SARTORIUS

BIA separations

Simplifying Progress

Fast in-process analytics to enable robust mRNA manufacturing

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AIChE mRNA Technology Conference Boston, April 2024

Sartorius BIA Separations product and solution portfolio

BIA's Proprietary Product and Services Offering

CIMmultus Monolith production-scale columns

10 - 100x
higher capacity

up 3x
higher recovery

up to 3x
lower drug manufacturing cost

Column sizes
40L+

PATfix LC systems & CIMac Monolith process control columns

Higher integrity information at lower cost and better accuracy

Biocompatible

Achieves rapid, high-resolution separations in minutes

Fast and reproducible HPLC monitoring of large biomolecules

Process and method development services

The leading expert on large biomolecules and viral particles

Drives long-term, embedded customer relationships

Tailored services for each clinical phase up to and including production

50+ Process and Method development experts

World-class team

State-of-the-art facilities in Slovenia

See us at HPNE booth at the coffee breaks

BIA separations
Leaders in Monolith Chromatography

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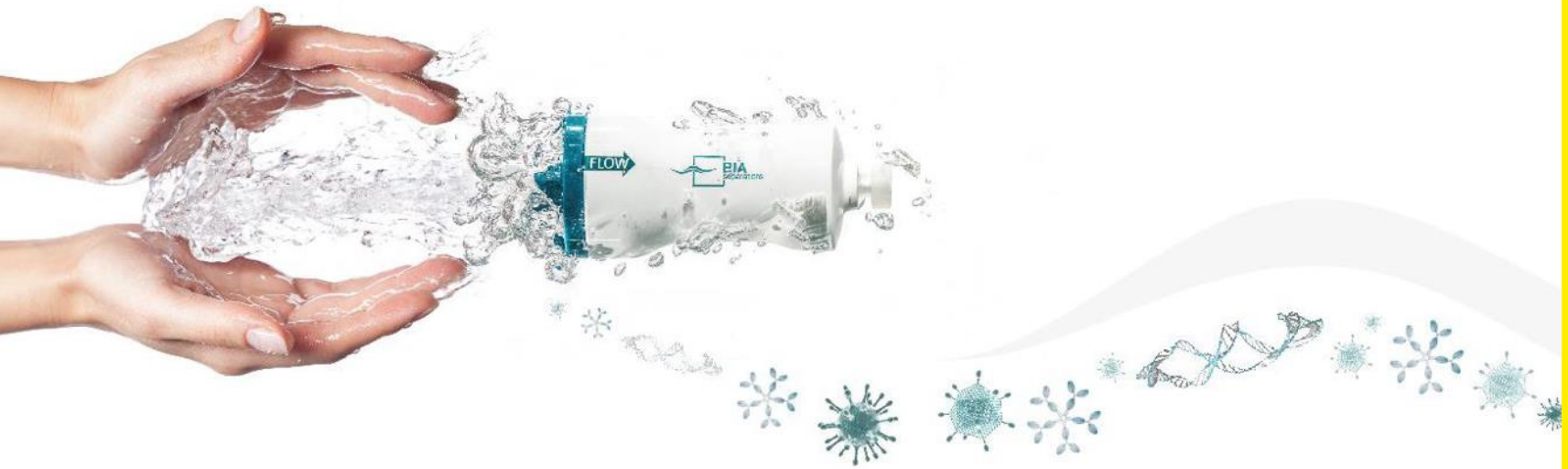

Convective Interaction Media (CIM) monolithic columns

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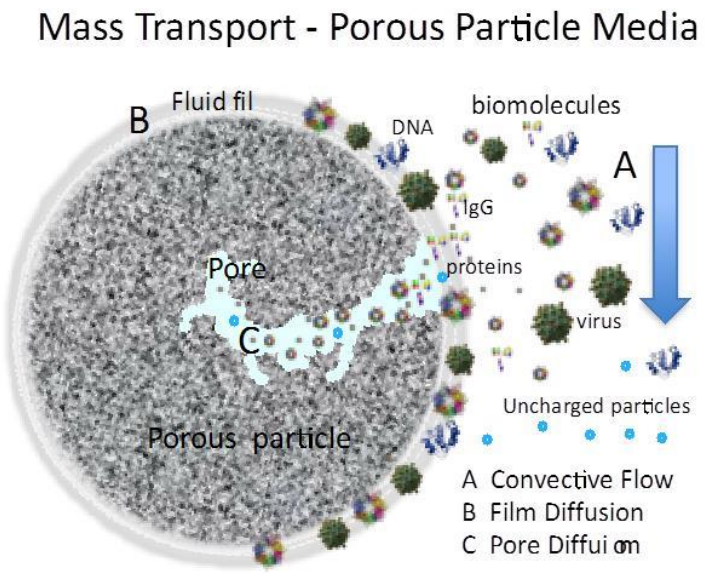
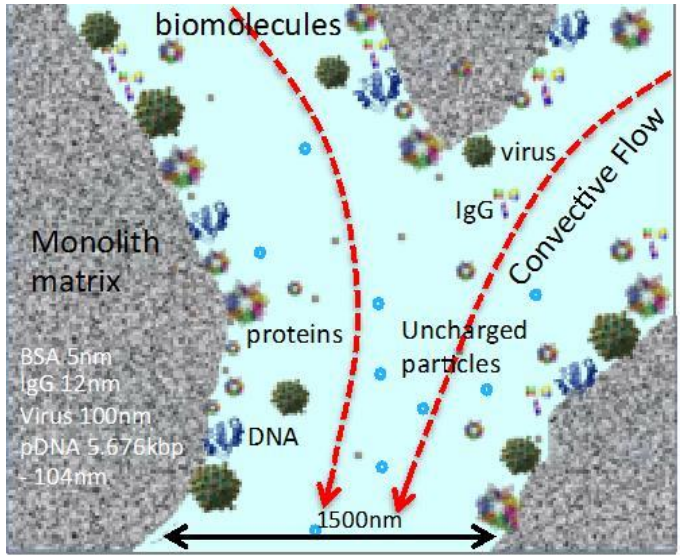
Rapid chromatography processes by convective mass transport





Traditional approach - Porous particle:

- Diffusive mass transport – slow process or **lower resolution**
- Pores too small – very **low capacity for large molecules**
- Counter current flow - shear forces – **lower yields**

Novel UNIQUE approach – Monolithic columns:

- Convective mass transport – **flow independent resolution and capacity**, very fast processes
- Accessible surface for large molecules – **high capacity**
- Laminar flow - **no shear forces – higher recoveries** of e.g. IgM, Lenti, Adeno, Vaccinia, Flu,...
- No diffusion and turbulent mixing - **better resolution**

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Leaders in Monolith Chromatography

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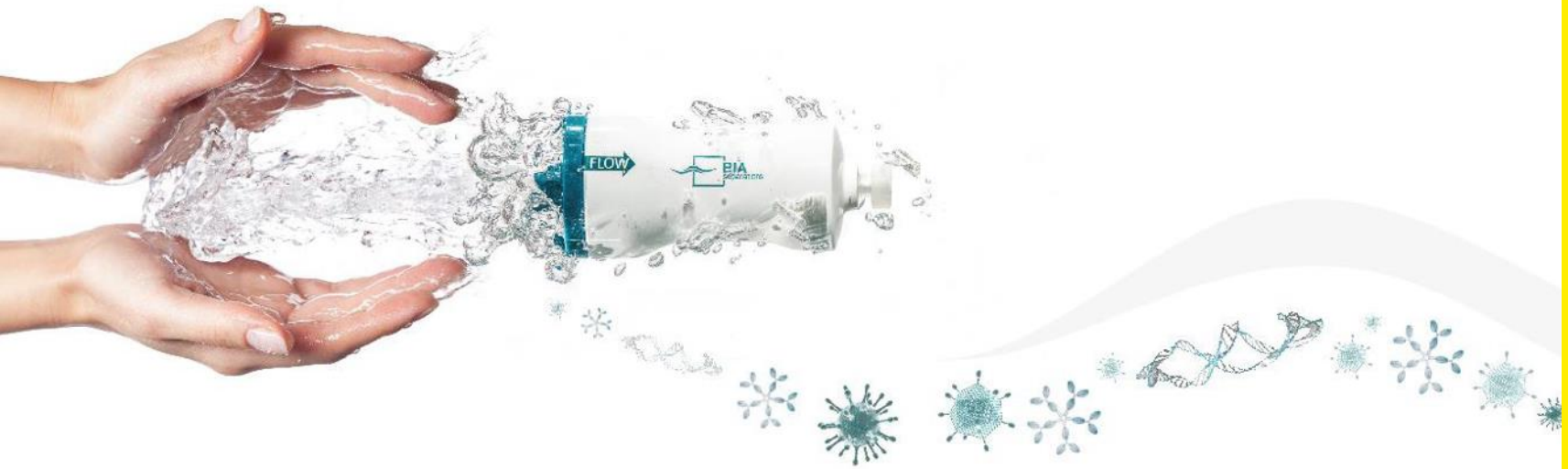

PATfix - fast method development and in-process control LC system

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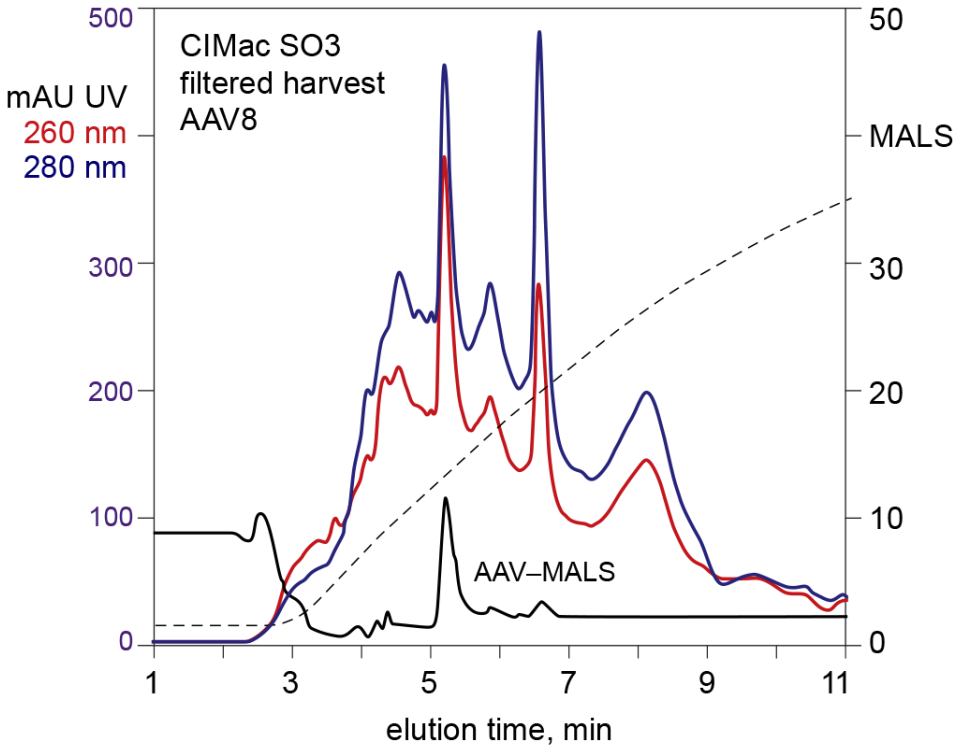
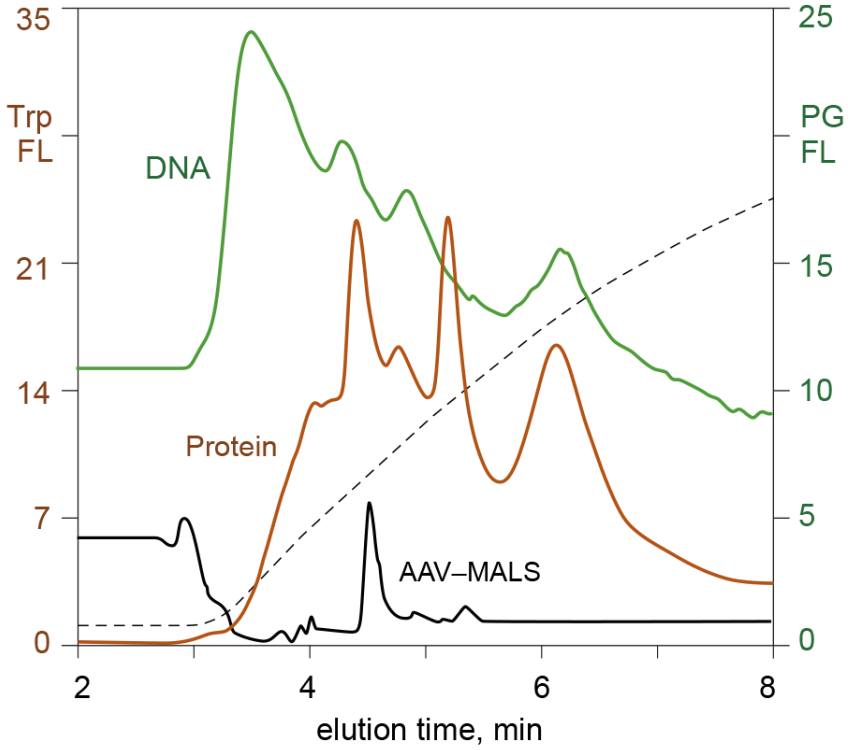
Fast process development enabled by multidetector LC system

PATfix LC with multiple detectors allows for sample characterisation in less than hour

Optional: novel, very reliable and affordable MLS detector, including the prep version





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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mRNA manufacturing optimisation using in-process analytics

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From *E. coli* to mRNA – typical workflow

Analytical workflow (PATfix pDNA; CIMac pDNA)

E.Coli Fermentation

Alkalizator $CaCl_2$ treatment

Filtration

CIMmultus DEAE

Linearization

CIMmultus C4 HLD

UF | DF

Linear pDNA

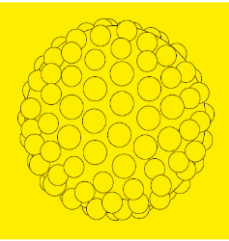
Linear pDNA

IVT in AMBR250 reactor

CIMmultus Toolbox
Oligo dT, PrimaS, **Swiper** / C4 HLD, SDVB

UF | DF

mRNA



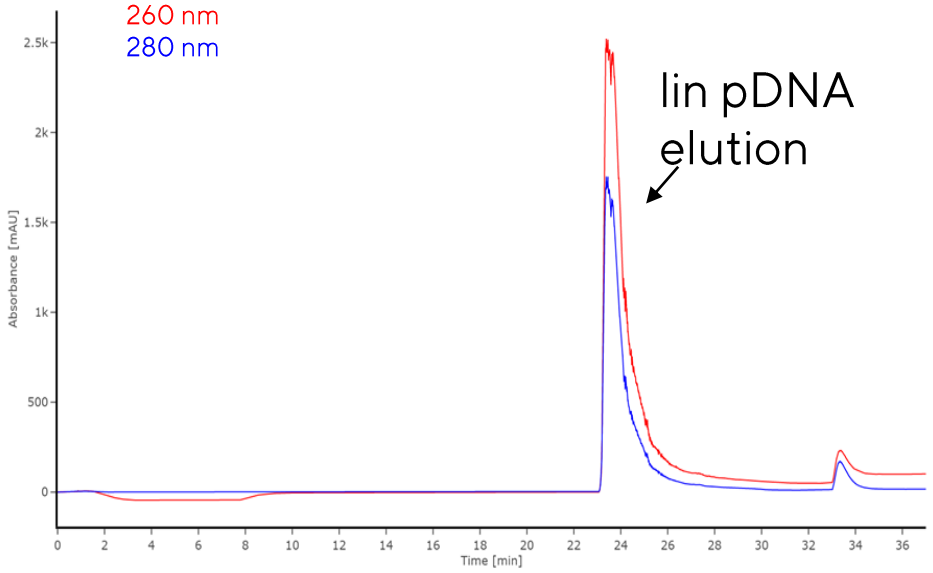
LNP

Analytical workflow (PATfix mRNA; CIMac PrimaS, CIMac Oligo dT, CIMac SDVB)

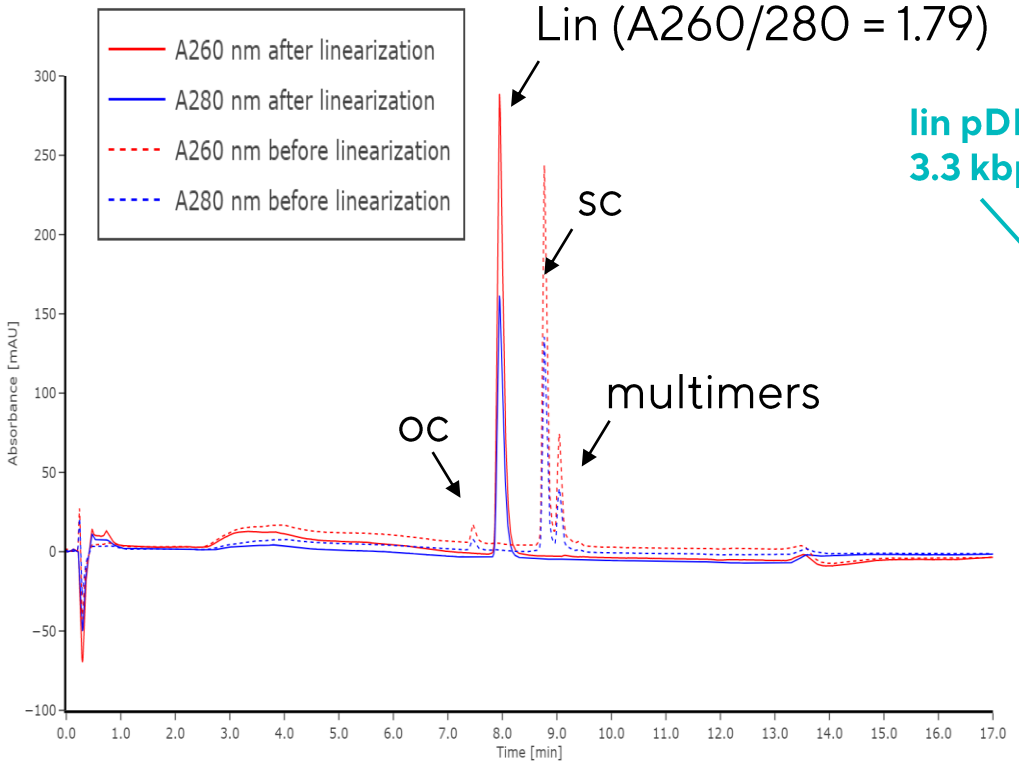
Purification of linear pDNA to lower the dsRNA

Preparative LC CIMmultus C4 HLD

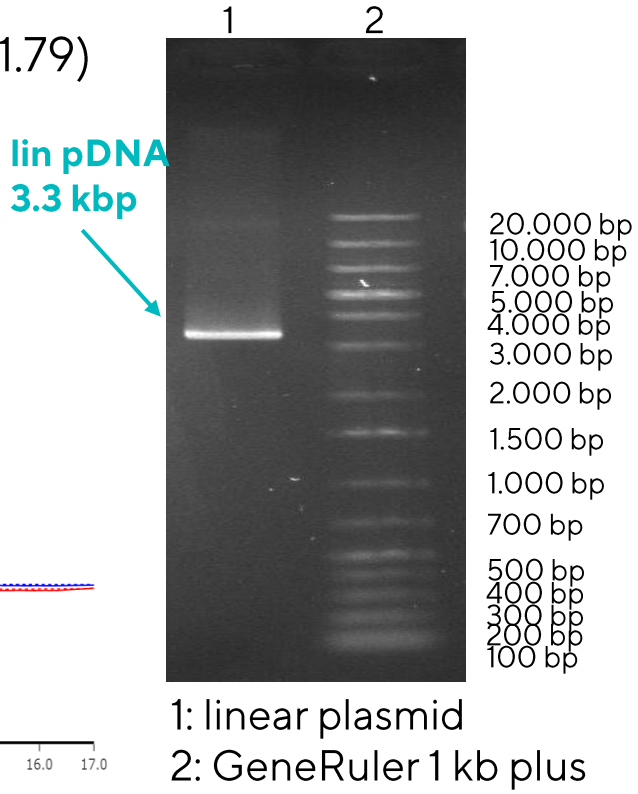
Efficient **protein** removal (proteins sticks to the column and elute in 1M NaOH or organic solvent only)



LC CIMac pDNA analytics



AGE

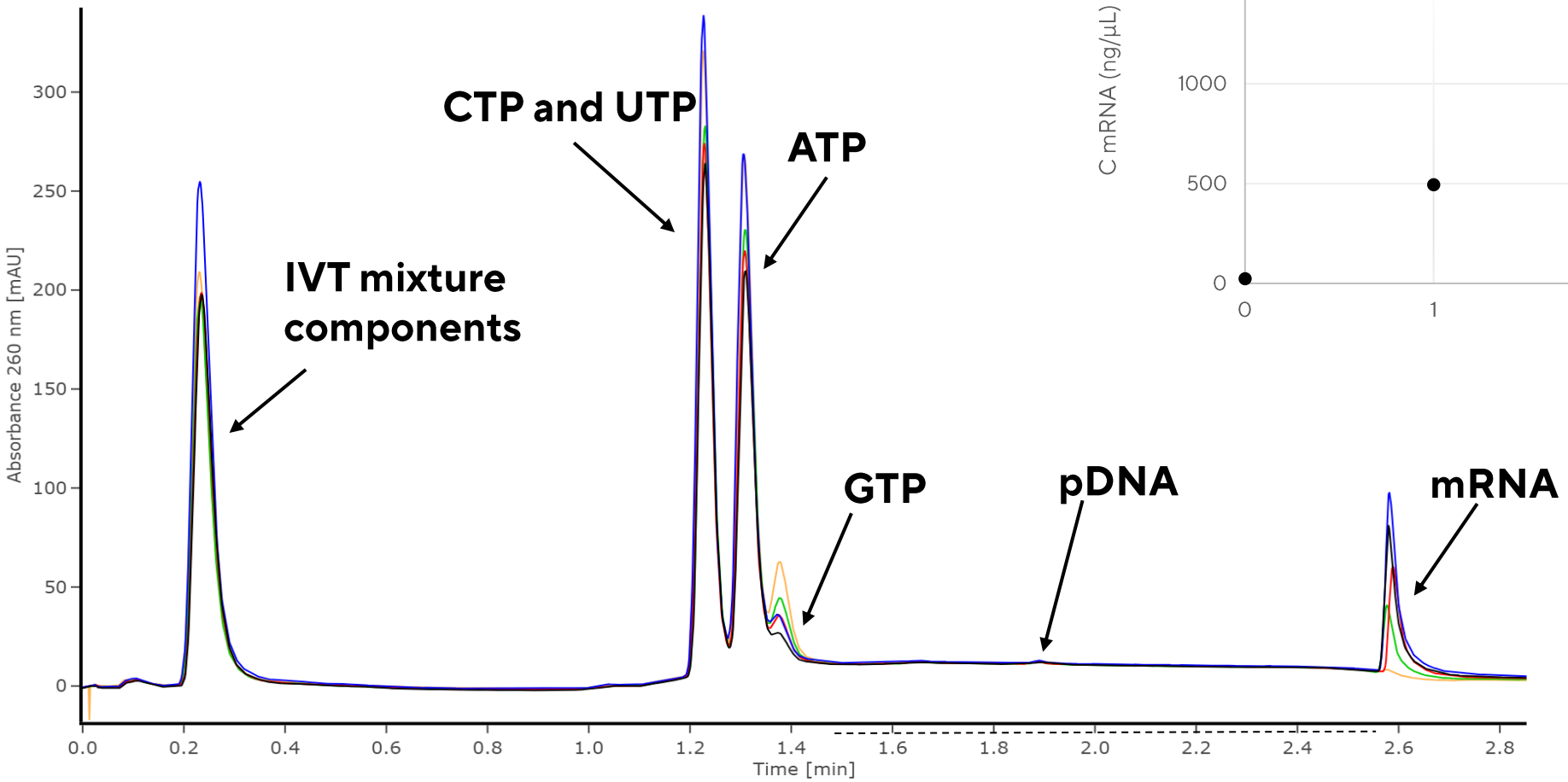


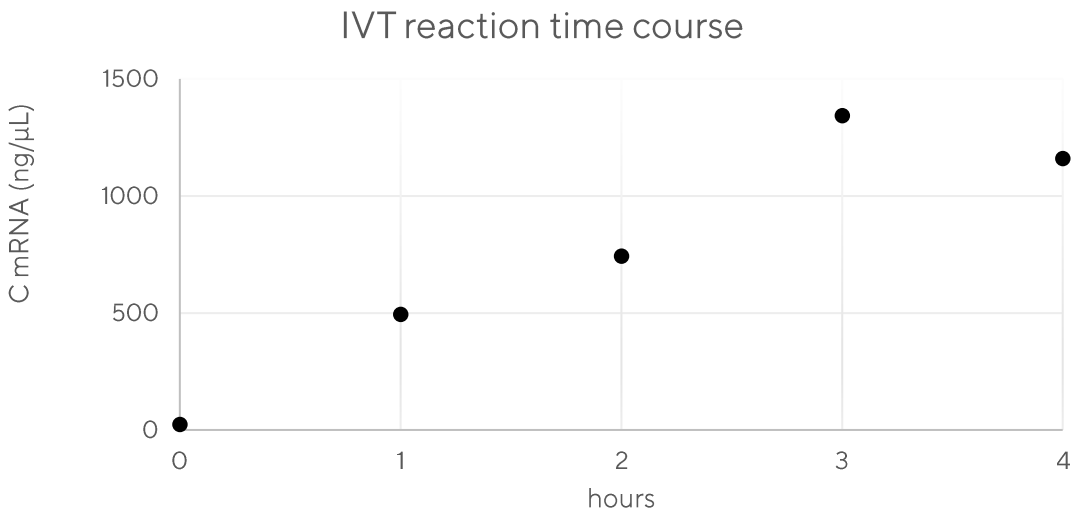
1: linear plasmid
2: GeneRuler 1 kb plus

Martínez et all "The results suggest that by employing a robust purification method for the initial linearized plasmids, such as hydrophobic chromatography, it is possible to obtain mRNA transcripts with lower dsRNA percentages…"*

*Front. Mol. Biosci., 29 September 2023, Sec. Molecular Diagnostics and Therapeutics, Volume 10, 2023, https://doi.org/10.3389/fmolb.2023.1248511

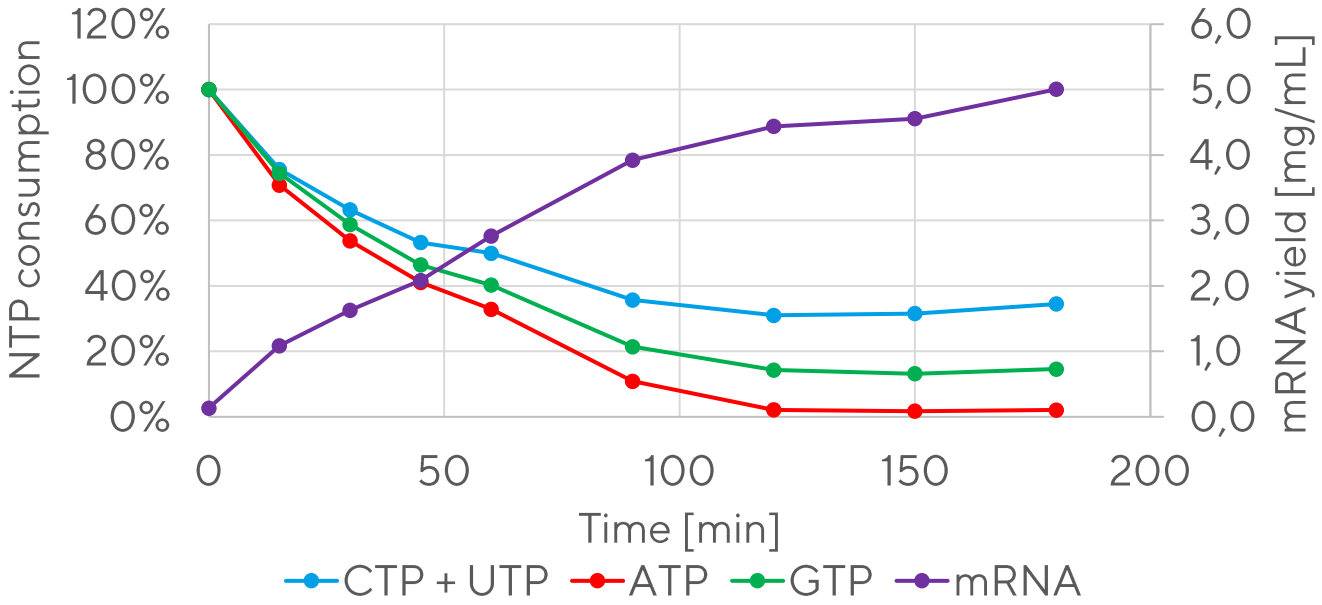
In-process control by CIMac PrimaS column to enable continuous process

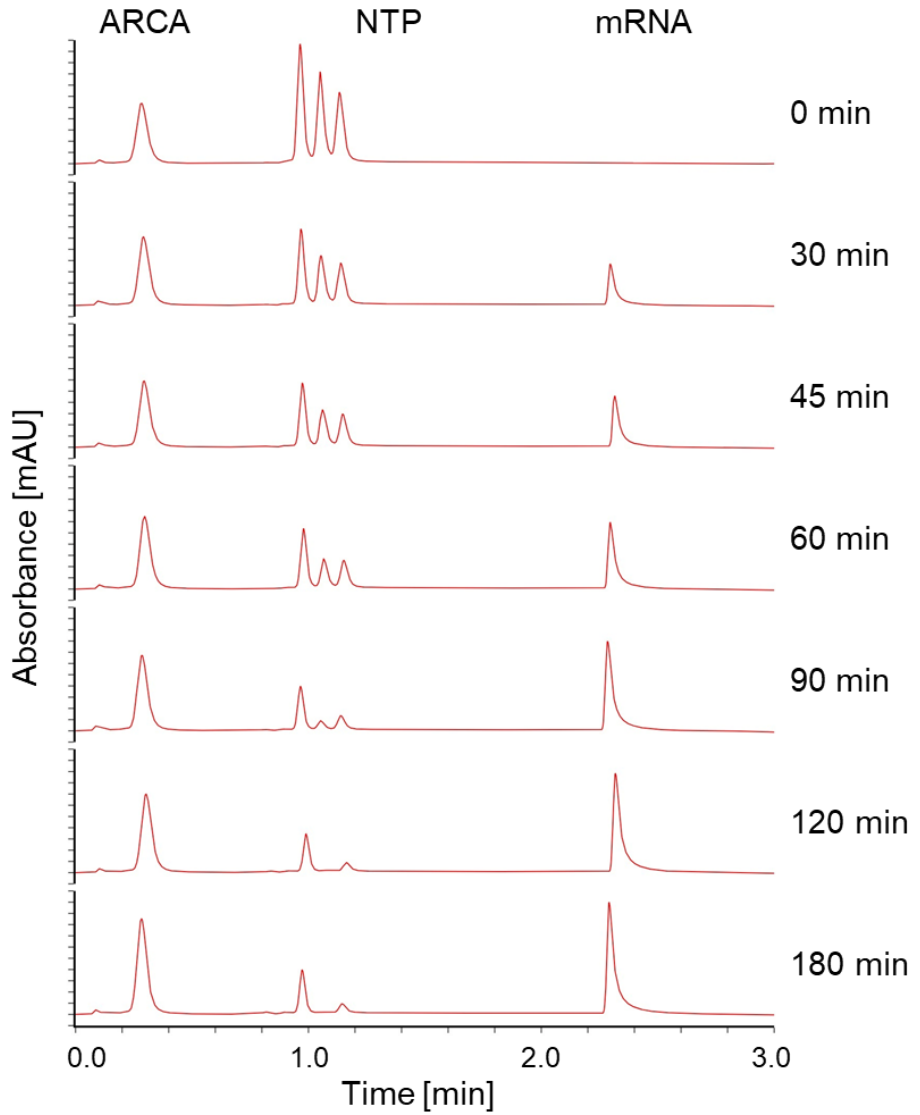




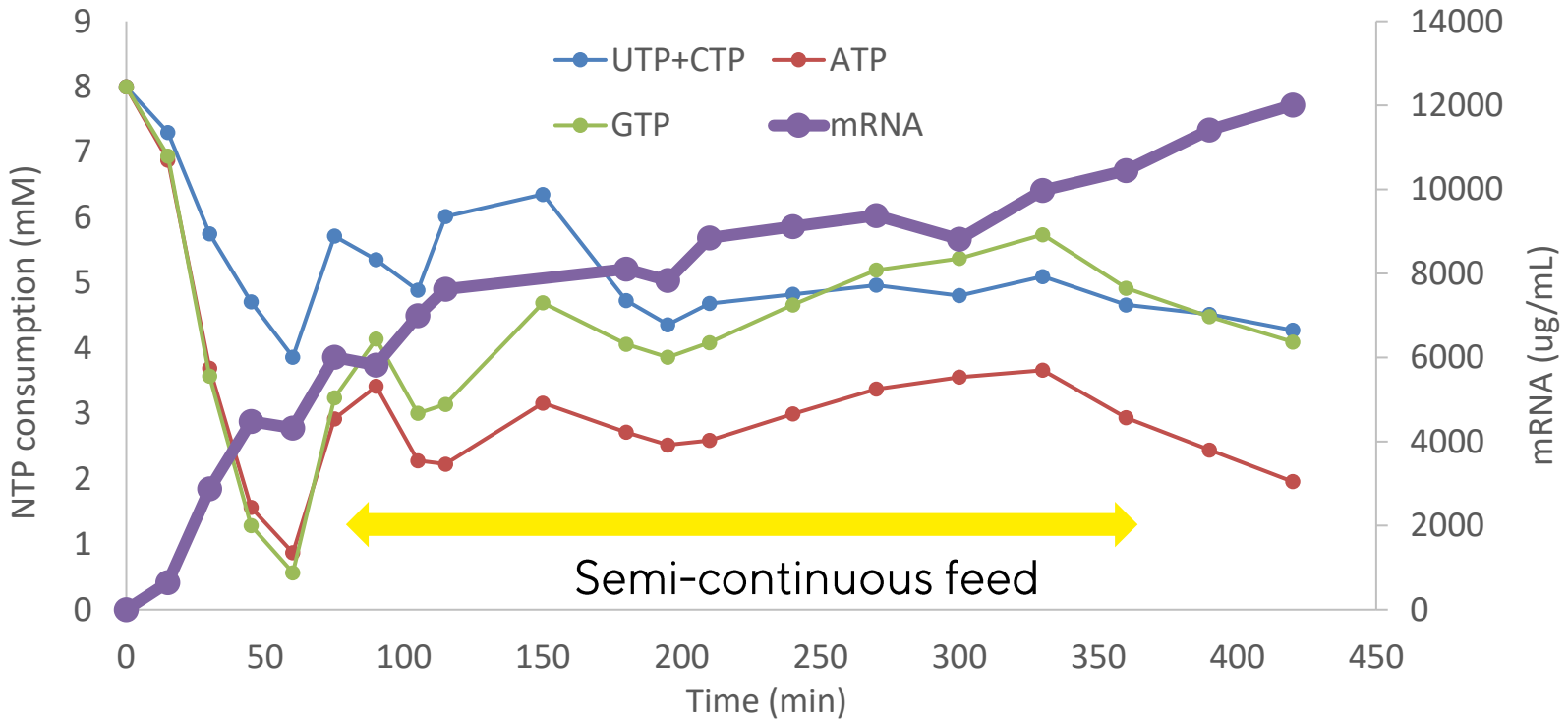
Building up IVT understanding with in-process LC analytics

- The IVT reaction can be monitored at-line **by CIMac PrimaS**
- mRNA production kinetics is monitored. Productivity maximum can be identified, to prevent degradation.
- Consumption of nucleotides and concentration of capping reagent can simultaneously be monitored (multiple capping reagents)
- Effects of feed addition can be studied





Fed-batch mRNA production optimisation using LC in-process control

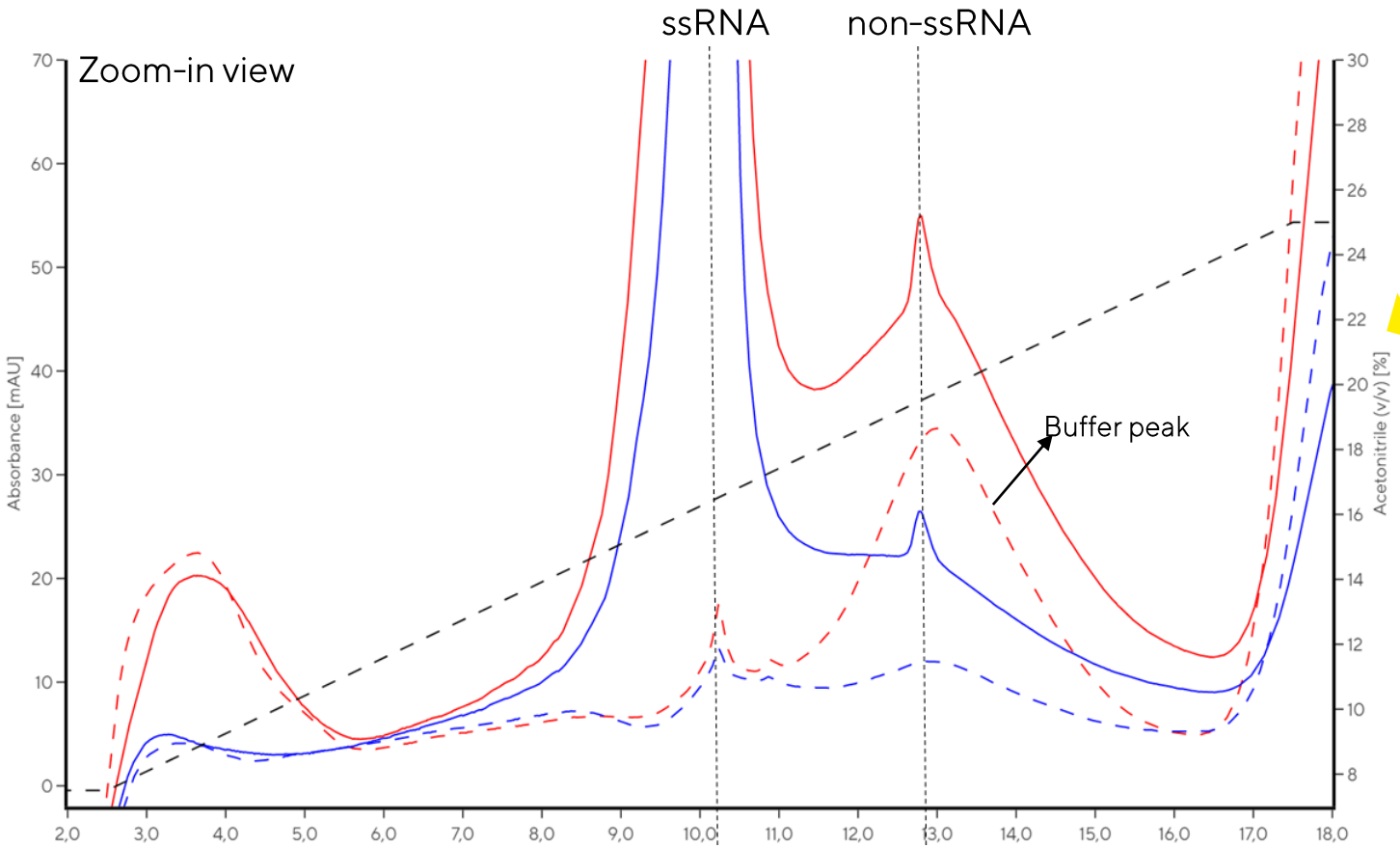


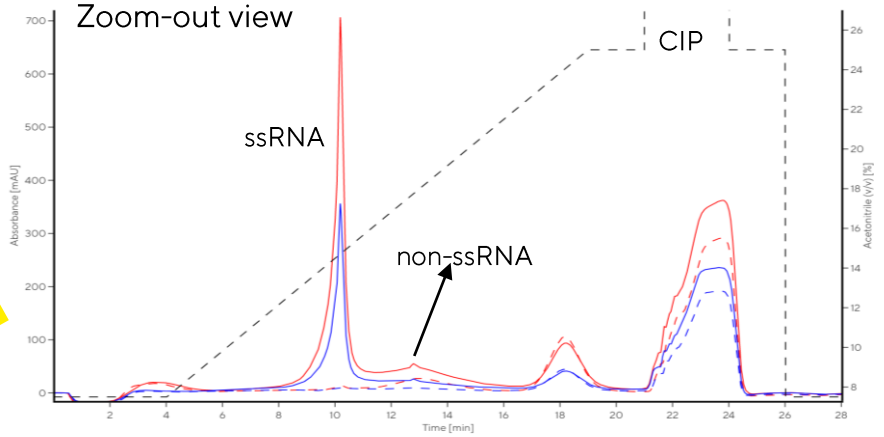
AMBR250 reactor

PATfix mRNA system

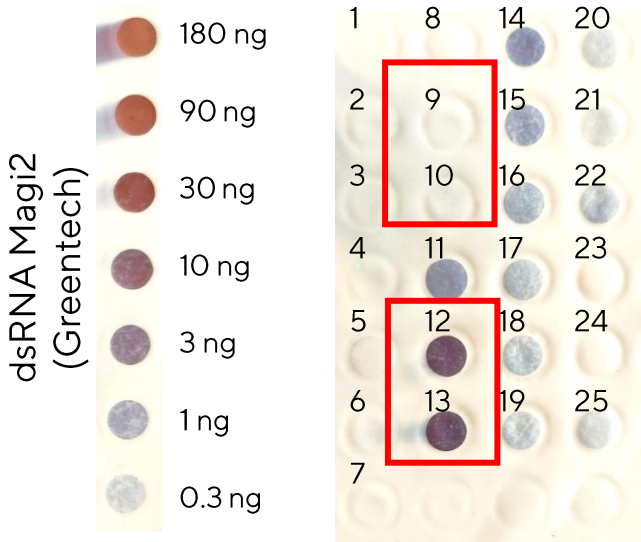
- 2g of mRNA produced in a single batch **(>11mg/mL yield)** by coupling automated reactor system (AMBR250) with PATfix LC analytics.
- Real-time monitoring of NTP in reaction container.

LC analytics of dsRNA removal using CIMac SDVB column





Dotblot from SDVB fractions



Buffer A: 100 mM TEAA; pH 7.0; Buffer B: 100 mM TEAA, **25% ACN**; pH 7.0;
Buffer C: 100 mM TEAA, **90% ACN**; pH ~7.0
Thermostat: **60°C**

Collection of fractions:
Every minute from 1 min to 25 min (e.g. 11=from 11min to 12min)

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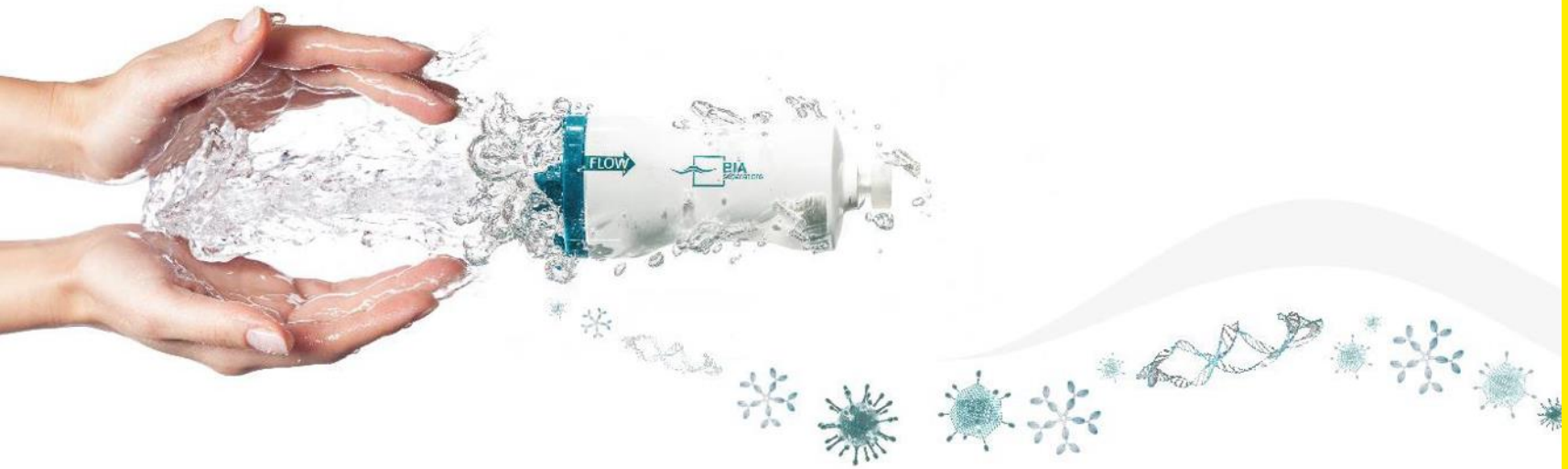

In-process analytics of mRNA encapsulation using LC

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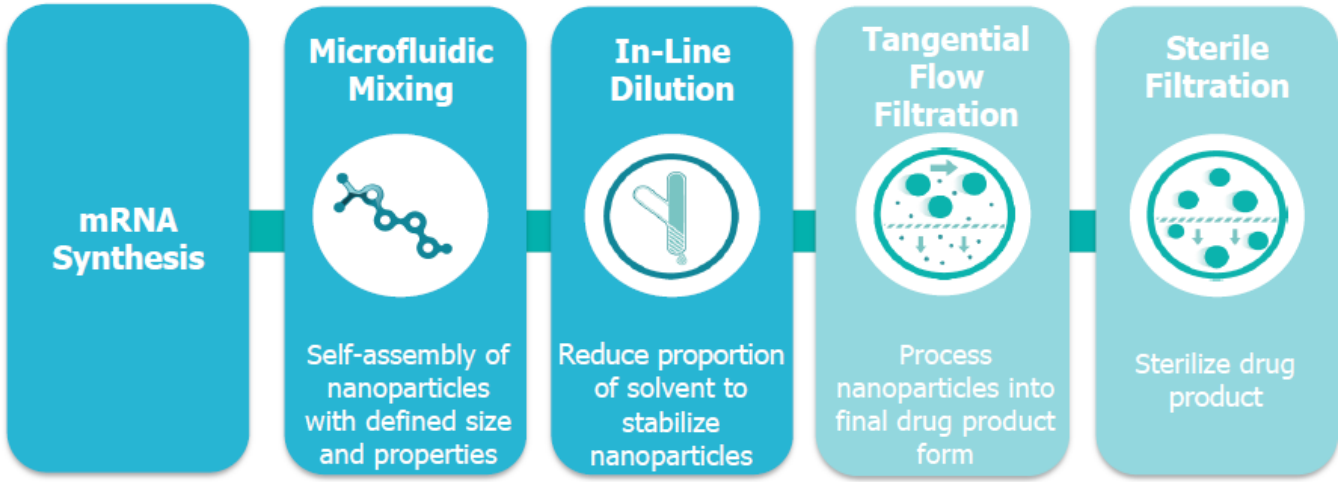
State of the art LNP synthesis



Ref: J. Coleman: Scalable Manufacturing Platform for RNA-LNP Vaccine Development. Boston, May 22

- LNPs are produced in high ethanol concentrations (25 – 33 %)
- Ethanol concentration immediately diluted to <6%
- Buffer exchange and concentration typically performed with TFF
- Disadvantages:
  - No clearance of free mRNA (retained by membrane with LNPs)
  - High shear force generated by TFF leads to low recovery of the process (‘the stress applied is from processing LNPs /.../ e.g. by TFF. During a typical TFF process, the LNP dispersion is exposed to a variety of hydrophobic interfaces, shear forces, and turbulence. [US20210145982A1]
  - Adhesion of LNP onto PES membrane of TFF device (Sakurai, Hada & Harashima, Journal of Biomaterials Science, 2017)

Encapsulation efficiency analysis using PATfix Switcher – flow chart

Direct injection of LNP formulations; no sample pre-treatment, dilution with loading buffer only

OH column

OH buffers

Sample → Analytical Autosampler → OH Pump → OH Column → Valve [1] → 2 → Waste

Valve [1] → 1 → MALS Detector

SDVB column

Inline dilution (mixing ratio 9:1 SDVB:OH) → SDVB Column → UV-Vis Detector → Valve [2] → 2 → Waste

SDVB Column: 60 °C

Valve [2] → 1 → CAD Detector

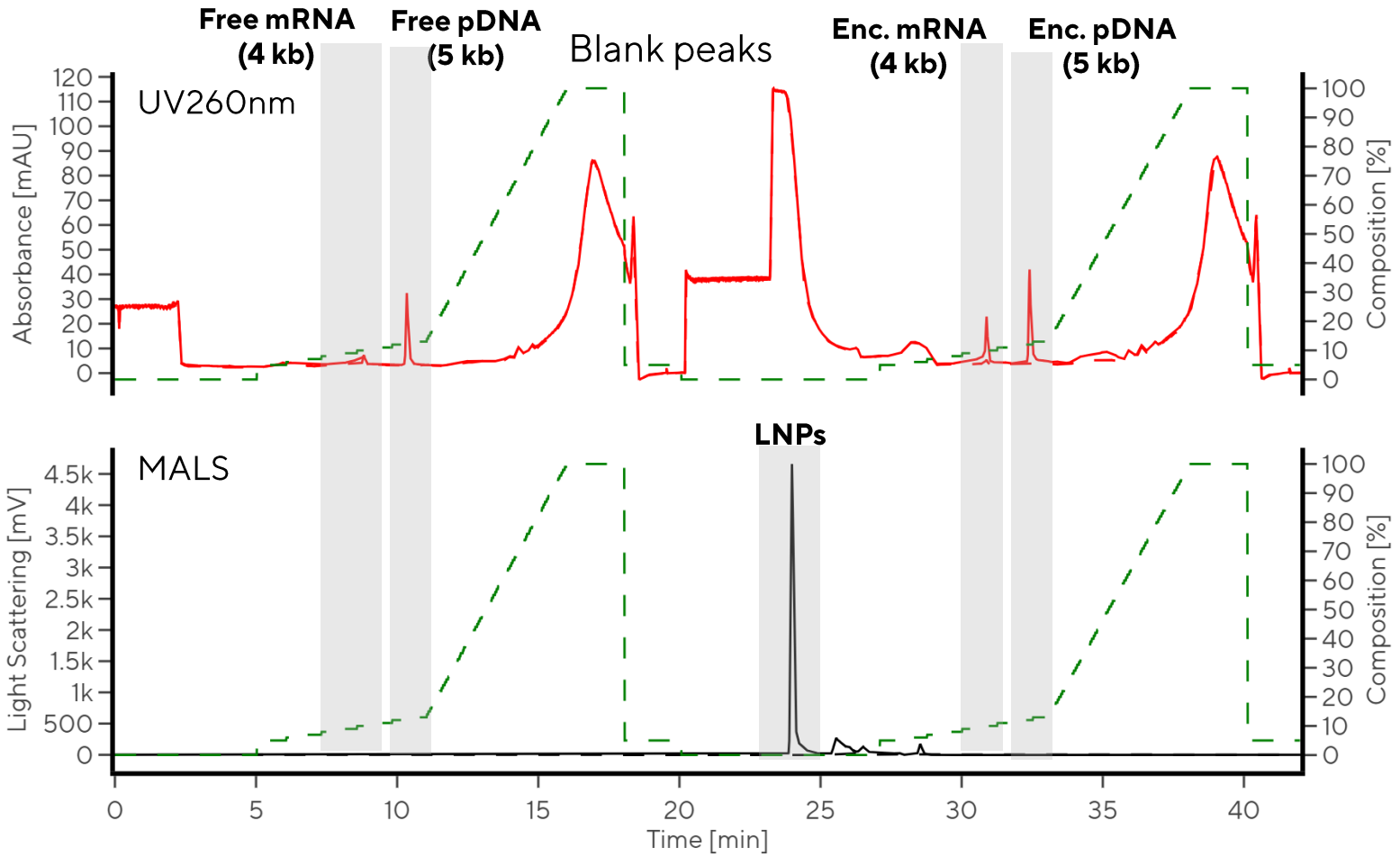
SDVB Pump ← SDVB buffers

Encapsulation efficiency analysis using PATfix Switcher – conditions

- First step capture of LNPs on OH column and capture of free mRNA on SVDB column
- Then degrading LNPs and capture of mRNA and free lipids on SVDB column
- Then gradient to separate mRNA and lipids

Column	CIMac OH 0.1 (6)	CIMac SVDB 0.3 (2)
Pore volume	0.1 mL	0.3 mL
Pore diameter	6 µm	6 µm
Detectors	MALS	UV, CAD
LC system	PATfix	PATfix
Loading Buffer A	200 mM Sodium citrate, 15 mM TRIS, pH 8	200 mM TEAA, 7% MeCN
Elution Buffer B	200 mM TEAA, 7% MeCN	98% MeCN
Cleaning Buffer C	1M NaOH	98% MeCN

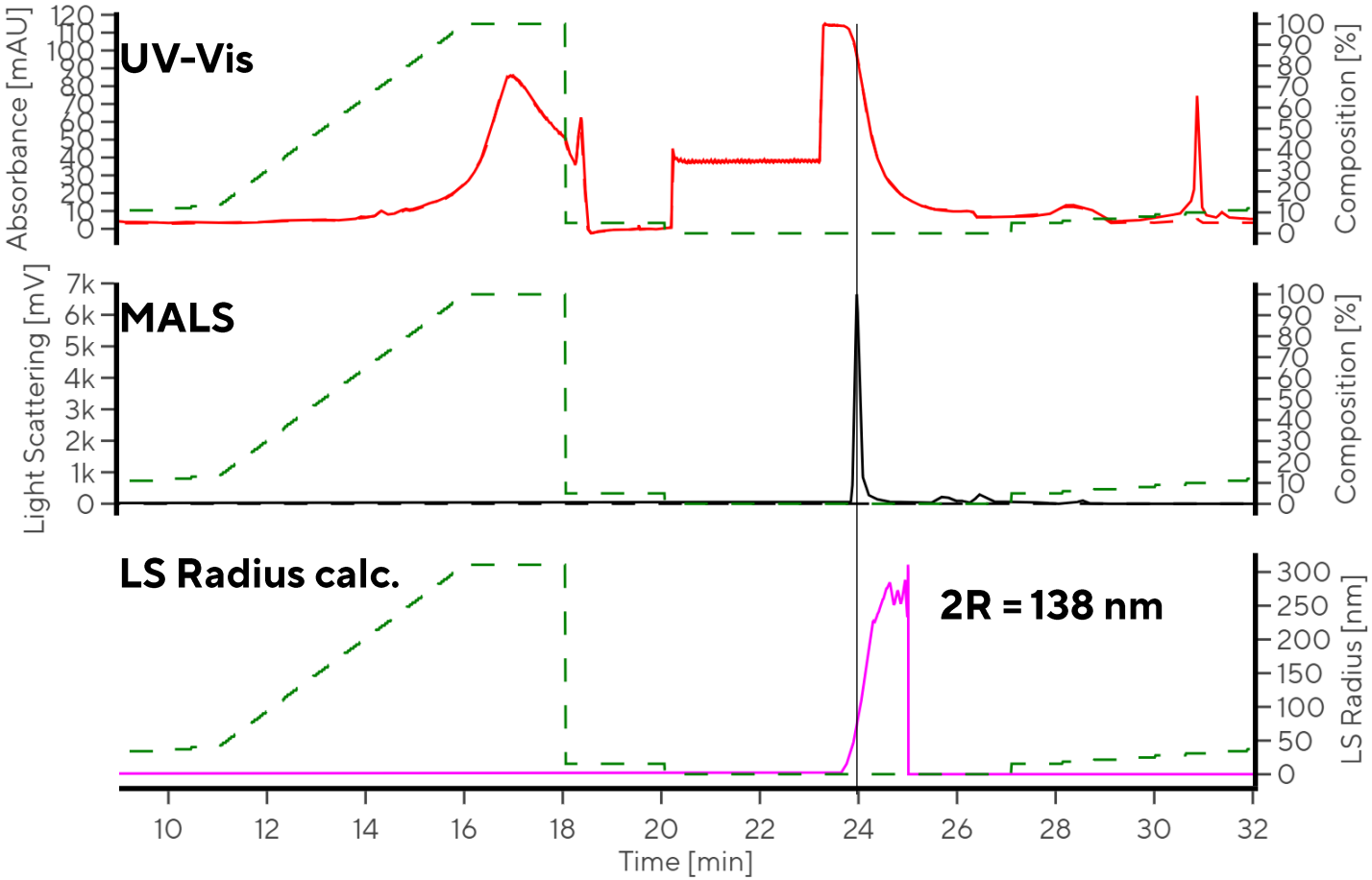
Encapsulation efficiency analysis using PATfix Switcher - chromatogram

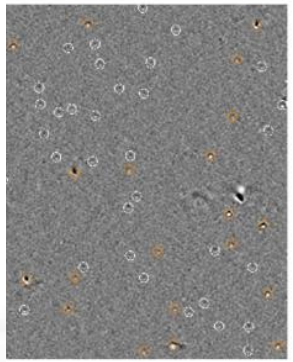


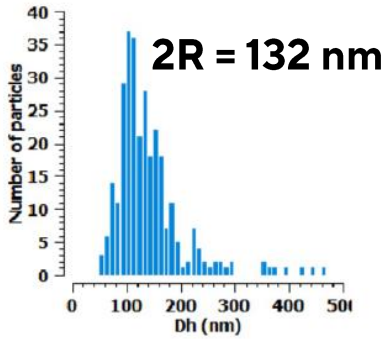
LNP mRNA + pDNA

- LNP mRNA + pDNA formulation was analysed with PATfix LNP Switcher
- **Capable of separating different free and encapsulated nucleic acids!**
- **Amount of the LNP can be determined using MALS detector**

PATfix Switcher - mRNA/LNP size distribution analytics

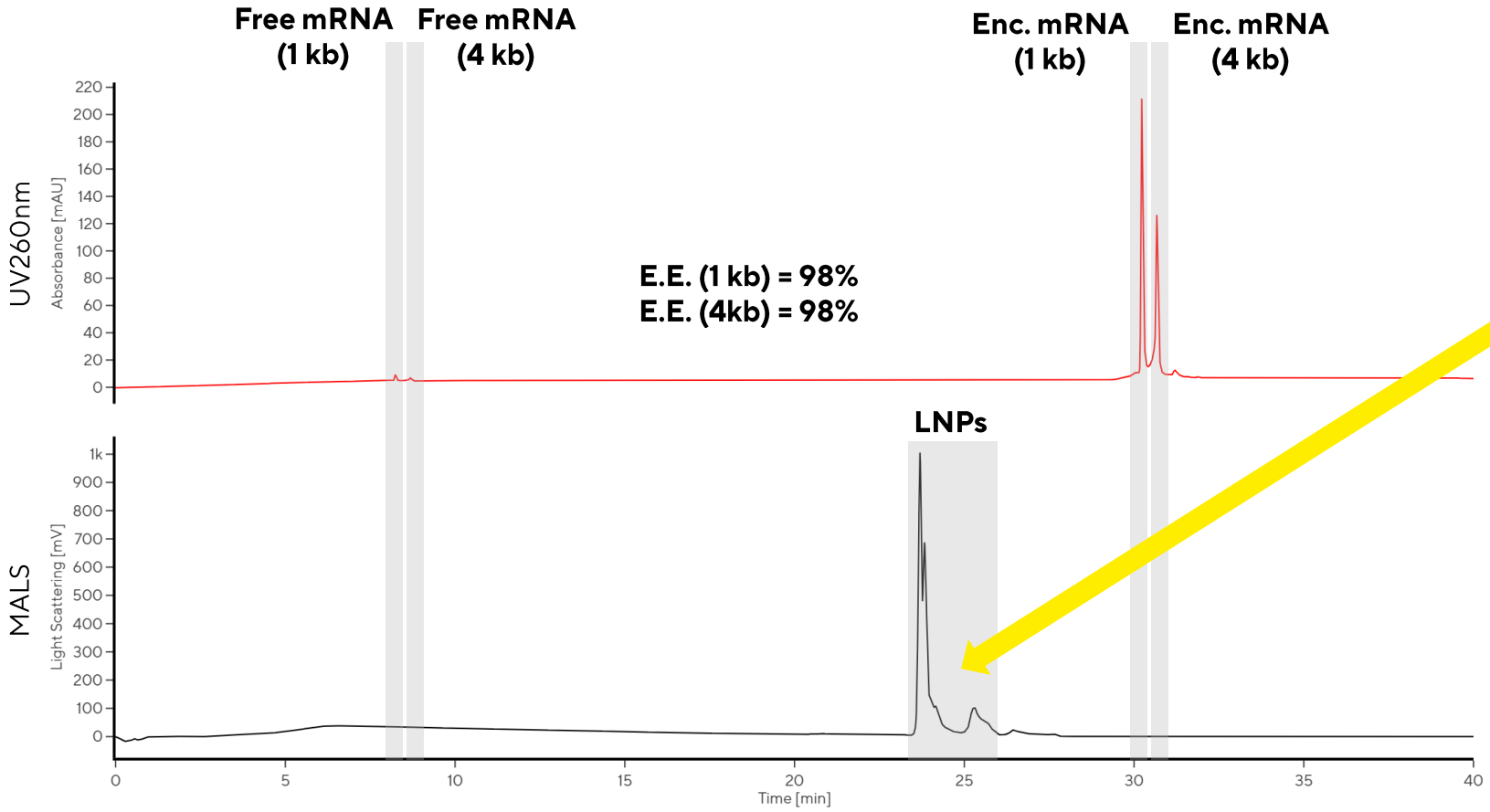






- LNP Switcher was used for size determination of LNP mRNA particles
- Radius was determined by modelling different scattering angles using Berry model
- In agreement with Videodrop (similar to NTA method) measuring the size and concentration of nanoparticles in real time and in one drop

Encapsulation efficiency PATfix Switcher analysis – heterogenous LNPs



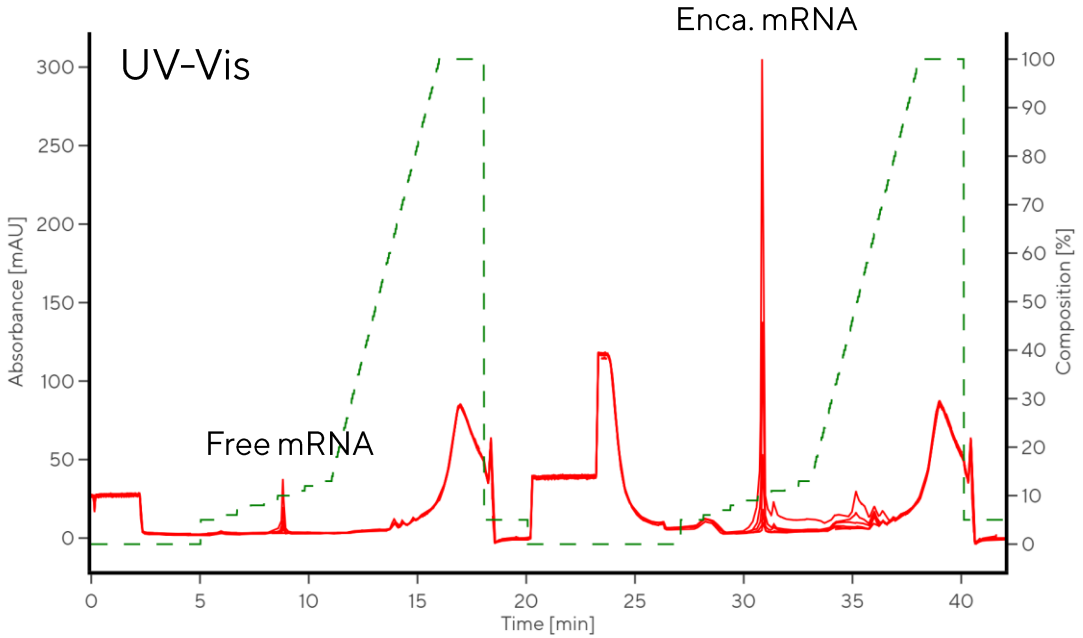
LNP mRNA 1 kb + mRNA 4kb

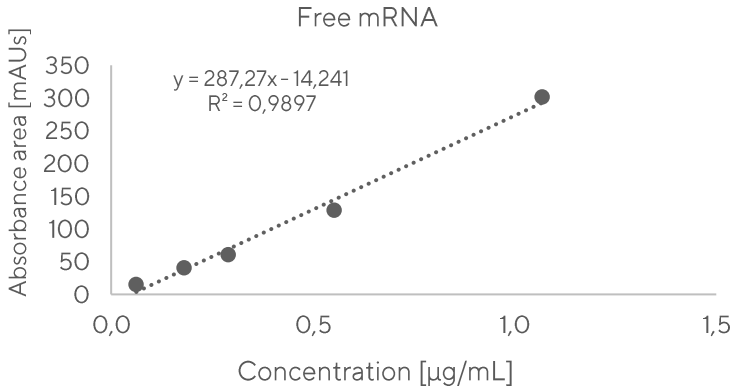
- Very efficient encapsulation
- **But heterogenous LNPs .....**

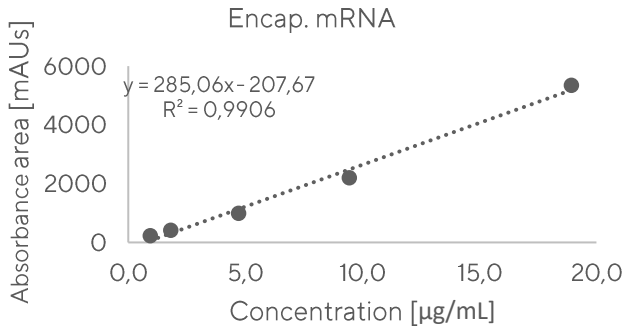
Encapsulation efficiency analysis using PATfix Switcher - LOD/LOQ

- Concentration of mRNA: 1, 2, 5, 10, 20 µg/mL (theoretical concentration of encapsulated mRNA)
- Concentration of each species was calculated from encapsulation efficiency

Species	LOD [ng]	LOQ [ng]
mRNA	2	7







Encapsulation efficiency PATfix Switcher analysis – comparison with RG

Quant-it™ RiboGreen method

- RiboGreen dye is used for detection and quantification of nucleic acids
- Triton X-100 is applied for LNPs opening
- There is no significant difference in the results obtained with these two techniques

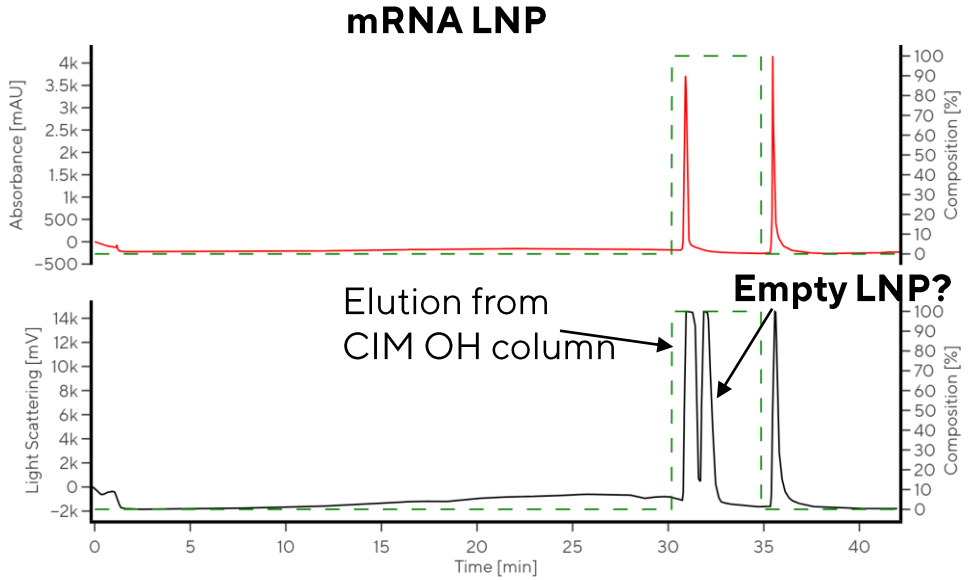
Comparison of encapsulation efficiency results obtained by LNP Switcher and Quant-it™ RiboGreen assay:

Sample	% encapsulation	
	LNP Switcher	Quant-it™ RiboGreen
LNP mRNA	**72**	**71**

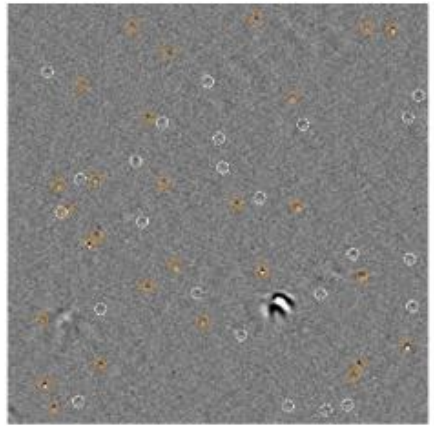
LNP PATfix Swither method can be used as orthogonal to RiboGreen method

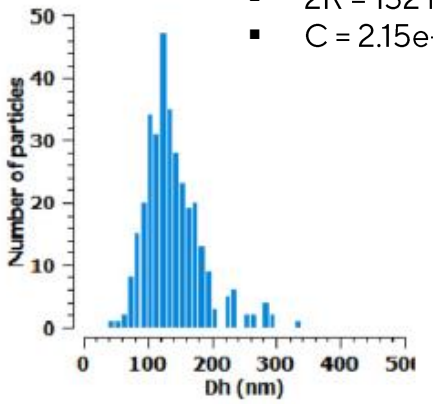
LNP fractionation using size separation on monolithic OH column

- Crude LNPs mRNA were loaded on CIMac OH column
- Elution was analysed by Videodrop
- **Size distribution – significant heterogeneity**
- Almost **no difference** in terms of size and size distribution of LNPs mRNA



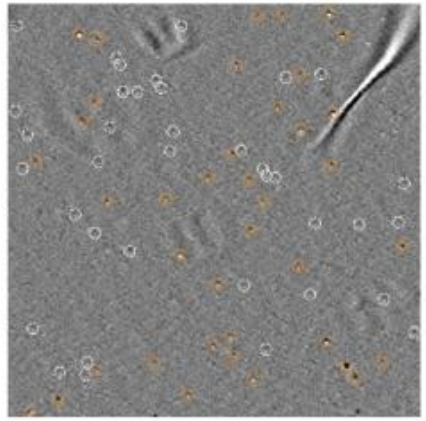
Crude sample

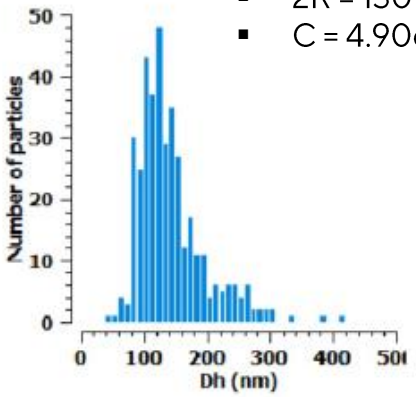




- 2R = 132 nm
- C = 2.15e+9 part/mL

Elution from OH column

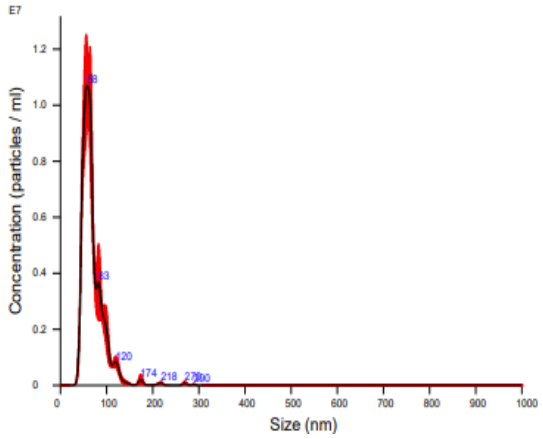




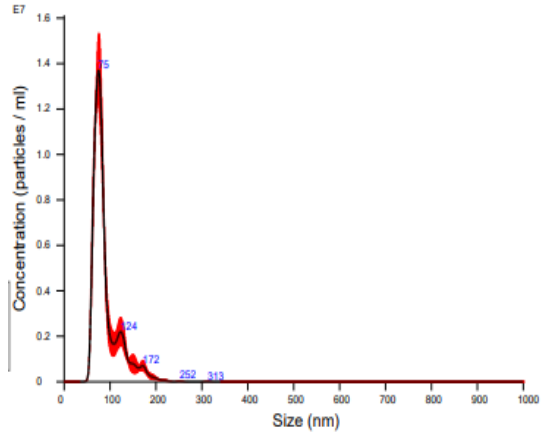
- 2R = 130 nm
- C = 4.90e+9 part/mL

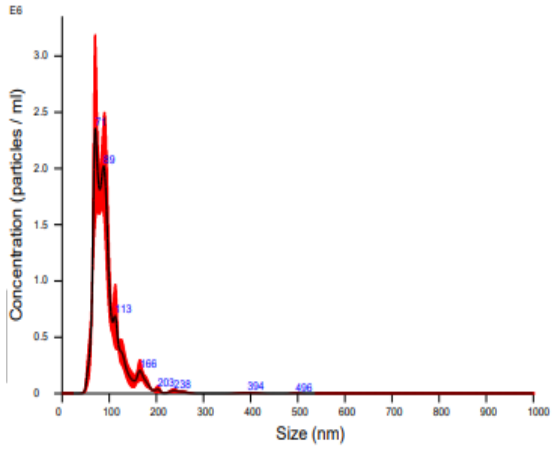
LNP stability is affected by buffer compositions

Crude LNP

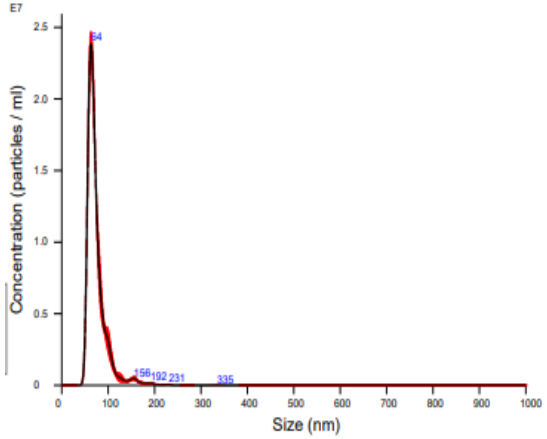


BE into 150 mM NaCl, 300 mM sucrose, pH 7.4

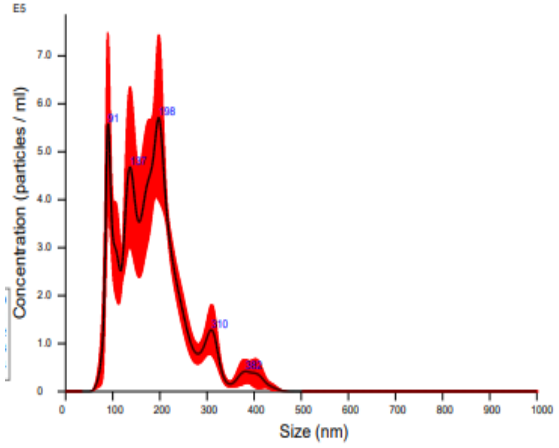




BE into 150 mM sodium citrate, 300 mM sucrose, pH 7.4

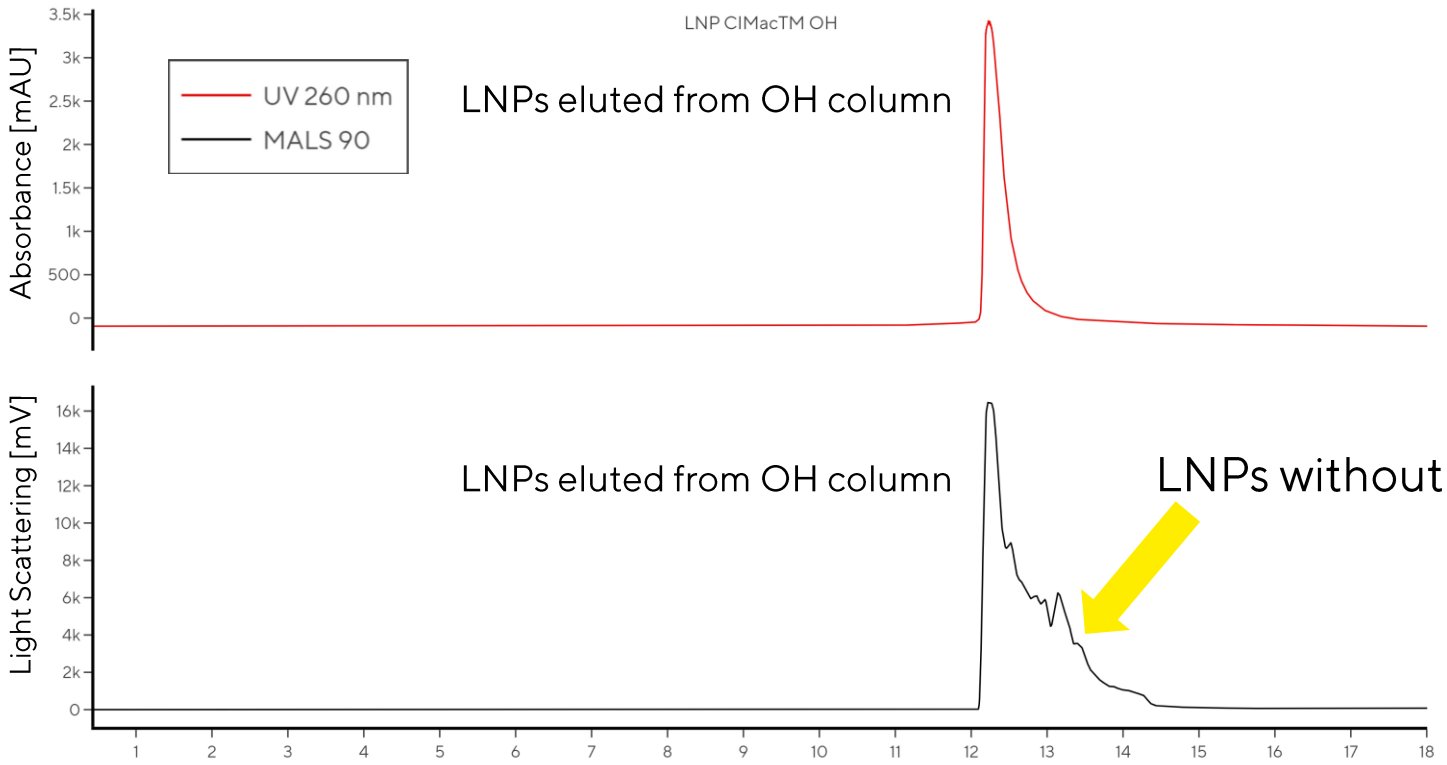


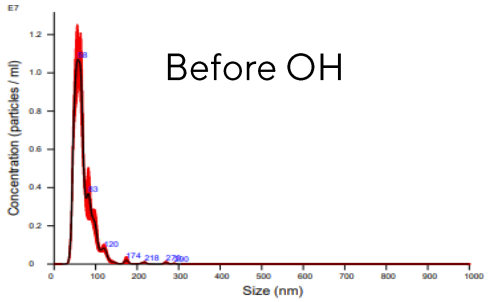
Fresh



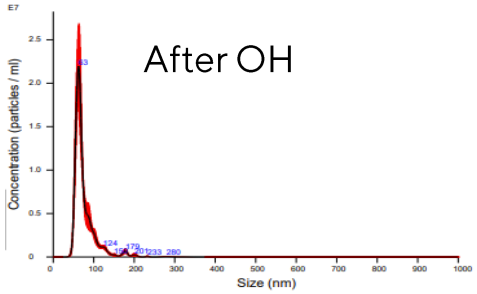
After freezing at -20 °C

Monolithic OH column allows for LNP heterogeneity analysis





Before OH



After OH

- LNPs bind and elute efficiently to and from the CIMac OH column.
- Size distribution remains intact.
- Monolithic OH column orthogonal to NTA

Conclusions

- **High purity of raw materials** and in-process control of the IVT process allow for high transcription number, much purer mRNA and lower consumption of expensive reagents.
- No **robust bioprocess** can be developed without support of proper analytical methods.
- Data of analytical methods might be misleading. **Orthogonal methods** are needed for accurate process understanding and its control.
- LNP PATfix Swither method can be used as **orthogonal to RiboGreen method**.
- PATfix analytics of the LNP size can be used as **orthogonal to DLS/NTA/Videodrop methods.**
- Most important, orthogonal analytics and in-process control enables **safer product and cheaper mRNA/LNP manufacturing processes.**

Thank you for your attention!

Some images and schemes were created using in BioRender.com for the purpose of conference presentation.

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