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BIA separations

Simplifying Progress

Acceleration of AAV manufacturing process development by using fast USP and DSP HPLC analytics

Aleš Štrancar

BPI, September 28, 2021

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 and for 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.

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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

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Expert DSP Bioprocess Knowledge

>30 pDNA, mRNA, virus DSP cGMP processes tech transferred to CMOs, sponsors, including Corona.

Product impurities are one of the key reasons for treatment side effects. High purity is therefore mandatory for product safety.

- pDNA including Corona, purity is THE key for better transfection and purer mRNA
- Minicircle DNA (shorten the pDNA)
- ssRNA and dsRNA, platform process from E.coli to mRNA including Corona
- Adeno virus, more than 20 years experience, including Corona
- AAV (all serotypes, > 20 tested)
- Influenza virus (all serotypes)
- Vaccinia/MVA
- Exosome
- Bacteriophage
- VLPs including Flu and Corona
- IVIG
- IgM and many more

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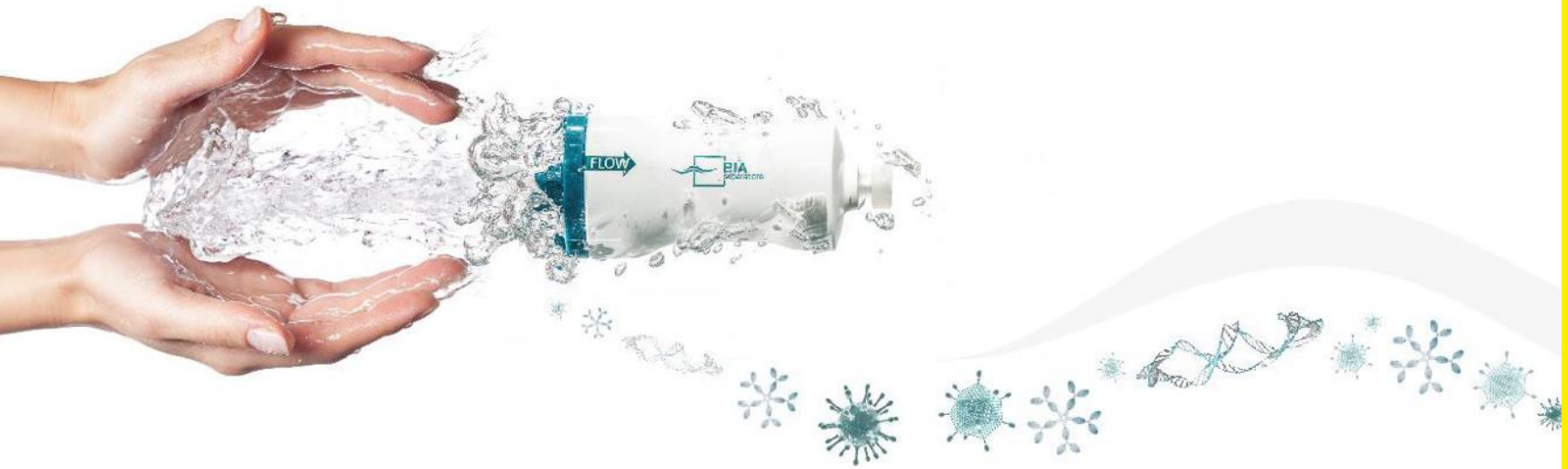

PATfix™ Fast method development and in-process control HPLC system with unique software

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CIMac analytical columns for PAT HPLC - no carry over of contaminants or viruses

Available:

- CIMac™ QA
- CIMac™ DEAE
- CIMac™ SO3
- CIMac™ EDA
- CIMac™ pDNA
- CIMac™ Adeno
- CIMac™ AAV empty/full

Soon to come:

- CIMac™ AAV total
- CIMac™ Lenti
- CIMac™ Vaccinia

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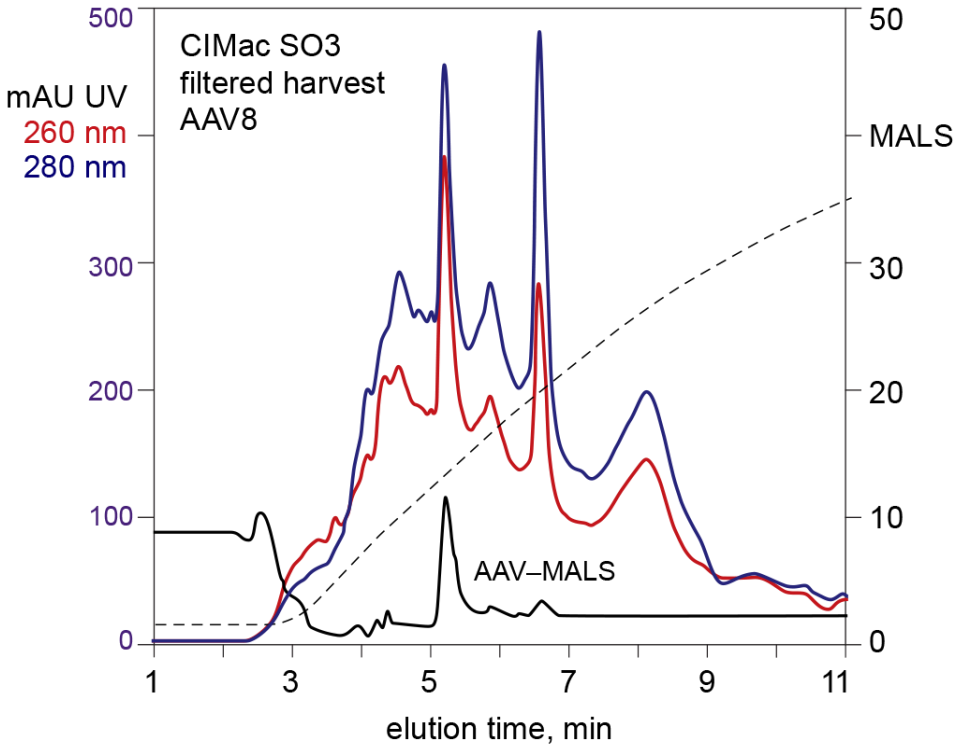
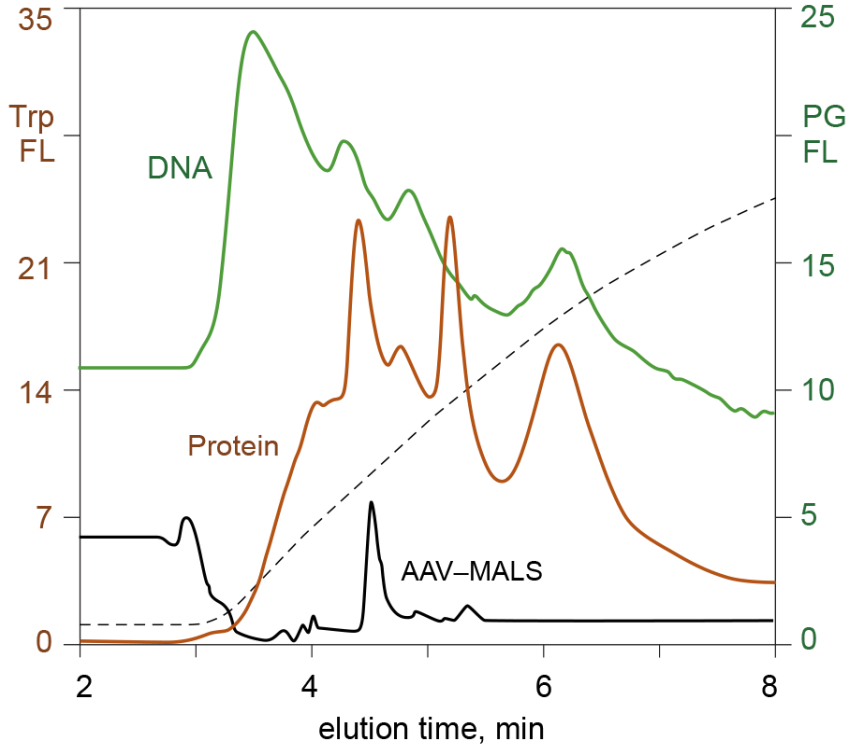
To enable fast process development high performance analytics is mandatory

PATfix HPLC with multiple detectors allows for sample characterisation in an hour.





PATfix and CIMac ➔
Process understanding

Cation exchange does not discriminate empty from full capsids but it still provides fast characterization of total AAV and contaminant content. UV wavelength ratios provide a hint about relative DNA and protein distribution but **fluorescence enables direct quantitative comparison**.

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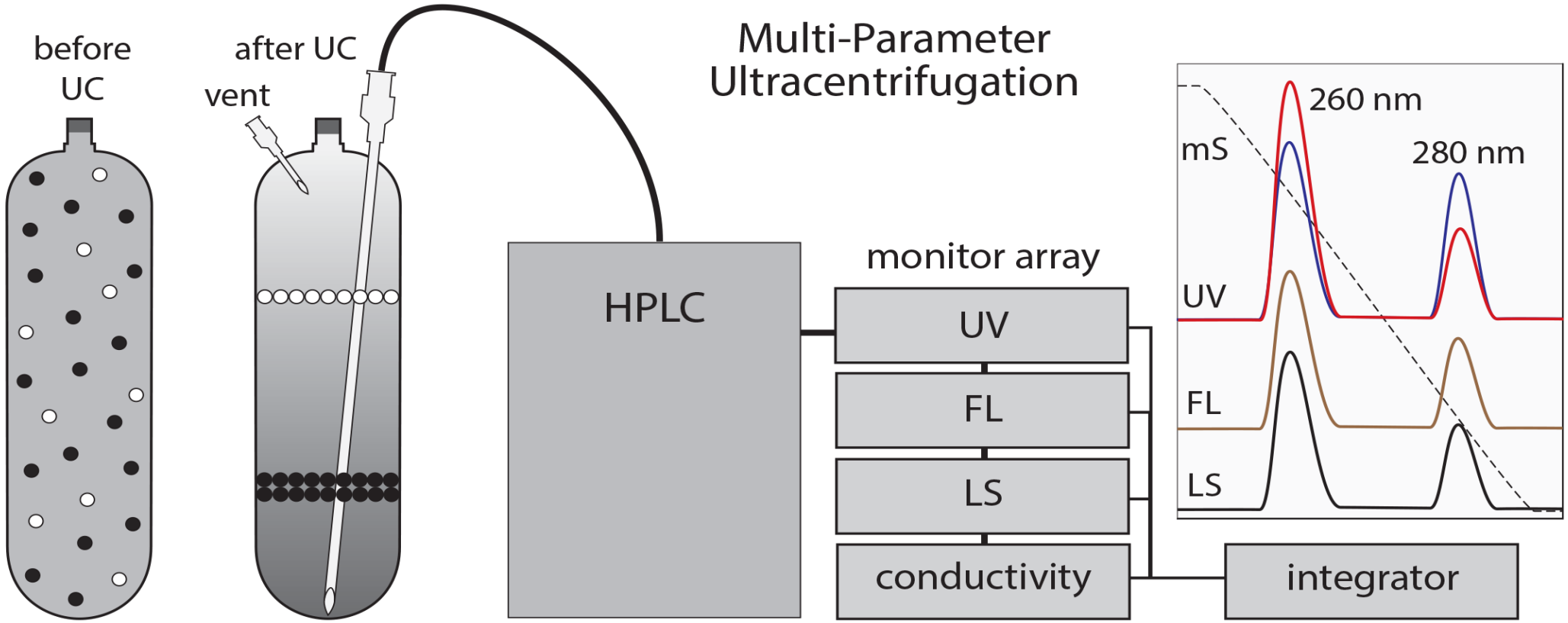
Centrifugram – analysis of ultracentrifuge fractions by HPLC detectors

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



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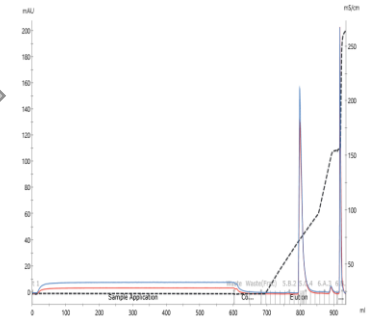
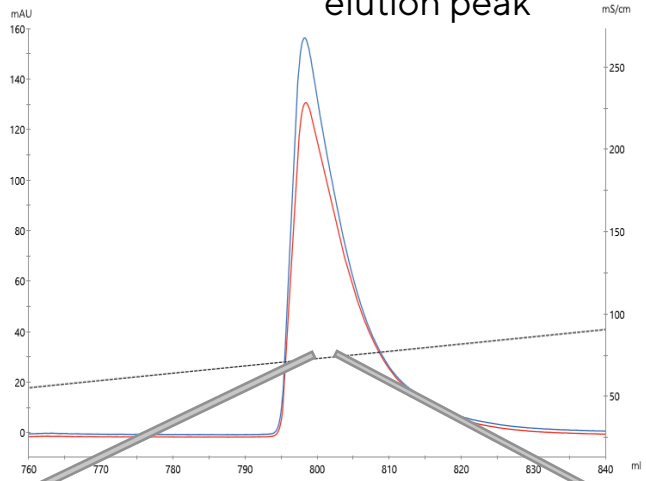
Orthogonal tools to study AAV particles distribution – HPLC : ultracentrifuge

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



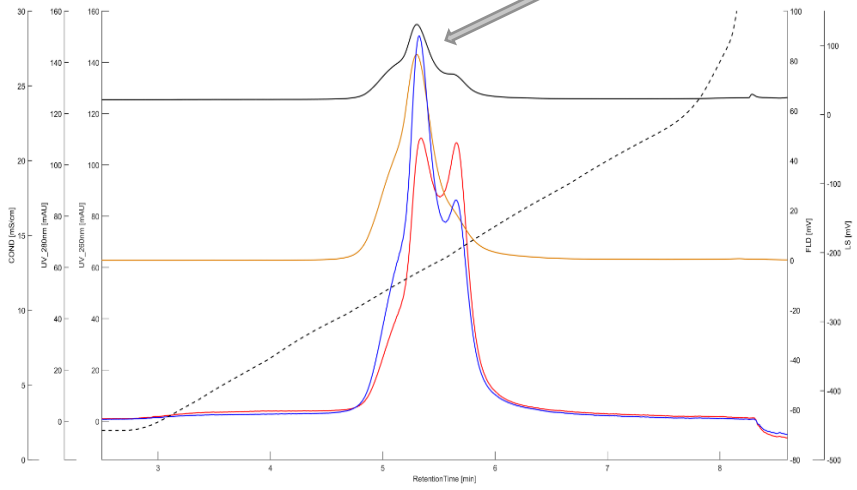
Zoom-in

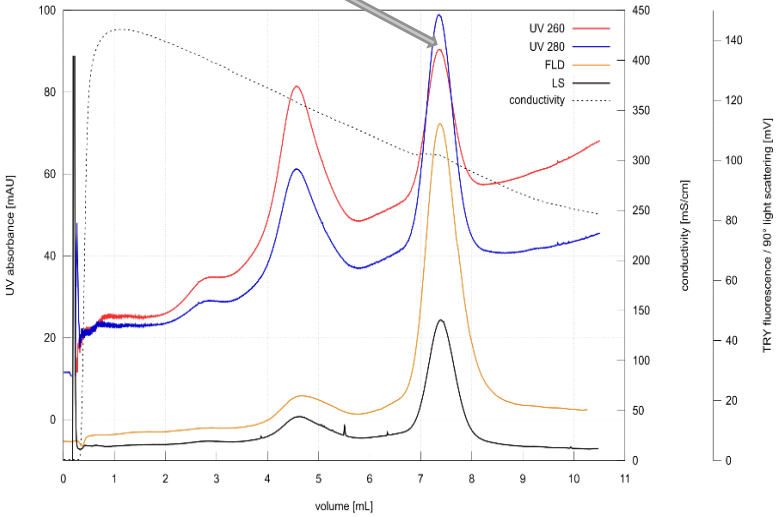
cation exchange elution peak



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**

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Analysis od USP samples by using columns switching PATfix system

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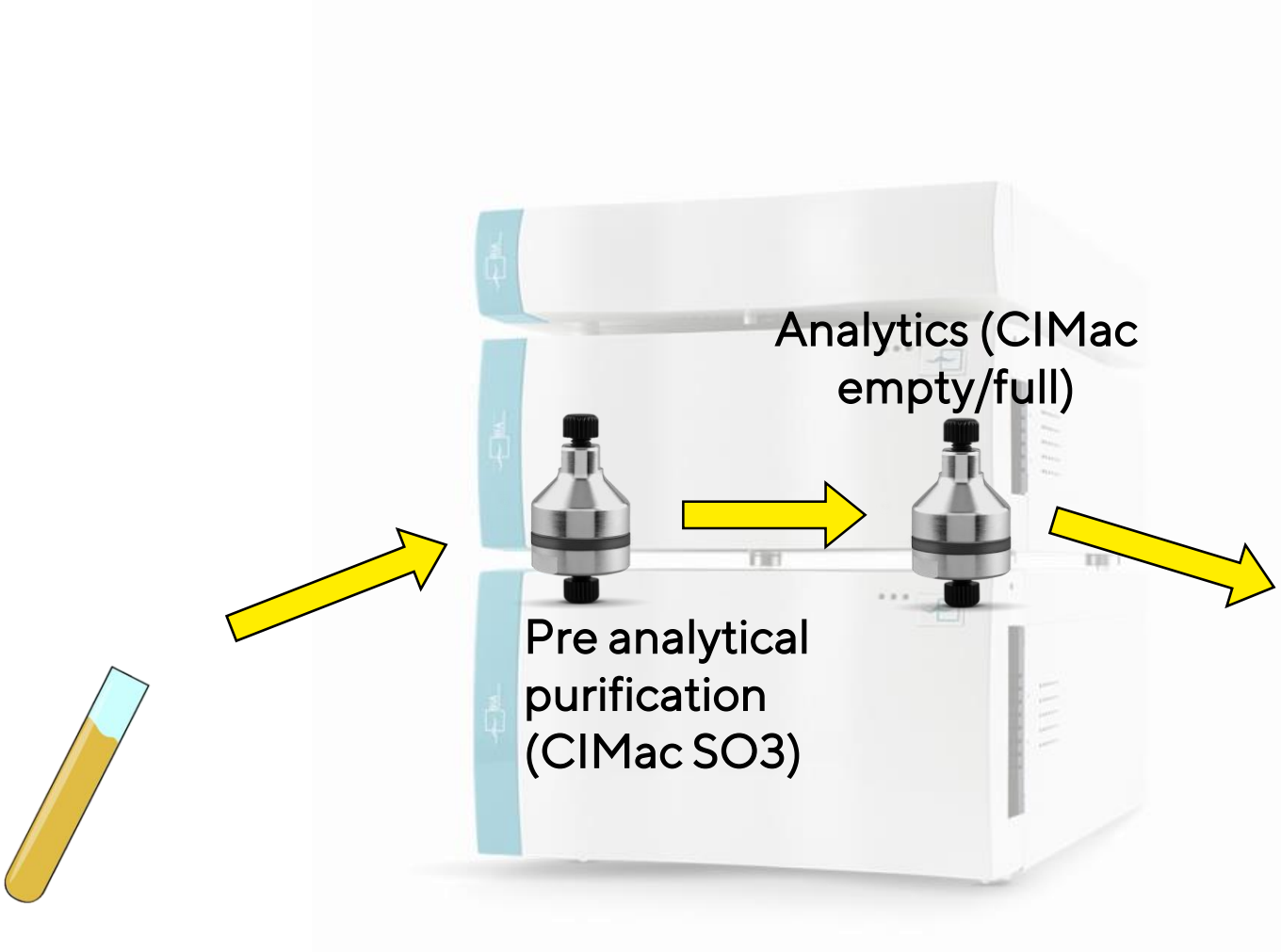
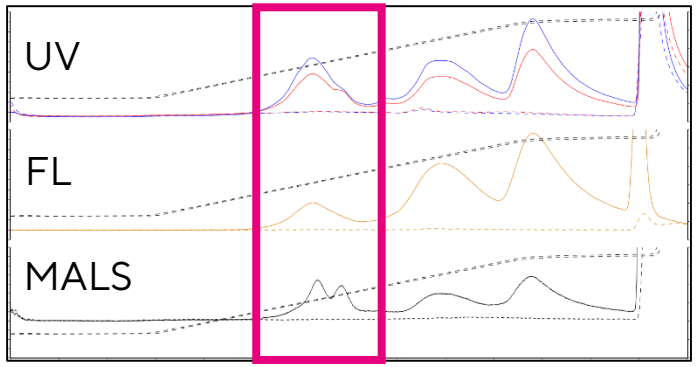
Two columns switching method



- **Two columns switching** PATfix configuration
- In-process analysis of empty/full ratio during production in **about 30 min**
- Best sensitivity and calculations using **MALS signal**



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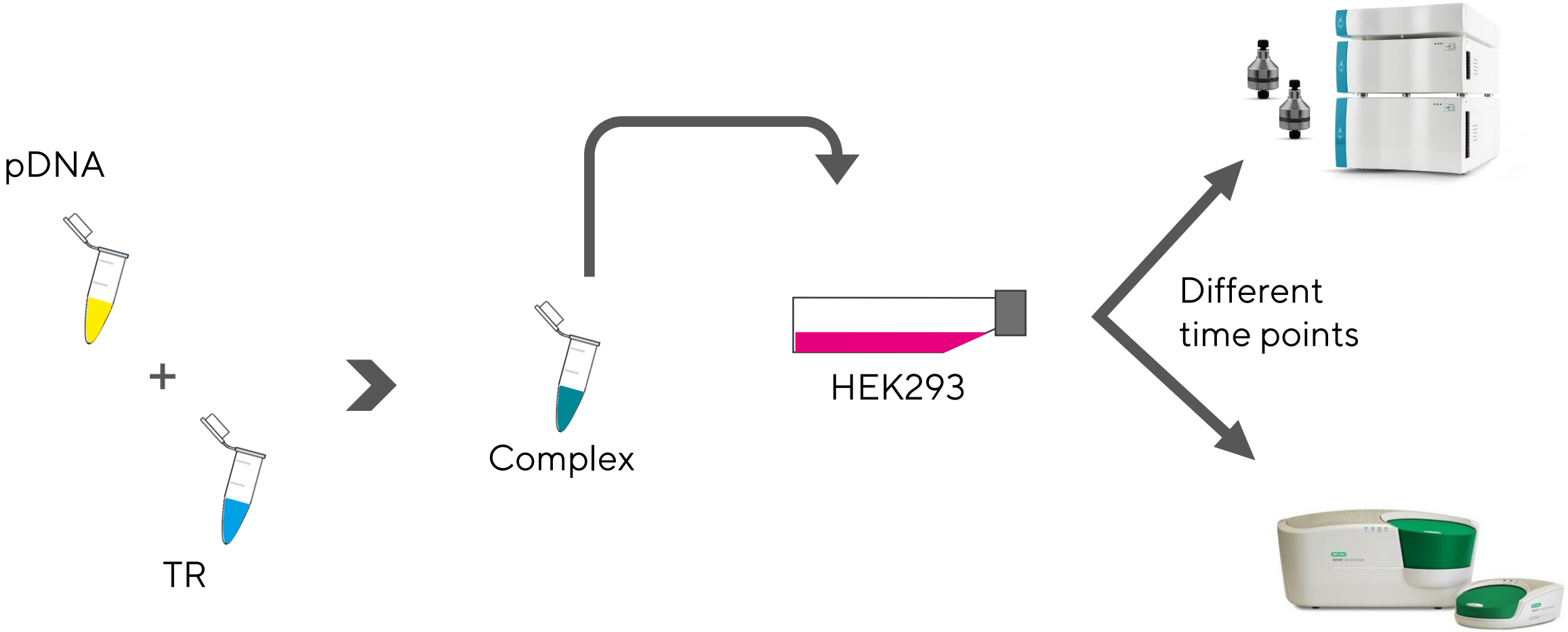
AAV case study: Vector capsid production kinetics



Cells and medium were collected **separately.**

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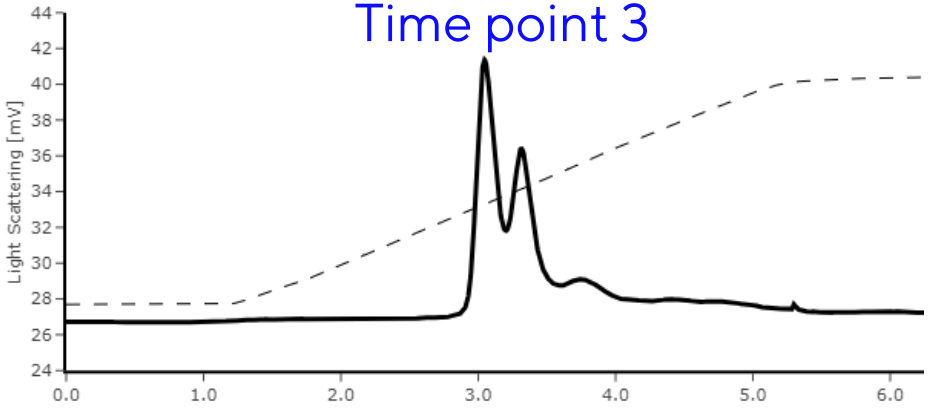
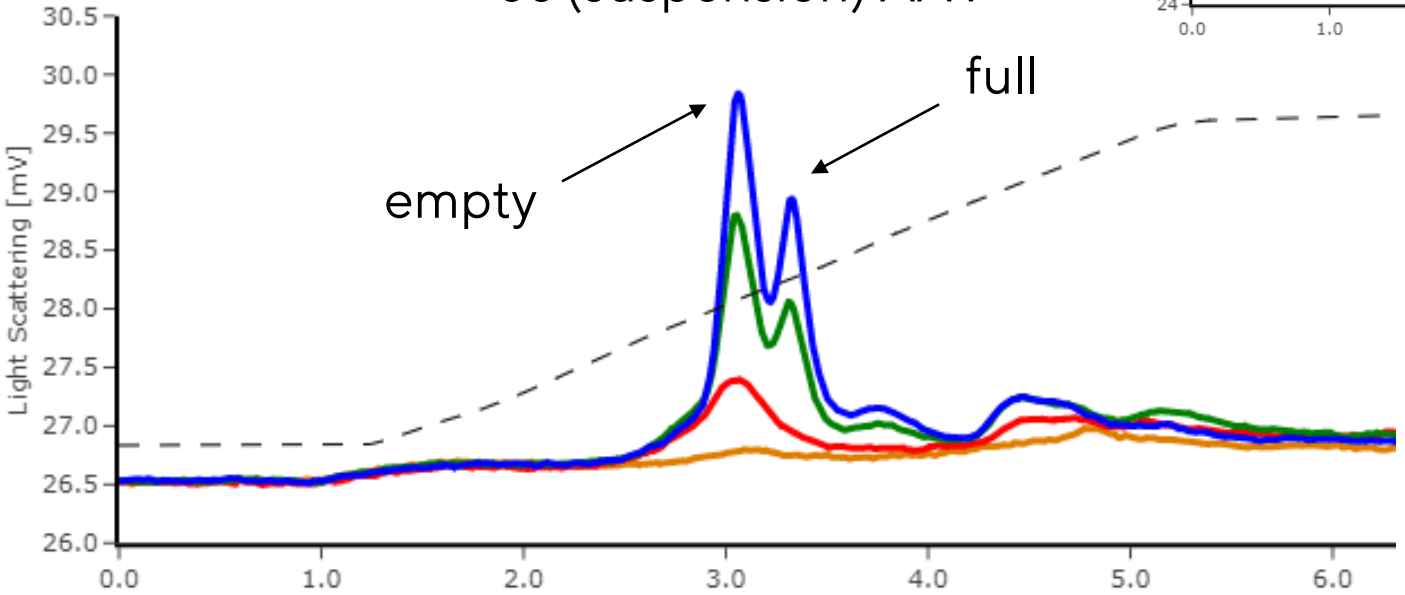
AAV case study: E/F capsid production kinetics

- Analytical chromatogram
- MALS signal:
  - Time point 0
  - Time point 1
  - Time point 2
  - Time point 3

ic (intra cellular)AAV at Time point 3



ec (suspension) AAV



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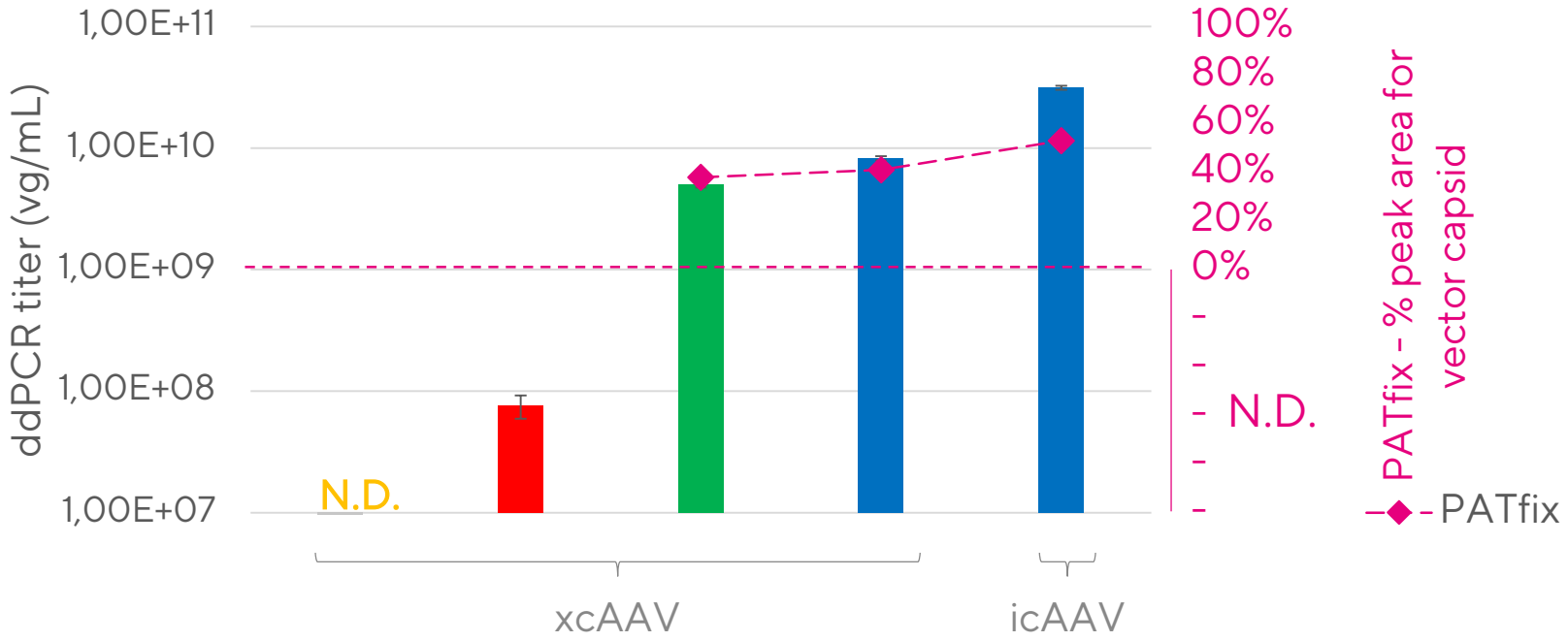
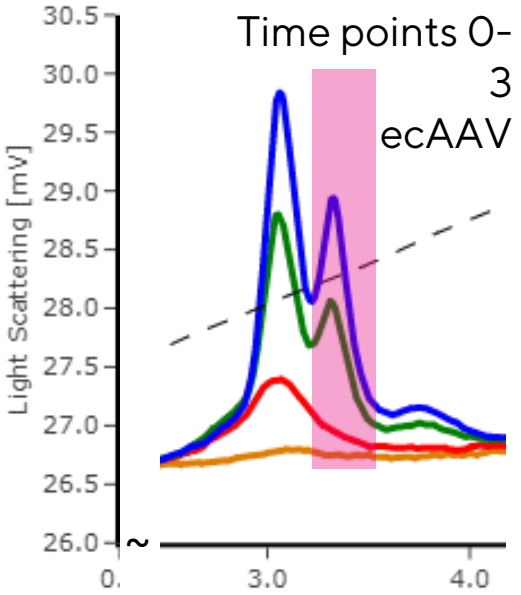
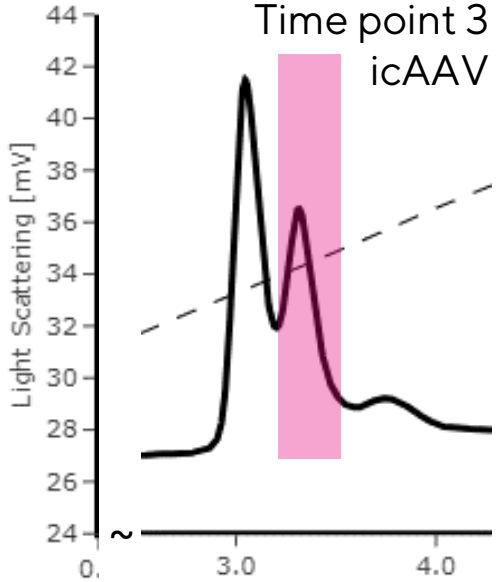
AAV case study: E/F capsid production kinetics



Time point 0; Time point 1; Time point 2; Time point 3 (end of the process)





PATfix and ddPCR data well aligned for the full capsid

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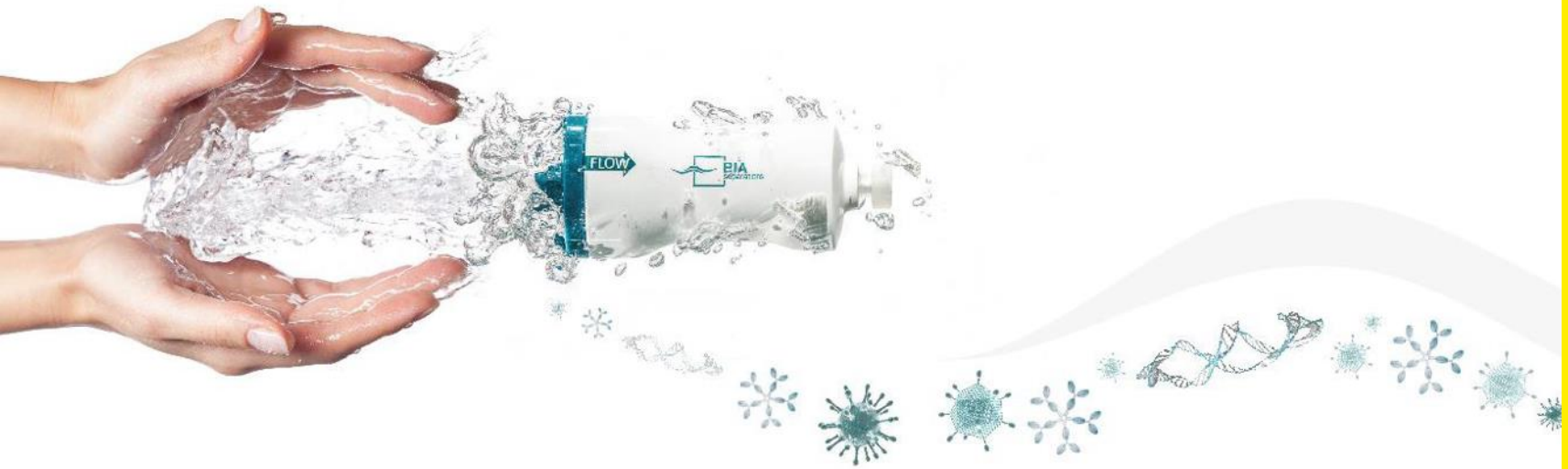

DSP process development using PATfix system analytics to allow for safer product manufacturing

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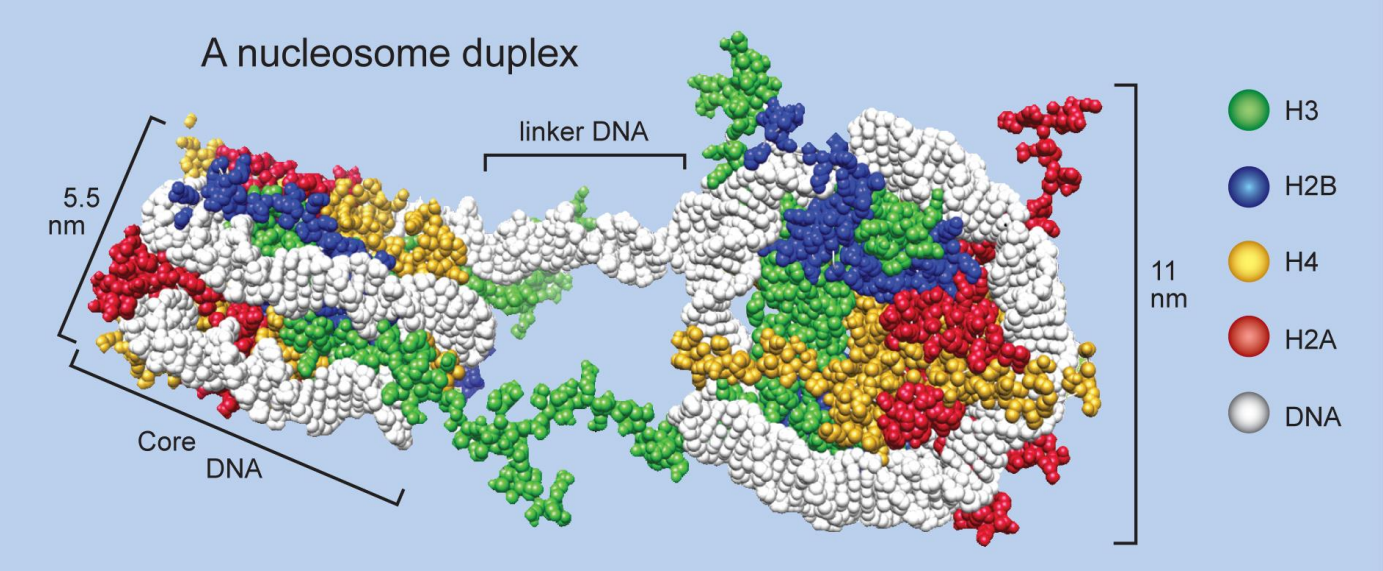
Intracellular produced AAV - after cell lysis sample is reach with Chromatin

For robust process and to reach ultra low residual DNA/RNA proper management of Chromatin structures is mandatory.

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.

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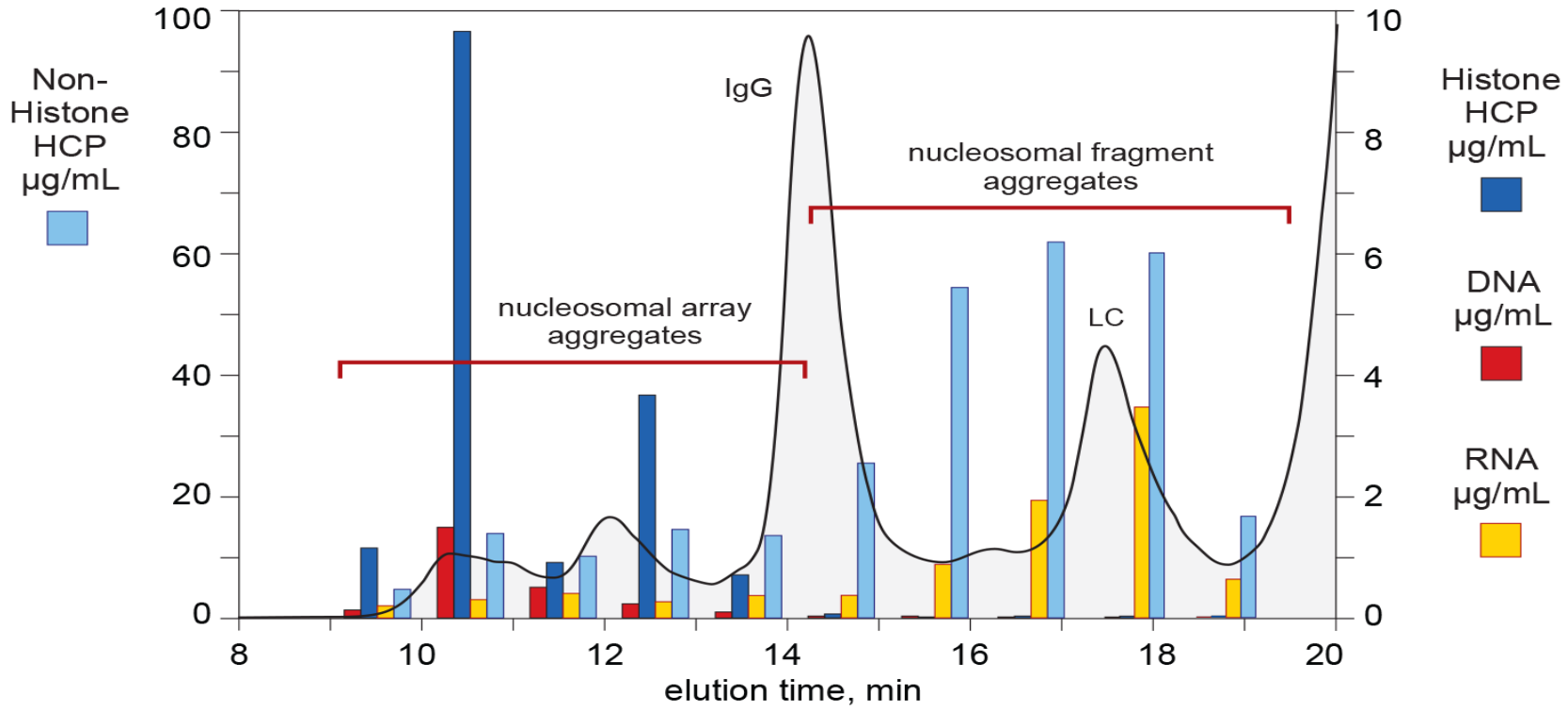
Chromatin from the CHO cell lysate – relatively low amount compared to the IgG

Filtered CHO Harvest containing prospective biosimilar Herceptin™

Analytical SEC. Host DNA by ddPCR. Histones and other HCP by ELISA.



Chromatin aggregates built on nucleosomal arrays range in size from 10–400 nm. Aggregates 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. Note the different scale for non-histone HCP.

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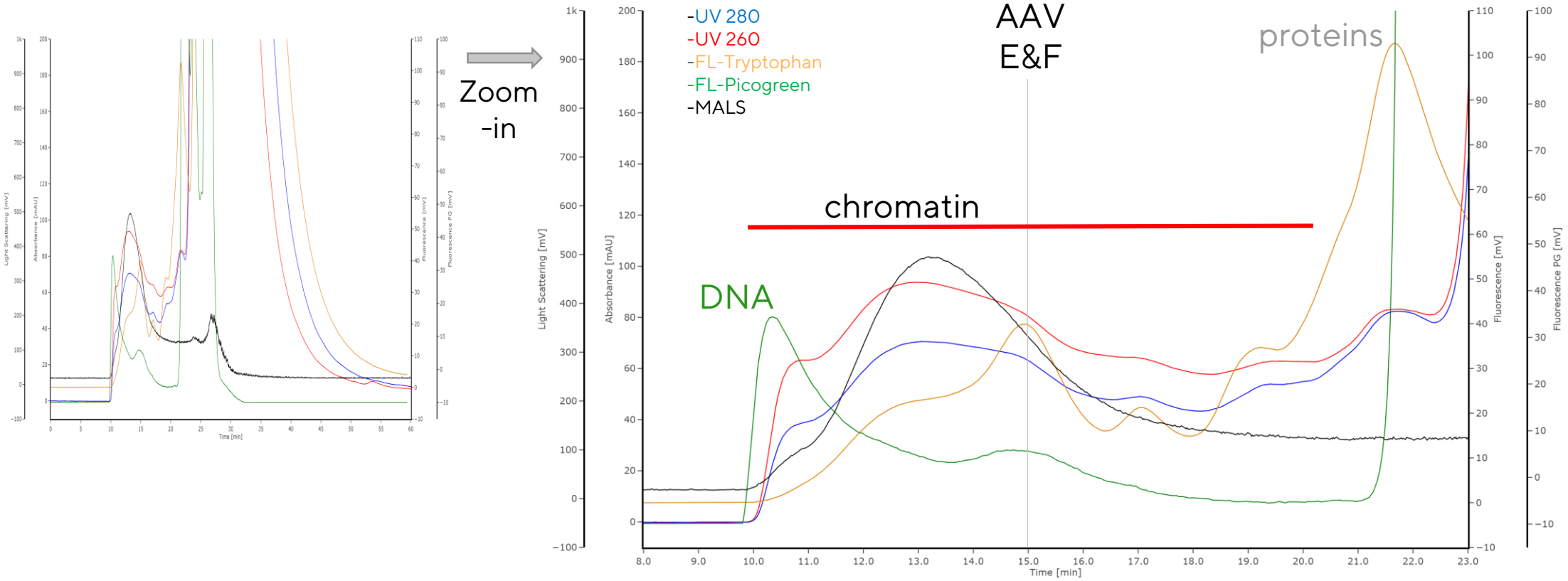
Low AAV concentration (compared to IgG) inflates the Chromatin-to-product ratio

PATfix HPLC with multiple detectors – very powerful tool for Chromatin detection



Analytical HPLC SEC: TSKgel™ G4000SWxl. 0.5 mL/min. Sample prestained with Picogreen™. Intrinsic tryptophan fluorescence amplifies sensitivity for proteins about 20-fold over UV and enables direct visualization of the AAV.

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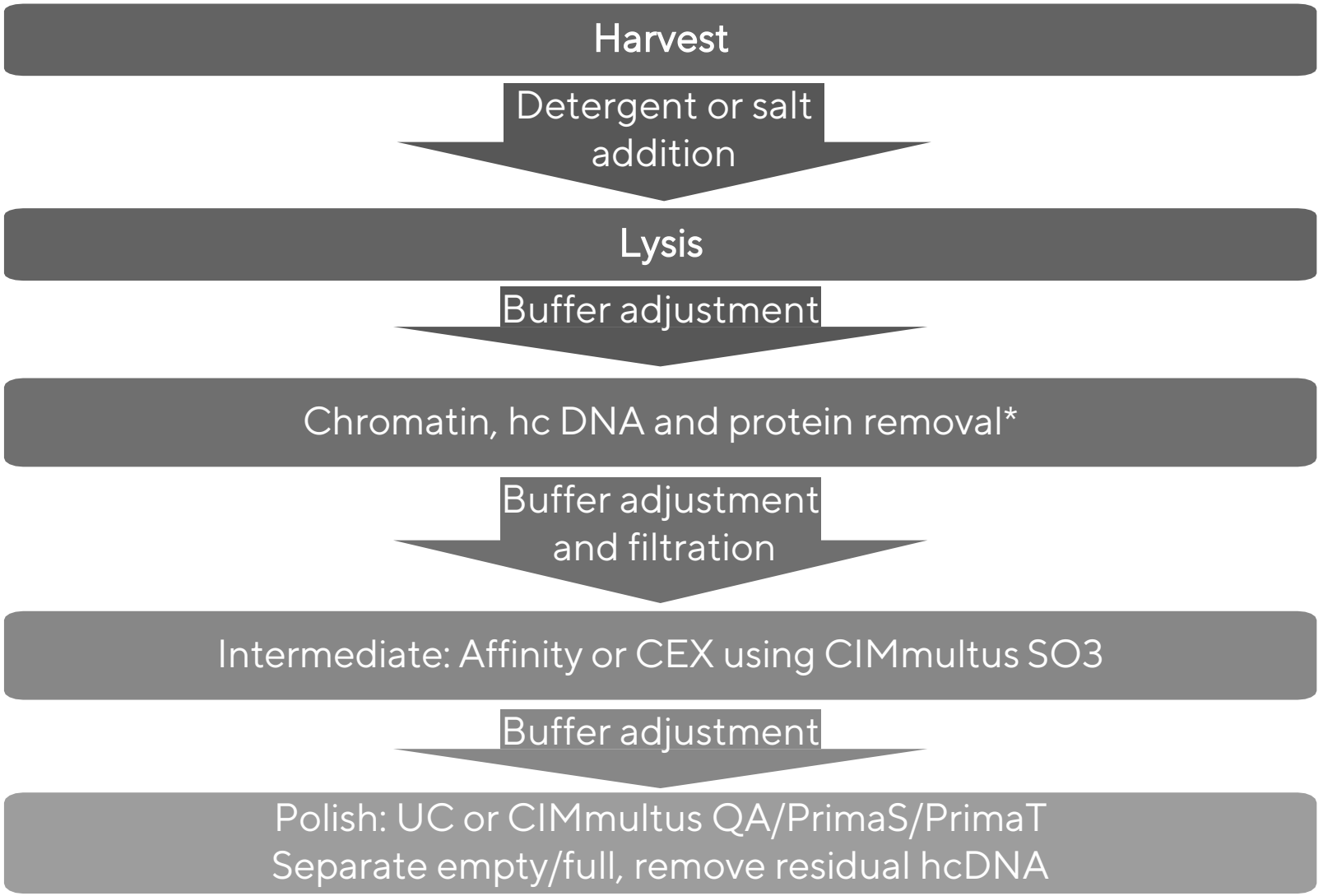
Chromatin removal options



***Multiple options, alone or combined:**

- Flocculation/DNAse treatment (Y/N)
- TFF/DNAse treatment (Y/N)
- **Solid phase extraction/DNAse treatment (Y/N)**
- DNAse treatment (Y/N) followed by acidic precipitation and direct load on the SO3 column

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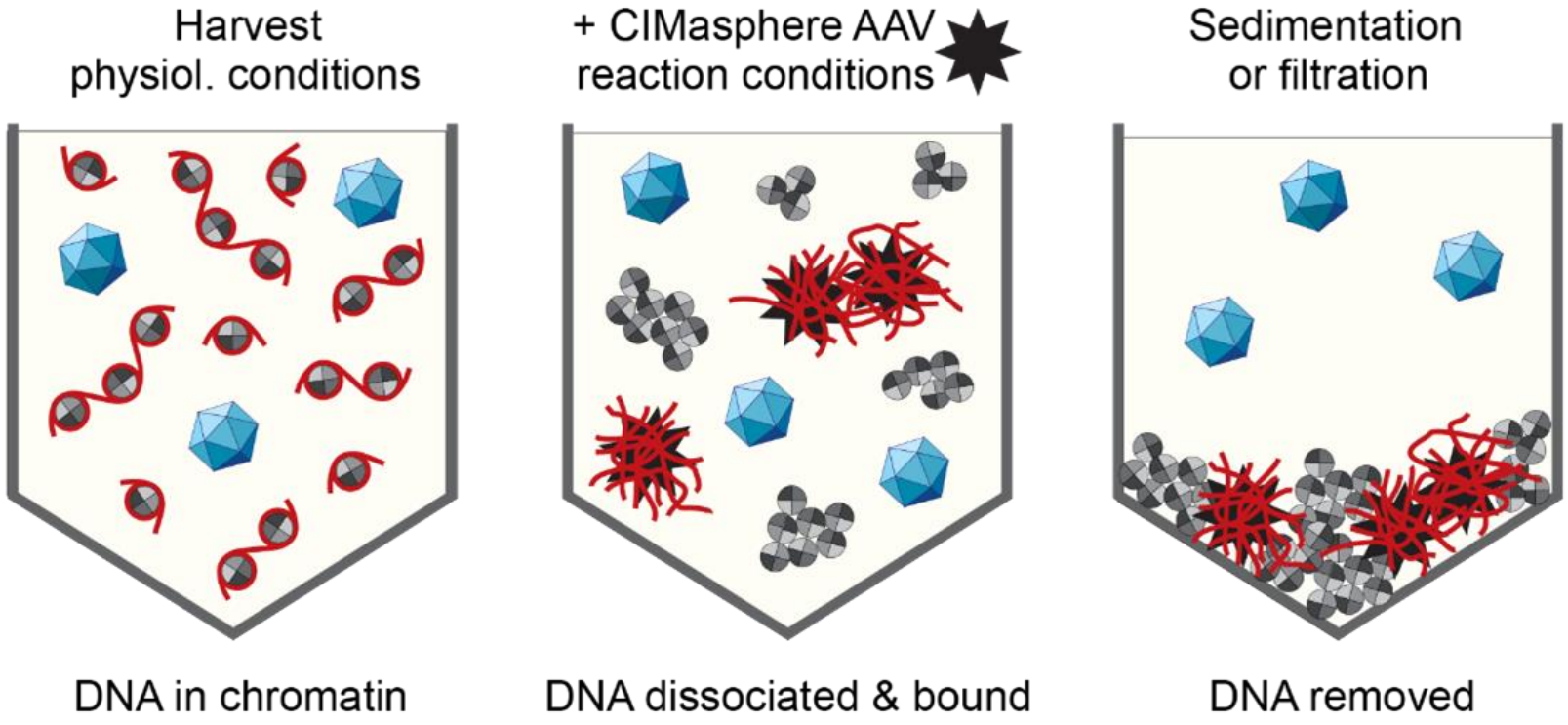
Example: Chromatin Solid phase extraction (SPE) using particles

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

After incubation the particles are removed by any convenient method; centrifugation, membrane or depth filtration.

CIMasphere™ AAV-extracted from 100 mL SF9 lysate, AAV2/8



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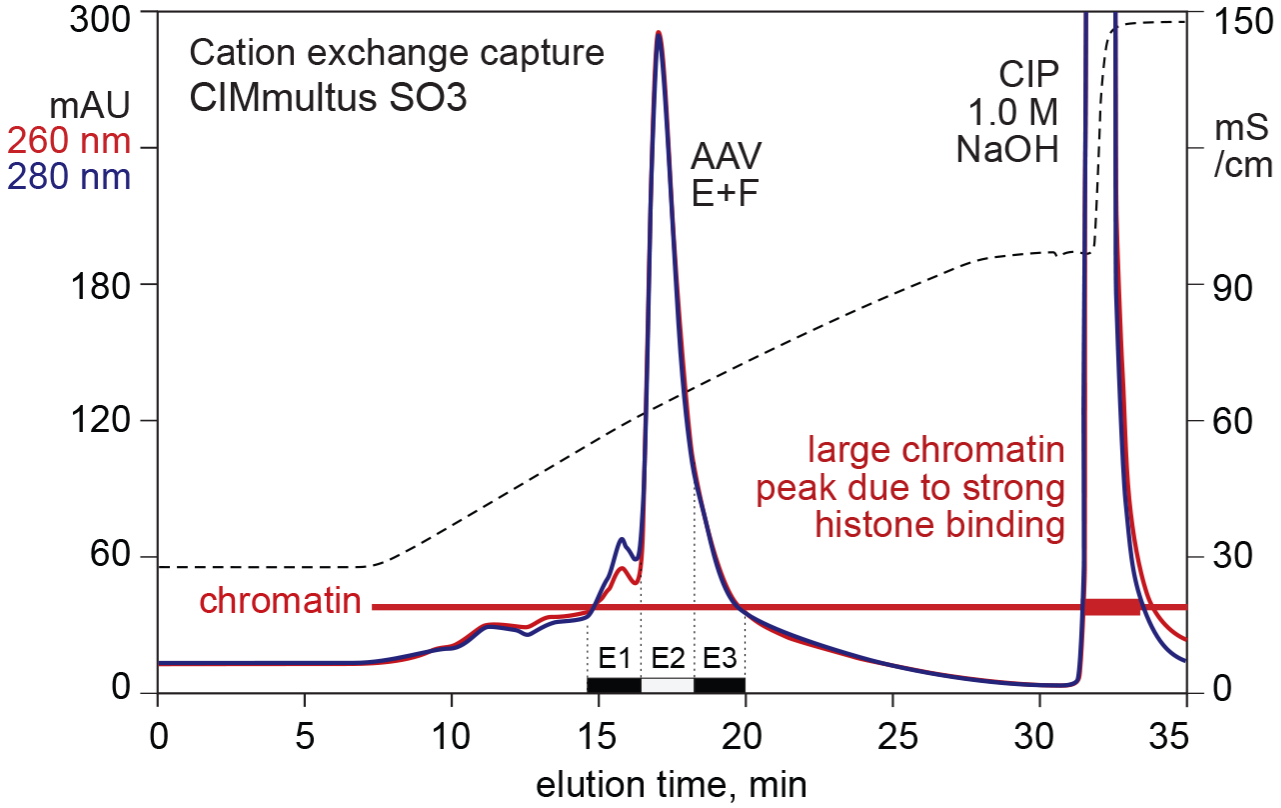
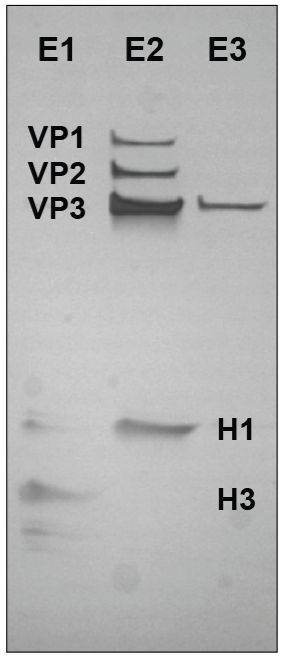
Post SPE capture of the AAV by cation exchange chromatography

Residual Chromatin left after solid phase extraction binds strongly to SO_3 because of its histone component, but it carries a lot of DNA with it. **Removing that DNA enables better separation of empty and full capsids.**





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

CIMasphere treatment also lowers viscosity and improves filterability, so that low titre samples can be concentrated much more effectively.

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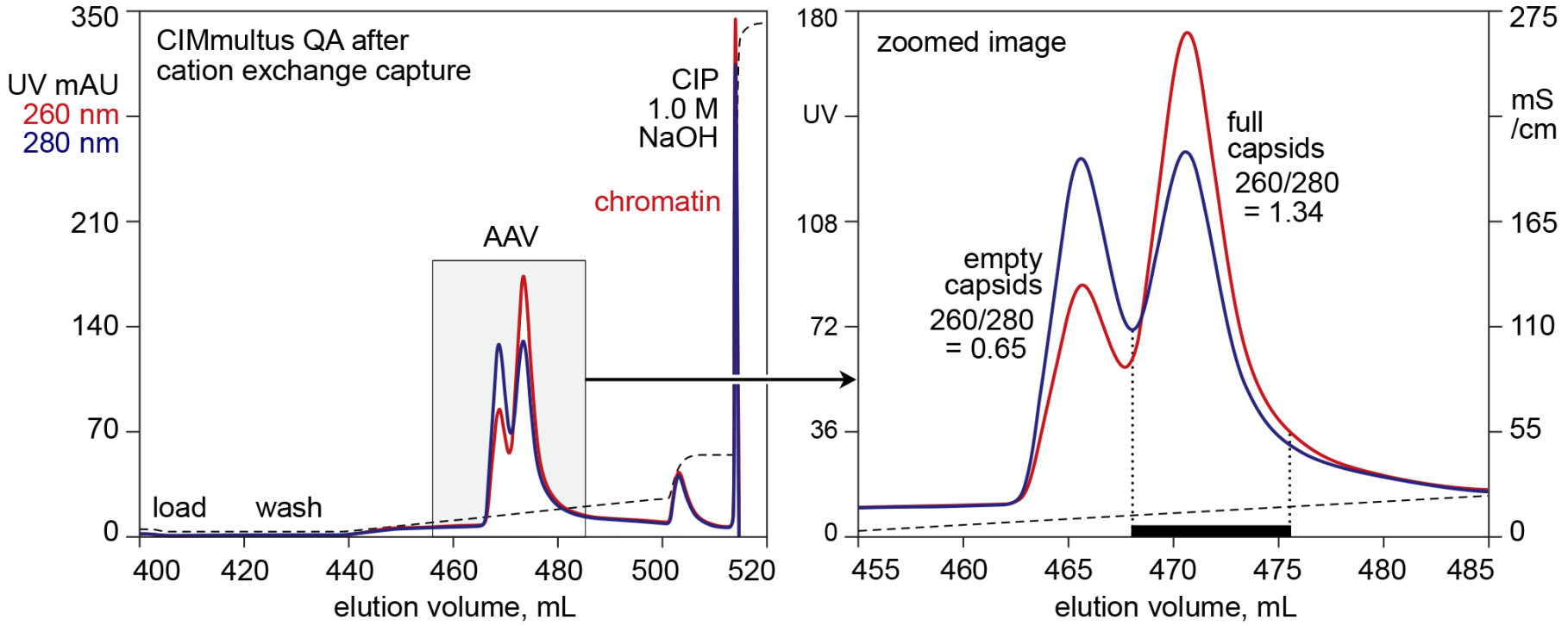
AAV Empty/Full separation AAV by anion exchange chromatography with Mg^{2+}

CIMmultus SO3 pre-purified sample loaded on the QA column.



CIMmultus QA, 1 mL, 2 µm channels, 10 CV/min (600 CV/h). **Separation of empty and full AAV capsids by anion exchange chromatography with a salt gradient is described in US patents US9198984B2 and US20160040137A1.**

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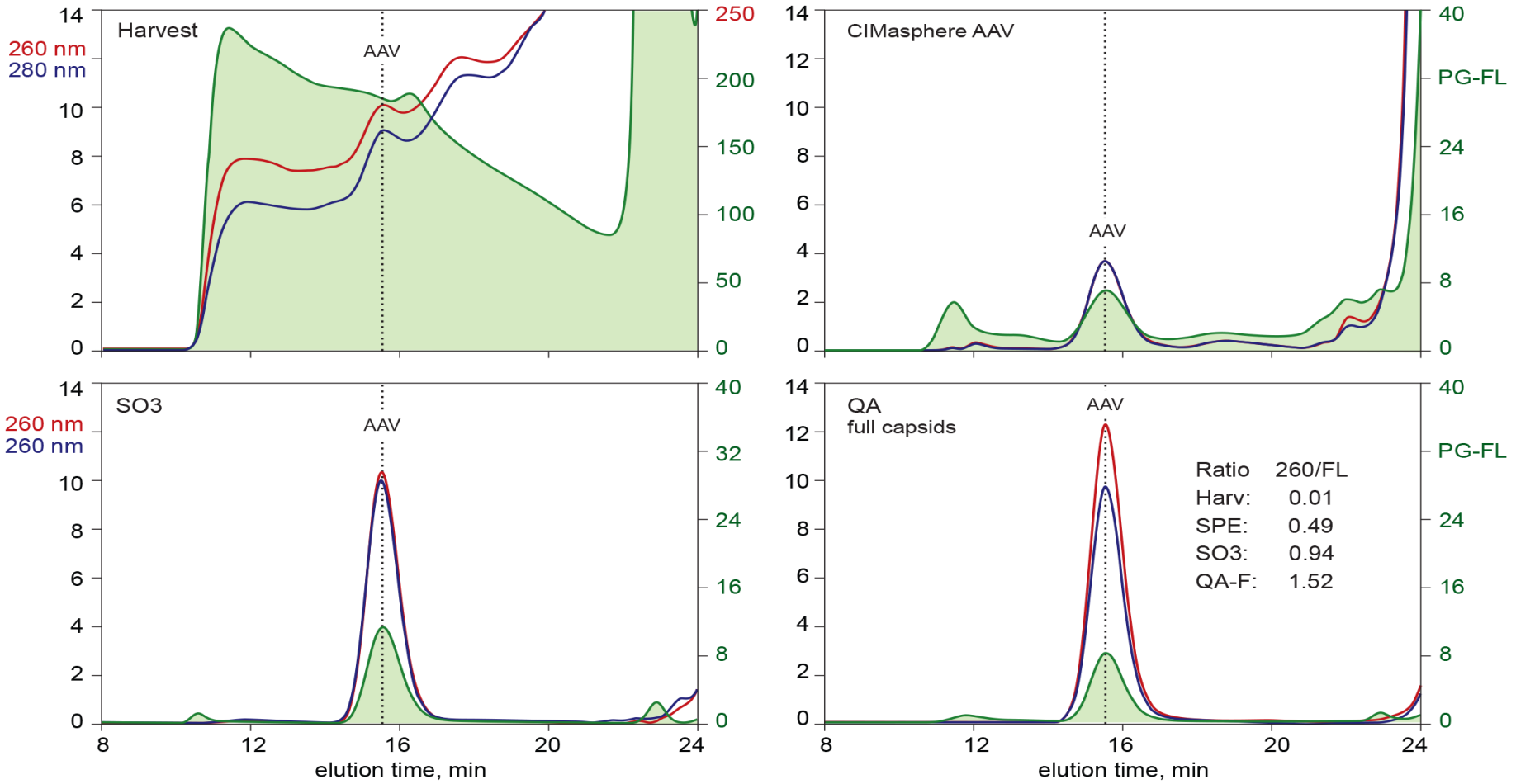
Monitoring DNA reduction across process steps

Samples prestained with Picogreen, analysed by HPLC SEC with UV and FLD. TSKgel™ G4000SWxl. 0.5 mL/min



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Conclusions

- Novel **PATfix column switching method** allows for working with complex USP samples during the process and can detect the product of interest as well it's impurities at the same time.
- PATfix column switching method allows for **studying virus expression kinetics**.
- HPLC based methods allow for **USP and DSP link-up**.
- Pre-staining analytical samples with **Picogreen enables SEC and ion exchange methods to provide easy and sensitive monitoring of DNA content** across purification processes and enables safer product manufacturing.
- **Ultracentrifugation and chromatography support orthogonal analysis**. Combined with multiple detectors, both provide deeper insight into sample composition.

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Acknowledgements

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Thank you for your attention!

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