BIA separations

Better purification. Faster.

Analytical Methods: The Path to More Efficient Processes & Safer Gene Therapy Products

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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
Vero cell bank

DSP bioprocess knowledge – no royalties on

- 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
- Adeno virus
- AAV **(all serotypes, > 20 tested)**
- 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**

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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

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Fast method development and in-process control using HPLC system with multiple detectors

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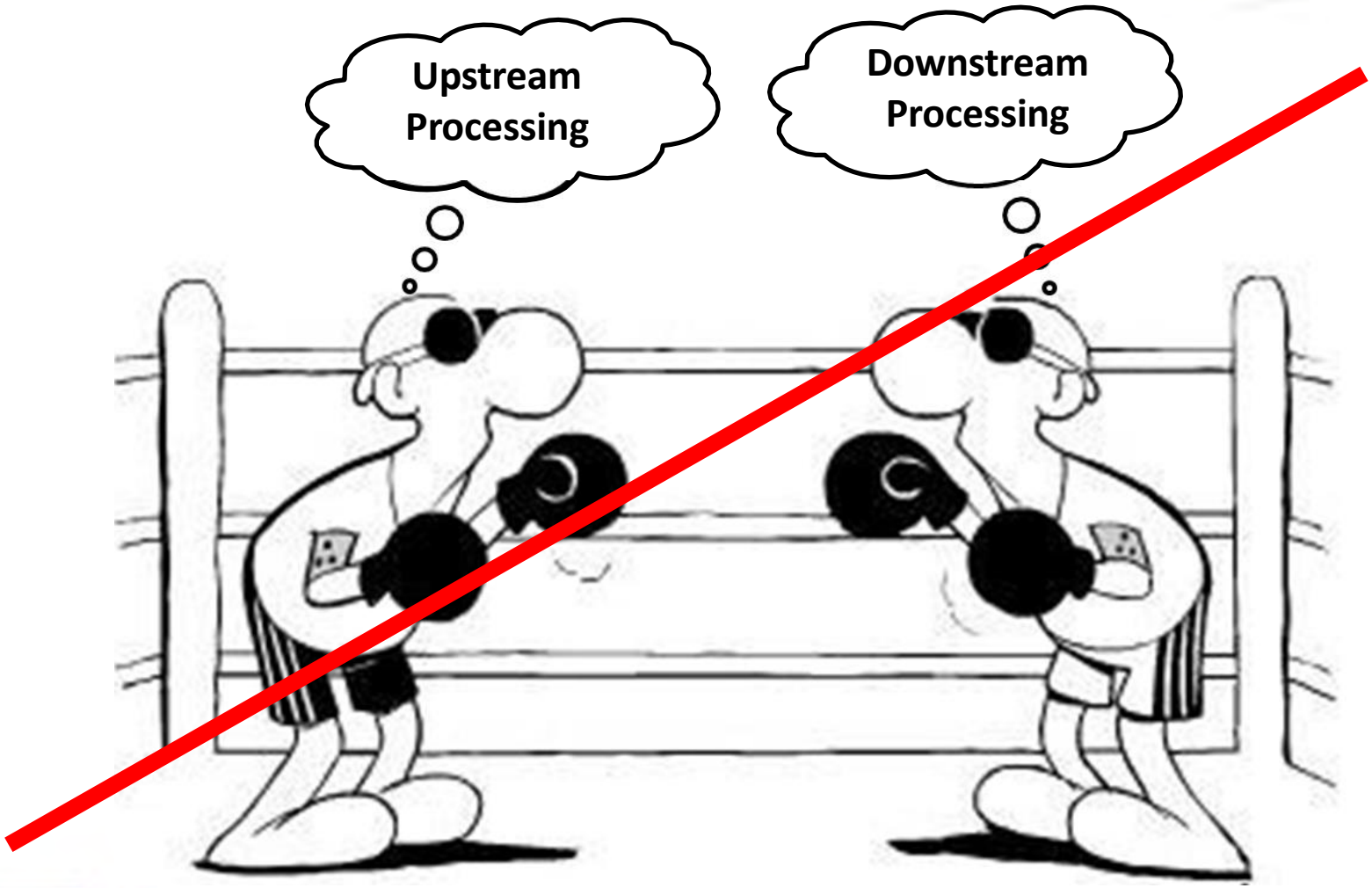
Guidance from analytics is crucial to harmonize USP and DSP processing – should be just ONE process



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HPLC. The key component for efficient process development and in-process control

CIMac™

inCyght

for chromatography

Fast quantitative process monitoring to reveal sample composition and reveal trends.

Custom tailored system to meet requirements of bioanalytical HPLC techniques.

Integrates multiple detectors: UV (multiple wavelengths), Fluorescence (multiple wavelengths), **Multi-angle light scattering (MALS)**, pH, conductivity.

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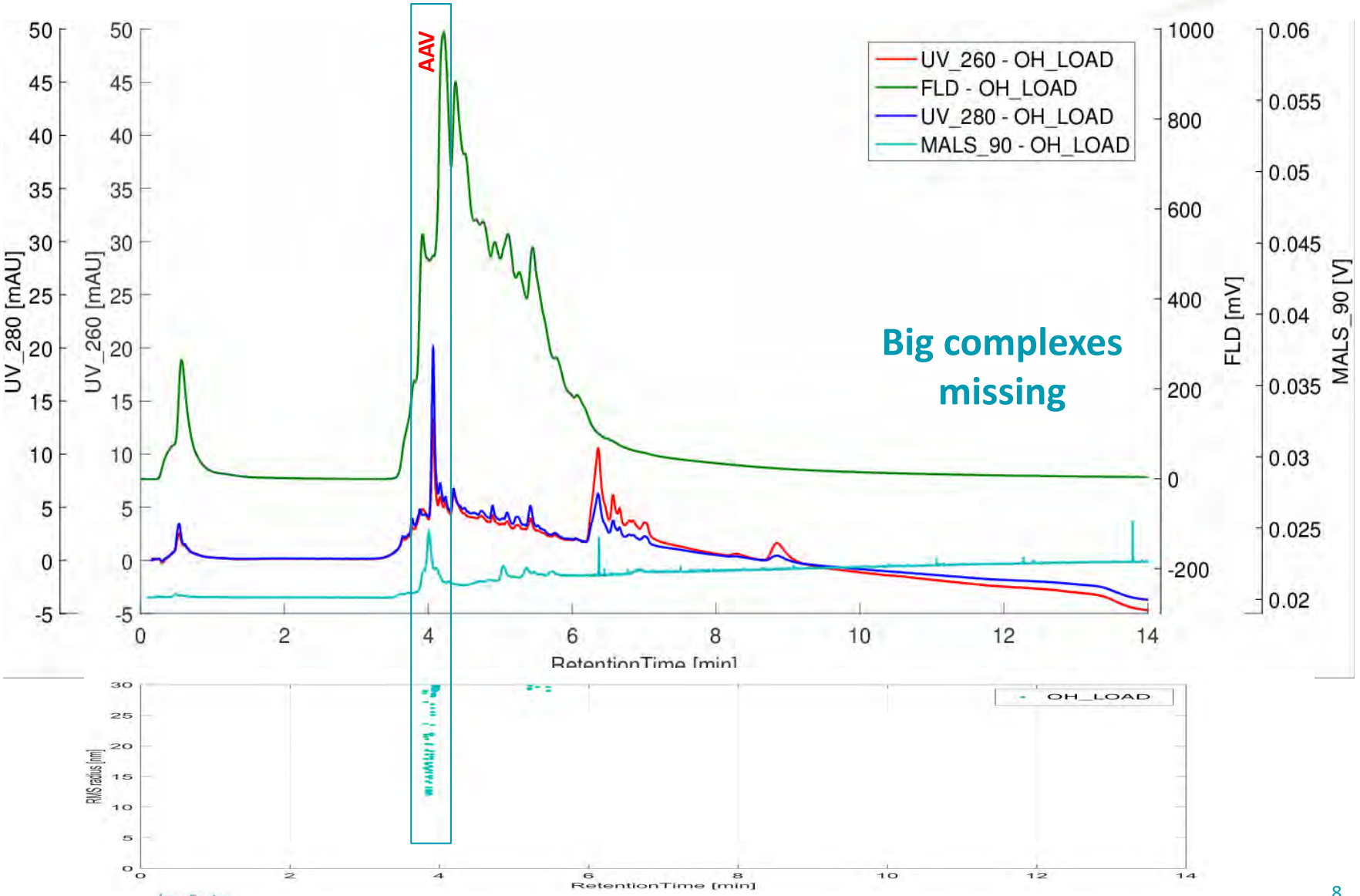
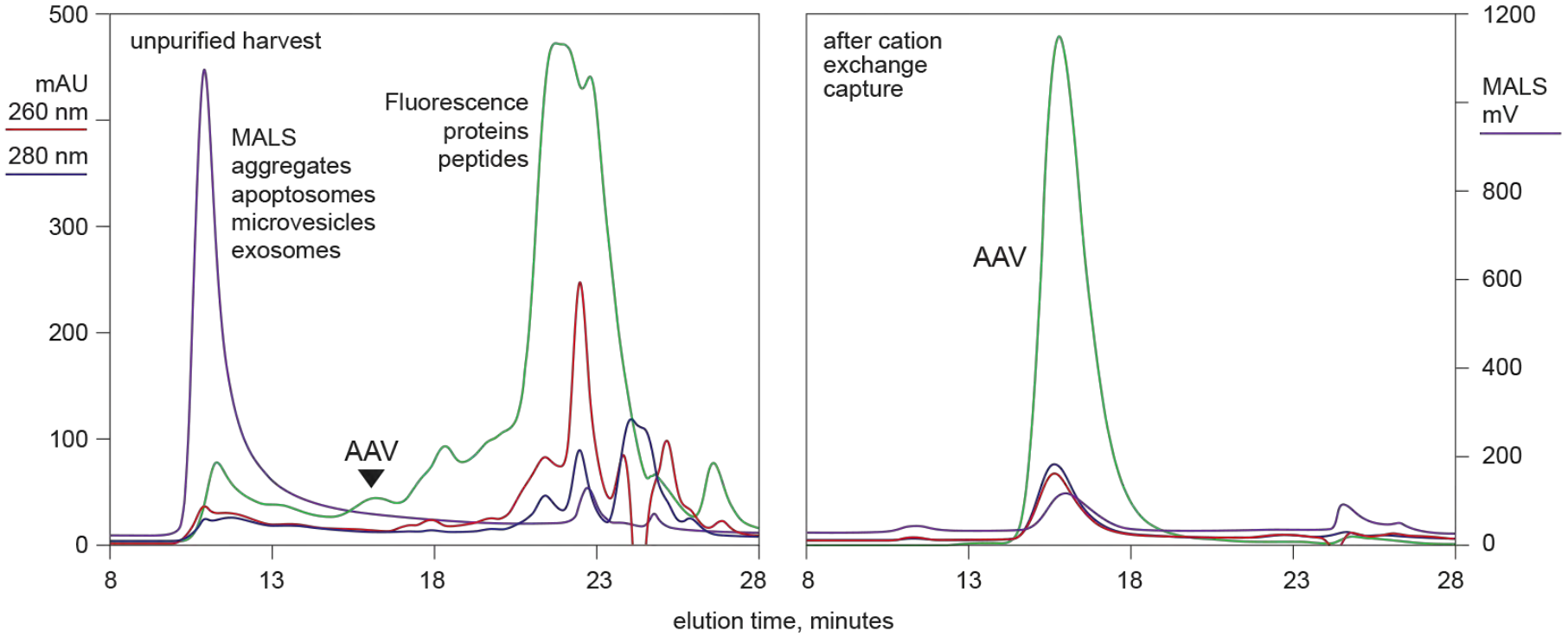
Fast process development using anion exchange HPLC in linear gradient: *SO3 step – AAV harvest*



Complexes are the source of residual DNA/RNA in the product - Need for orthogonal analytical methods: *Characterization by analytical SEC*



Fluorescence enables direct detection of virus in harvest. MALS highlights large contaminants at early process stages and confirms the identity of AAV at later process stages.

Thanks to Stephen M. Kaminsky and Hyunmi Lee, Belfer Gene Therapy Core Facility, Department of Genetic Medicine, Weill Medical College of Cornell University, NY

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AAV manufacturing platform process – towards ultra low residual DNA/RNA – safety 1st

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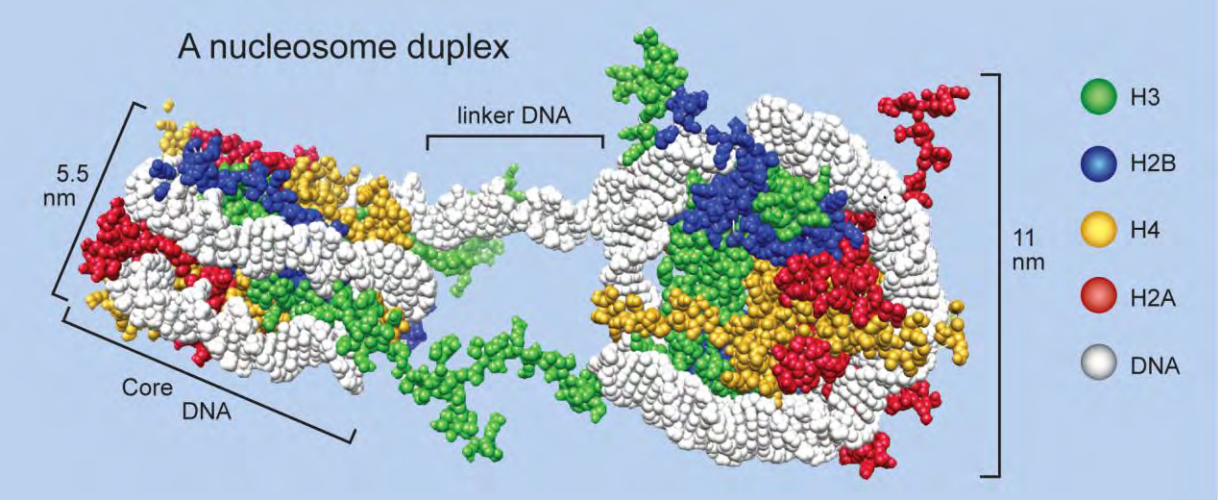
What is Chromatin?
Leaks from all columns if not very strongly bound

The basic structural subunit of Chromatin is a nucleosome

It consists of a histone octamer wrapped with 1.6 turns of DNA; about 150 bp



Histones are extremely hydrophobic and highly positively charged, with isoelectric points ranging from 9 to 11. DNA has a pK of about 2.6.

The net charge of Chromatin is roughly neutral but its exposed components still retain their extreme charge characteristics. Both also participate in metal affinity, hydrogen bonding, and van der Waals interactions.

Part of the hcDNA might not be detectable by the PCR as the DNA is not accessible by the primer

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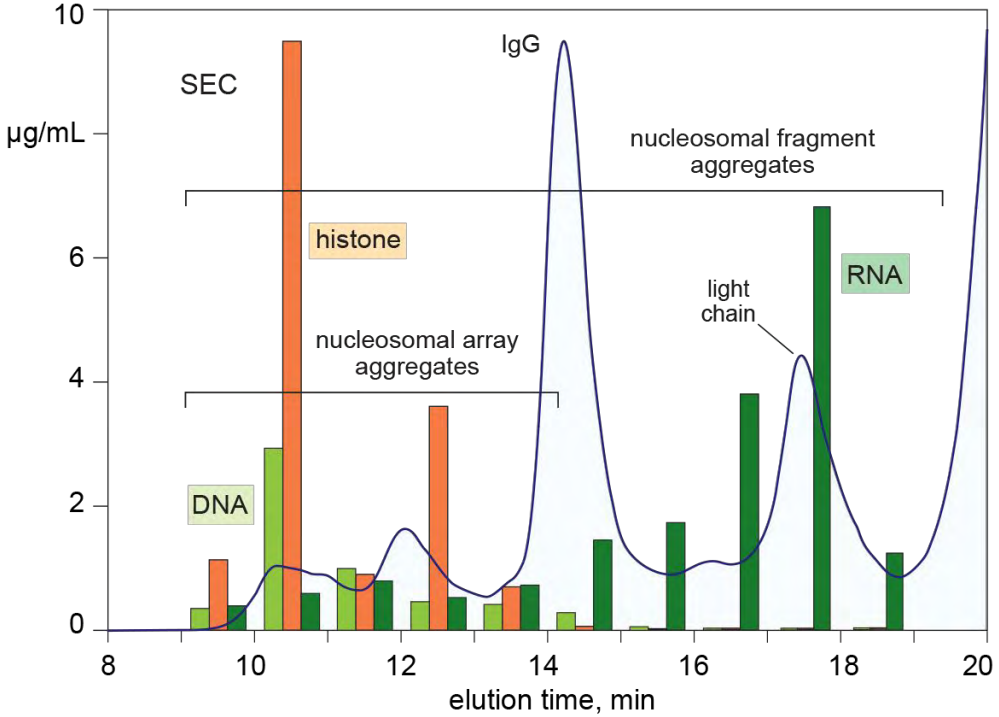
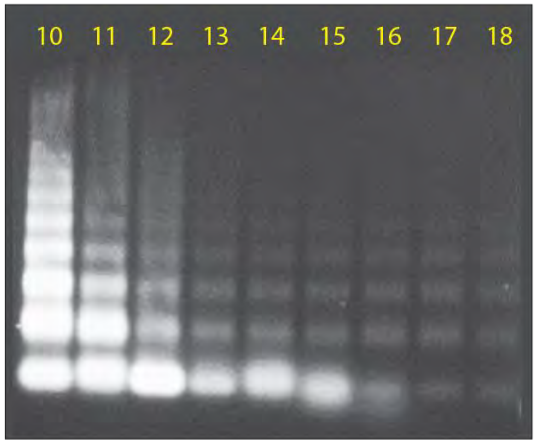
Key task to reach ultra low residual DNA/RNA is proper management of Chromatin structures

Filtered CHO Harvest containing prospective biosimilar Herceptin™

Analytical SEC. DNA by ddPCR. RNA by Ribogreen. Histones by ELISA.





Agarose electrophoresis after SEC in PBS. Poor resolution shows the chemical stickiness of chromatin. Good resolution needs 2 M NaCl to suppress nonspecific binding.

Chromatin complexes built on nucleosomal arrays range in size from 10–400 nm. Complexes based on nucleosomal fragments range from 2–10 nm. Arrays and fragments both act as nucleation centers for accretion of non-nucleosomal proteins and RNA.

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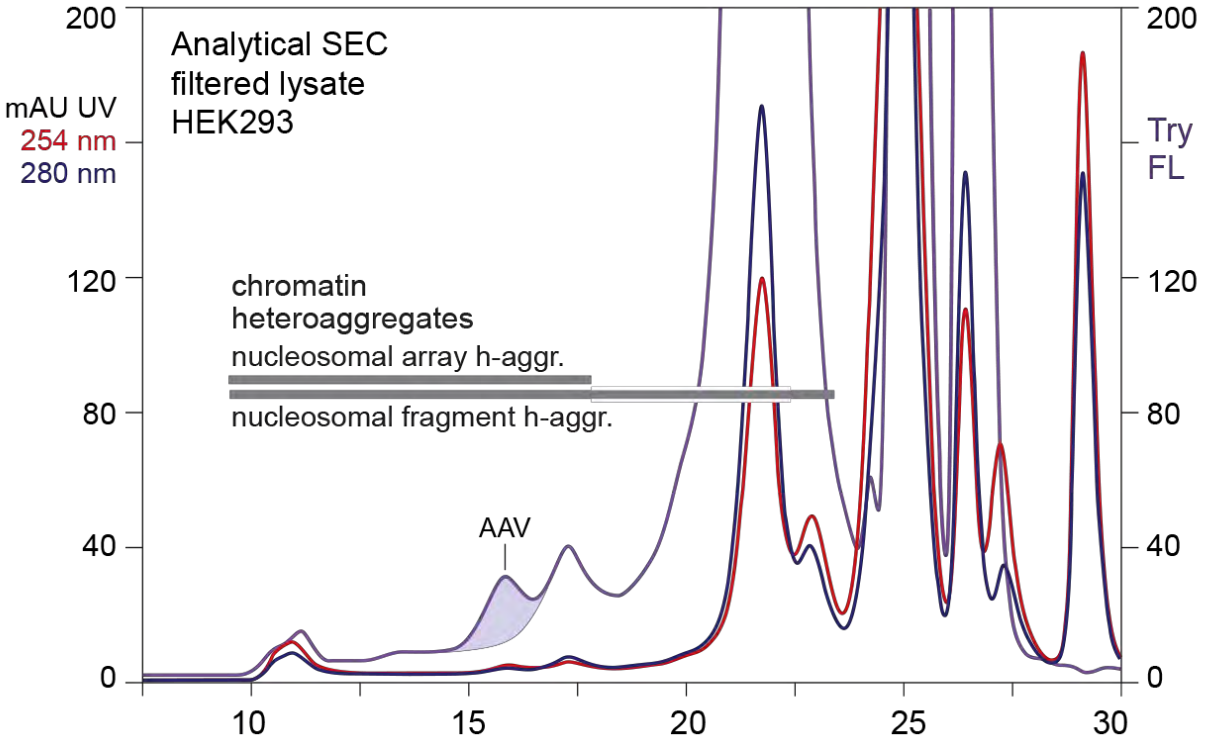
Take into account the Chromatin/virus product ratio - AAV not visible under the UV comparing to the IgG

Chromatin in virus harvests has the same basic characteristics as it has in IgG harvest, but viruses are, unlike IgG, in the harvest in very tiny amount

Analytical SEC with monitoring of intrinsic tryptophan fluorescence



Chromatin is distinguished by 260/280 ratios from about 1:1 to 2:1, depending on relative DNA dominance. Proteins are 280 dominant. Fluorescence gives high sensitivity for proteins but not for DNA.

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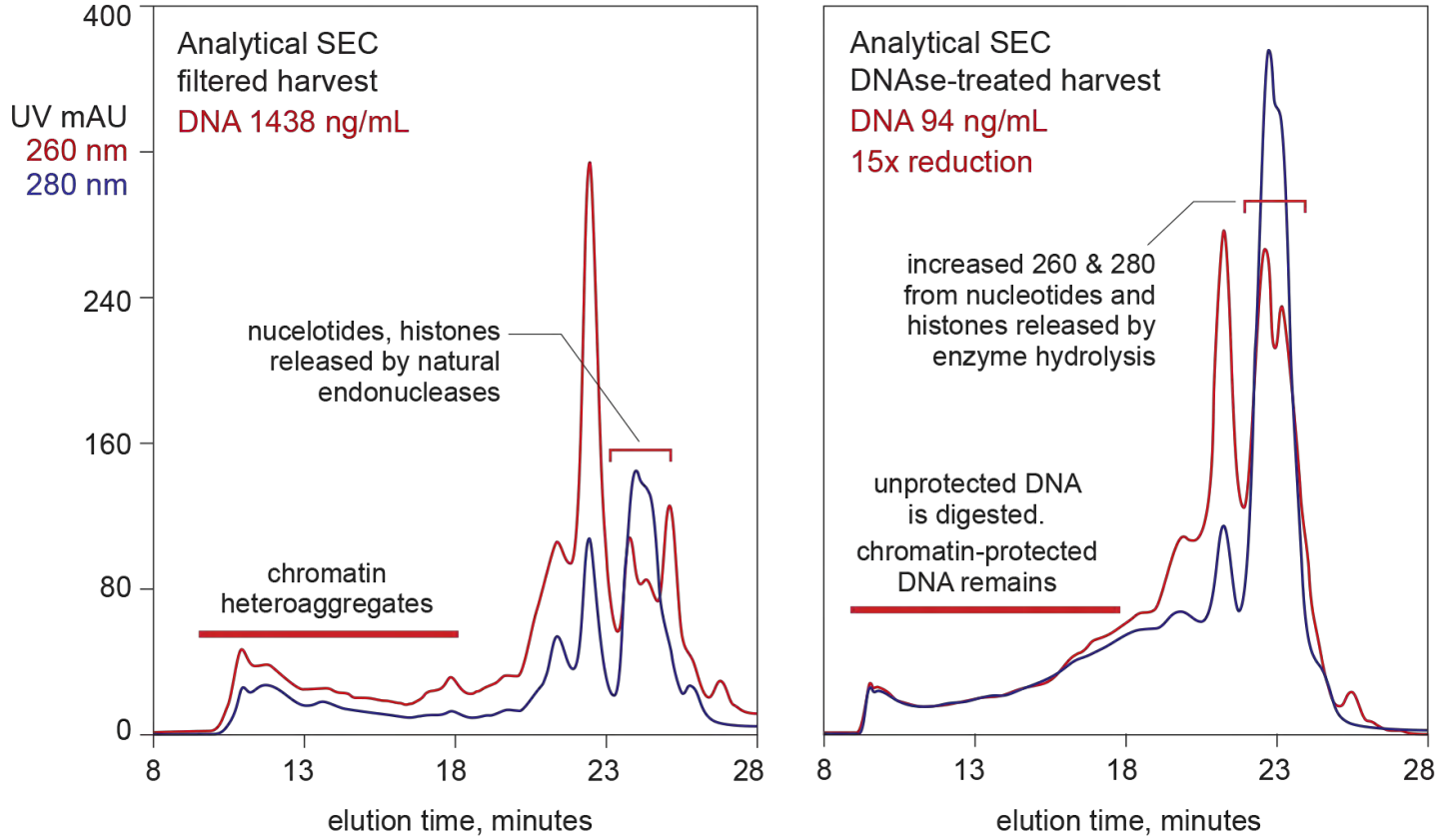
Strong association with histones protect DNA to be digested - nucleases eliminate accessible DNA only, PCR can not detect the DNA inside the Chromatin



Red trace, 260 nm. Blue trace, 280 nm. DNA values by picogreen.

Part of the hcDNA might not be detectable by the PCR as the DNA is not accessible by the primer.

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Celebrating 20 Years of Innovation

High performance manufacturing platform for all AAV serotypes – strong CEX and strong AEX should be used to eliminate residual Chromatin structures

AAV Harvest or Lysate

Chromatin extraction with CIMasphere AAV

filter, optional TFF

Cation exchange capture with CIMmultus SO3

adjust pH, optional TFF

Polishing & empty capsid removal with CIMmultus QA or E/F

Clinical quality AAV

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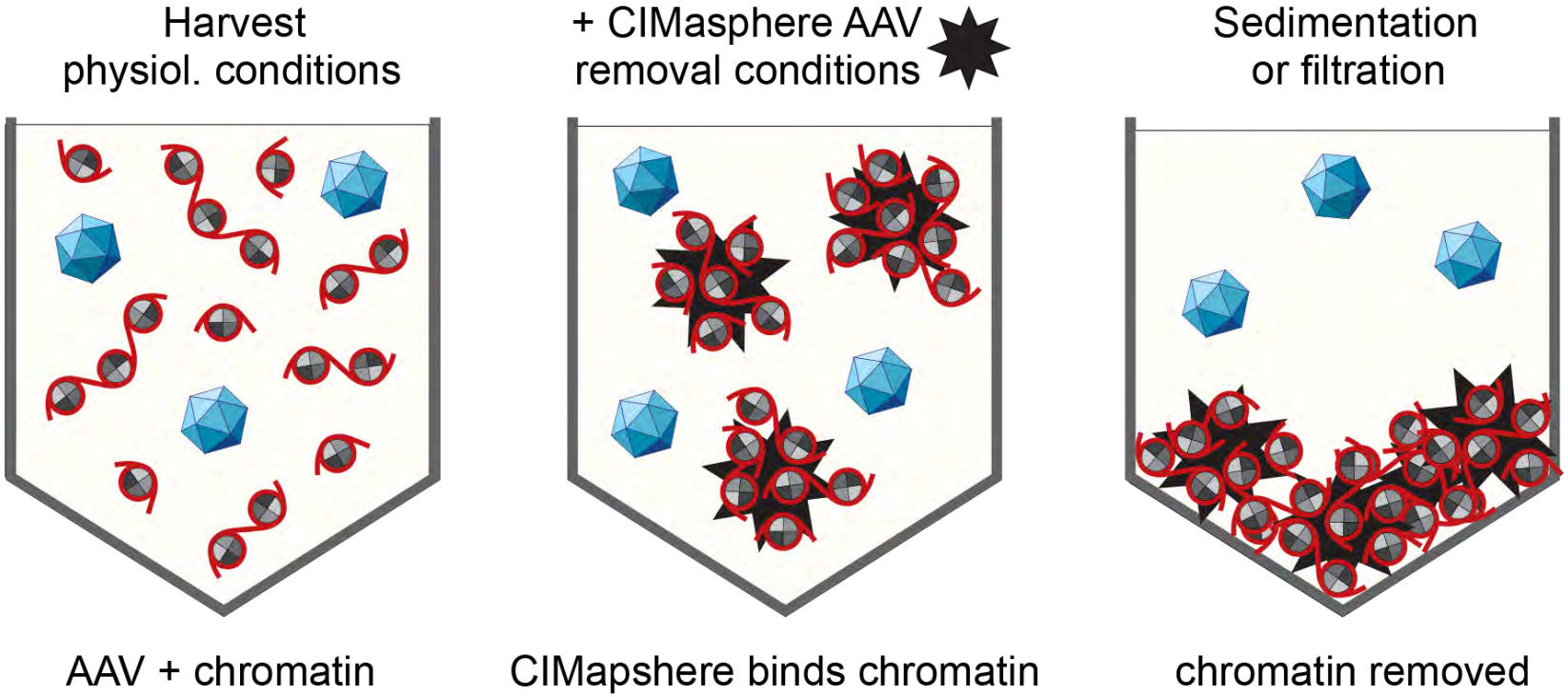
Chromatin reduction should take place in advance of chromatography if in high amount (when cells lysed)

CIMasphere AAV for advance DNA extraction from all serotypes

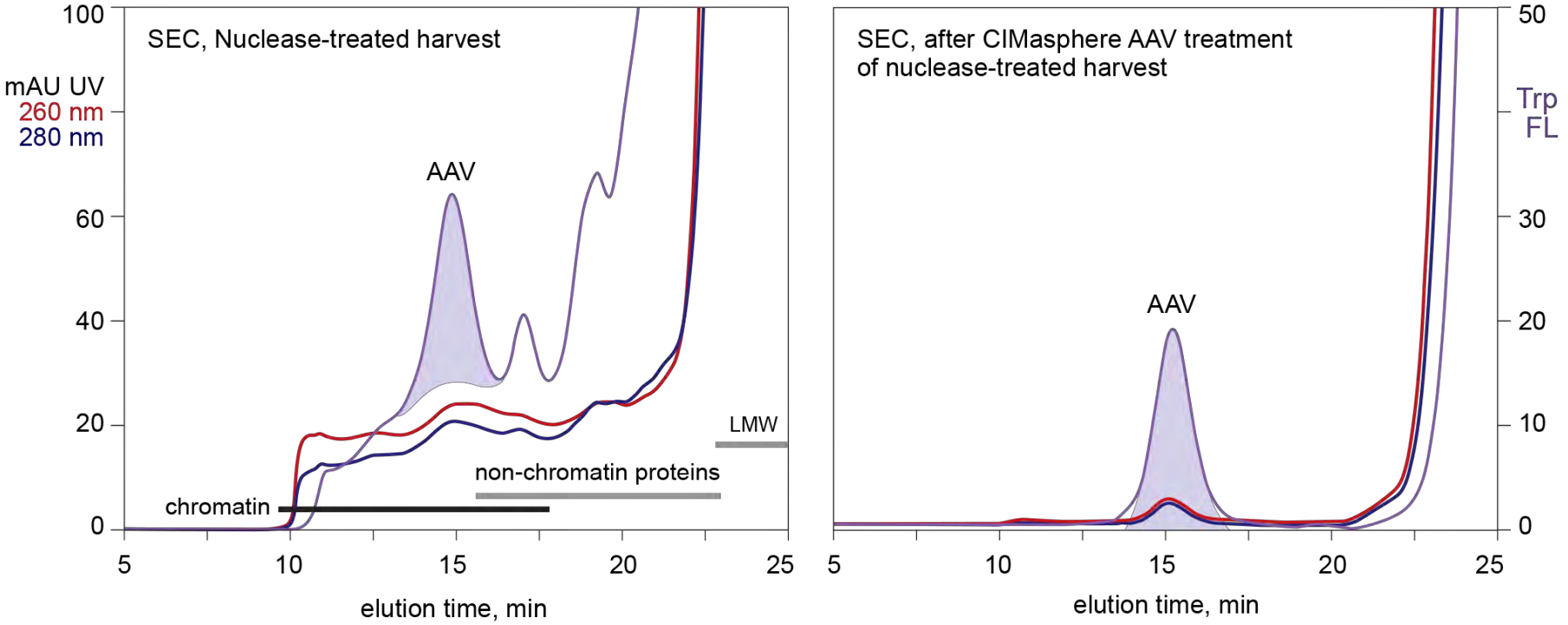
CIMasphere is a particulate solid phase that binds DNA so strongly it becomes dissociated from its pre-existing associations



Advance chromatin reduction from filtered harvest

CIMasphere AAV processing of nuclease-treated SF9 lysate, AAV 2/8

Analytical SEC with AAV monitoring by intrinsic tryptophan fluorescence



Nuclease enzymes lyse accessible DNA but they work poorly with Chromatin because DNA's strong histone associations protect it. CIMasphere AAV targets DNA in the form of Chromatin and co-removes associated host contaminants.

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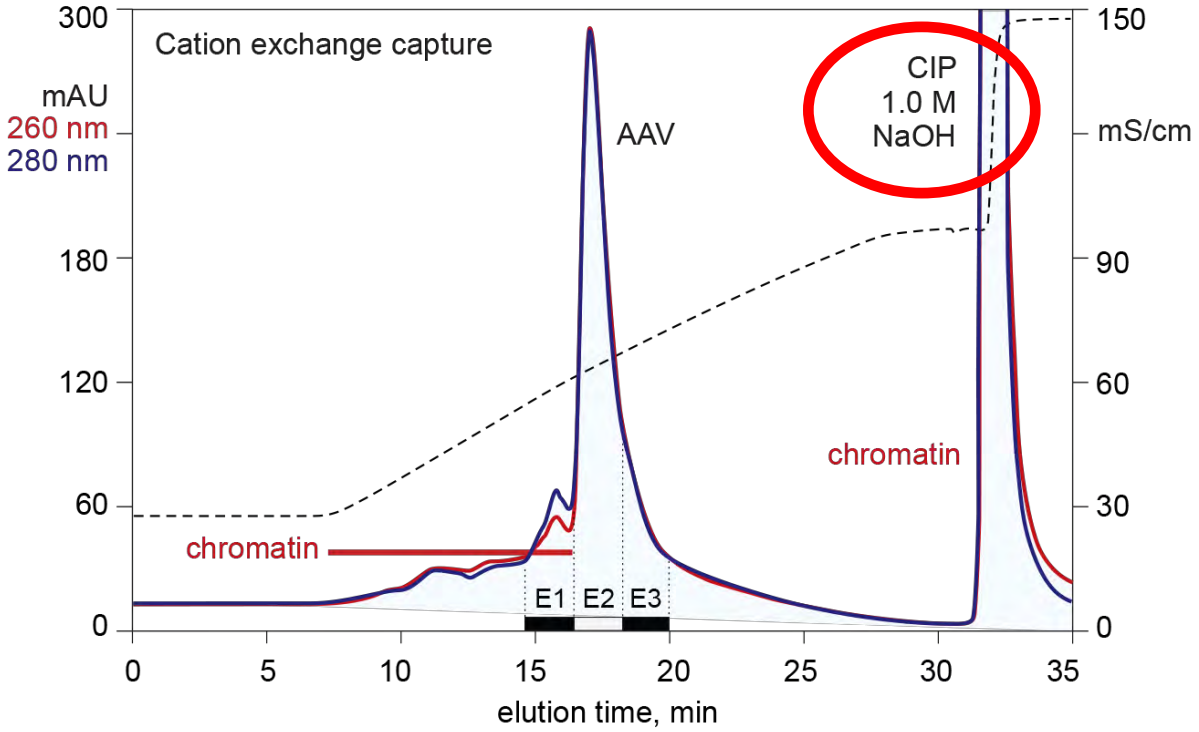
Capture by strong cation exchange chromatography - loading 1-2 CV/min – no concentration needed

100 mL SF9 lysate, AAV2/8, post-extraction with CIMasphere AAV

CIMmultus SO3, 1 mL, 2 µm channels, 10 CV/min



Chromatin left after solid phase extraction binds strongly to SO3 because of its histone component. Its removal at this step is essential for enabling good separation of empty and full capsids by anion exchange.

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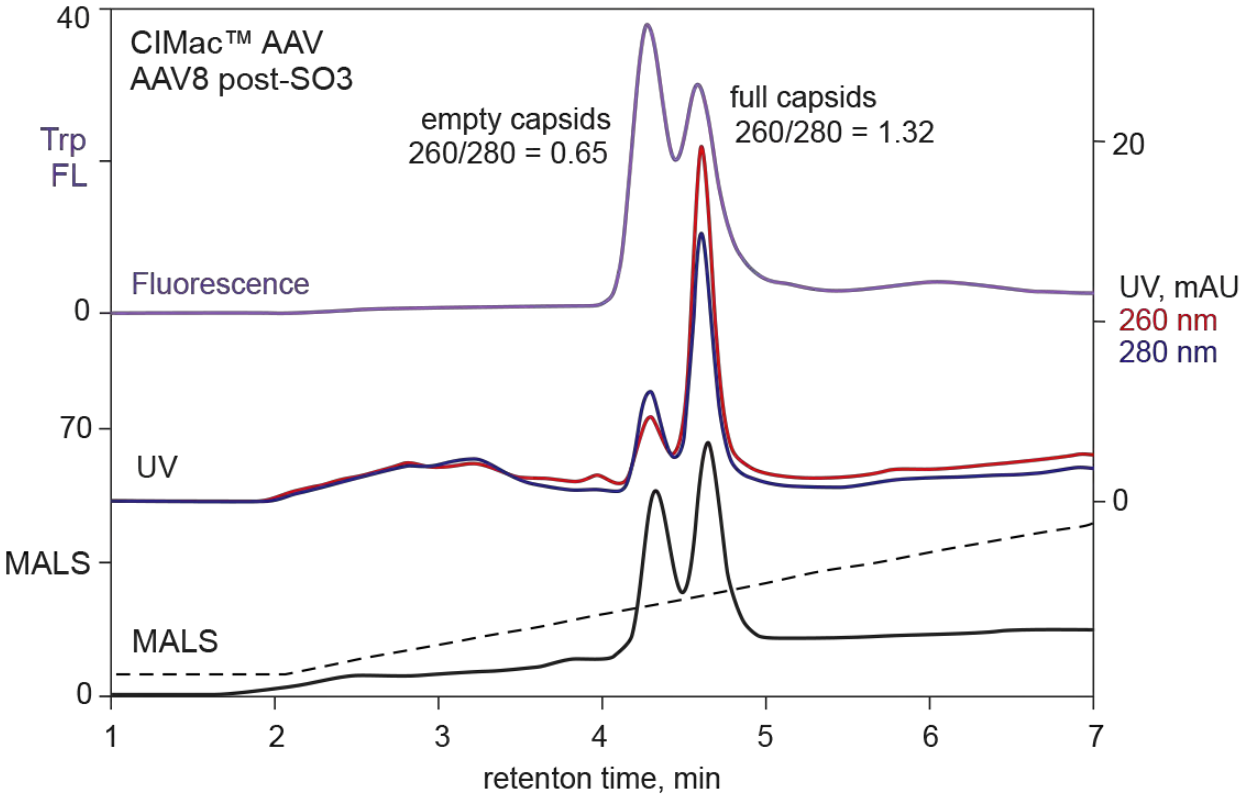
SO3 eluate analytical characterization of empty/full content and residual impurities

Analytical comparison of full and empty capsids after cation exchange

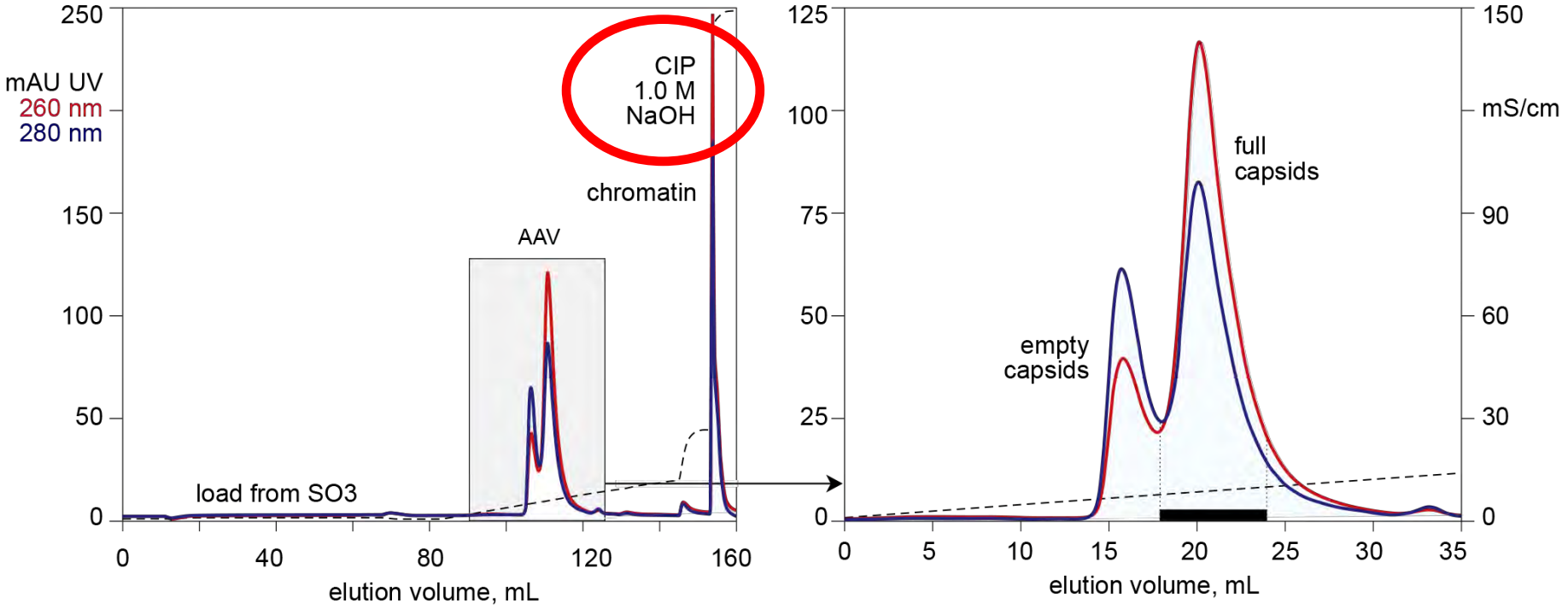


Fluorescence supports reliable estimates of empty/full proportions because it measures only capsid proteins. UV overestimates full capsids because UV absorbance by DNA inflates protein measurement. MALS requires adjustment for particle size and mass.

Polishing by strong anion exchange chromatography

AAV2/8, CIMmultus SO3-purified, diluted and titrated

CIMmultus QA, 1 mL, 2 µm channels, 10 CV/min



Anion exchange chromatography on monoliths supports effective separation of empty and full capsids for most serotypes *See Fu et al, Hum. Gene Ther. Met. 30 (2019) 144–152.*

The CIP peak here is much smaller compared to cation exchange because most of the chromatin was removed in the prior steps.

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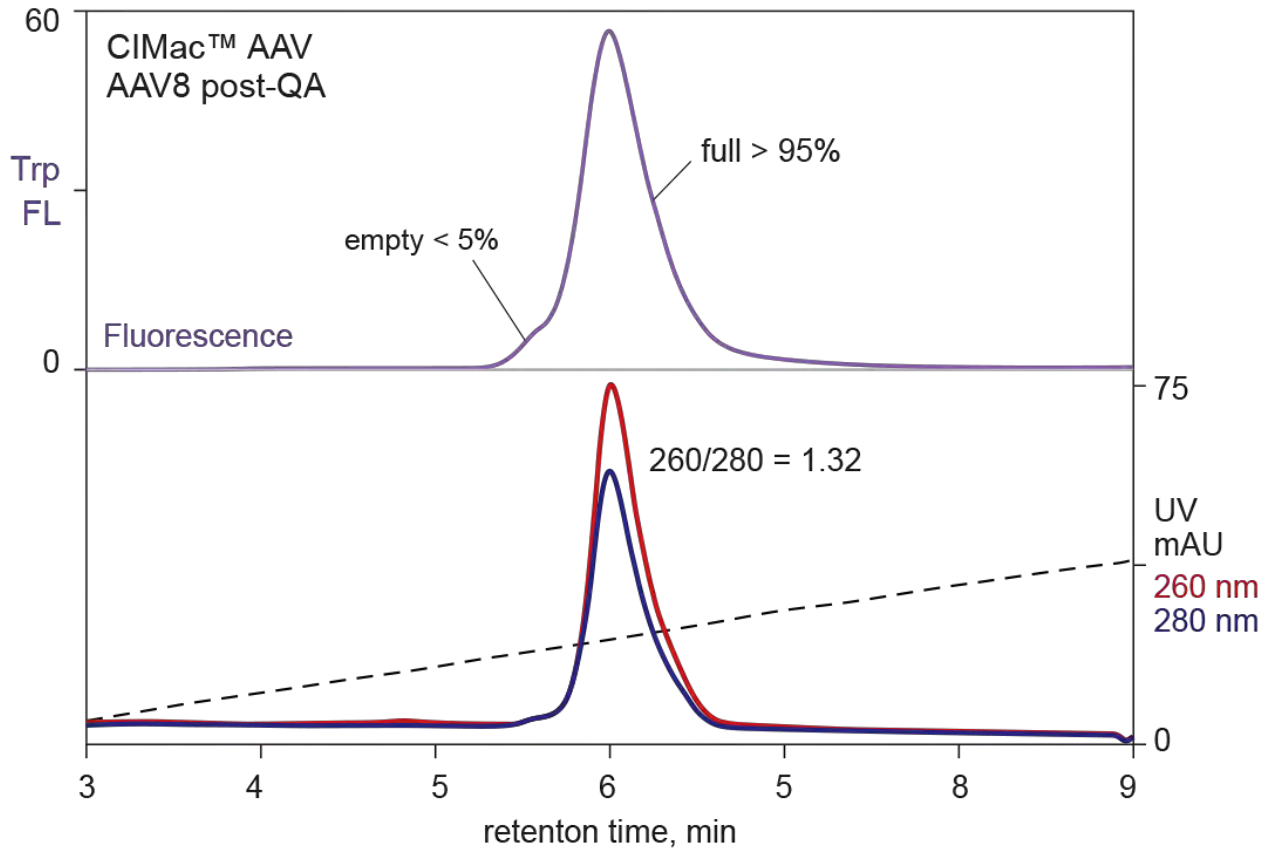
Analytical characterization of empty/full content

Analytical determination of % full capsids after anion exchange



Fluorescence supports reliable estimates of empty/full proportions because it measures only capsid proteins. UV overestimates full capsids because UV absorbance by DNA inflates protein measurement.

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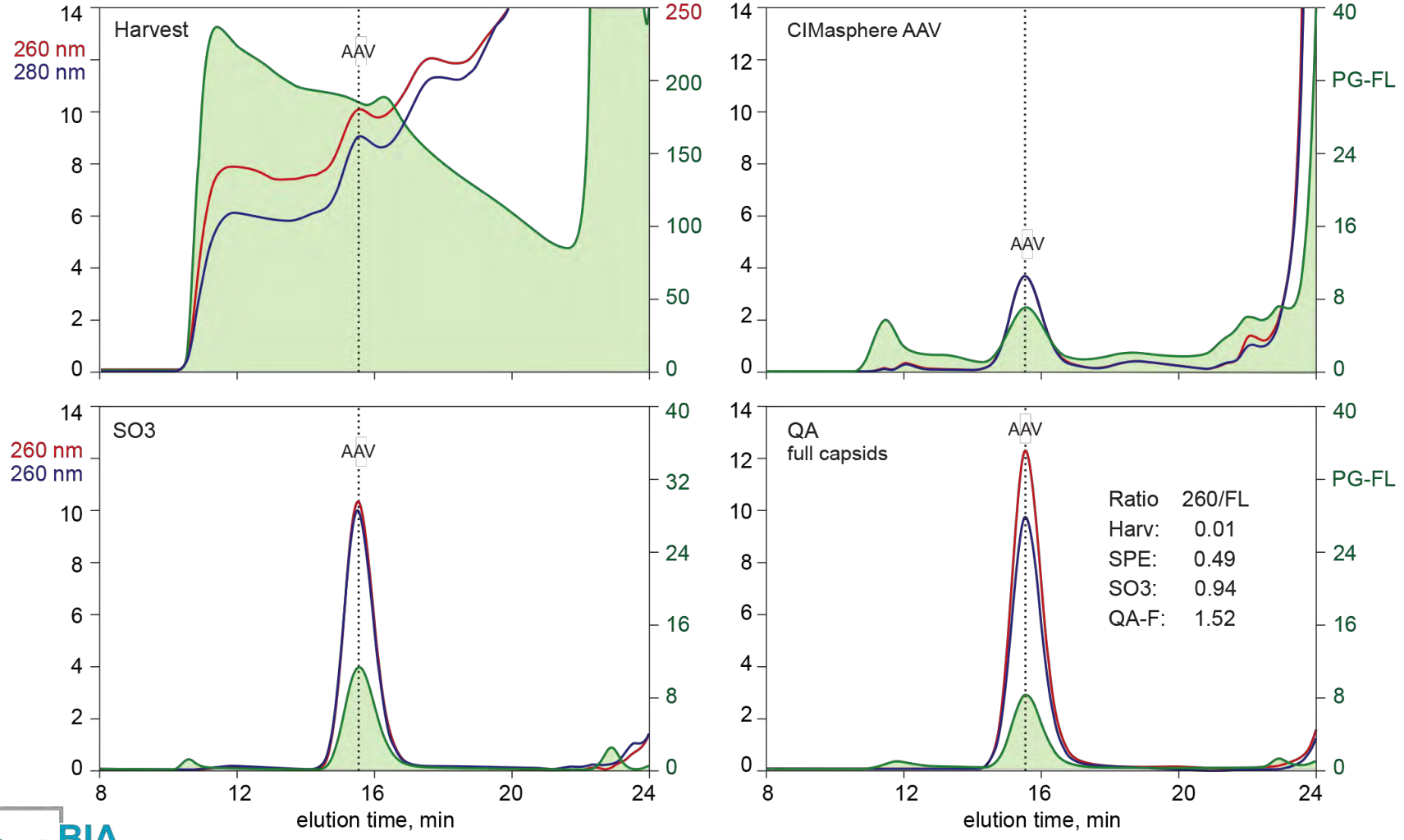
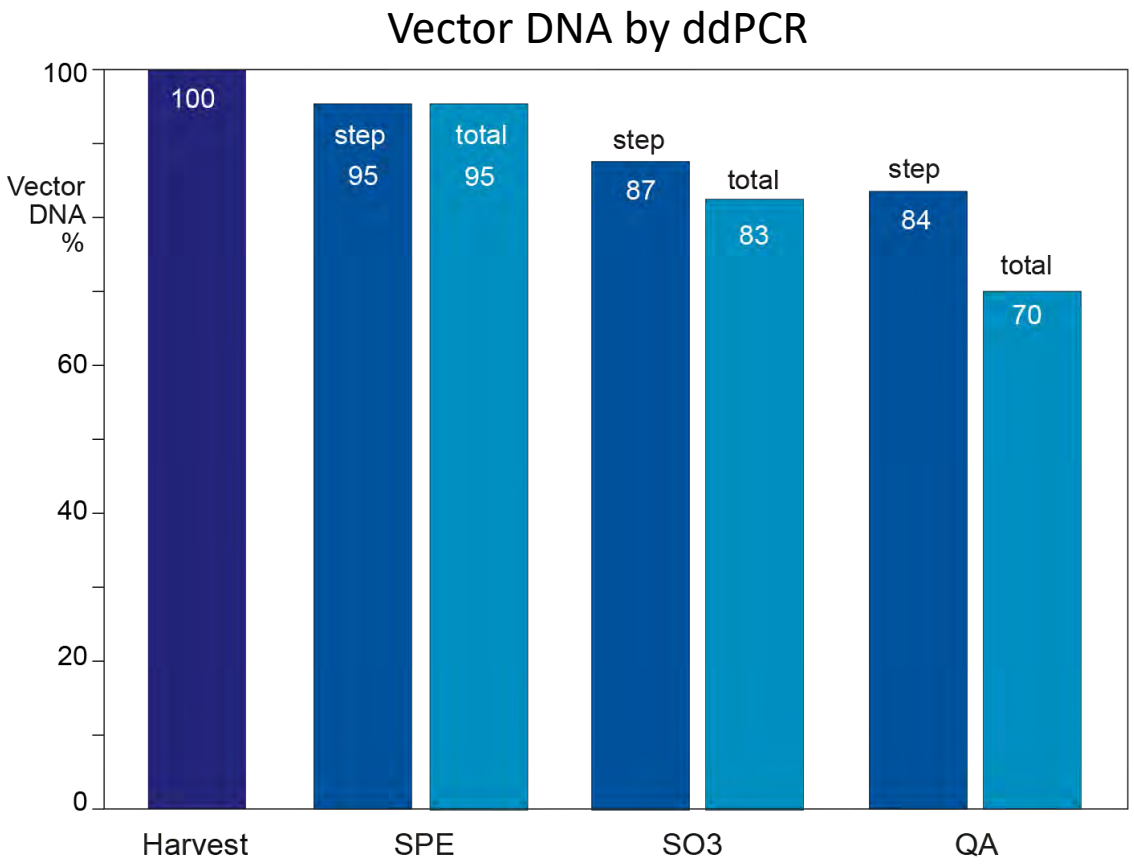
Hidden DNA removal - from harvest to full AAV

Process snapshot.

DNA/protein complexes (Chromatin) size distribution by picogreen-SEC-UV/FLD



AAV process recovery – high recovery is cornestone for process robustness



Recovery varies according to AAV titer, media formulation, cell culture duration at harvest, and lysis method. Cleaner higher titer cultures give higher recoveries; **dirtier lower titer cultures give lower recoveries.**

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Empty/Full AAV determination – for accurate results orthogonal methods are requested

WORK IN PROGRESS (influence of the serotype, source (cell line), method used, purity, residual DNA/RNA,… on the E/F result):

AAV serotype	client/partner	sample	cryoTEM Partner A	cryoTEM Partner B	AUC	HPLC (UV, FLU, MA)
AAV10	Partner 1	before QA				
AAV2/8	Partner 2	QA LOAD/QA E				
AAV9	Partner 3	QA LOAD/QA E				
new partners welcome						

Ratio ELISA : PCR not selective and not tested

Ref also to: Xiaotong Fu at all, Human Gene Therapy, 2019

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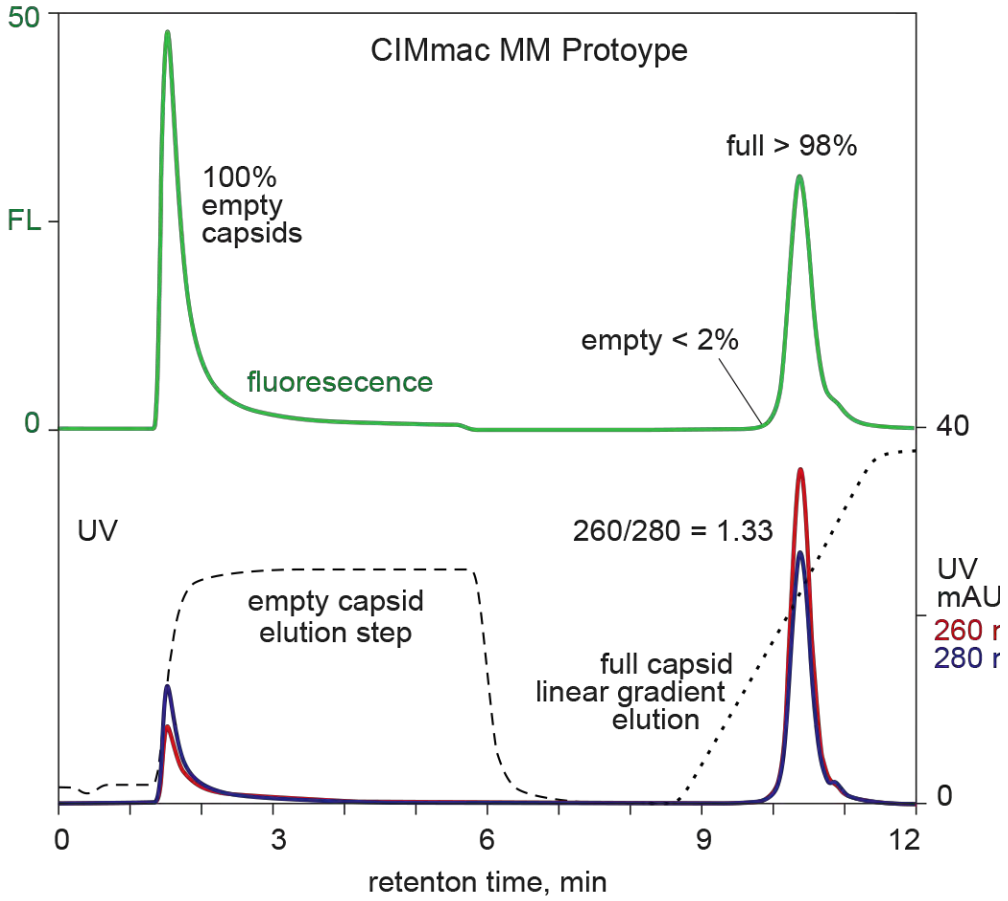
Celebrating 20 Years of Innovation

Emerging technology for empty AAV removal

A new monolith for chromatographic removal of empty capsids.

Sample: SO3-purified AAV 2/8.



Capsids are loaded. Empty capsids are eluted with a dedicated elution step. Then full capsids are eluted with a different dedicated step. Beta columns are scheduled for introduction on a limited basis in early 2020.

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Helpful hints

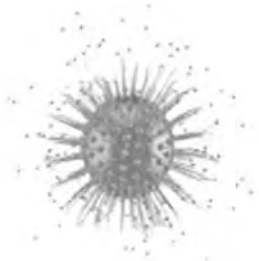
- Get in the habit of including the cleaning peak (1M NaOH) on all your chromatograms. It is a clue you cannot afford to overlook.
- Consider that the other things that never made sense might relate to the **host DNA and its fragments being bound to virus exteriors.**
- Apply analytical methods that reveal it so you can track it and develop methods to eliminate it.
- **Managing Chromatin structures is the path towards safer biotech products and robust processes.**
- **Use orthogonal analytical methods – do never trust single method results.**

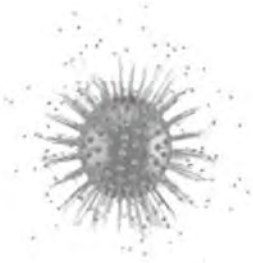
BIA separations

We invite you to join us at MSS2020, the 9th International Symposium on Monoliths.

Purification and analytics

OF DNA AND RNA, VACCINES, GENE THERAPY VECTORS, ANTIBIOTIC REPLACEMENTS (PHAGES), LARGE PROTEINS, NANOPARTICLES, ENZYME REACTORS, EXOSOMES AND OTHER VESICLES





For the latest information and registration, please visit the MSS homepage at:
www.monolith-events.com

15th-19th of June 2020

Portoroz, Slovenia

MSS 2020

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 Jasmina Puc, Spela Persic, Timotej Zvanut, and Andrej Mihevc, for development of valuable new analytical methods.

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