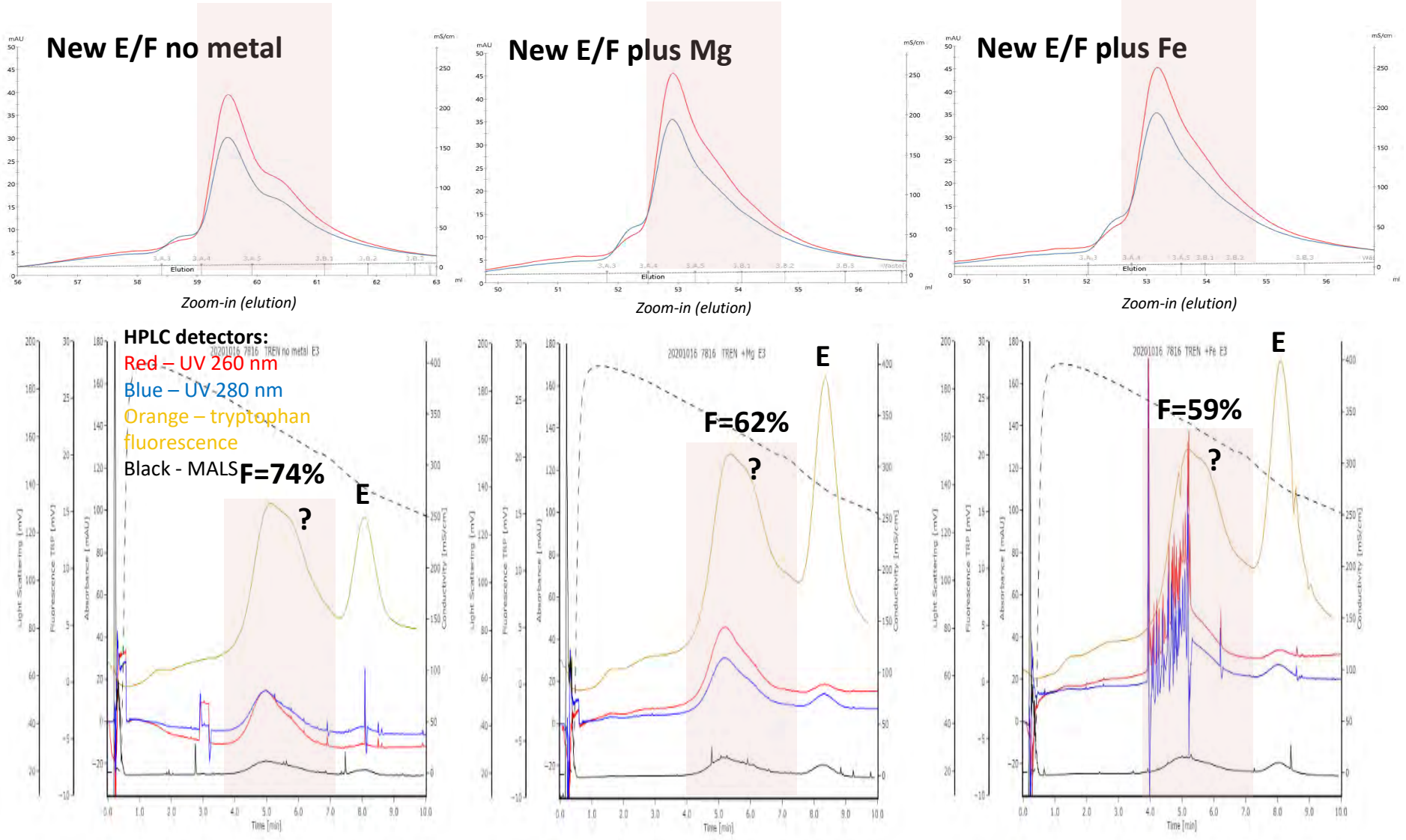


Column: CIMmic™ New E/F column (2 µm ID channels); Buffer A: 50 mM TRIS, 12 mM boric acid, 1% saccharose, 0.1% Poloxamer, pH 9.0;
Buffer B: Buffer A + **50 mM $MgCl_2$**; Gradient: 0 % B to 50 % B in 50 CVs; Flow rate: 10 CV/min

Higher CIP with Fe ligand may indicate stronger DNA removal.

BIA separations

Using ultracentrifuge to check the purity of full capsids

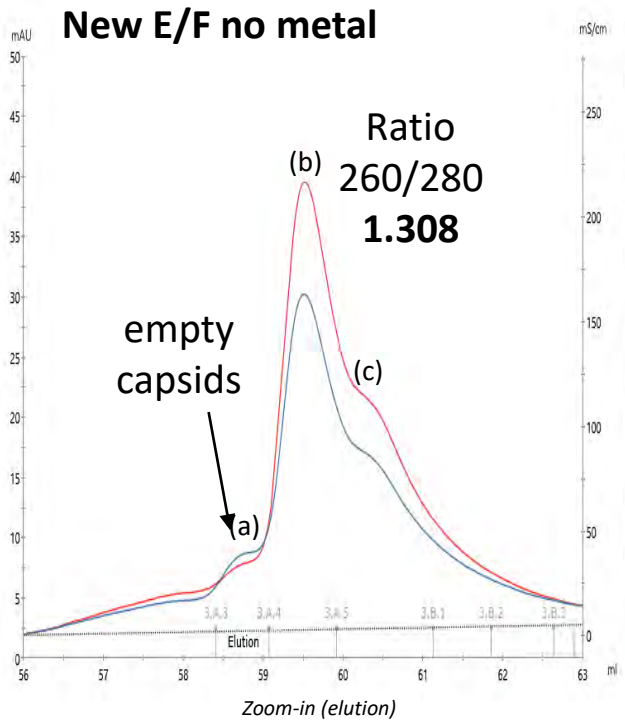


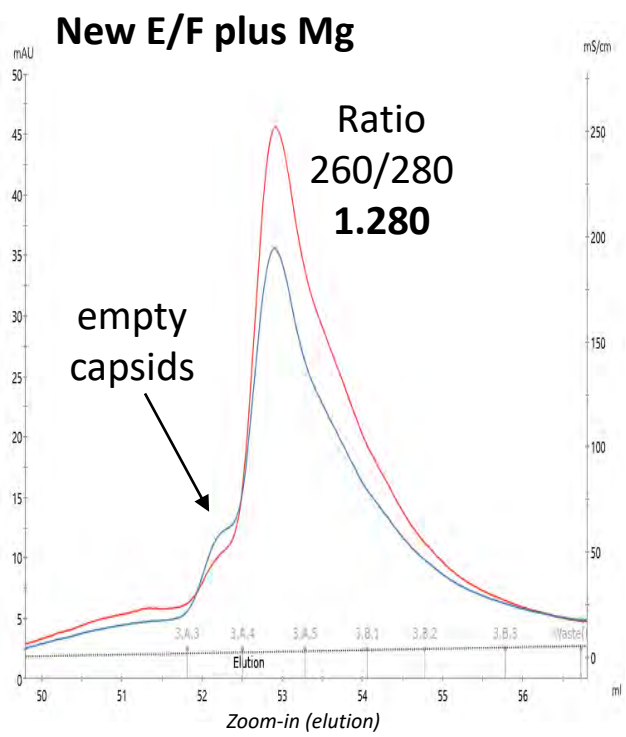
Further investigations needed to understand the heterogeneity of the full peak

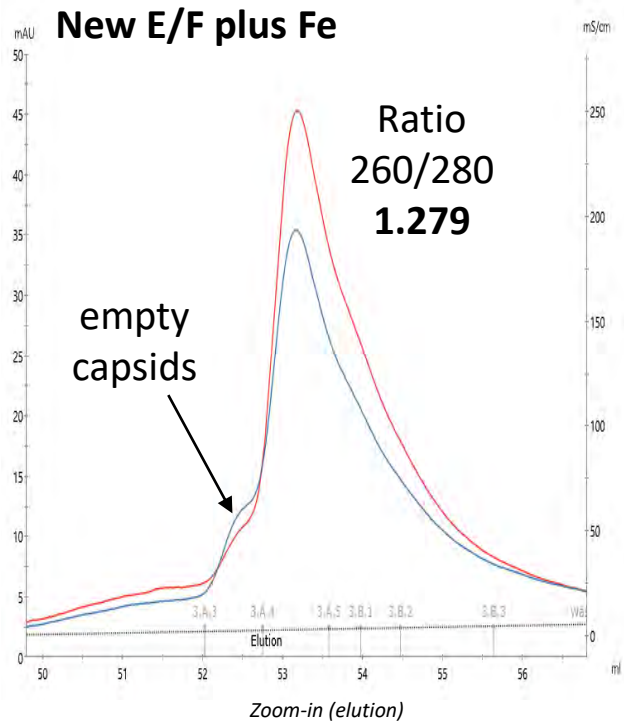
BIA separations

Influence of Mg^{2+} and Fe^{3+} loading of CIM New E/F column on separation of AAV capsids – zoom-in

Filtered lysate AAV2/8 from Sf9; TFF/Kryptonase treatment, SO3 purif.







		Mg^{2+} concentration in elution buffer at [mM]		
		peak start (a)	peak apex (b)	peak shoulder (c)
CIM New E/F column	no-metal	15,9	18,35	20,75
	Mg	14	16,8	18,35
	Fe	14	16,5	18,5

BIA separations

CIM New E/F column - yield and purity

New E/F no metal		VECTOR GENOME (ddPCR)		
		date:	14.10.2020	
Fraction	Volume (ml)	vector genome concentration (vg/ml)	vector genome in fraction (vg)	% of full capsids
CEX-44 E2				
LOAD	9,00	7,1E+10	6,4E+11	100%
New E/F no metal				
E2 (empty)	0,68	8,3E+09	5,6E+09	1%
E3 (full)	2,11	1,9E+11	3,9E+11	61%
E4 (tail)	1,81	3,4E+10	6,2E+10	10%
E5 (empty)	0,78	8,4E+10	6,5E+10	10%
CIP	0,99	under limit of det.		
		SUM recovery total		**82%**
		SUM recovery fraction		**71%**

New E/F + Fe		VECTOR GENOME (ddPCR)		
		date:	15.10.2020	
Fraction	Volume (ml)	vector genome concentration (vg/ml)	vector genome in fraction (vg)	% of full capsids
CEX-44 E2				
LOAD	9,00	7,1E+10	6,4E+11	100%
New E/F + Fe				
E2 (empty)	0,74	9,9E+09	7,3E+09	1%
E3 (full)	2,18	2,3E+11	5,1E+11	79%
E4 (tail)	2,53	4,0E+10	1,0E+11	16%
E5 (empty)	0,69	5,2E+10	3,6E+10	6%
CIP	1,81	under limit of det.		
		SUM recovery total		**102%**
		SUM recovery fraction		**95%**

New E/F + Mg		VECTOR GENOME (ddPCR)		
		date:	14.10.2020	
Fraction	Volume (ml)	vector genome concentration (vg/ml)	vector genome in fraction (vg)	% of full capsids
CEX-44 E2				
LOAD	9,00	7,1E+10	6,4E+11	100%
New E/F + Mg				
E2 (empty)	0,71	9,9E+09	7,0E+09	1%
E3 (full)	1,59	2,6E+11	4,2E+11	66%
E4 (tail)	2,73	4,4E+10	1,2E+11	19%
E5 (empty)	0,67	5,1E+10	3,4E+10	5%
CIP	1,34	under limit of det.		
		SUM recovery total		**91%**
		SUM recovery fraction		**84%**

	V (fraction) [ml]	c (total DNA) [ng/ml]	c (total DNA in CIP) [ng]
New E/F no metal_CIP	0,99	4,02	3,98
New E/F + Mg_CIP	1,34	27,31	**36,60**
New E/F + Fe_CIP	1,81	19,82	**35,87**

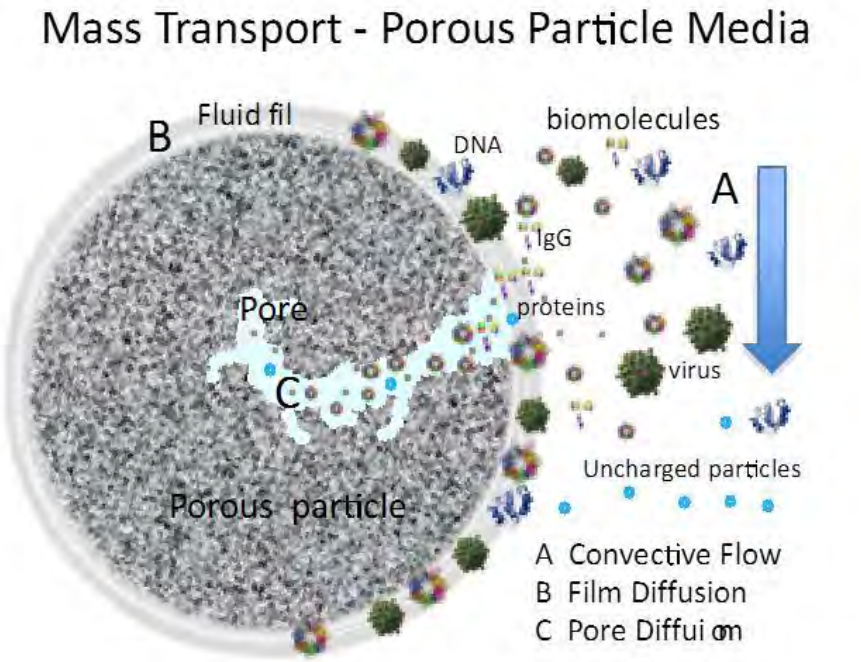
CIM New E/F without Mg or Fe ligand provides better purity but lower yield and poorer DNA removal

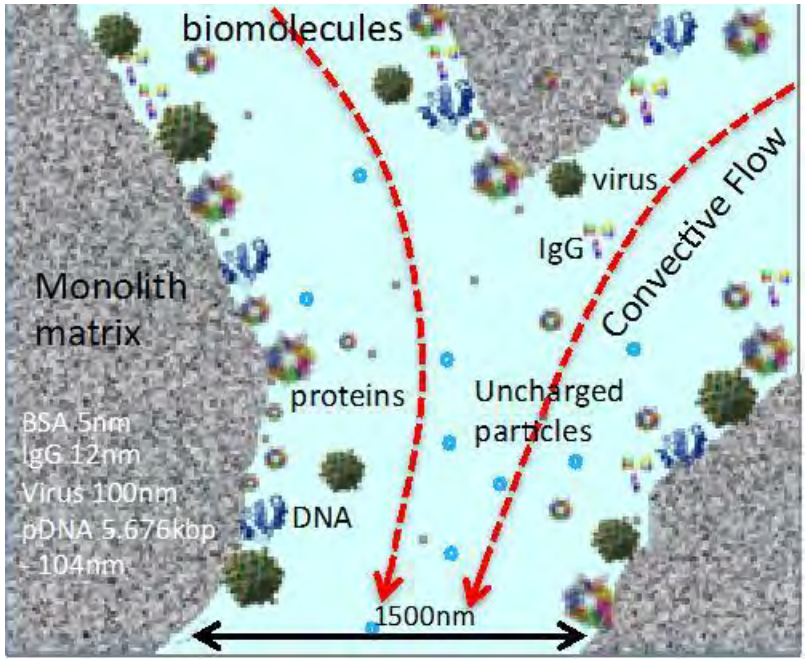
BIA separations

CIM monolithic column batch-to-batch and scale-up manufacturing robustness verification - Specimen introduction

BIA separations

CIM monolithic columns – no diffusion constraints = easy scale-up





Traditional approach - Porous particle:

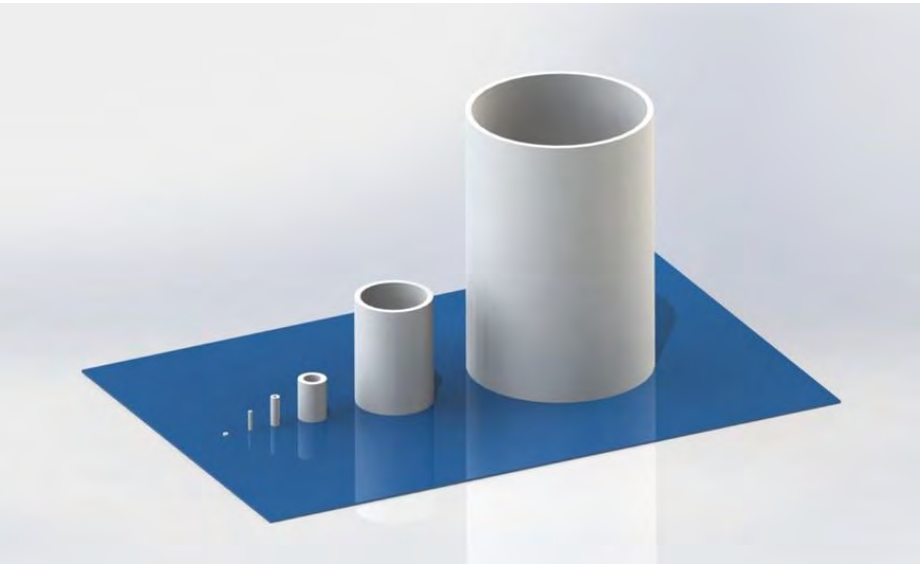
1. Diffusive mass transport – slow process or lower resolution
2. Pores too small – very low capacity
3. Counter current flow - shear forces – lower yields

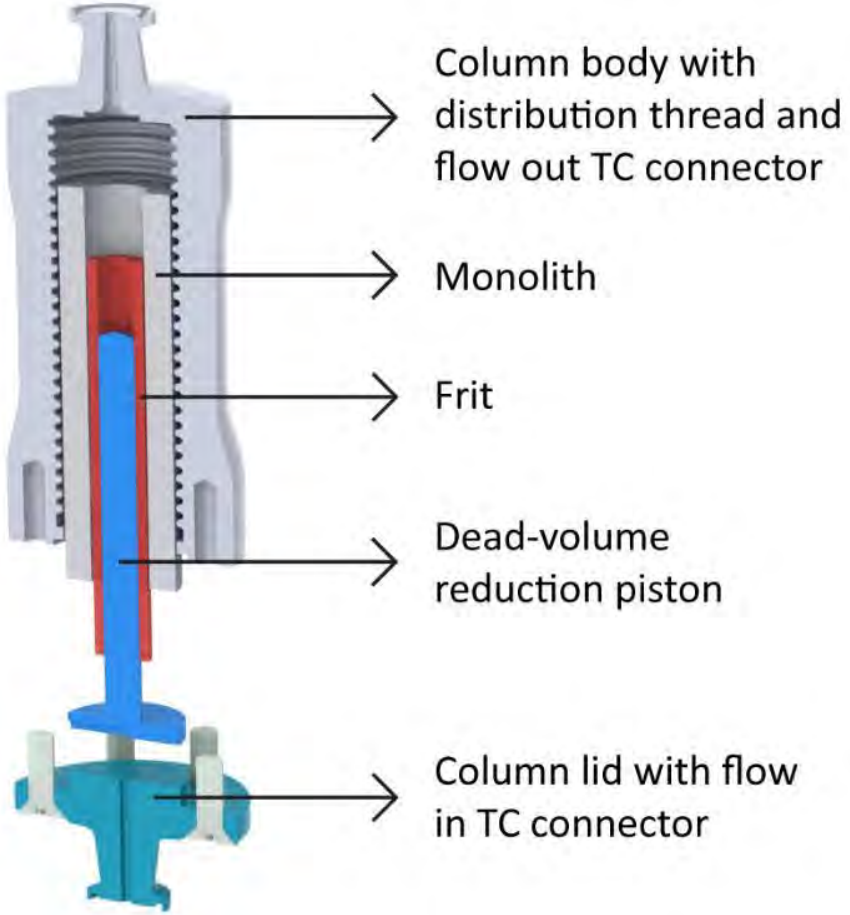
Novel UNIQUE approach – Monolithic columns:

1. Convective mass transport – flow independent resolution and capacity, very fast processes
2. Accessible surface for big molecules – high capacity
3. Laminar flow - No shear forces – better yields of e.g. IgM, Lenti, Adeno, Vaccinia, Flu,...
4. And better resolution due to lack of diffusion and no turbulent mixing

BIA separations

CIM monolithic column – single piece of tubular highly inter-channeled polymer





BIA separations

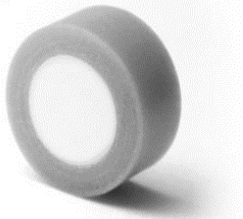
CIMmultus columns Specimen

Predicting the characteristics of the whole large-scale column from small piece of material from the same column by SPECIMEN

80, 800, 8000 mL columns

0.2 mL disks

Specimen

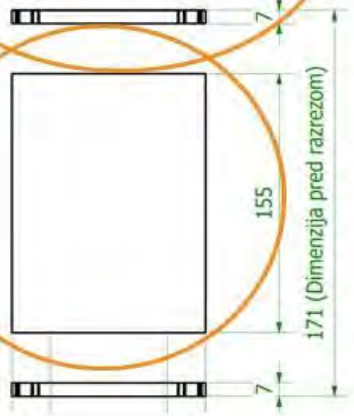


Specimen column

CIMmultus columns Specimen assembly

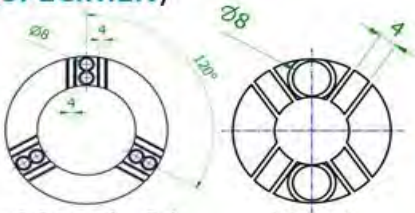
1) Polymerization and modification of 80, 800, 8000 mL monoliths

2) Cutting monolithic rings above and below the final column dimensions



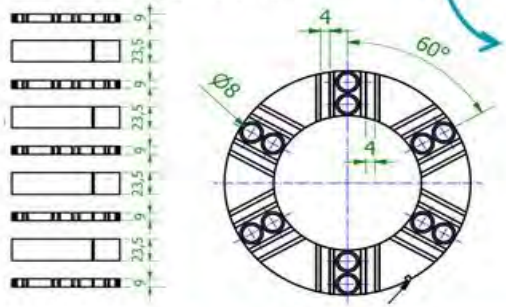
3) Preparing 0.2 mL disks out of the rings = CONTROL DISKS (SPECIMEN)

4) Testing CONTROL DISKS (SPECIMEN):
- Chromatography
- Mechanical properties



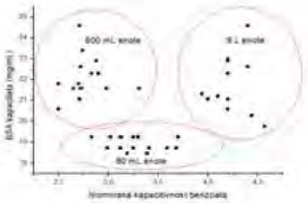
5) Packing and chromatographic testing of 80, 800, 8000 mL columns

6) Unpacking and cutting the rings along the column heigth, followed by 0.2 mL disk drilling from different positions

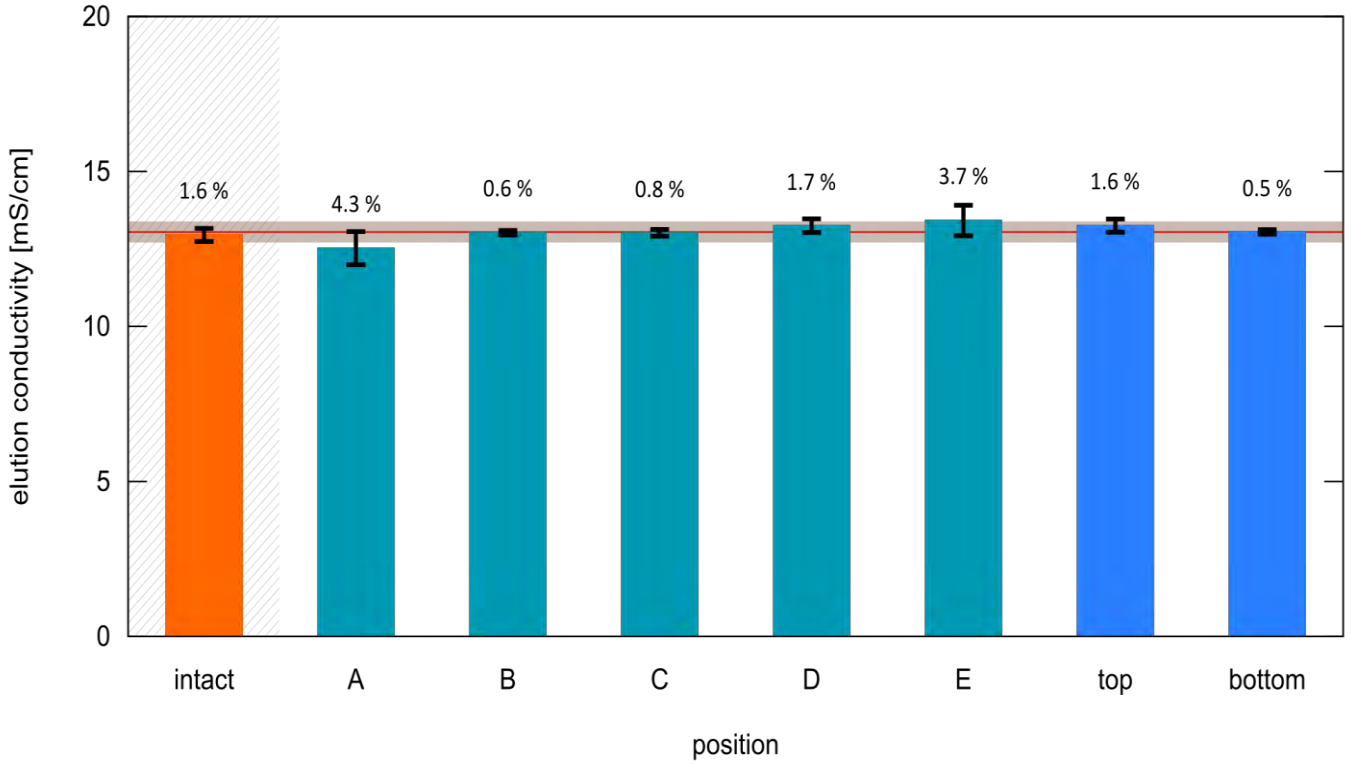


7) Testing DRILLED DISKS:
- Chromatography
- Mechanical properties

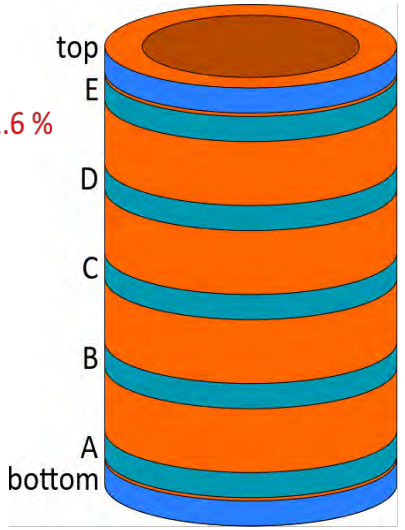
8) Data evaluation



800 ml CIMmultus column homogeneity tested by Full/empty AAV separation



13.0 ± 2.6 %

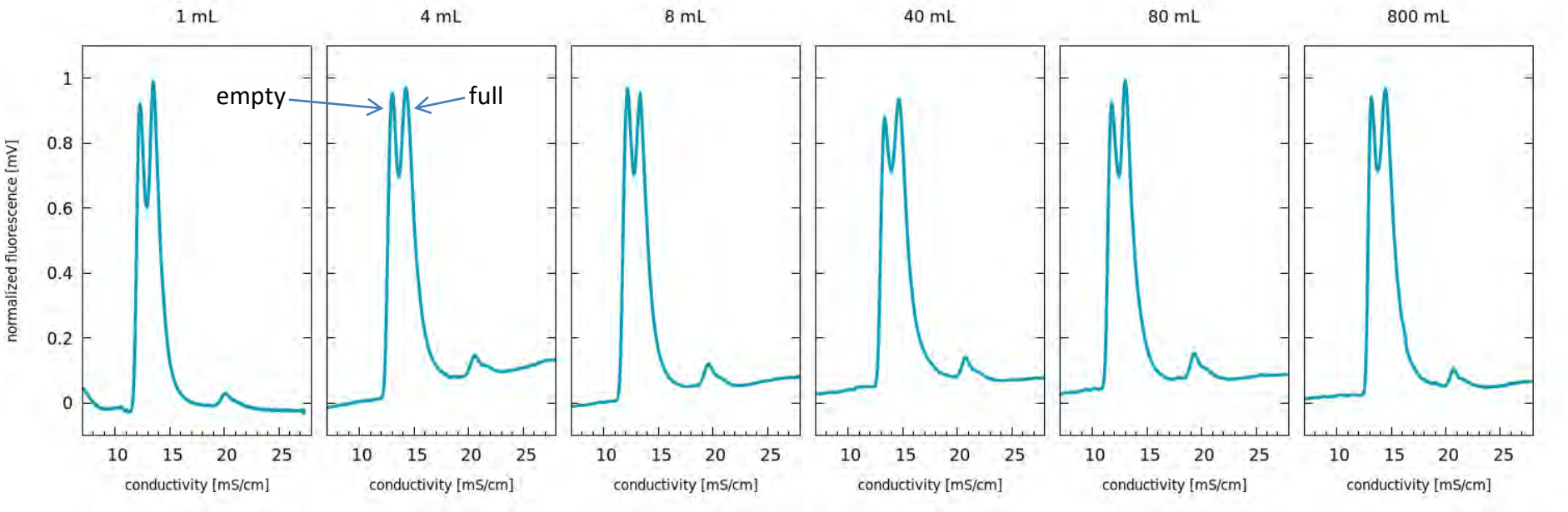


RSD value of 2.6 % of average elution conductivities of column sample disks (position A-E) indicate outstanding homogeneity of the column. There is no statistically significant difference between sample disks (A-E) and control disks (bottom, top).

The top and bottom disks of the column (Specimen) accurately represent column characteristics and can be used as scale-down models.

BIA separations

Empty – full AAV separation scale-up using Isoconductivity approach



Isoconductivity* approach (gradient duration in CV is preserved) was used to scale up Empty/Full separation method from 1 mL to 800 mL column.

Chromatography conditions:

- Buffer A: 50 mM TRIS, 2 mM MgCl2, 0.1 % poloxamer, pH 9.0
- Buffer B: Buffer A + 500 mM NaCl pH 9.0
- Gradient: 22 CV from 0 % B to 50 % B
- Flow rate: 2 CV/min – 1 mL, 4 mL, 8 mL, 40 mL, 1 CV/min – 80 mL, 800 mL
- Sample: SO3 purified AAV2/8 (GFP insert)

Yamamoto et all. Journal of Chromatography A, 1065 (2005) 45–50

Conclusions

- CIM monoliths enable a **fast**, **high capacity, high yield and high resolution** platform for purification of full AAV capsids.
- AAV preparations contain full, partially full, mixed, empty capsids, all with similar surface properties. **Orthogonal chromatography methods are needed to develop effective purification protocols.**
- **Full AAV particles may be heterogenous**. Further investigations are needed to understand the nature and safety of each of them.
- **AUC centrifugrams** are orthogonal and complementary to HPLC for studying capsid heterogeneity.
- Column reproducibility from batch to batch and across scales is **essential for robust process. Introduction of Specimen allows for monolith consistency check**. Reproducibility of operating temperature, buffer preparation, and equipment configuration across process scales are equally important.

BIA separations

Acknowledgements

We thank our valued collaborators: Stephen Kaminsky and Hyunmi Lee, Belfer Gene Therapy Core Facility, Department of Genetic Medicine, Weill Medical College of Cornell University, New York; The University of Nantes, Center for Translational Therapy for Genetic Diseases, UMR 1089, Nantes, France; The International Center for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy; and The National Institute of Biology (NIB), Ljubljana, Slovenia. From BIA Separations we thank Timotej Zvanut, and Andrej Mihevc, for development of valuable analytical methods.

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