SARTORIUS

BIA separations

Simplifying Progress

In-Process Analytics of AAV vector capsid production

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Gene Therapy Analytical Development Meeting, 25-27. May 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 > 140 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

Testimonials

" 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. "

Andy Stober,

Senior Vice President of
Technical Operations for AveXis

AAV vector capsid production

- Critical optimization target in AAV-based gene therapy products
- Current techniques are more geared towards DSP – some are highly sensitive to impurities
- Need for fast and reliable in-process analytics to access AAV vector capsid production during the USP

Analytical switching method using PATfix™

Fast at-line HPLC based system

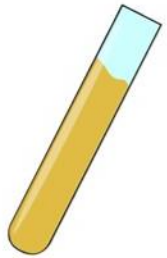
Enables differentiation of vector capsid and empty capsid

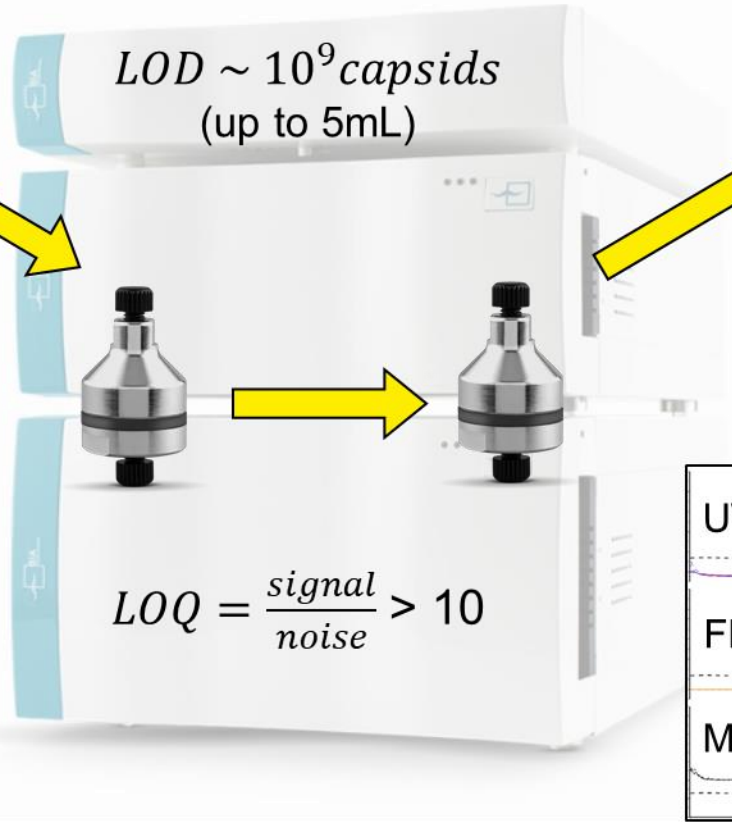
Suitable for complex samples, such as cell media and lysates

Multiple detectors available (UV, MALS, Fluorescence)

PATfix™ analytical switching method

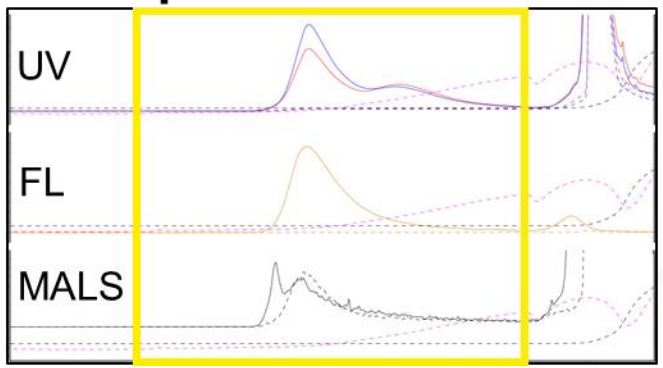
Sample from PD or production run



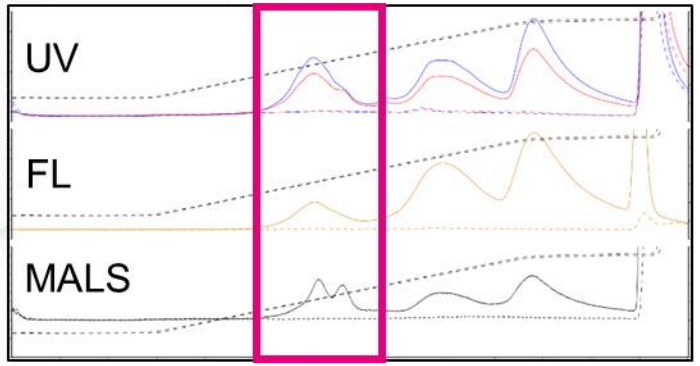


- E/F ratio
- Relative titer (based on MALS)
- Absolute titer (calibration curve needed)
- Additional info from various detectors (UV260, UV280, Trp, PicoGreen)

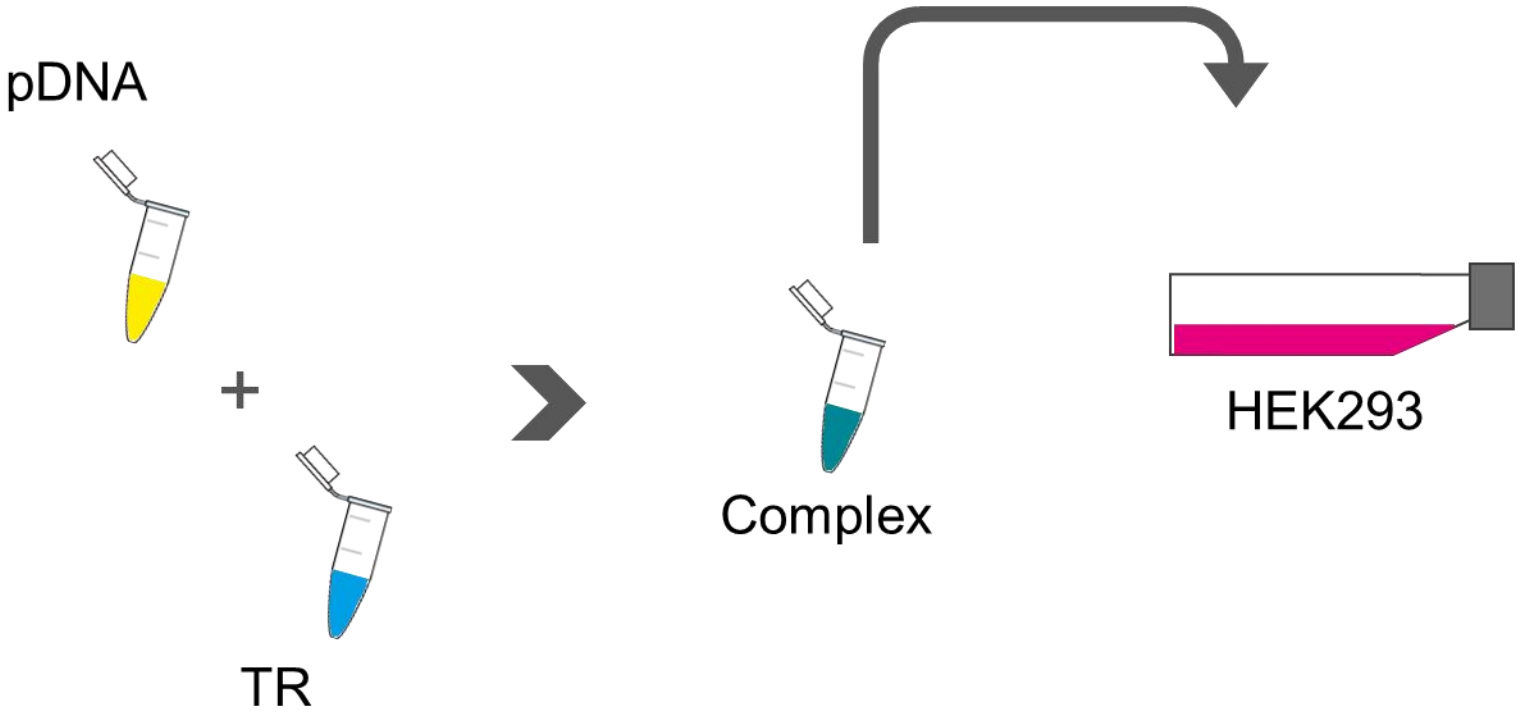
Pre-analytical purification



Analytics



AAV case study: transfection reagents (TR)

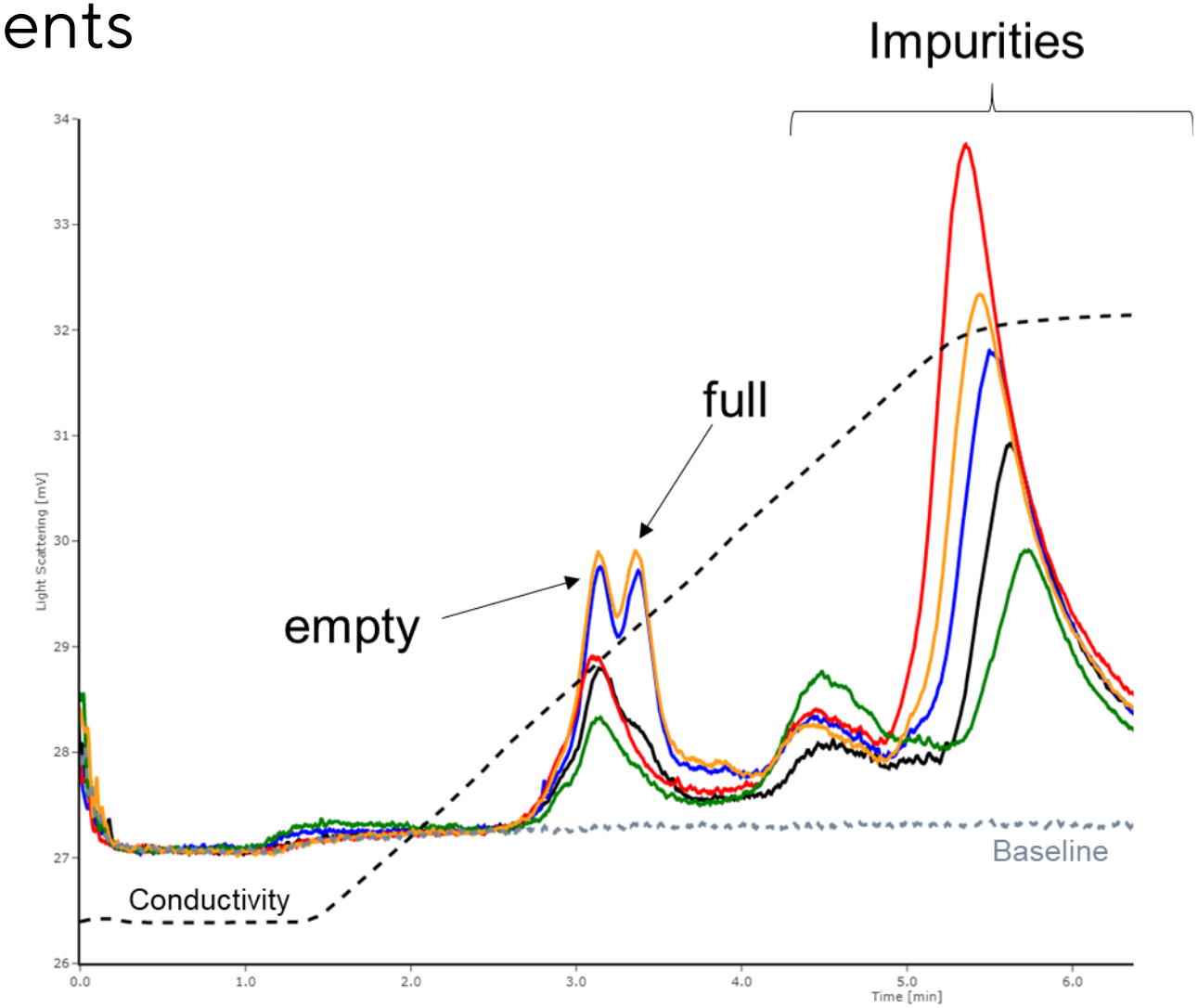


Cells and medium were collected **together.**

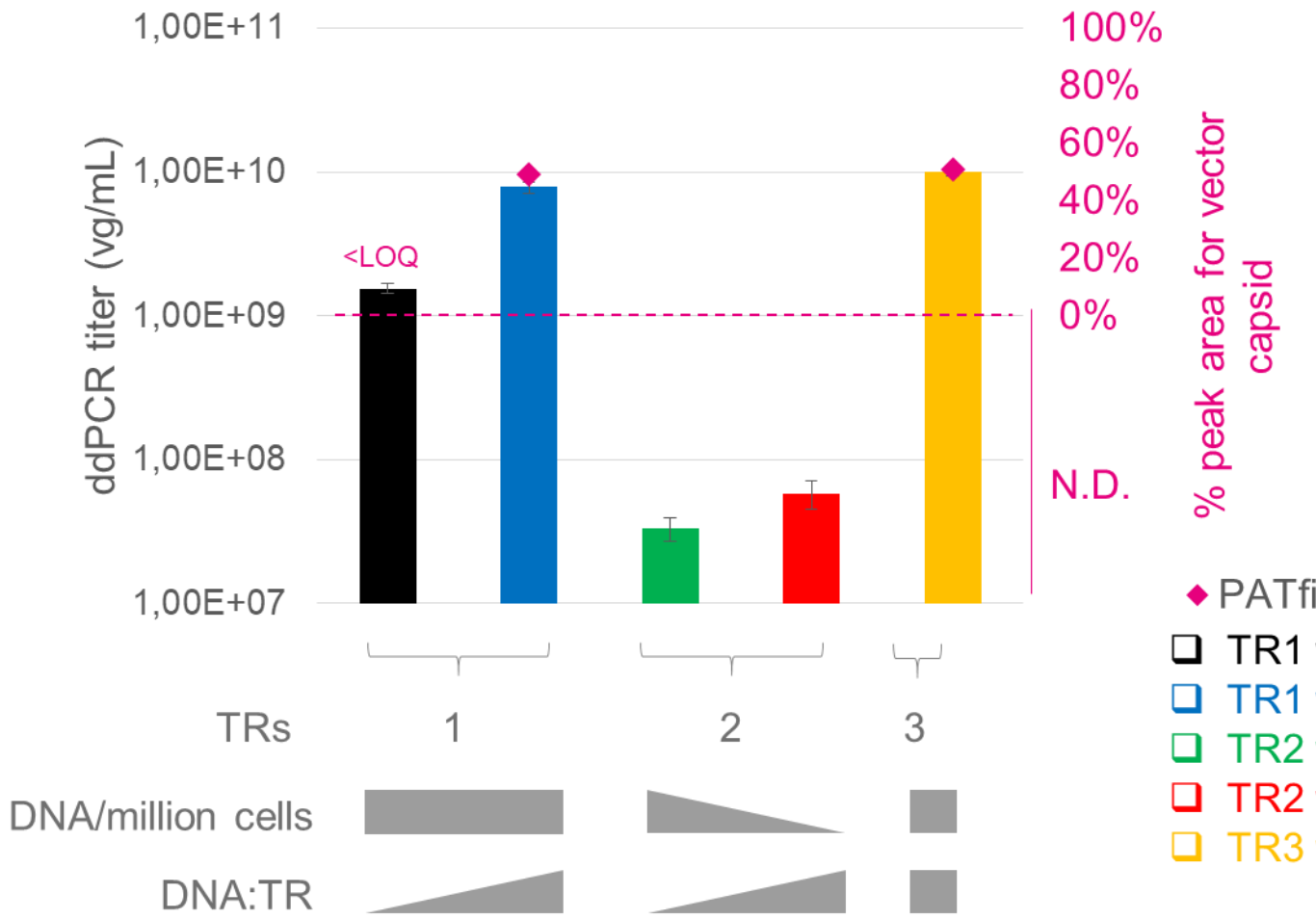
AAV case study: transfection reagents

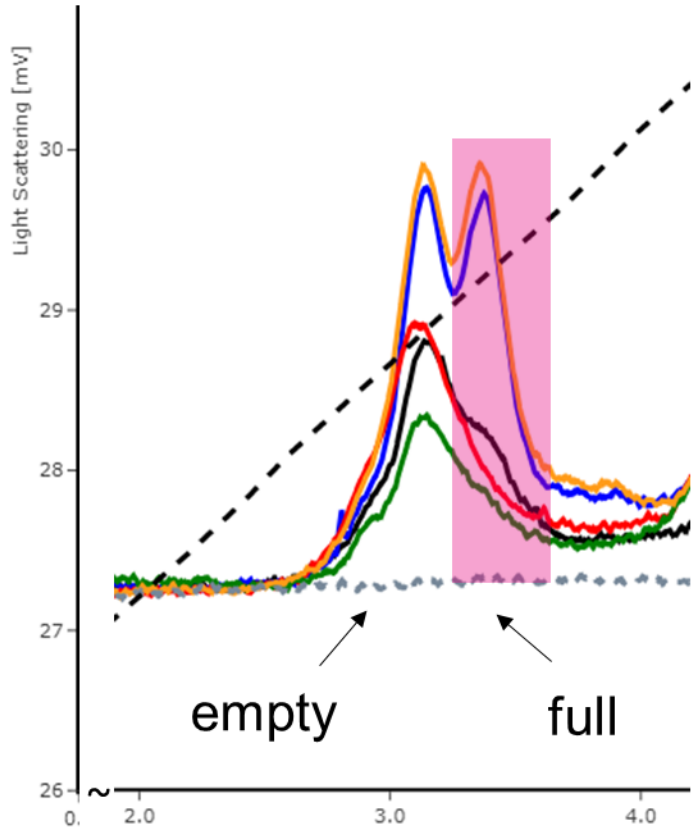
Analytical chromatogram

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



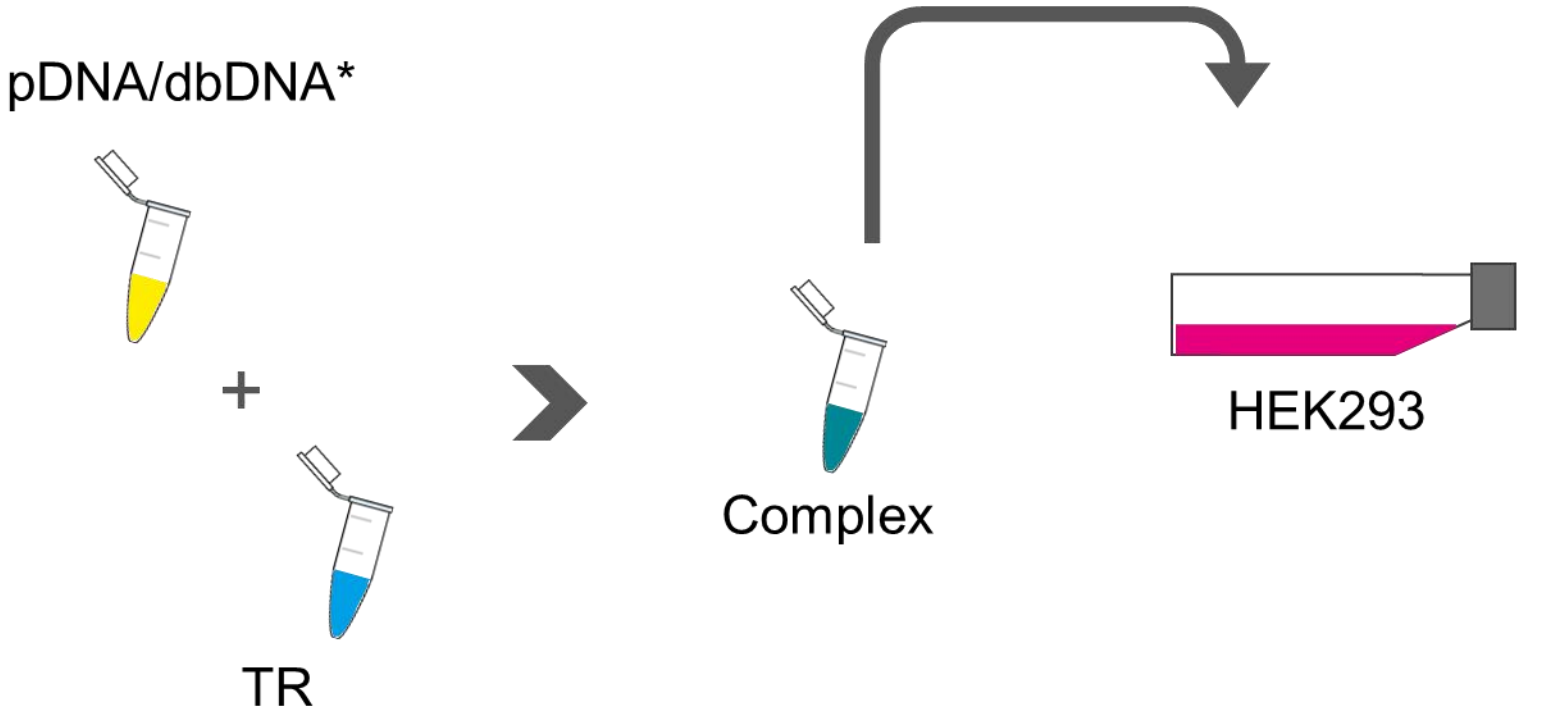
AAV case study: transfection reagents (TR)





- ◆ PATfix
- ❑ TR1 with lower DNA:PEI ratio
- ❑ TR1 with higher DNA:PEI ratio
- ❑ TR2 with higher DNA:PEI ratio
- ❑ TR2 with lower DNA:PEI ratio
- ❑ TR3 with previously optimised DNA:PEI ratio

AAV case study: DNA

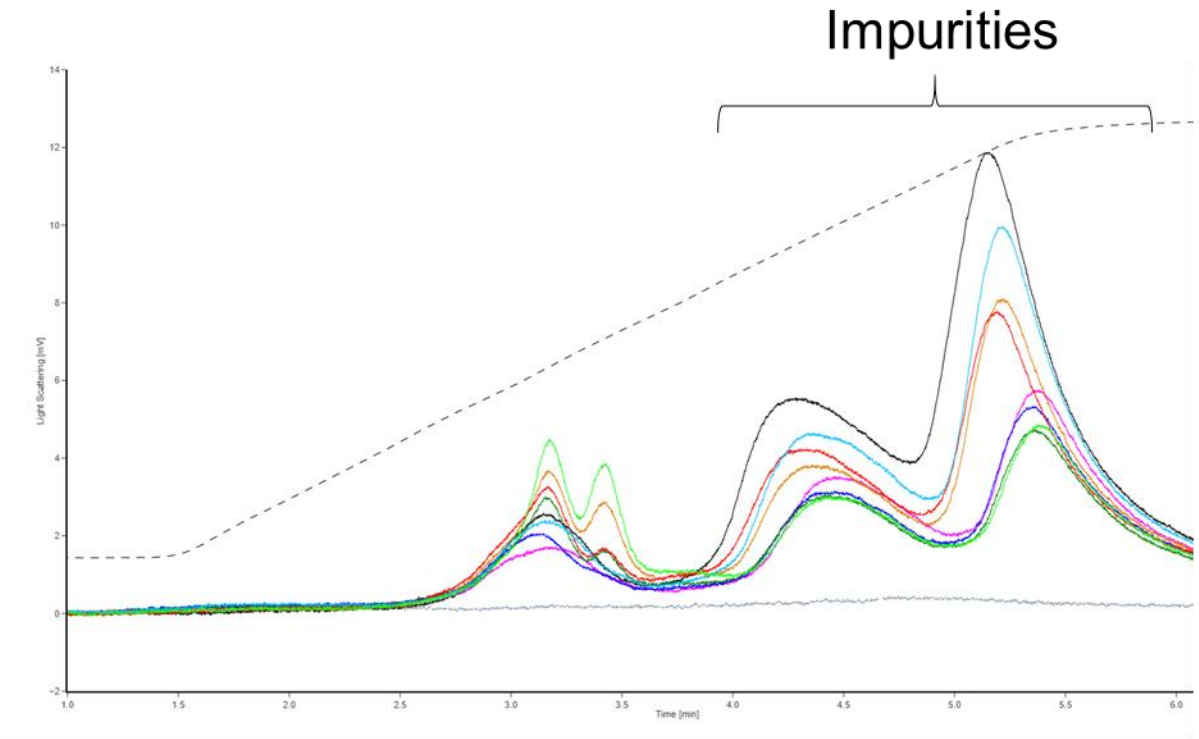


* *Molarity matched.*

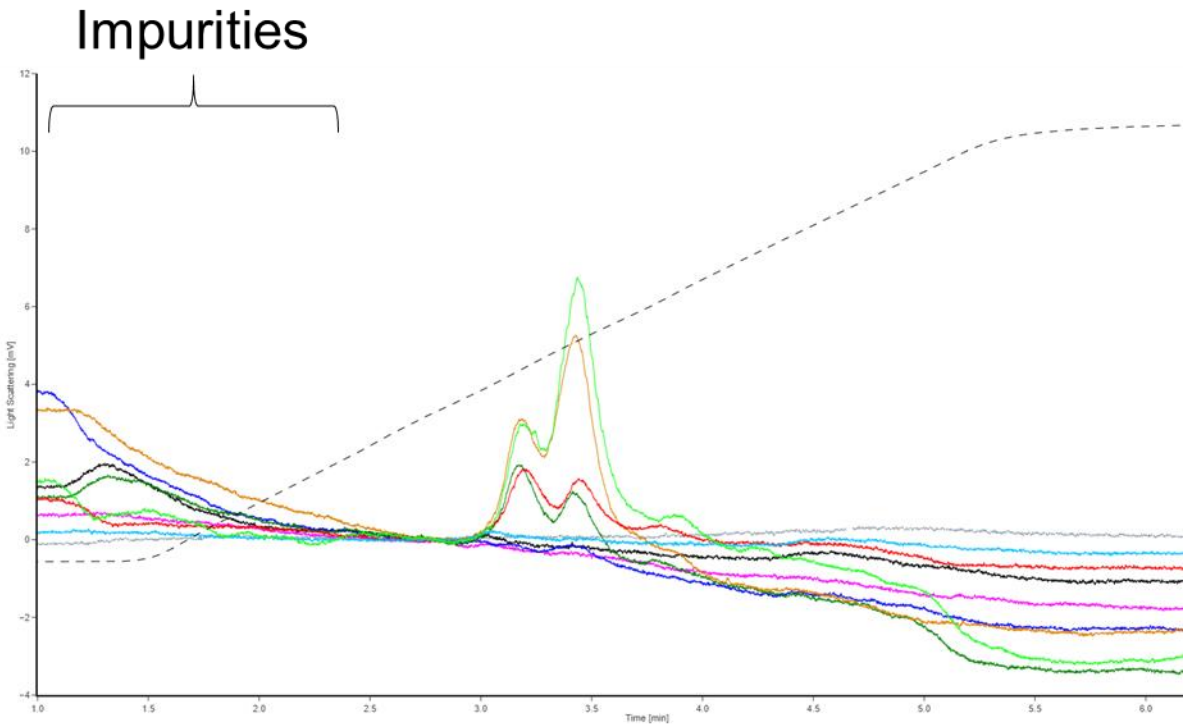
Cells and medium were collected **separately.**

AAV case study: DNA

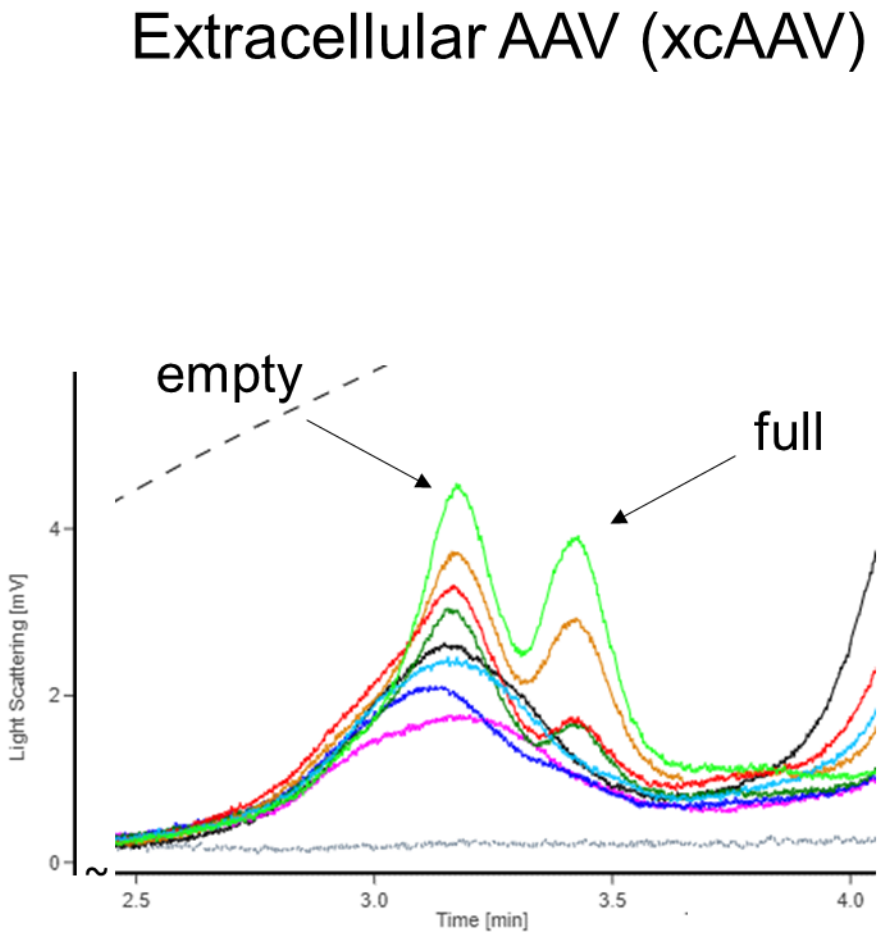
Extracellular AAV (xcAAV)

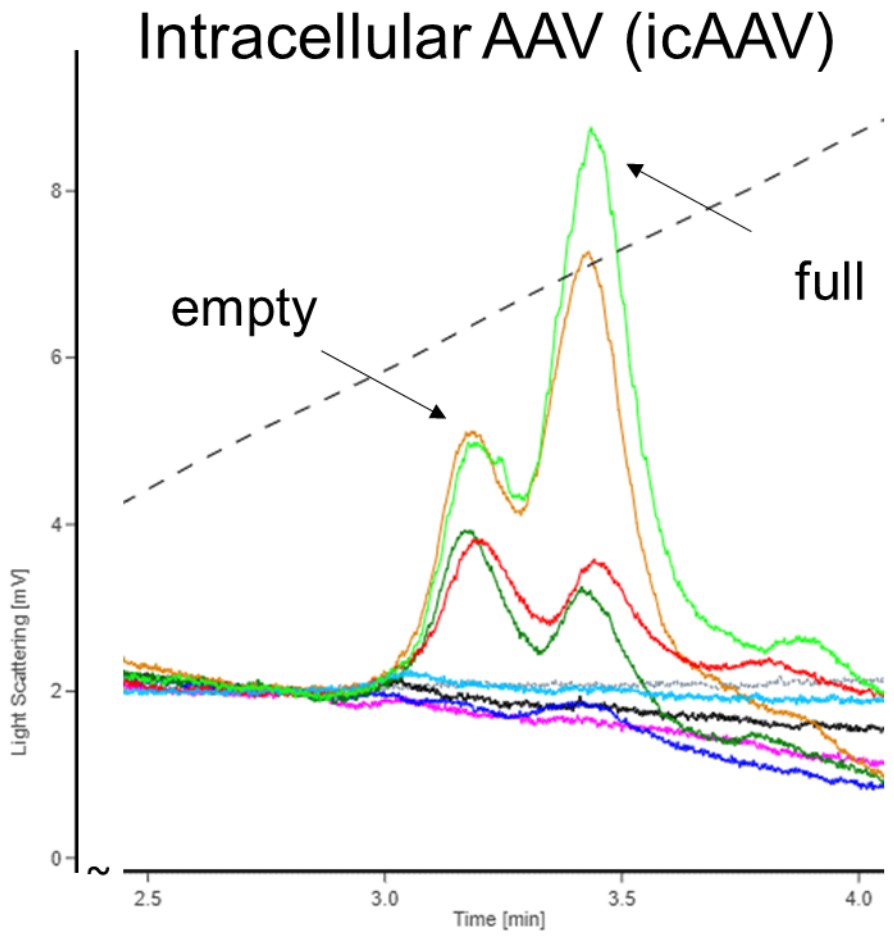


Intracellular AAV (icAAV)

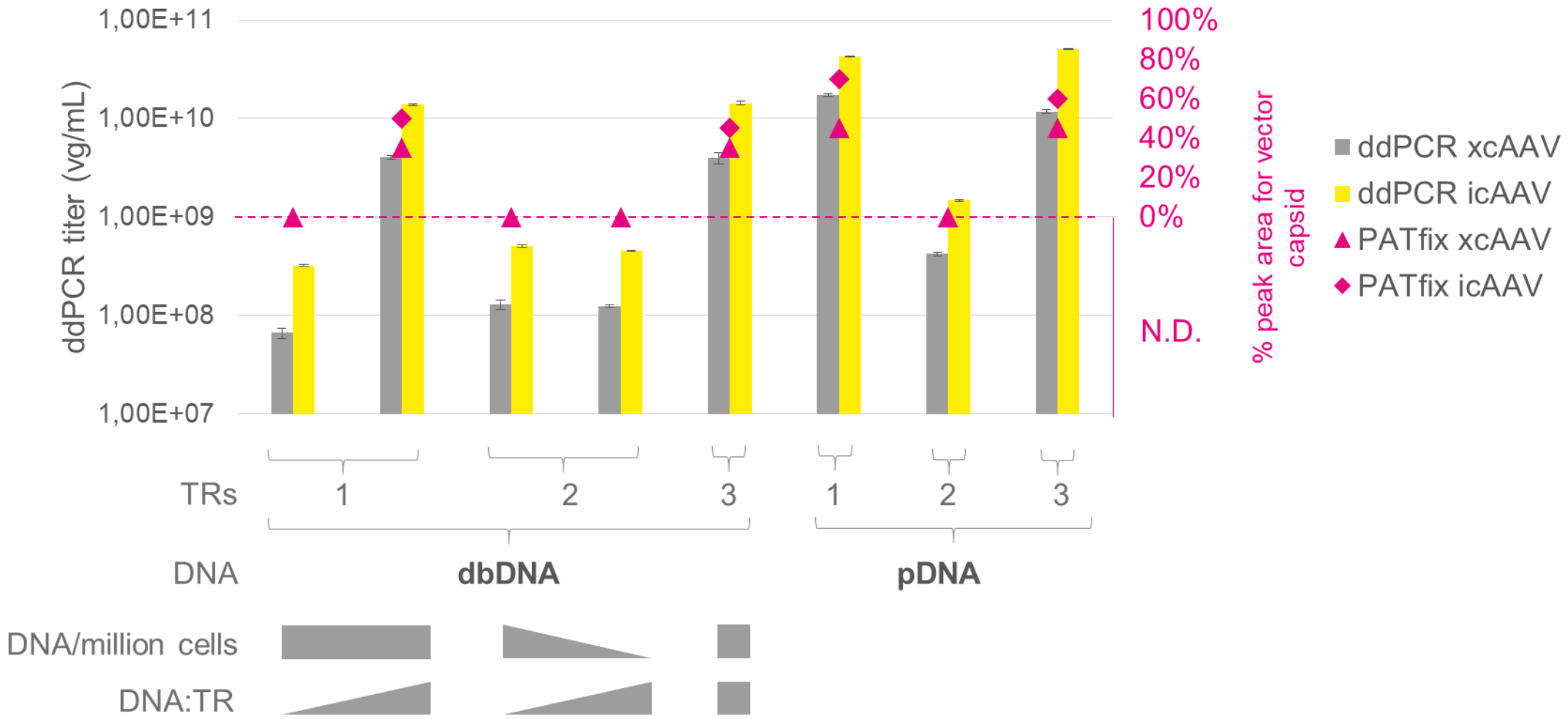


AAV case study: DNA

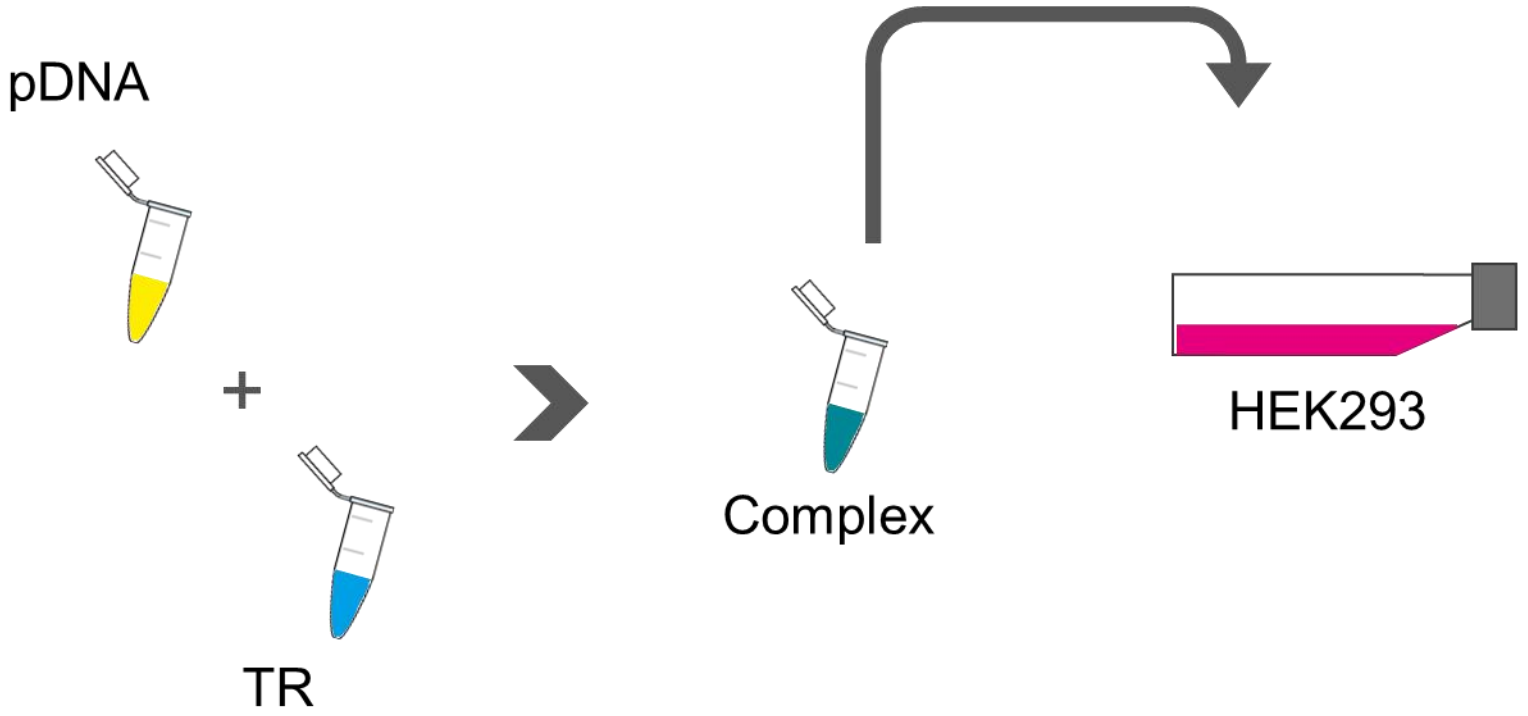




AAV case study: DNA



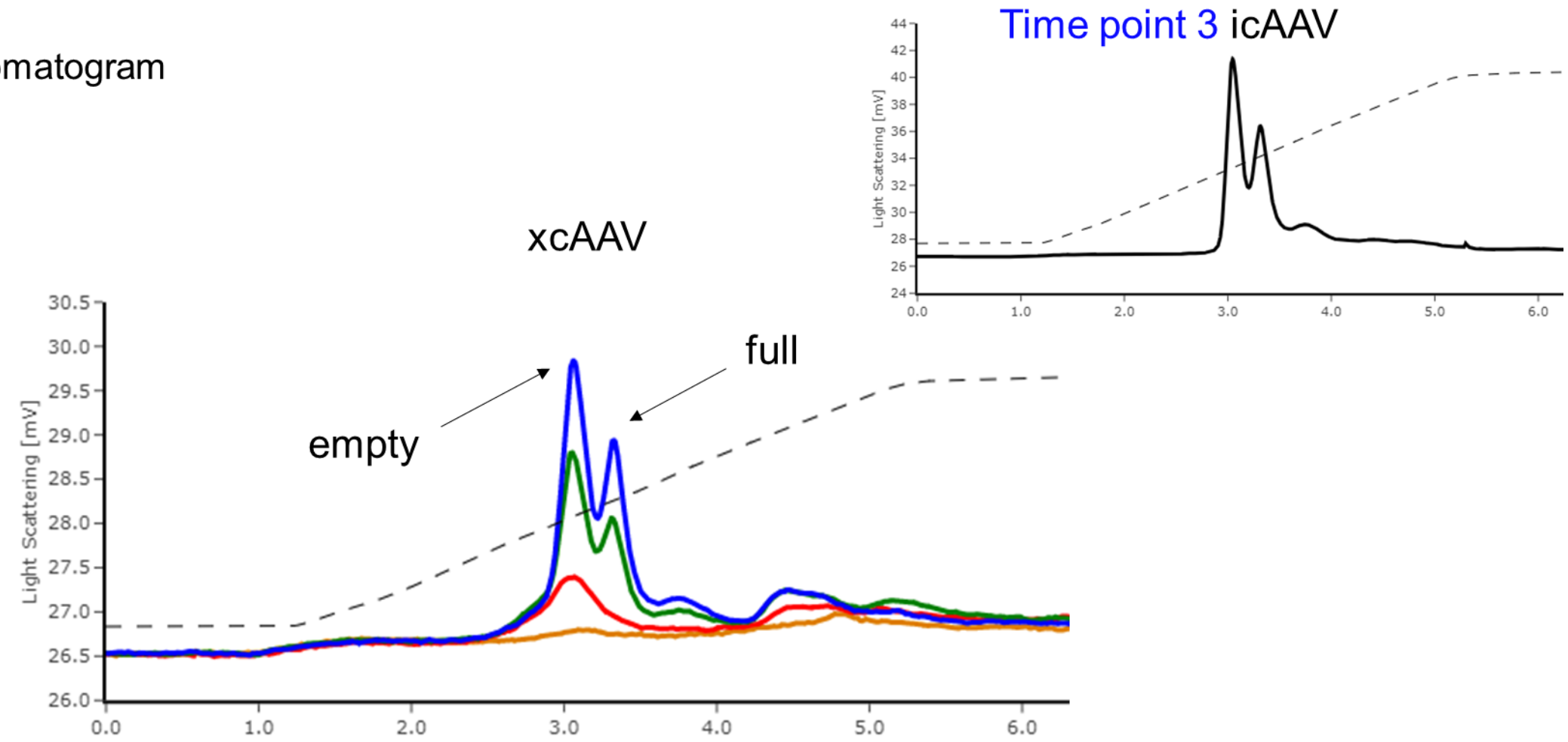
AAV case study: Vector capsid production kinetics



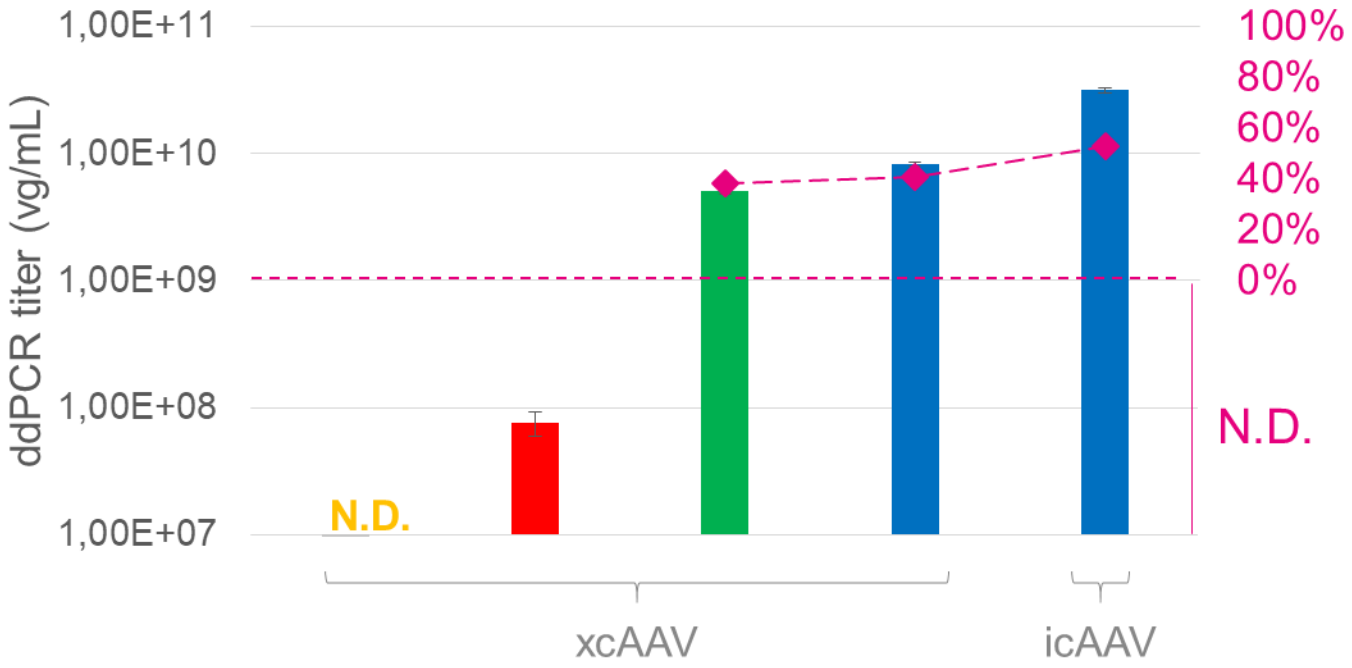
Cells and medium were collected **separately.**

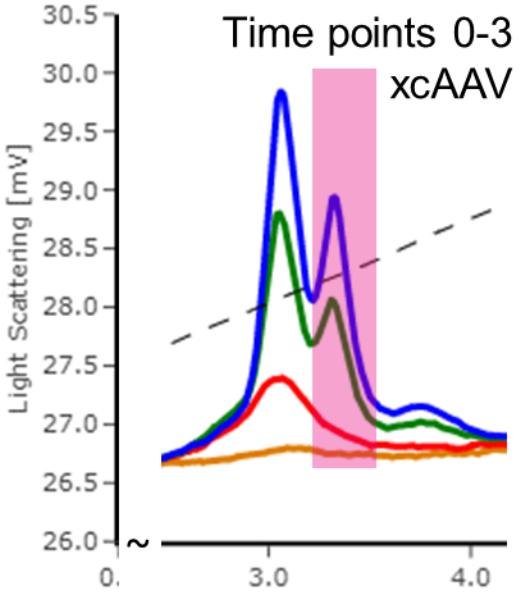
AAV case study: E/F capsid production kinetics

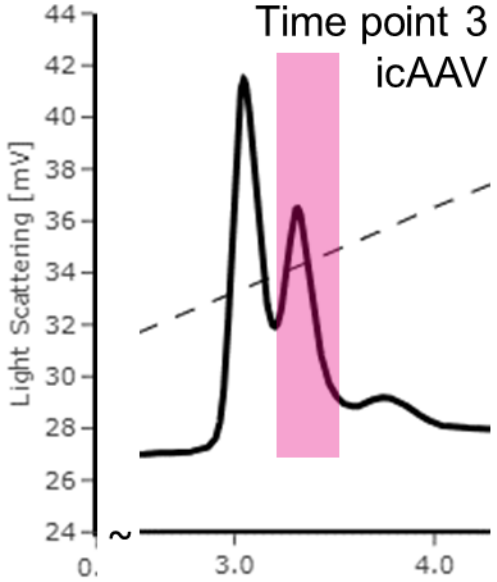
- Analytical chromatogram
- MALS signal:
  - Time point 0
  - Time point 1
  - Time point 2
  - Time point 3



AAV case study: E/F capsid production kinetics







Benefits of PATfix switch analytics

- Orthogonal and complementary analysis to PCR
  - Shows both empty and full capsids
  - Relative titer can be estimated without any additional data
  - Absolute titer can be determined through calibration curve, like for qPCR
  - Possibility to couple with fluorescence detectors for more information
- Suitable both for upstream process development and production runs
- Faster and more informative tool for in-process control than PCR
  - One run is about 1hr long, compared to 2.5hrs for qPCR and 2.5 + 1hrs for ddPCR
  - Gives more information about the sample, not just vector capsid titer
  - Allows quick and informed decision making (example: when to stop the process and start harvesting)

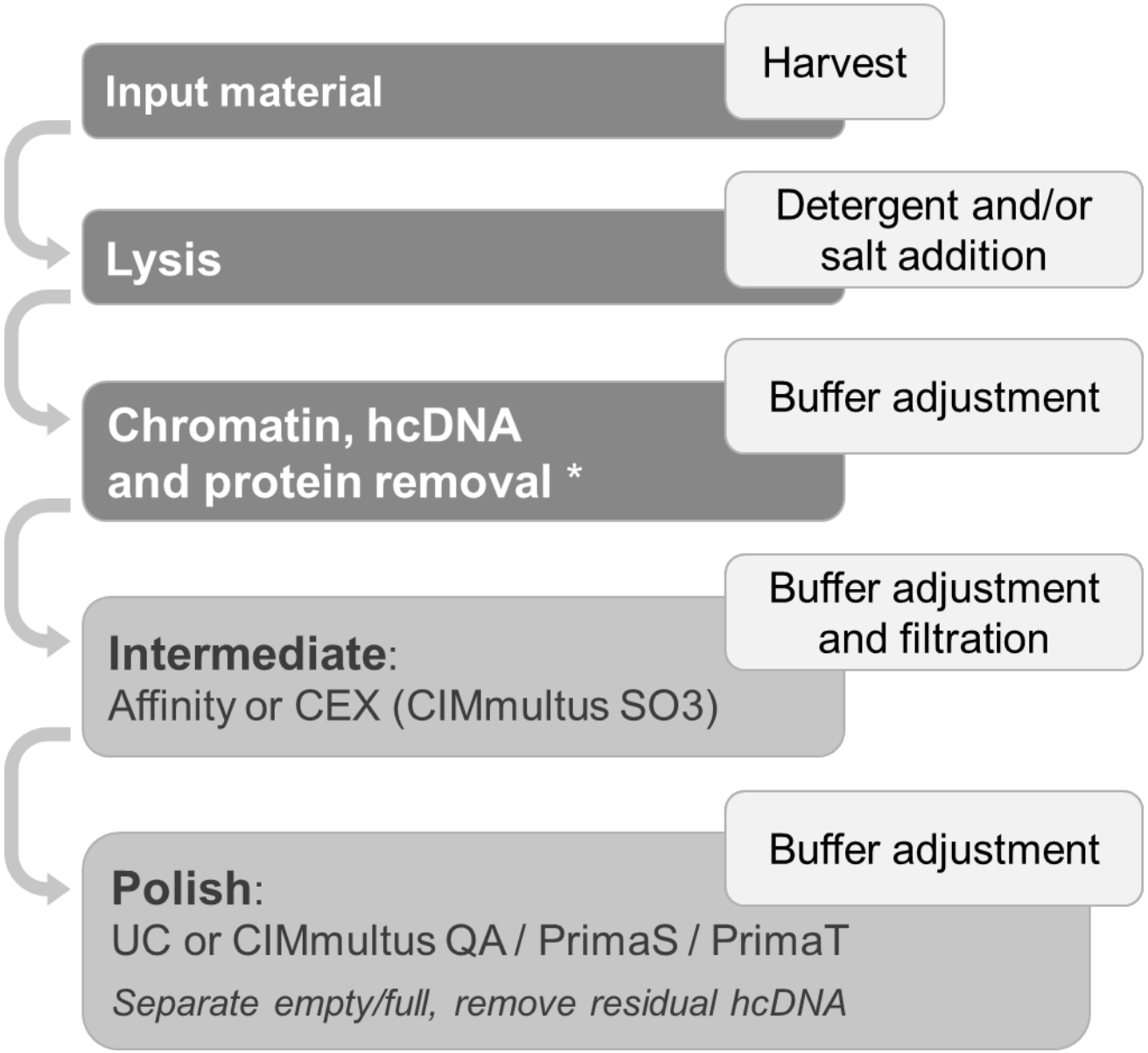
CIMac analytical columns for PAT HPLC - no carry over of contaminants or viruses

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



Purification insights

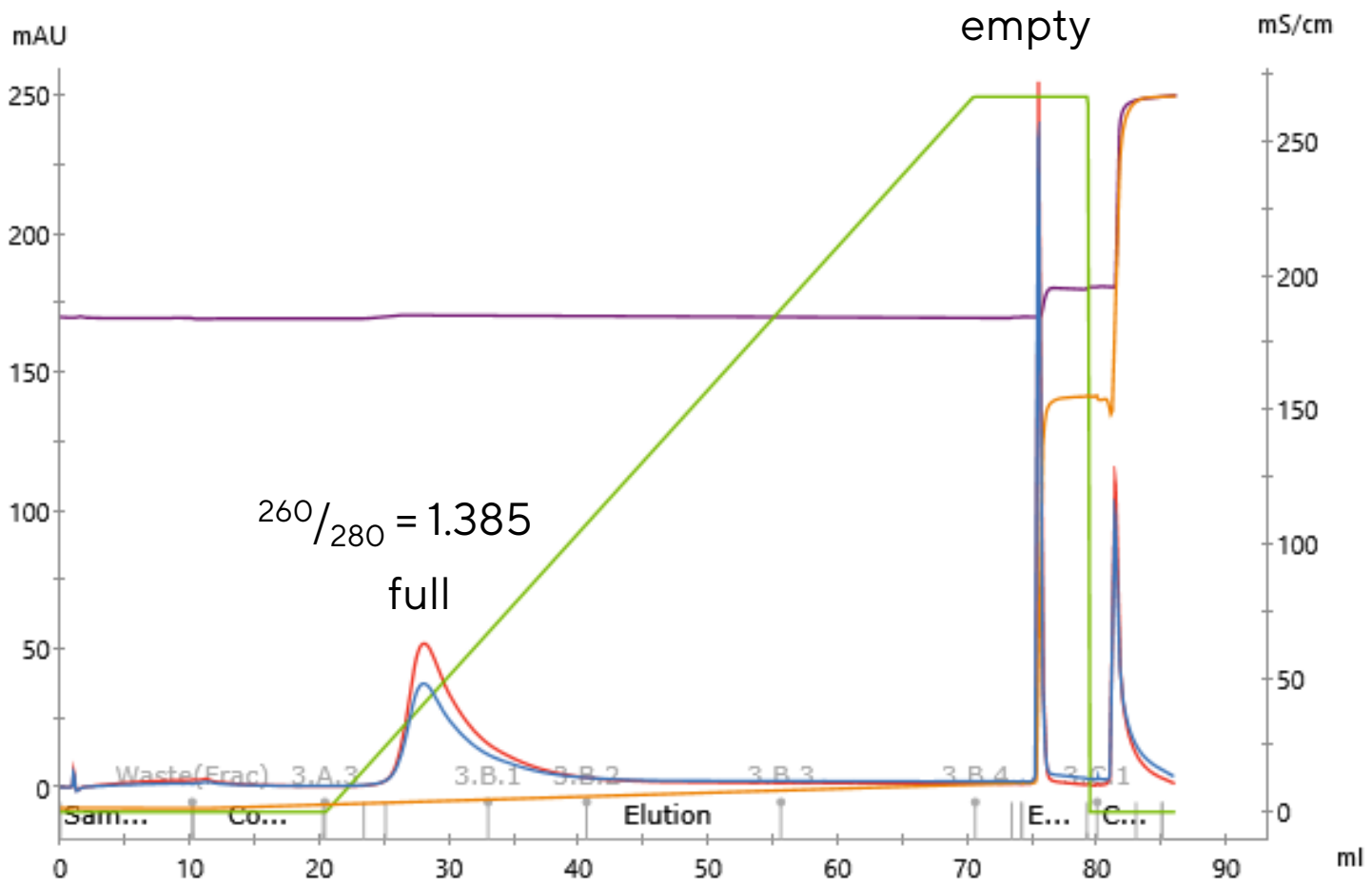
*** Multiple options, alone or combined:**

- 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

Purification insights

CIM PrimaT

- new multimodal ligand for separation of empty and full AAV9 capsids



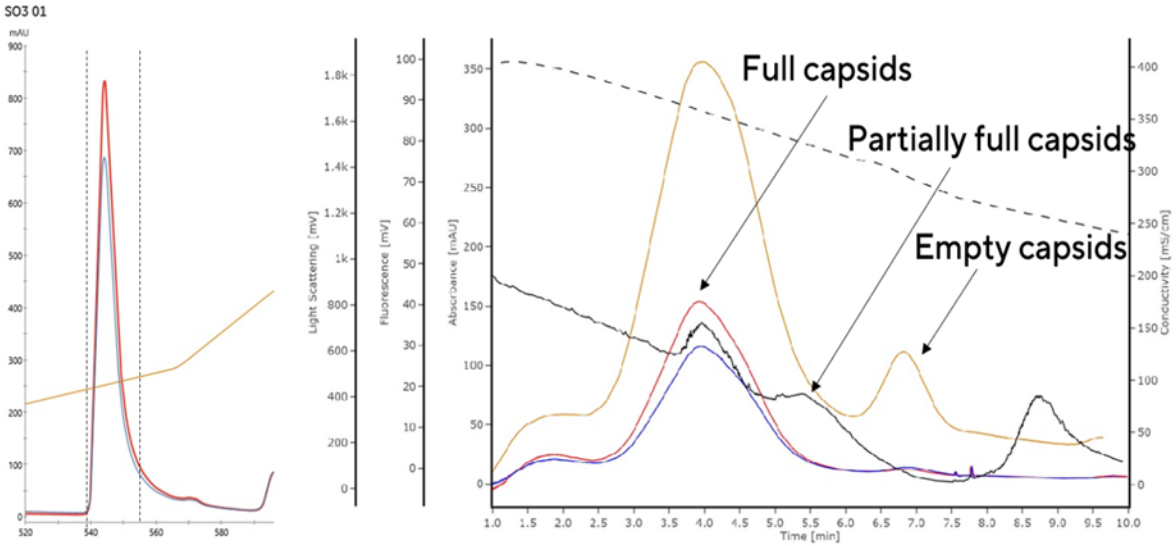
https://www.biaseparations.com/en/library/posters/1108/cim-primat-new-multimodal-ligand-for-separation-of-empty-and-full-aav9-capsids

AAV9 full capsid purity check using ultracentrifuge

Ultracentrifuge analysis in CsCl gradient of AAV with concentration 5E+11 vg (ddPCR).
Method: spin for 24h at 50000 xg. UV260, UV280, Trp, MALS.

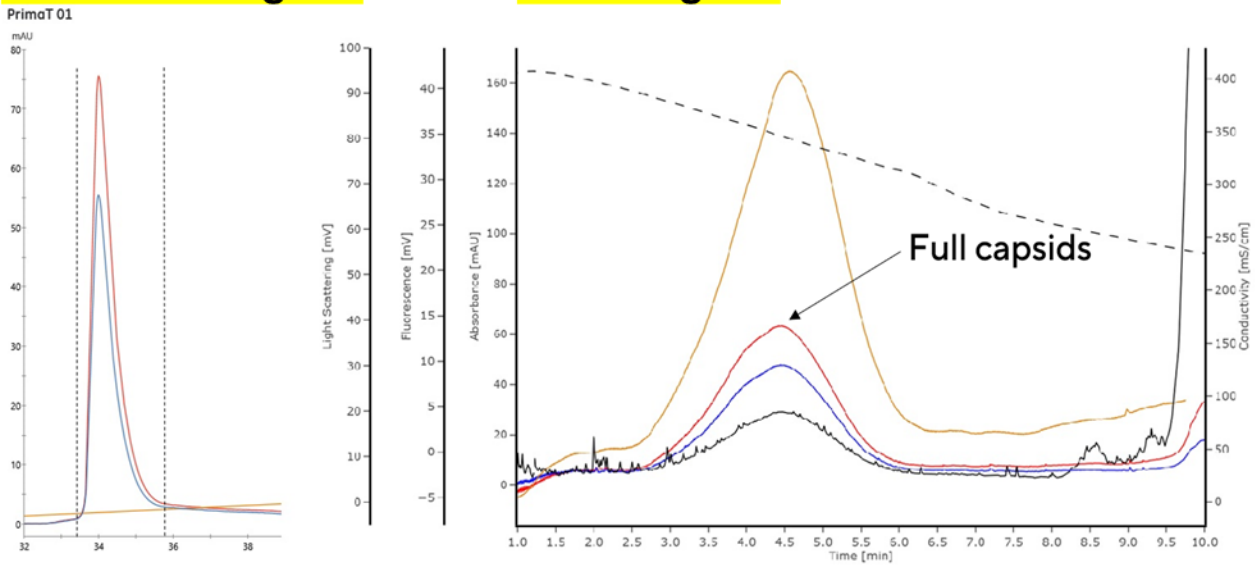
SO3 elution fraction loaded on ultracentrifuge

LC chromatogram **Centrifugram**



PrimaT elution fraction loaded on ultracentrifuge

LC chromatogram **Centrifugram**



Recovery of the PrimaT column: 100% by ddPCR

Thank you for your attention!

Please visit our virtual booth

PDF presentation available from:
https://www.biaseparations.com/en/library/seminars-webinars/1121/in-process-analytics-of-aav-vector-capsid-production

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