SARTORIUS

BIA separations

Simplifying Progress

Optimization of the AAV Expression Using Dedicated HPLC System

Ivana Petrović Koshmak, PhD

Late Stage Bioprocessing & Viral Vectors, 2-11 November 2021

Agenda

About BIA Separations

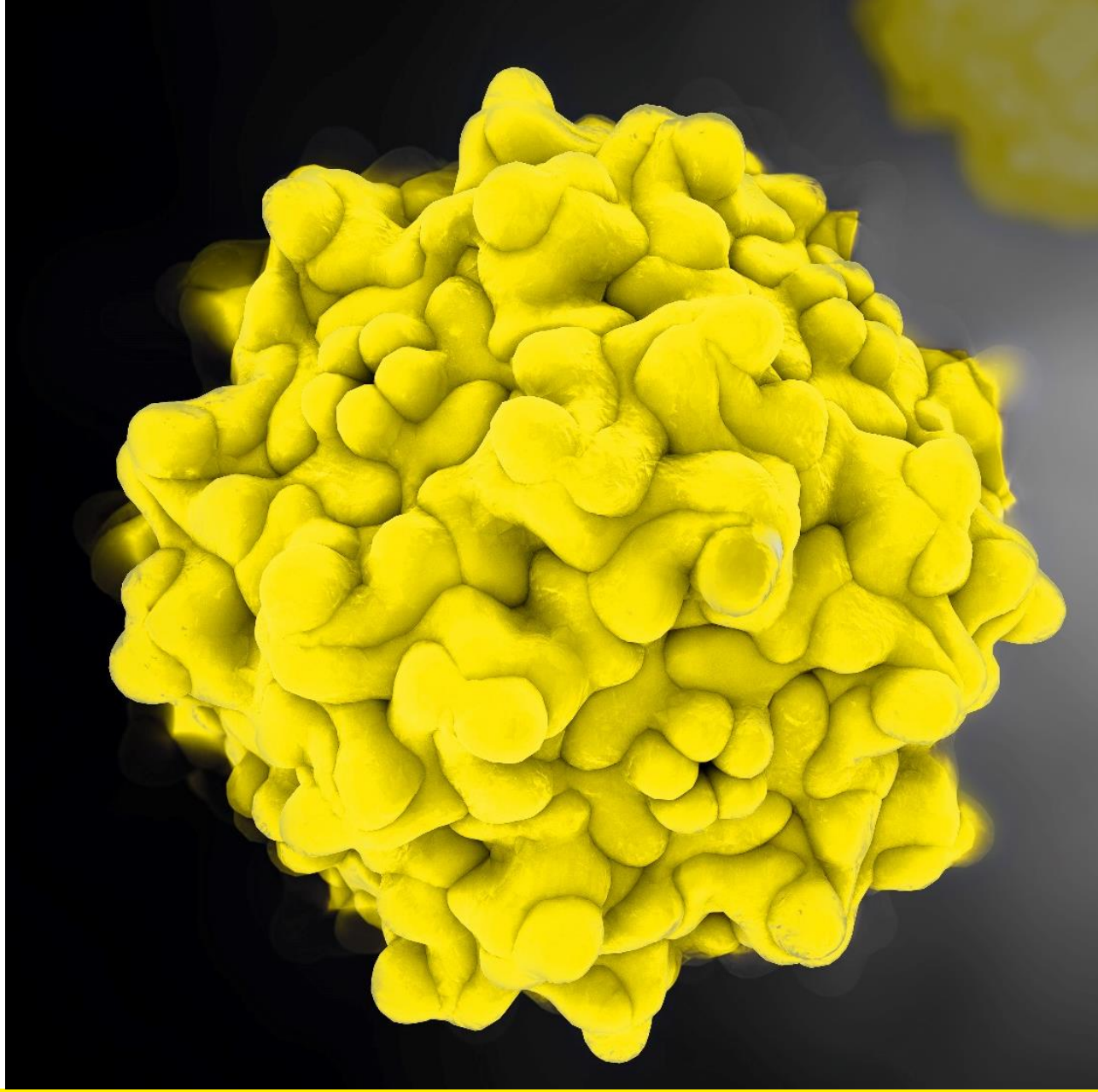
Control your ingreedients:
pDNA analytics

Control your background:
analytical profile of media, feed and cells

Trace your product and impurities:
orthogonal methods for complex samples

Novel PATfix column switching method:
AAV E/F expression profile

Conclusions



About BIA Separations

- The leading developer of monolith technology and the exclusive producer **of CIM® (Convective Interaction Media) monolithic chromatographic columns** for more than 20 years and with > 160 employees currently.
- A specialist in the purification of large biological molecules and viral particles for **gene therapy and the vaccine markets.**
- **Sartorius center of excellence in gene therapy** offers solutions for downstream process development and manufacturing, as well as analytical methods applicable to multiple large molecules, e.g. AAV, Adeno, Flu, pDNA, mRNA.
- **Supplies unique monolithic chromatographic columns** complimentary to porous particles and membranes.

SARTORIUS | Press Release

Göttingen, November 2, 2020

Sartorius closes acquisition of BIA Separations

- Combination of businesses creates a premium portfolio for advanced therapies
- Course set for successful integration

Expertise in Downstream Process Development

Impurities reduce the efficiency of transfection, transduction and IVT, and increase concerns for product safety

- **pDNA** and **mRNA**, ss and dsRNA, minicircle DNA (including SARS-CoV2 applications)
- **AAV** (all serotypes) and **Adenovirus** (including SARS-CoV2 applications)
- **VLPs** (including Flu and SARS-CoV2 applications)
- Influenza virus (all serotypes), Vaccinia/MVA
- IVIg, IgM and many more

>30 pDNA, mRNA and viral vector cGMP downstream processes tech transferred to clients & CMOs

Currently establishing Upstream activities (Adeno, AAV & other).

PATfix HPLC and CIMac analytical columns - no carryover of contaminants or viruses

Available:

- CIMac™ QA
- CIMac™ DEAE
- CIMac™ SO3
- CIMac™ EDA
- CIMac™ pDNA
- CIMac™ Adeno
- CIMac™ AAV E/F

HPLC-based in-process control for AAV production:

- QC of pDNA for transfection
- Recording background: media, feed and cells
- Total AAV, expression profile and E/F analytics of complex USP & DSP samples

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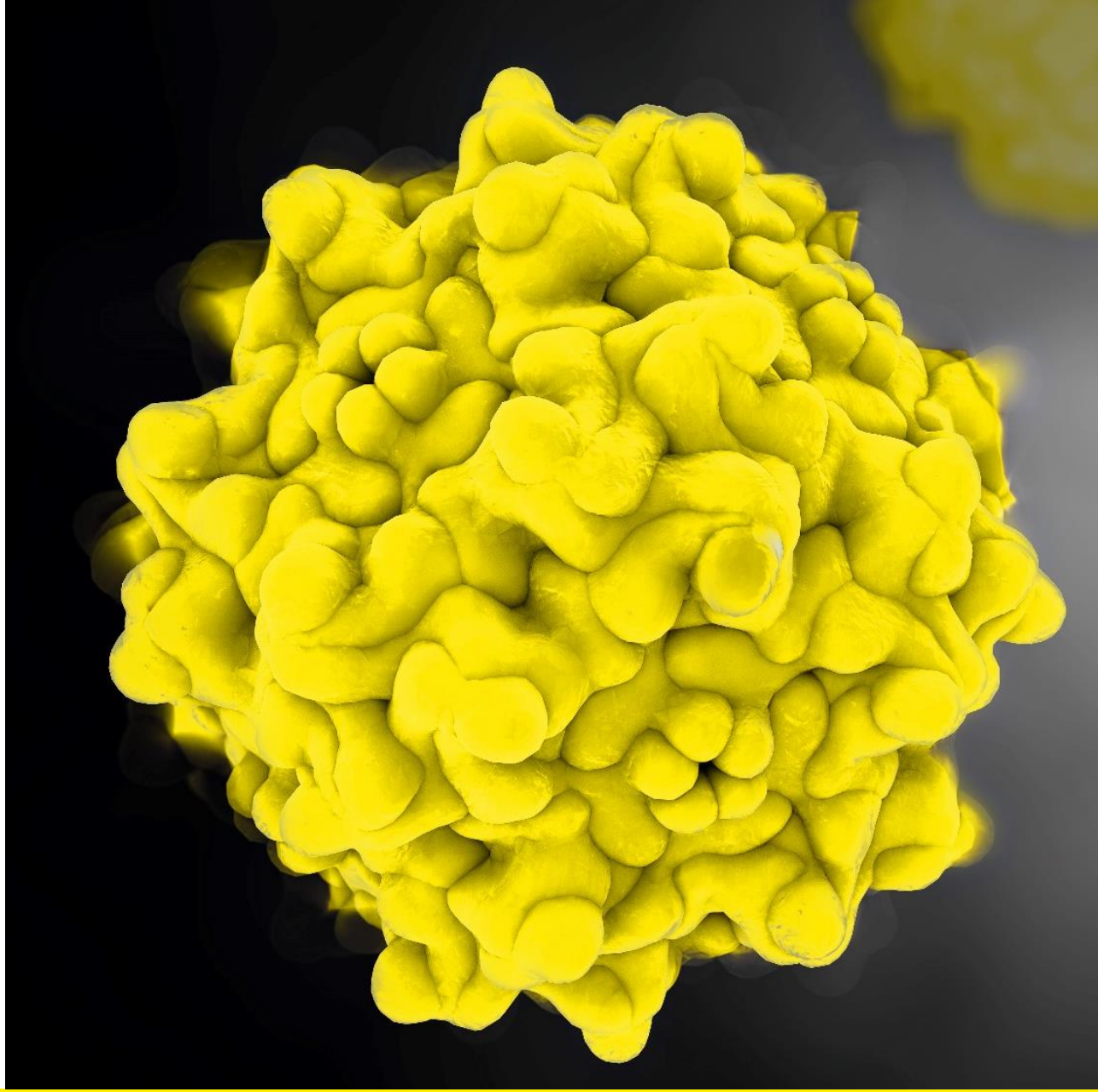
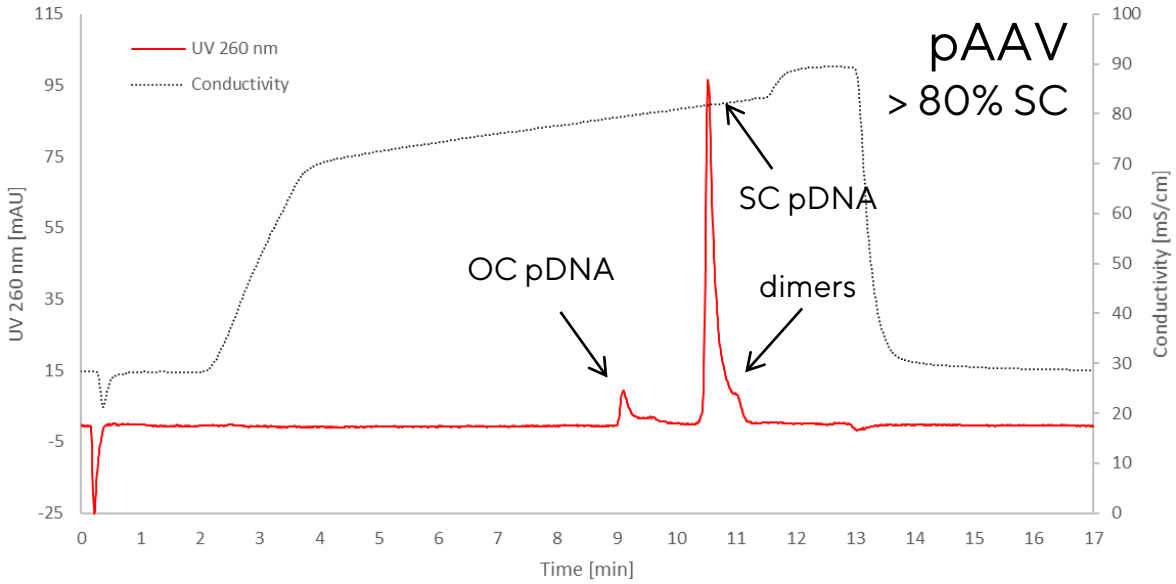


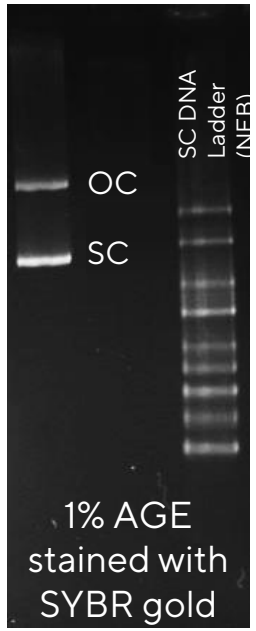
Plasmid DNA forms: OC/SC ratio in pDNA

Higher transfection efficiency is achieved with pure and highly supercoiled DNA.

Plasmid DNA was purified using CIMmultus DEAE and CIMmultus C4 columns without the SC enrichment step.

Samples were analysed by HPLC using CIMac pDNA column to detect the ratio between open circular (OC) and supercoiled (SC) forms.





Concentration (Nanodrop)	UV260/280	Endotoxins (LAL assay)
1,19 mg/mL	1,86	<2 EU/mg of pDNA

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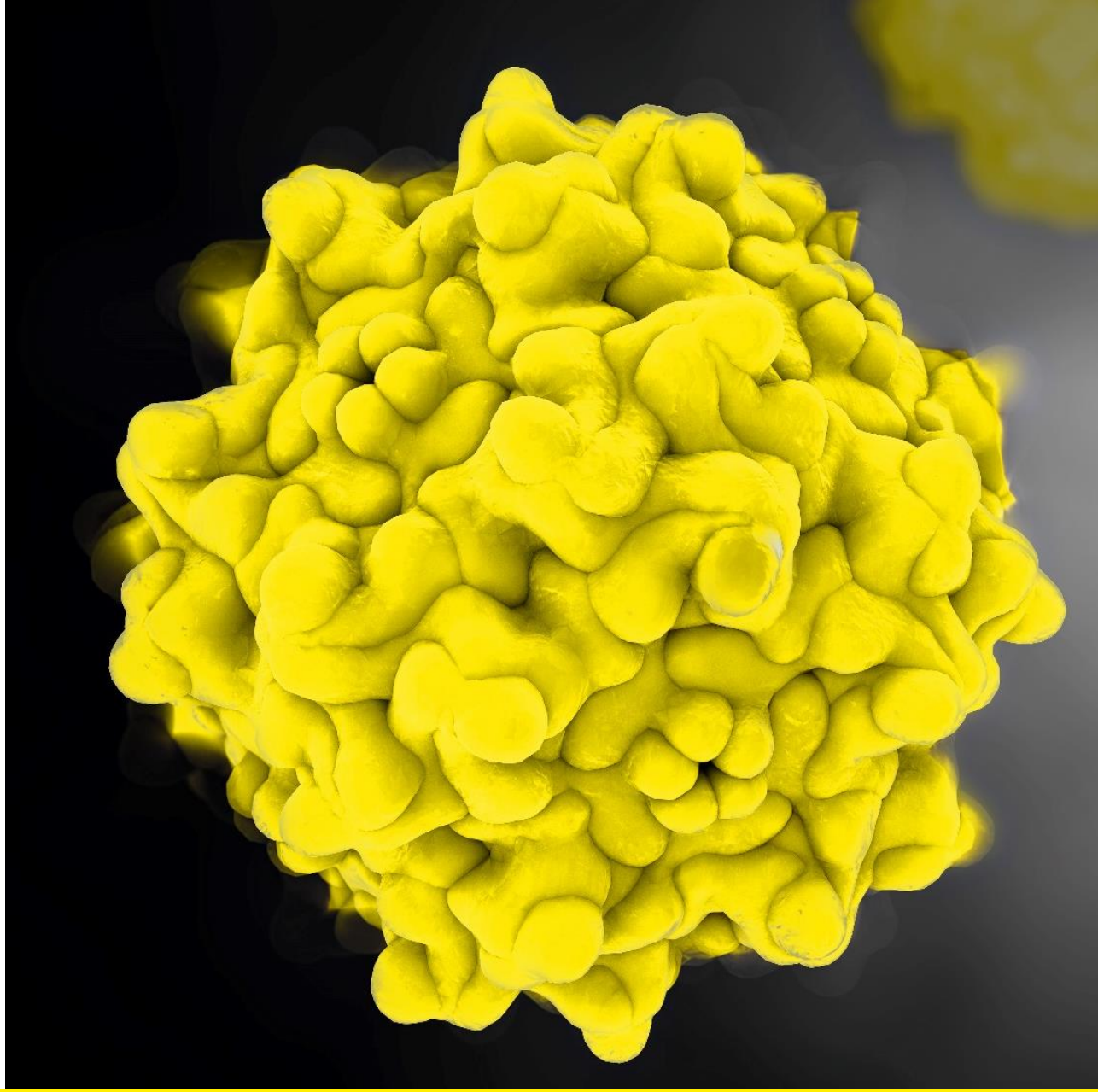
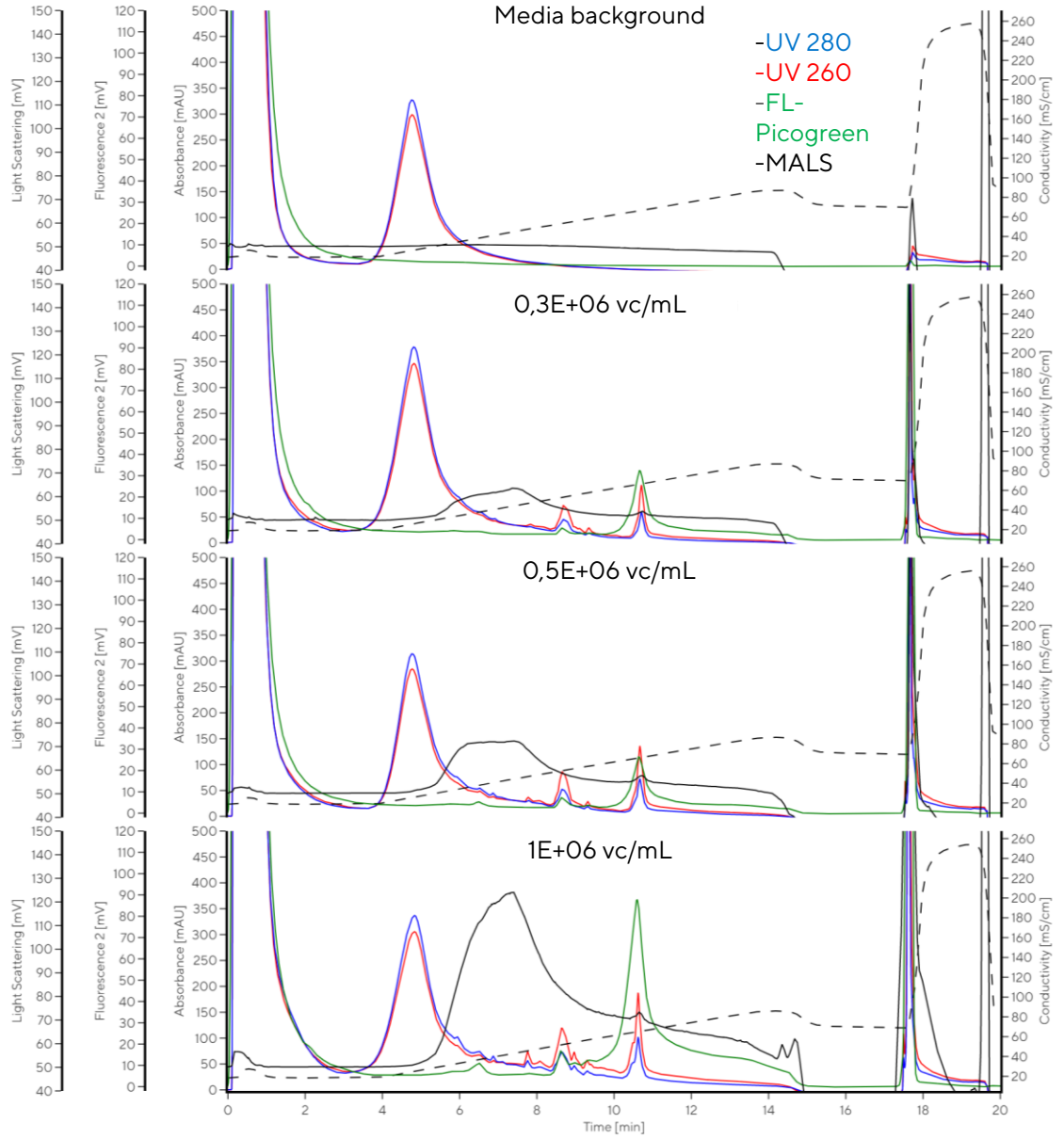


HPLC analytics: background

Suspension HEK cells were seeded at different density, collected after 4 days and lysed.

Samples were analysed by HPLC, using CIMac Adeno column.

This type of analytics shows distribution of impurities that can be expected in viral vector harvest.



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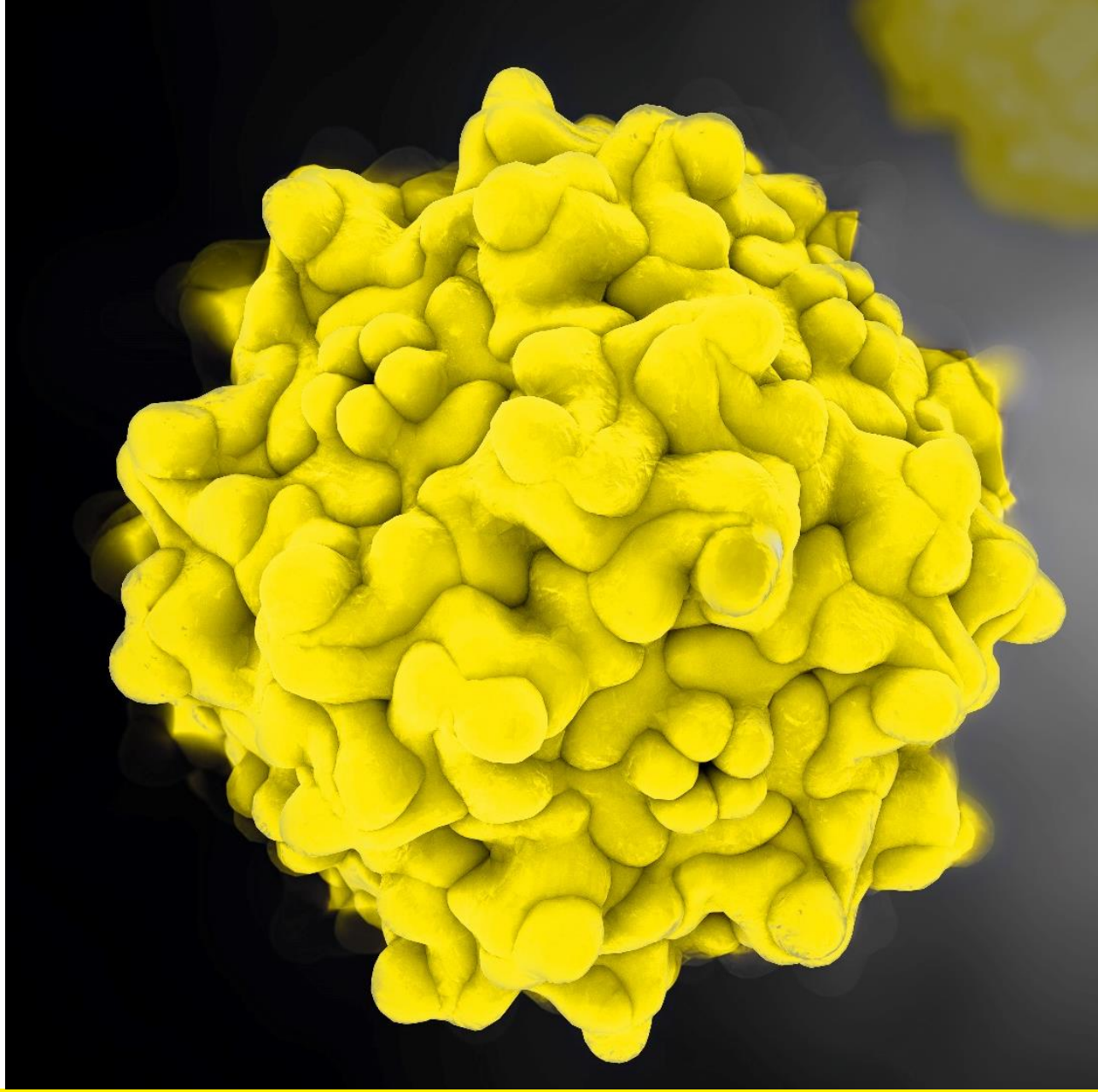


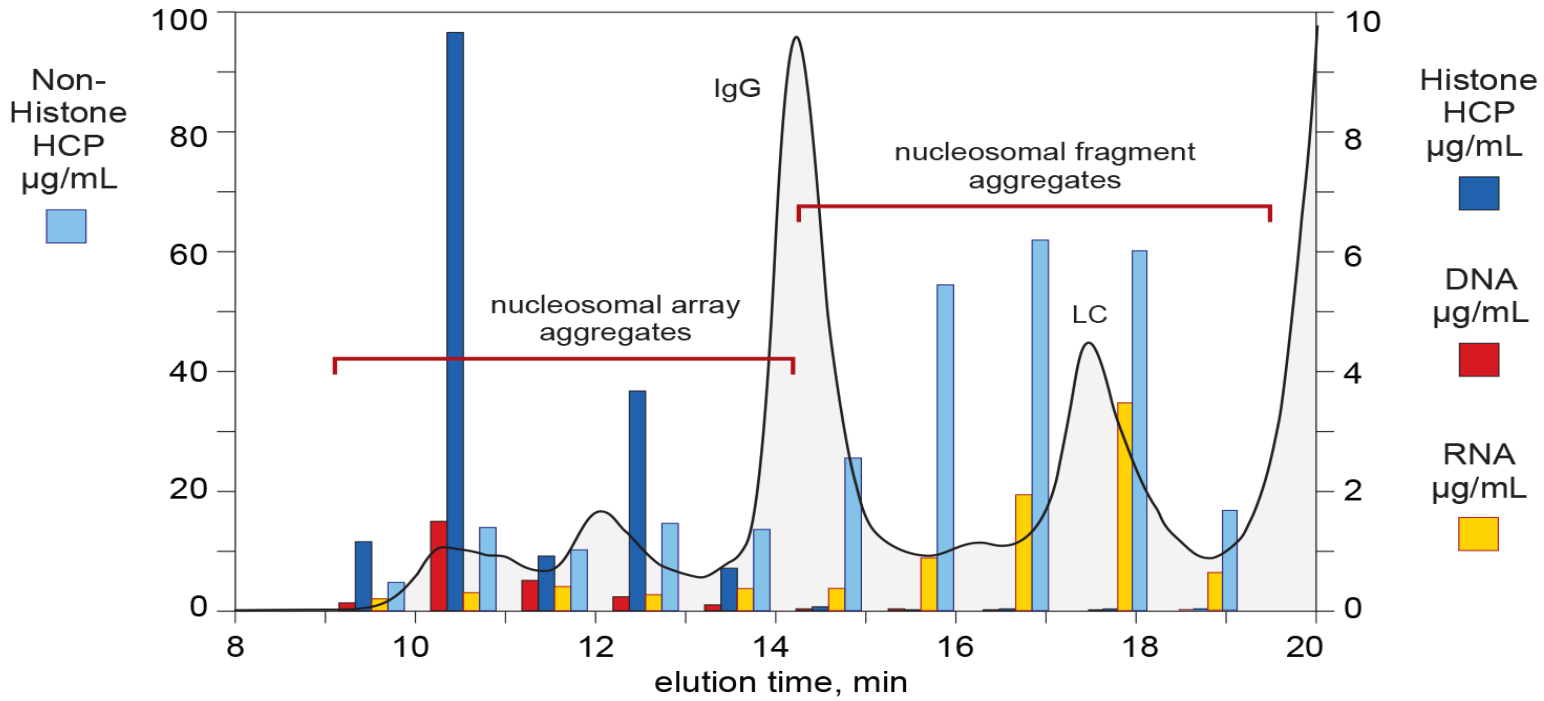
Comparison of AAV and mAb process: Host cell DNA (chromatin) is in low quantities compared to the product (IgG)

Filtered CHO Harvest containing prospective biosimilar Herceptin™

On the graph:

- Analytical SEC profile
- Host DNA by ddPCR
- Histones and other HCP by ELISA

Arrays and fragments both act as nucleation centers for accretion of non-nucleosomal proteins and RNA.

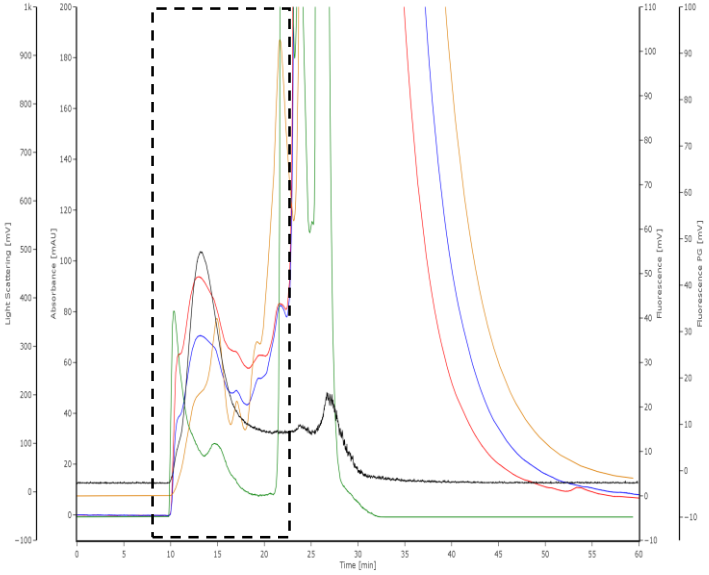


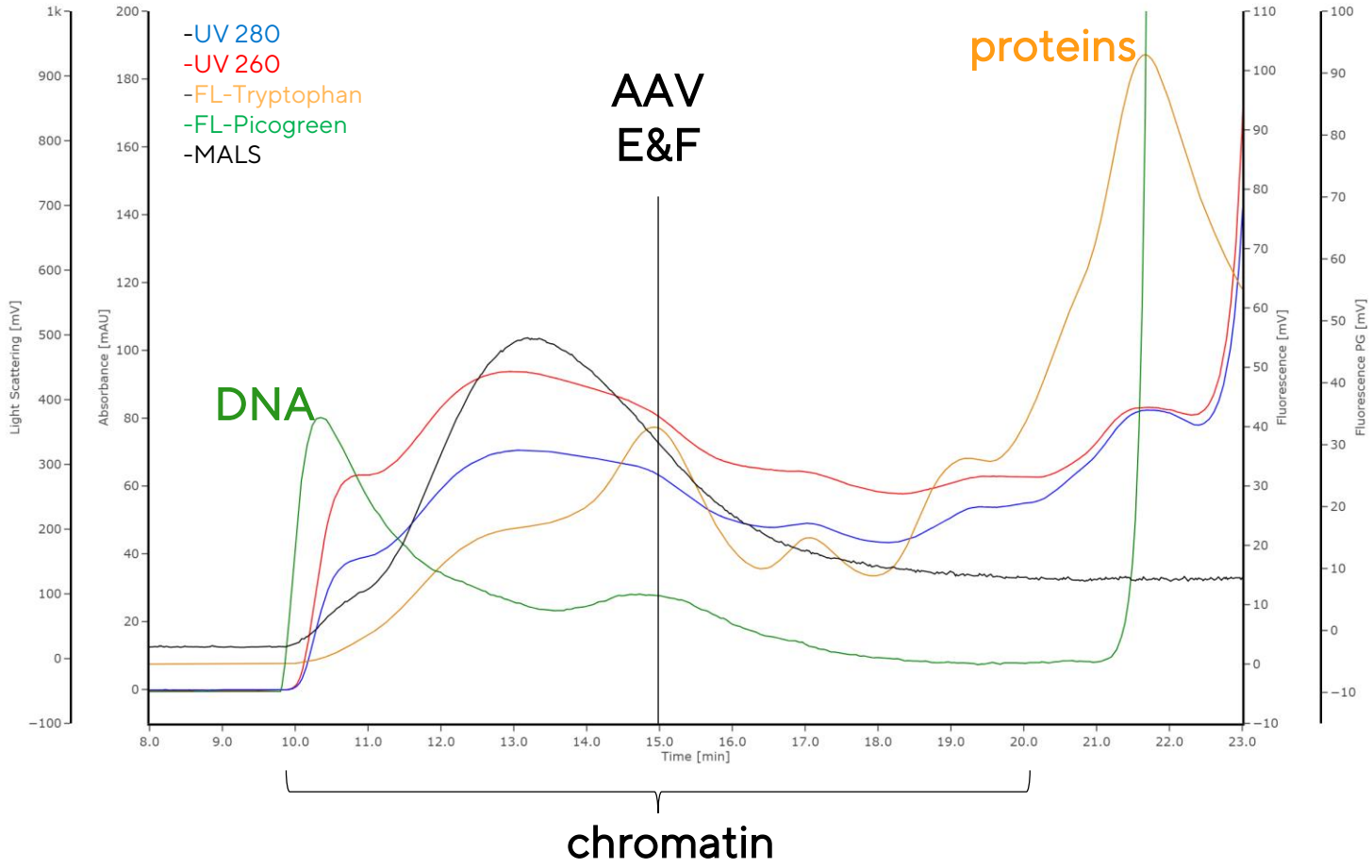
*Note the different scale for non-histone HCP.

Analytical HPLC: PATfix + SEC TSKgel™ G4000SWxl, 0.5 mL/min.

Comparison of AAV and mAb process: Low AAV concentration (compared to IgG) inflates the chromatin-to-product ratio

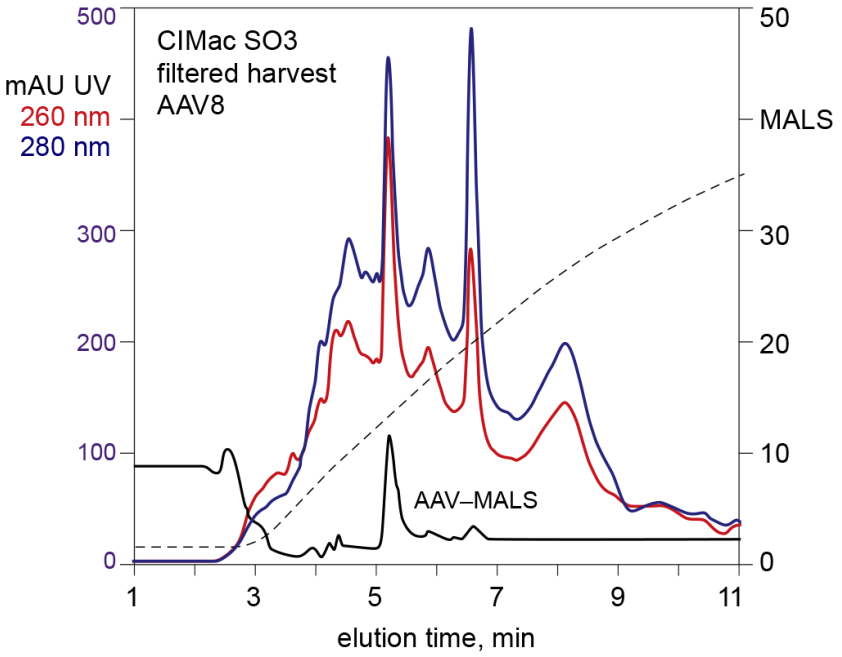
Sample prestained with Picogreen™. Intrinsic tryptophan fluorescence amplifies sensitivity for proteins about 20-fold over UV, and enables direct visualization of the AAV.

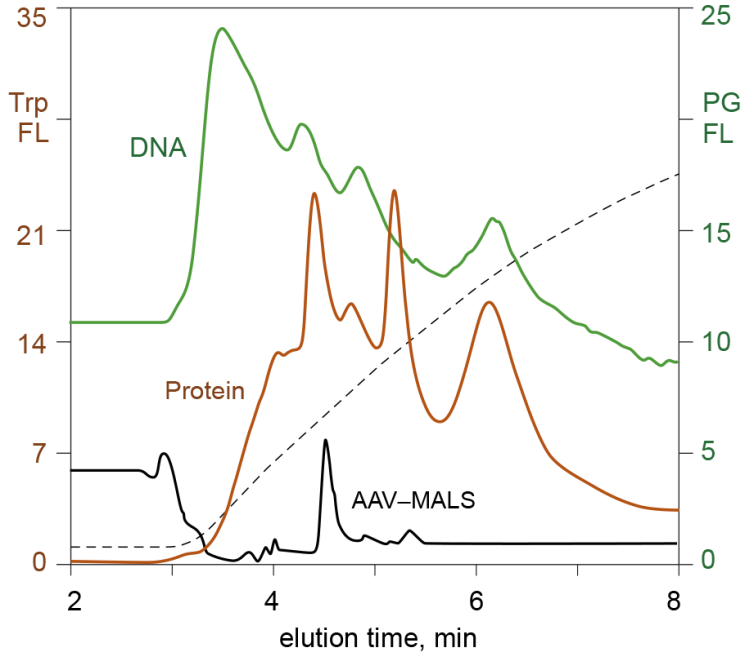




Cation exchange HPLC analytics for total AAV analytics

Cation exchange (CEX) provides fast characterisation of total AAV, supplemented with information about impurities (DNA & proteins):

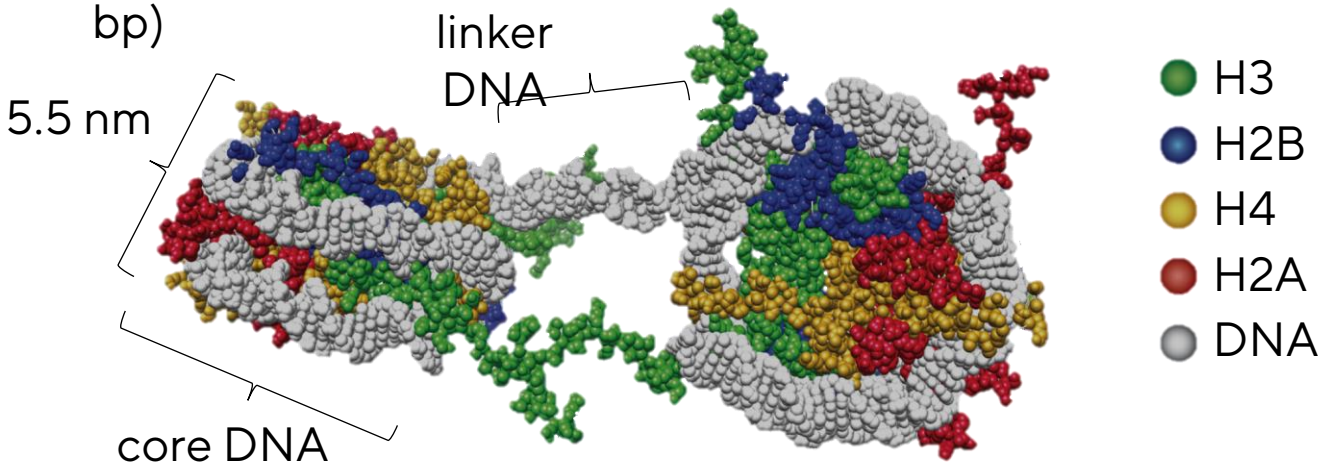




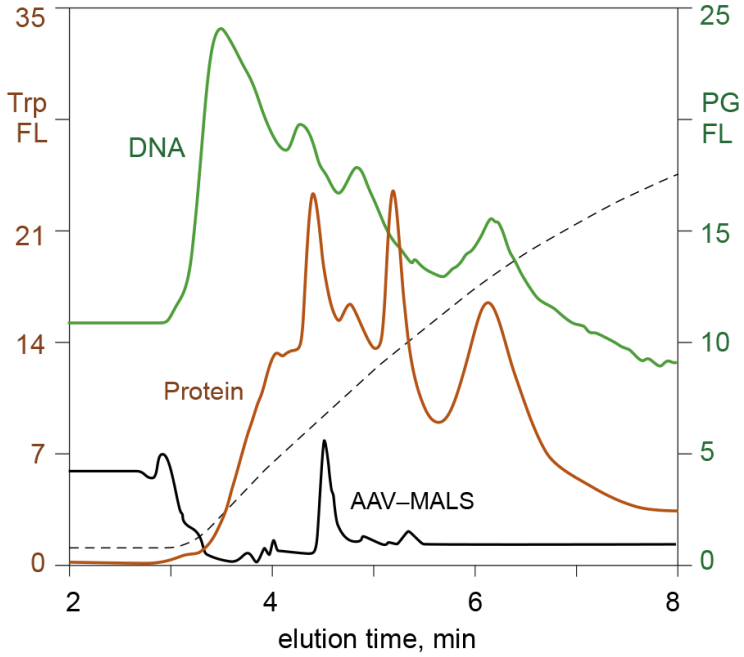
PATfix HPLC with multiple detectors allows for sample characterisation within an hour

Nucleosome – basic structural subunit of chromatin

Histone octamer with 1.6 turns of DNA (about 150 bp)



Filtered harvest (AAV8)
PATfix + CIMac SO3 column
Zoom in to elution gradient



- **Histones** are <u>extremely hydrophobic and highly positively charged</u>, 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 <u>extreme charge characteristics</u>. Both also participate in metal affinity, hydrogen bonding, and van der Waals interactions.

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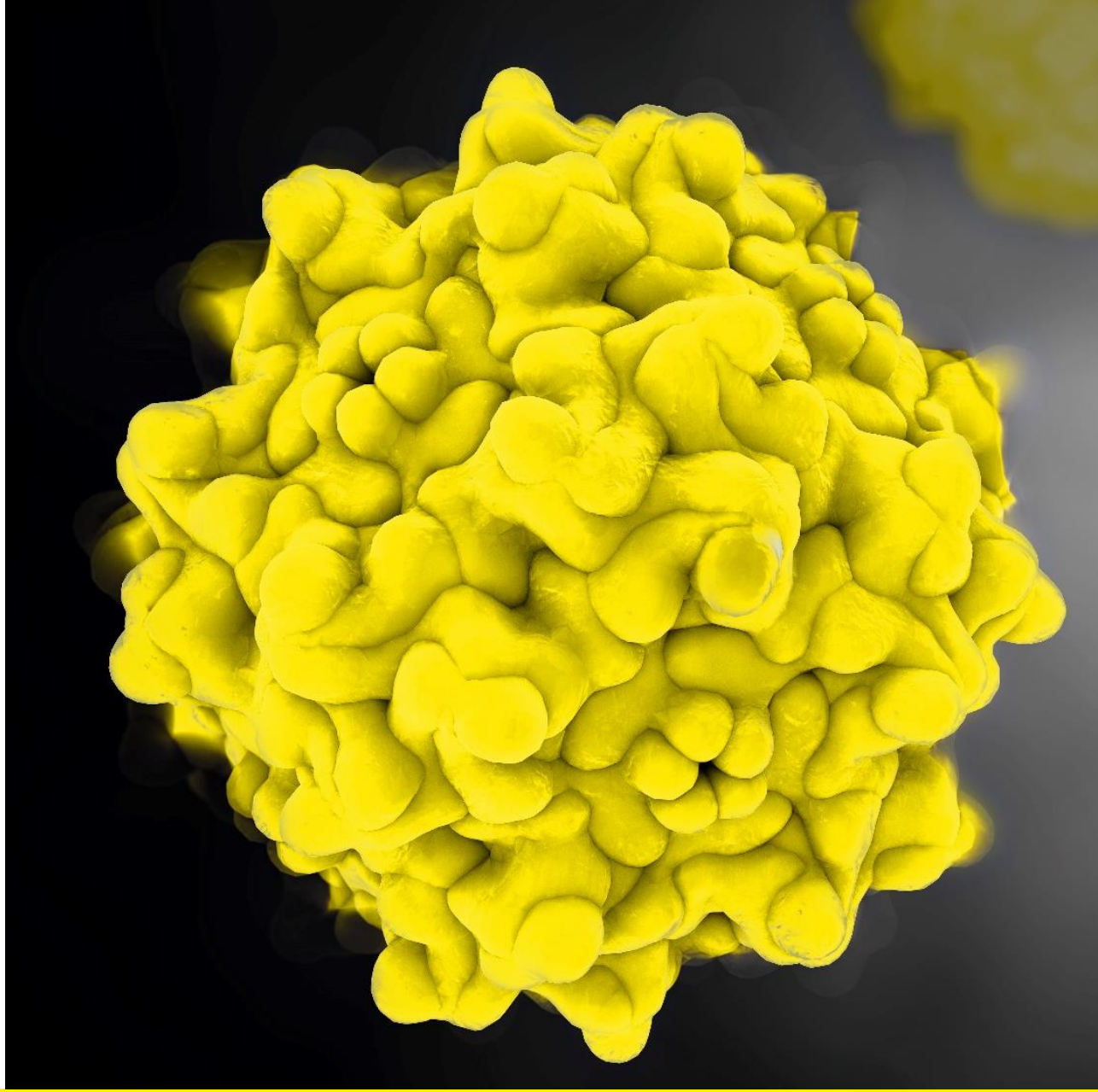
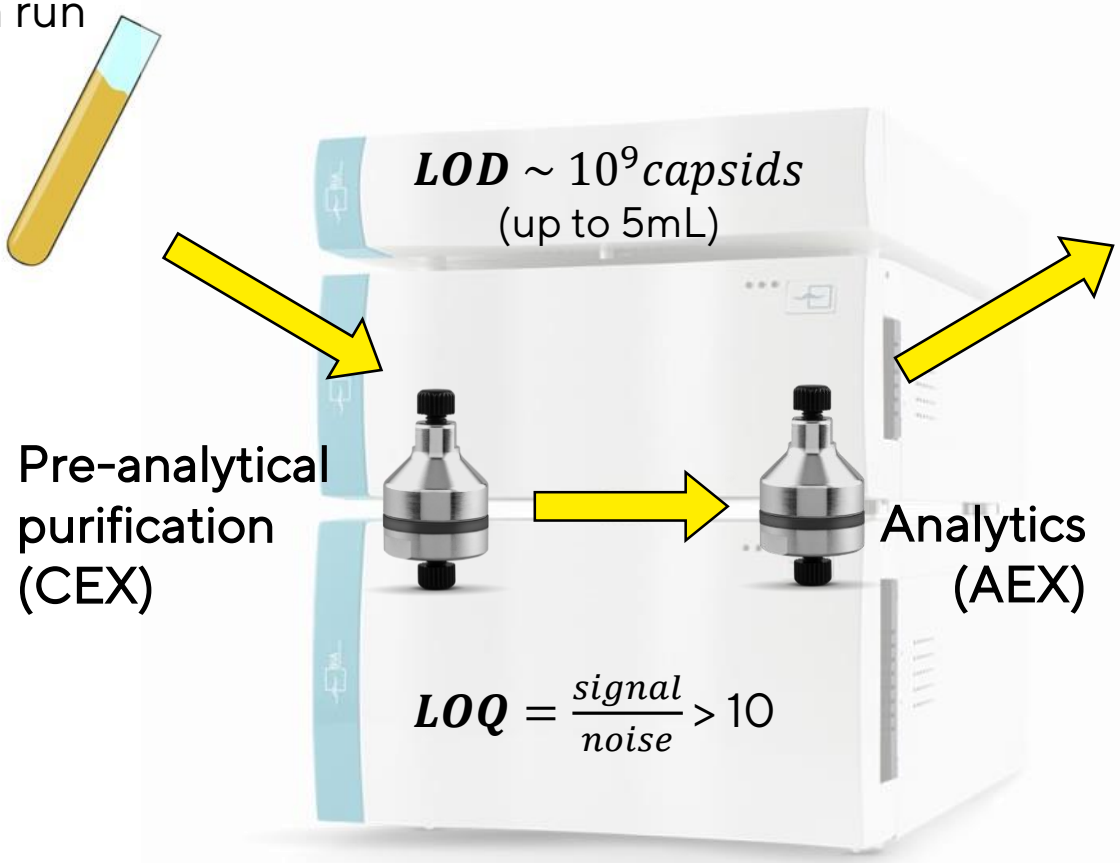
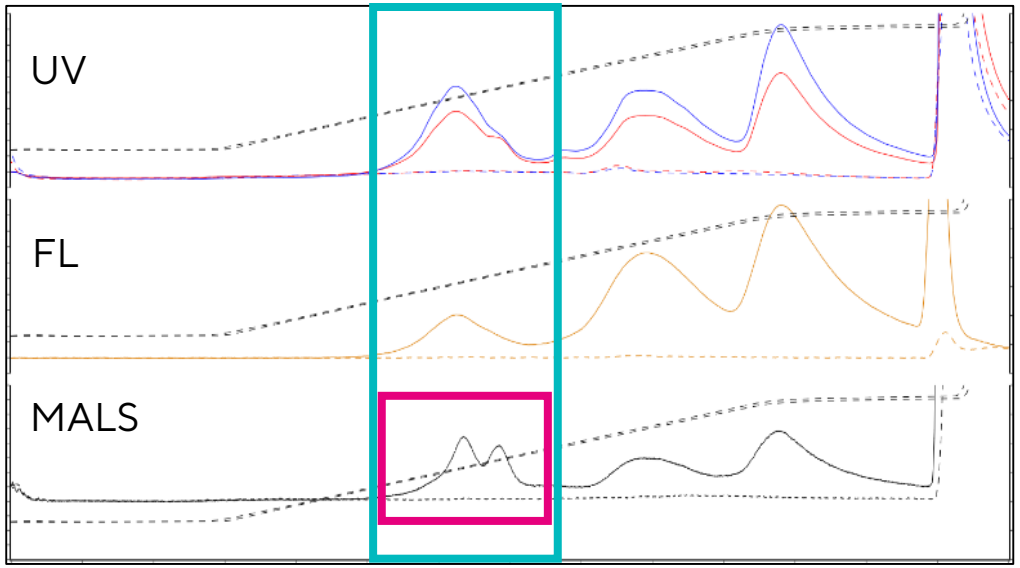


PATfix column switching method

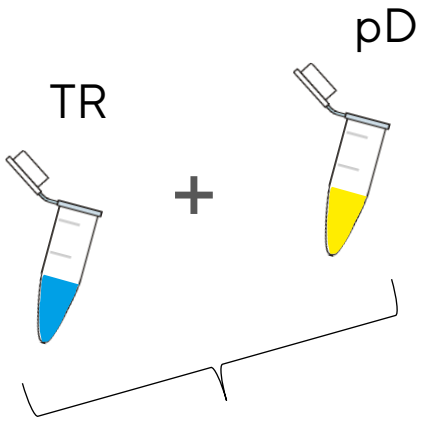
Sample from PD or production run





- E/F ratio
- Relative titer (based on **MALS**)
- Absolute titer (calibration curve needed)
- Additional info from various detectors (UV 260nm, UV 280nm, Tryptophan, Picogreen)

AAV USP: testing transfection reagents (TR)



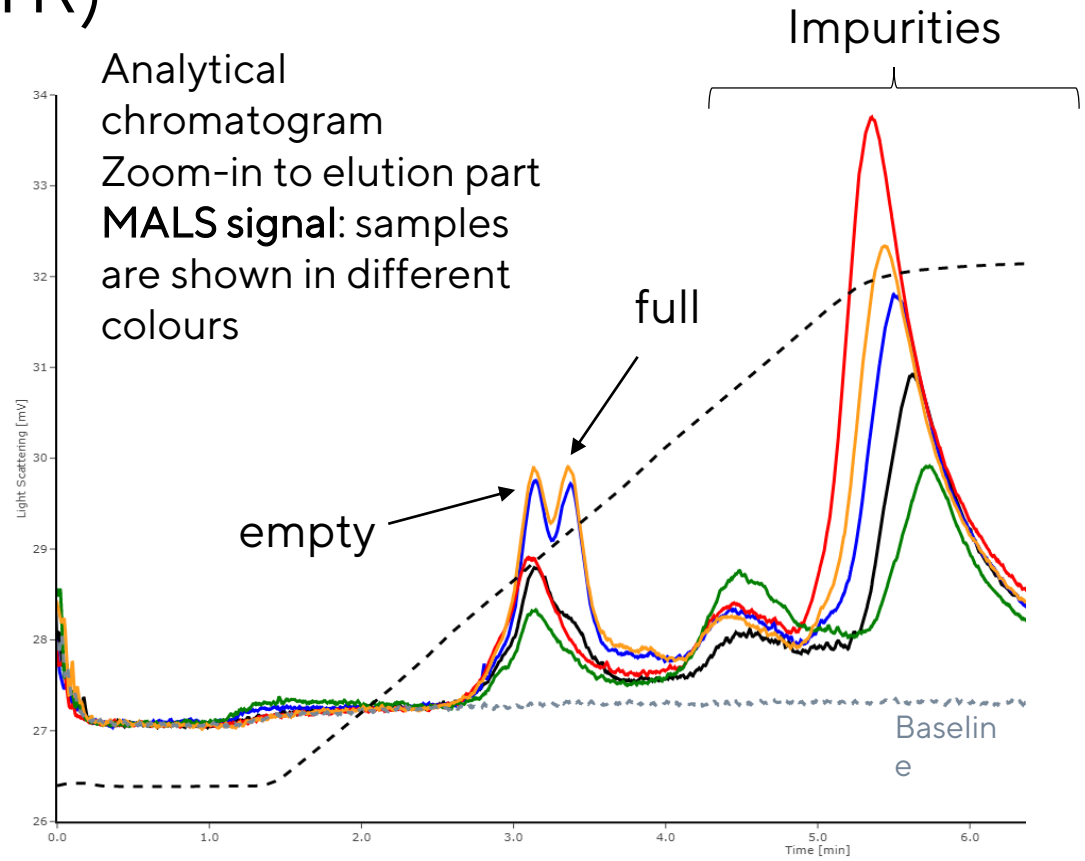
Complex

Transfected HEK293

After 72h

* Cells and media were collected **together and lysed**

Analytical chromatogram
Zoom-in to elution part
MALS signal: samples are shown in different colours



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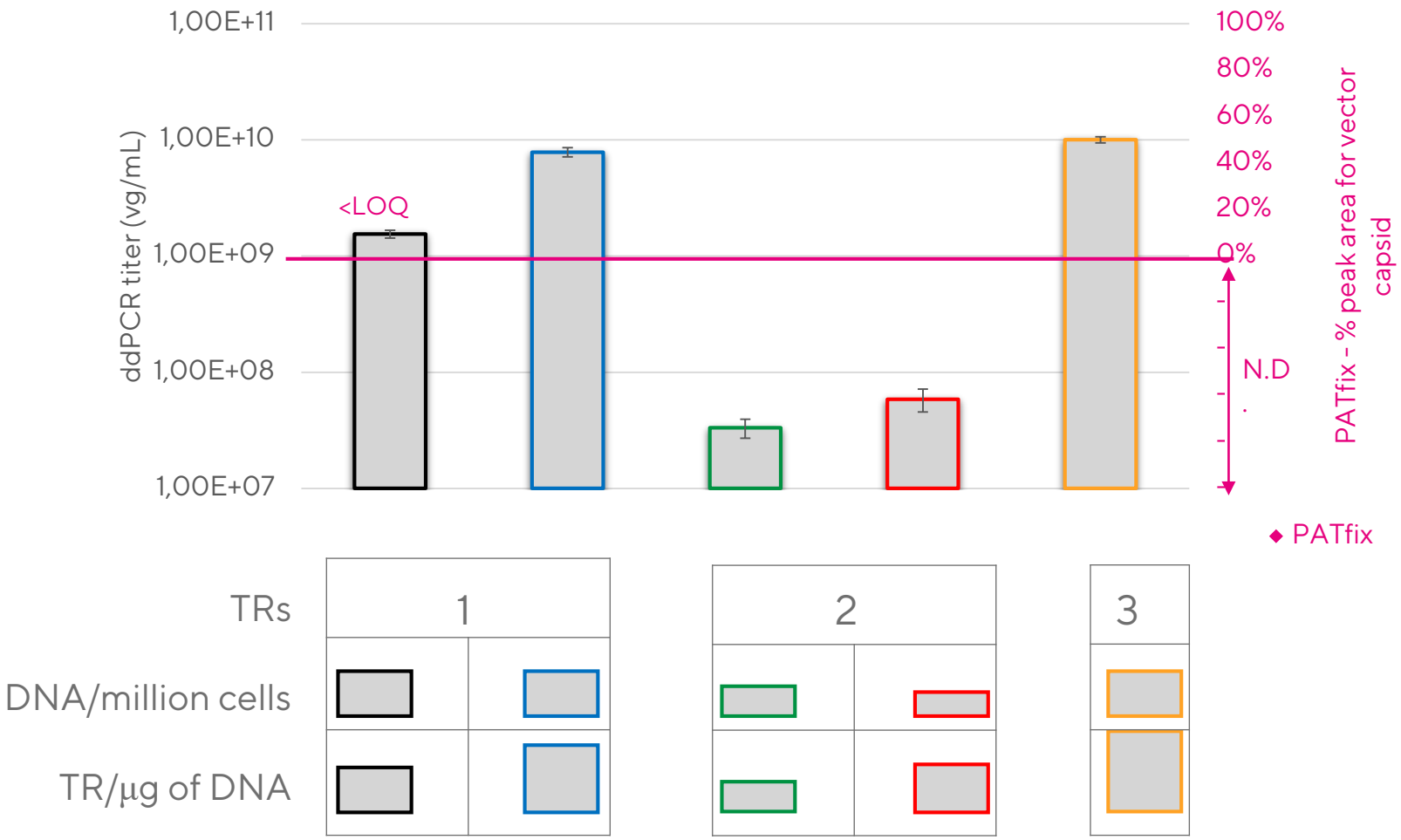
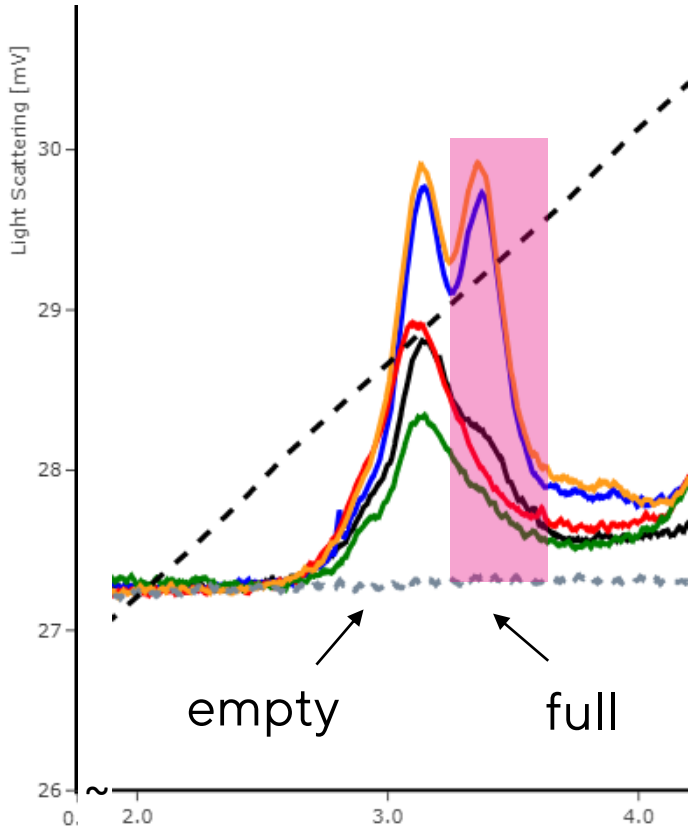
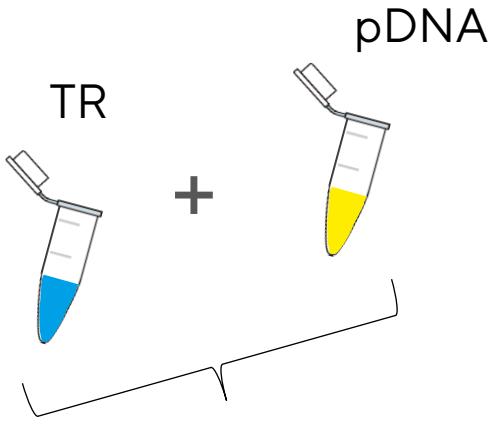




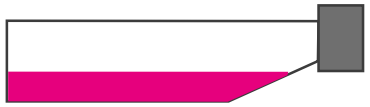
Analytical chromatogram
Zoom-in to elution part
MALS signal: samples are shown in different colours

AAV USP: E/F AAV production kinetics



Complex

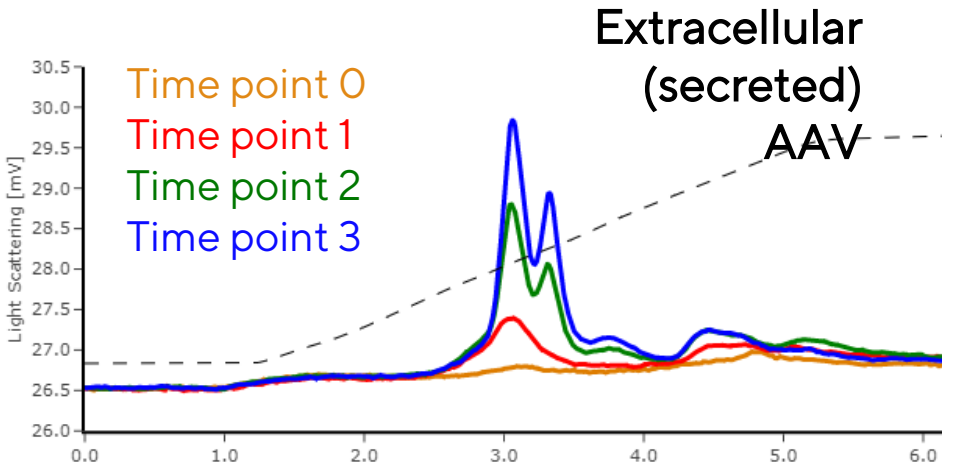
Transfected HEK293

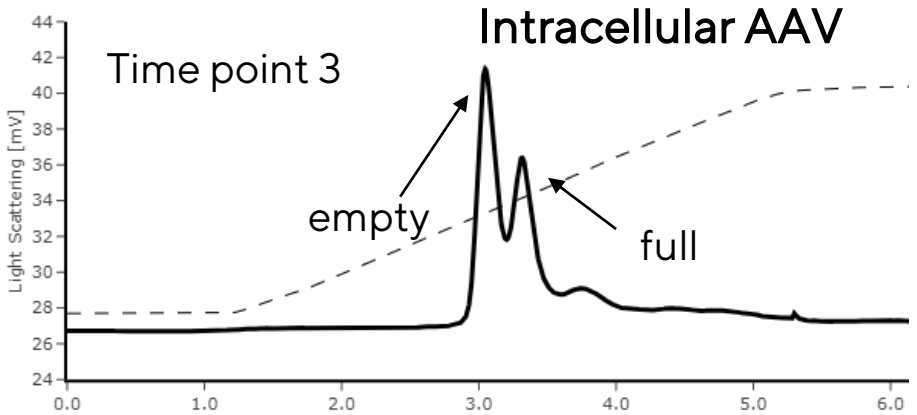


Analytical chromatograms
Zoom-in to elution part
MALS signal: time points are shown in different colours

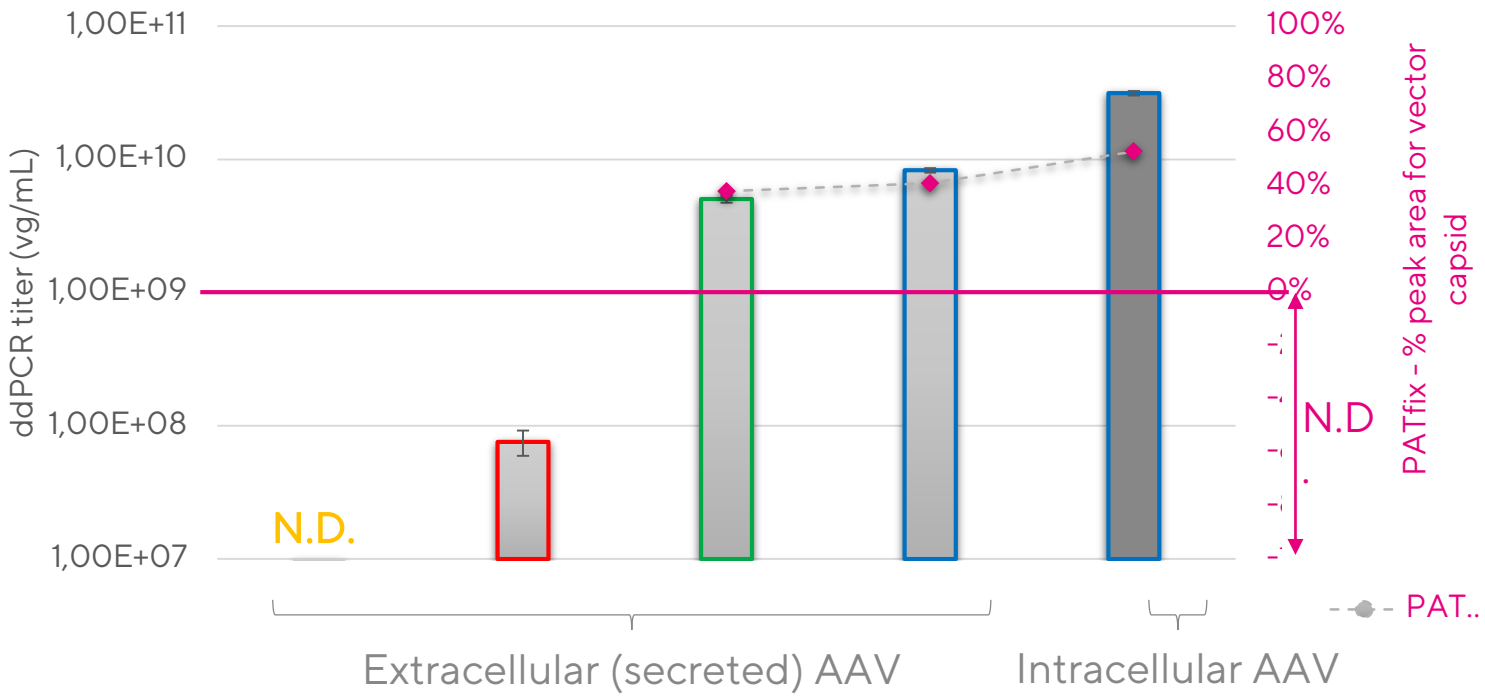
Different time points

* Cells and media were collected **separately and lysed**





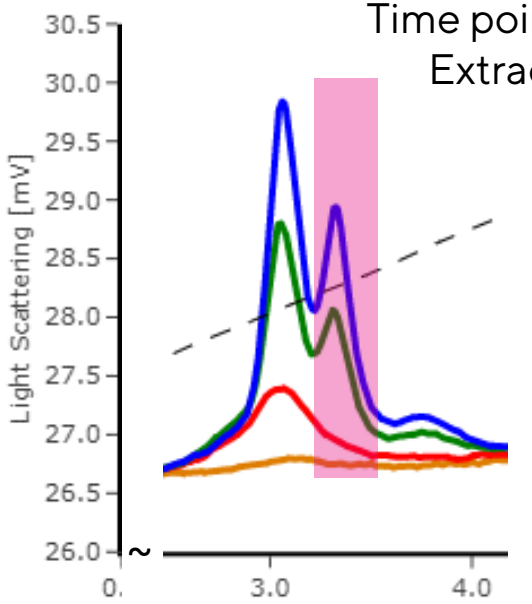
AAV USP: E/F AAV production kinetics



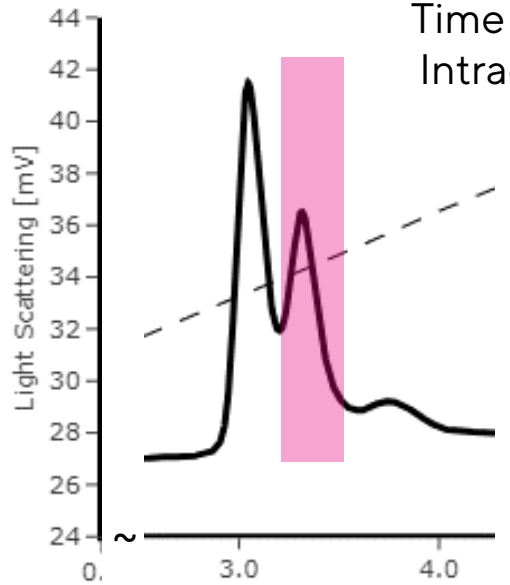
- ❑ Time point 0
- ❑ Time point 1
- ❑ Time point 2
- ❑ Time point 3

Analytical chromatogram, Zoom-in to elution part
MALS signal: samples are shown in different colours

Time points 0-3
Extracellular AAV

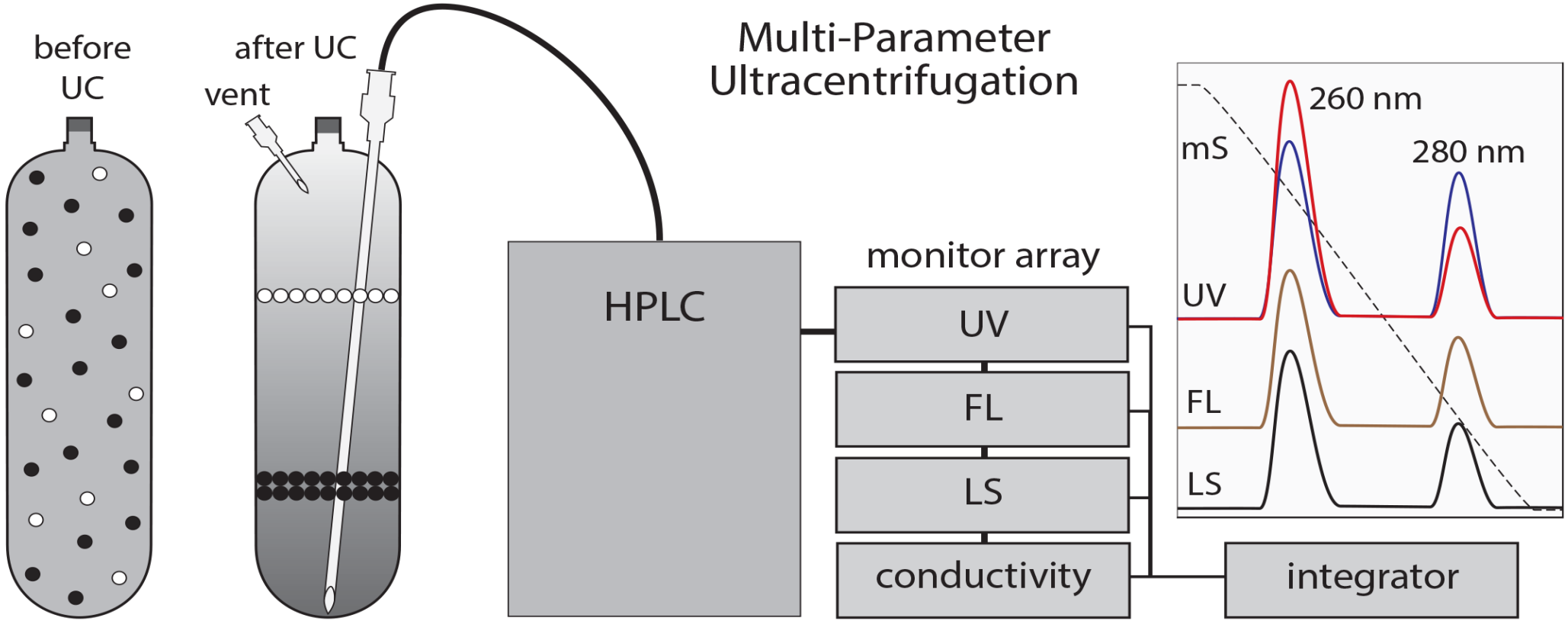


Time point 3
Intracellular AAV



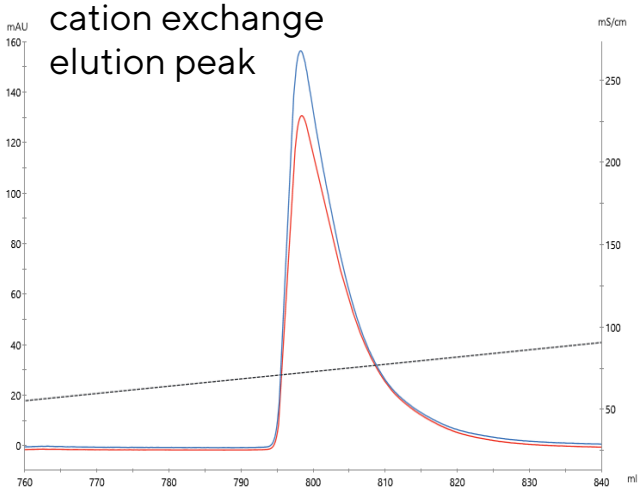
Centrifugram – analysis of ultracentrifuge fractions by HPLC detectors

Density gradient fractionation followed by stratigraphic analysis through an HPLC detector array:

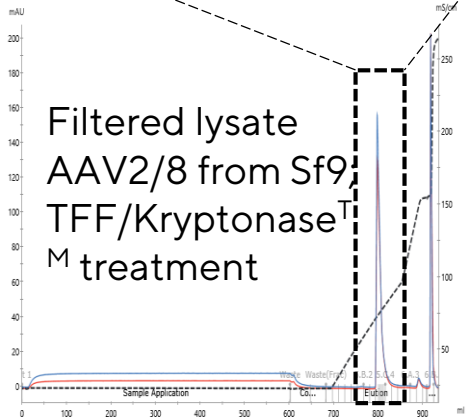


Centrifugram – analysis of ultracentrifuge fractions by HPLC detectors

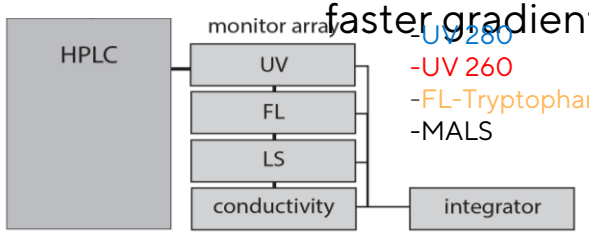
cation exchange elution peak



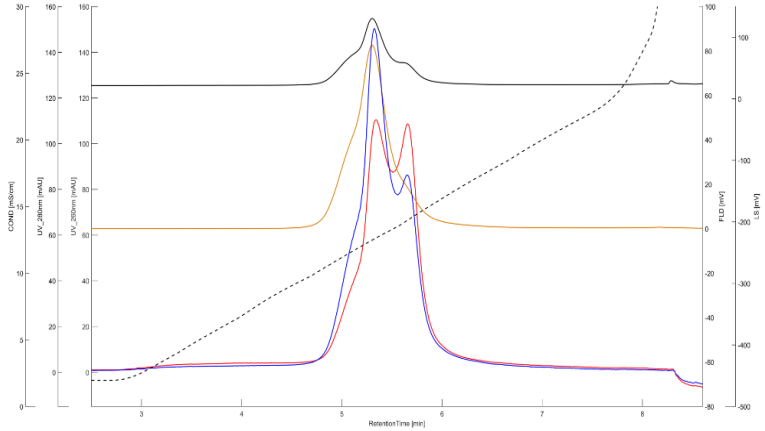
Filtered lysate AAV2/8 from Sf9; TFF/Kryptonase™ treatment



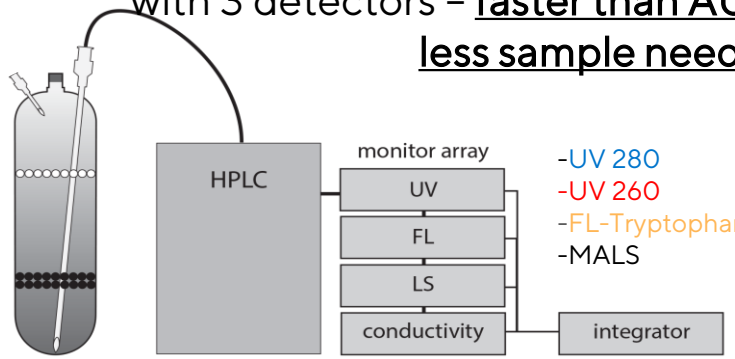
Chromatogram: HPLC with 3 detectors using CIMac QA column – faster gradient



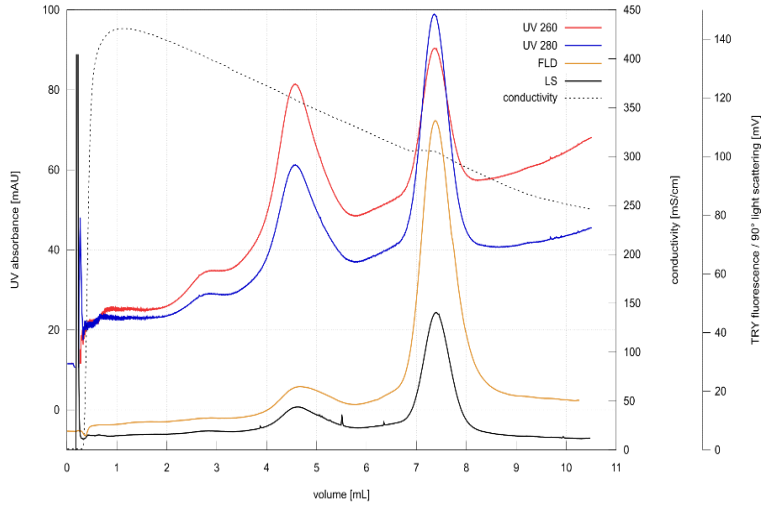
-UV 280
-UV 260
-FL-Tryptophan
-MALS



Centrifugram: CsCl ultracentrifuge with 3 detectors – <u>faster than AUC, less sample needed</u>



-UV 280
-UV 260
-FL-Tryptophan
-MALS



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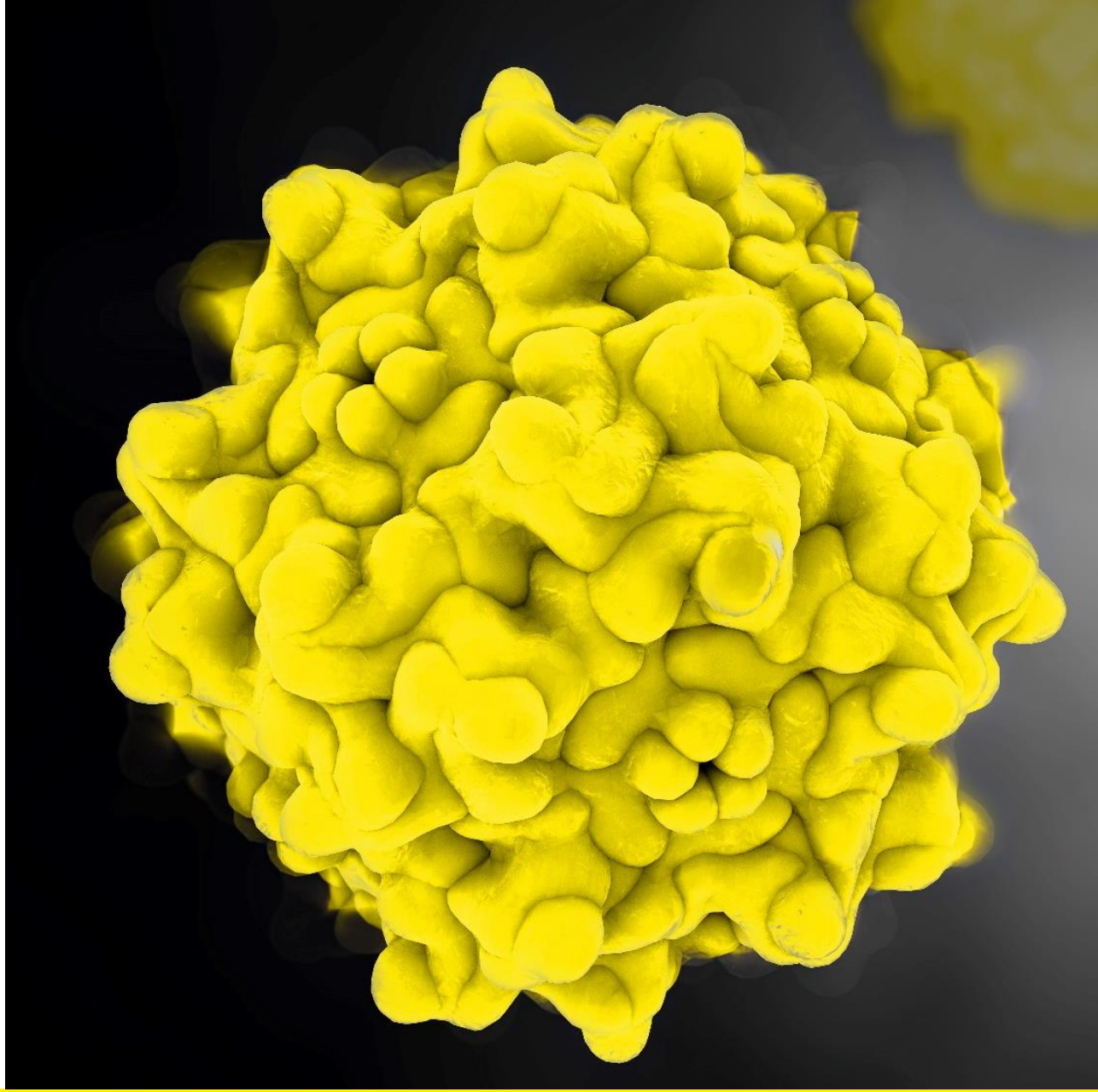

Conclusions

PATfix HPLC system combined with CIMac analytical columns has a wide range of application during AAV process, from **quality control of pDNA** used in transfection, to monitoring **AAV quantities** and **impurity content.**

Coupling of Ultracentrifuge with HPLC detectors gives additional insights on different AAV particle populations.

Novel PATfix column switching method can be used for E/F analytics and determining expression profile early at upstream and downstream PD stage, saving time and operating with small volumes

Orthogonal methods suitable for complex samples should be used in optimization of AAV expression:

- CEX analytics for total AAV
- ddPCR for vg
- UC (combined with HPLC) and PATfix switch for E/F ratio

Special thank you to:

BIA Separations Departments

- DNA and mRNA Process Development (PC2)
- Process Development for Viral Vectors and Vaccines (PC3)
- HPLC Analytical Method Development (PC4)

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  Belfer Gene Therapy Core Facility,
  Department of Genetic Medicine,
  Weill Medical College of Cornell University
- The University of Nantes,
  Center for Translational Therapy for Genetic Diseases
- ICGEB
- Cobik

Combining the sensitivity of the PATfix HPLC platform with the resolution of ultracentrifugation for AAV characterization

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* Corresponding author: blaz.bakalar@biaseparations.com

Open Access Article

Multiple-Monitor HPLC Assays for Rapid Process Development, In-Process Monitoring, and Validation of AAV Production and Purification

by Pete Gagnon *, Blaz Goricar, Nina Mencin, Timotej Zvanut, Sebastijan Peljhan, Maja Leskovec and Ales Strancar

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* Author to whom correspondence should be addressed.

Pharmaceutics **2021**, *13*(1), 113; https://doi.org/10.3390/pharmaceutics13010113

DNA Vaccines pp 167-192 | Cite as

Scale-Up of Plasmid DNA Downstream Process Based on Chromatographic Monoliths

Authors Authors and affiliations

Urh Černigoj, Aleš Štrancar

Research Article

Guanidine improves DEAE anion exchange-based analytical separation of plasmid DNA

Urh Černigoj, Jana Vidič, Ana Ferjančič, Urša Sinur, Klemen Božič, Nina Mencin, Anže Martinčič Celjar, Pete Gagnon, Aleš Štrancar,

First published: 26 September 2021 | https://doi.org/10.1002/elps.202100210

Thank you for your attention!

PDF presentation available from:
https://www.biaseparations.com/en/library/seminars-webinars/1137/optimization-of-the-aav-expression-using-dedicated-hplc-system

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