SARTORIUS

Simplifying Progress

Speeding mRNA Process Development & Securing Robust Manufacturing by Using Fast In-Process Analytics

September 2023

Sartorius BIA Separations Product and Solutions Portfolio

BIA's Proprietary Product and Services Offering

CIMmultus® Monolith production-scale columns

- **10 to 100 times** higher capacity
- **Up 3 times** higher recovery
- **Up to 3 times** lower drug manufacturing cost
- Column sizes **40L or more**

CIMac™ monolith process control columns

- **Higher integrity** information at **lower cost** and **better accuracy**
- **Biocompatible**

PATfix® solutions

- 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
- **More than 50** process and method development experts

World-class team

State of the art facilities in Slovenia

Experts in Express Bioproduct Manufacturing Process Development – No Royalties

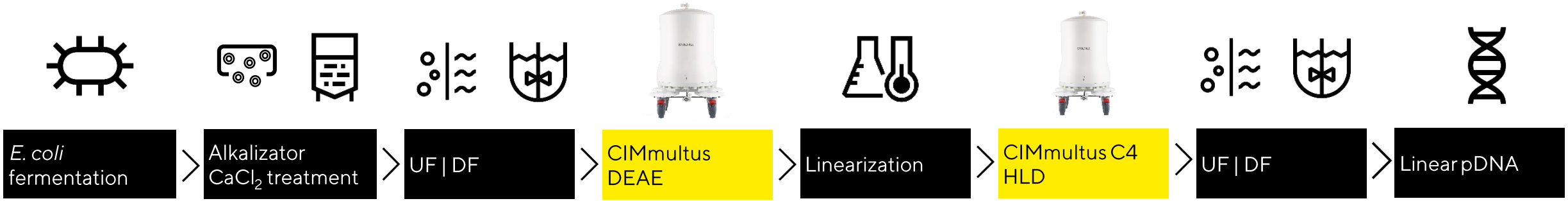
- pDNA including Corona, purity is the key for better transfection and purer mRNA
- Minicircle DNA (shorten the pDNA)
- ssRNA and dsRNA, platform process from E.coli to mRNA including Corona
- Adeno virus, more than 20 years experience, including Corona
- AAV (all serotypes, > 20 tested)
- Life influenza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

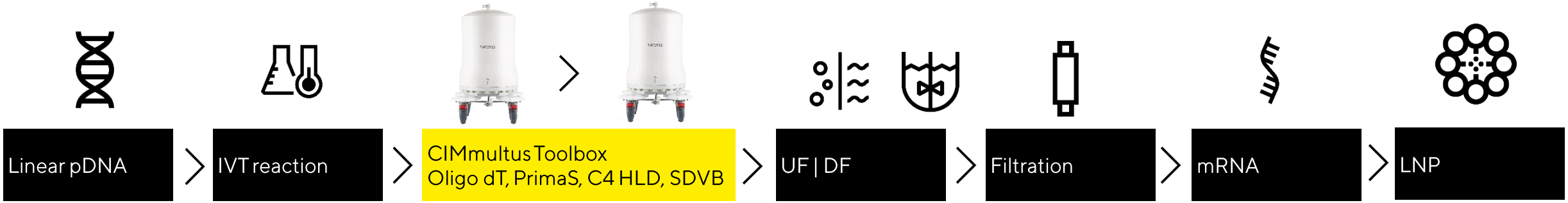
>50 pDNA, mRNA, virus DSP cGMP processes tech transferred to CMOs, sponsors, including Corona.

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

From *E. coli* to mRNA LNP | Sartorius One-Stop Shop for mRNA Manufacturing

Analytical workflow (PATfix pDNA; CIMac pDNA)





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

Testimonials: Need for Express Bioproduct Manufacturing Process Development, Not Possible Without Fast, Robust and Orthogonal Analytics

"We use BIA Separations Monolith columns for the purification of our mRNA drug substance. The Monolith columns come in multiple sizes to meet our needs from small scale product development work to large scale cGMP manufacturing runs. We have found the Monolith columns to provide high throughput and high purity while being very robust and reliable. In addition, BIA Separations has top notch customer service that never fails to impress."

Greg Kubczak, Director of Technical Services and Manufacturing at **Arcturus Therapeutics**

"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/Novartis**

"We are very pleased to be collaborating with BIA Separations / Sartorius in developing and tech-transferring optimized mRNA vaccine manufacturing processes. When time is of the essence, it is crucial to be able to rely on key partners whilst maintaining the highest level of quality in every aspect of our work. We are also very proud that our team was able to produce two kinds of genetic vaccines (DNA and mRNA) and we look forward to expanding our partnership with the BIA Separations' team on future projects."

Hong Thai Pham, CEO at **BioNet**

There is No Robust Bioprocess Without Using Orthogonal In-Process Analytical Methods

PATfix – pDNA and mRNA Validated Methods Provided With the System

- Analyses of various biomolecules; pDNA, mRNA, dsDNA, protein…
- Used for process monitoring or release of product and control
- Rapid chromatographic analytical methods (~3-15 min)
- HPLC analytics does not require expensive reagents
- Broad diversity of column chemistries; PrimaS, Oligo-dT, pDNA, SDVB…
- Various analytical techniques; pH, gradients, RP, SEC, HIC

No need to…

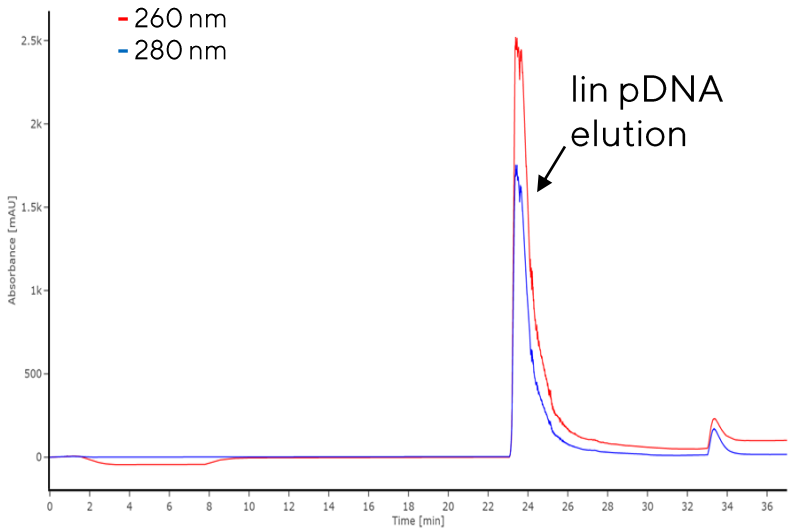
- Search for the right HPLC hardware
- **Selecting suitable column**
- Develop an internal standard
- **Develop/optimize the analytical method**
- Validate the method

Validated methods include:

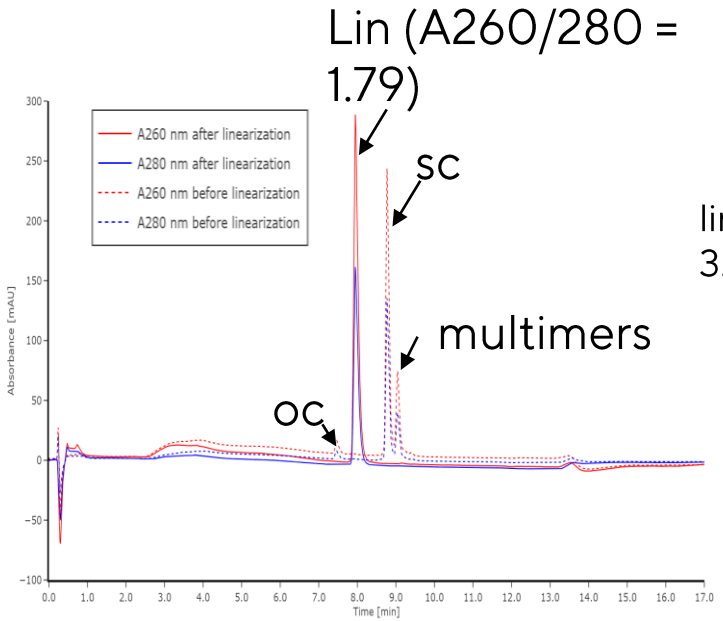
- pDNA purity and quantification
- mRNA IVT monitoring
- mRNA quantification
- mRNA purity/ds mRNA quantification
- Encapsulation efficiency and LNP purity soon to come

Pure pDNA, Free of Proteins, Requested to Produce mRNA With High Yields

Preparative CIMmultus® C4 HLD
Efficient protein removal (proteins sticks to the column and elute in 1M NaOH only)

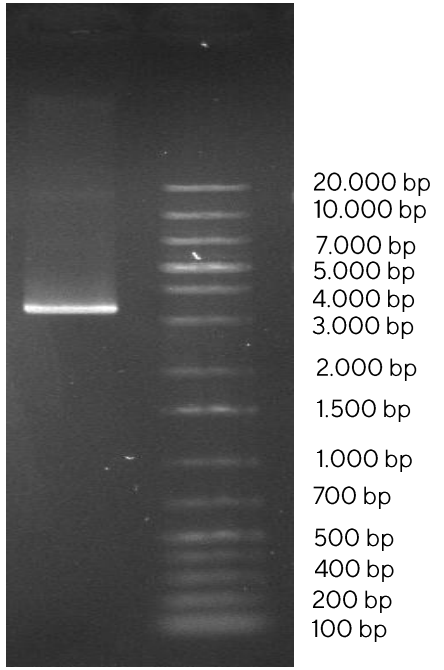


HPLC CIMac™ pDNA analytics



AGE

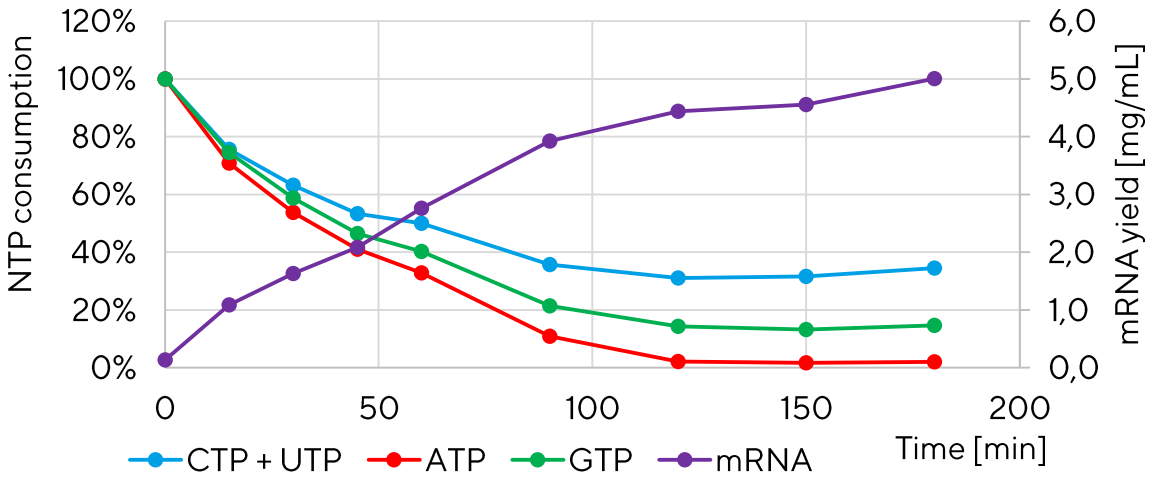
lin pDNA
3.3 kbp

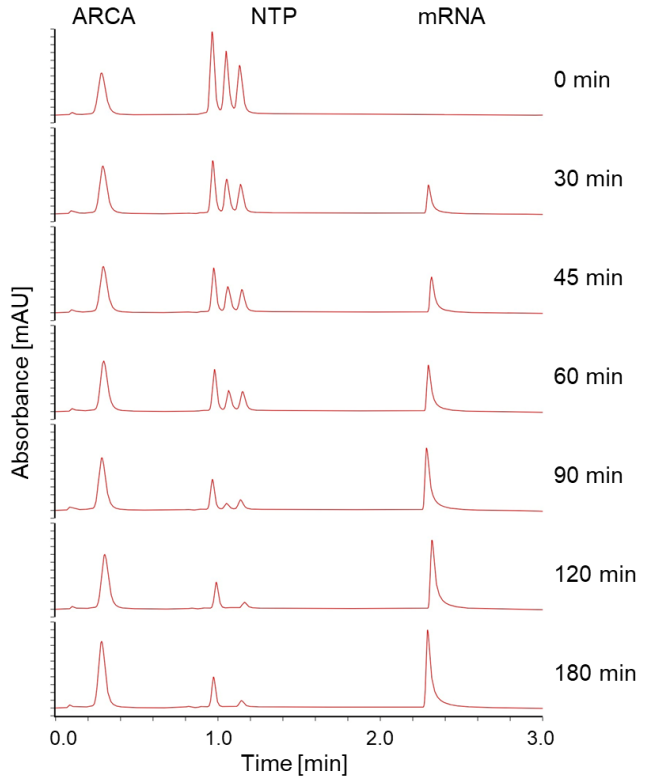


1: linear plasmid
2: GeneRuler 1 kb plus

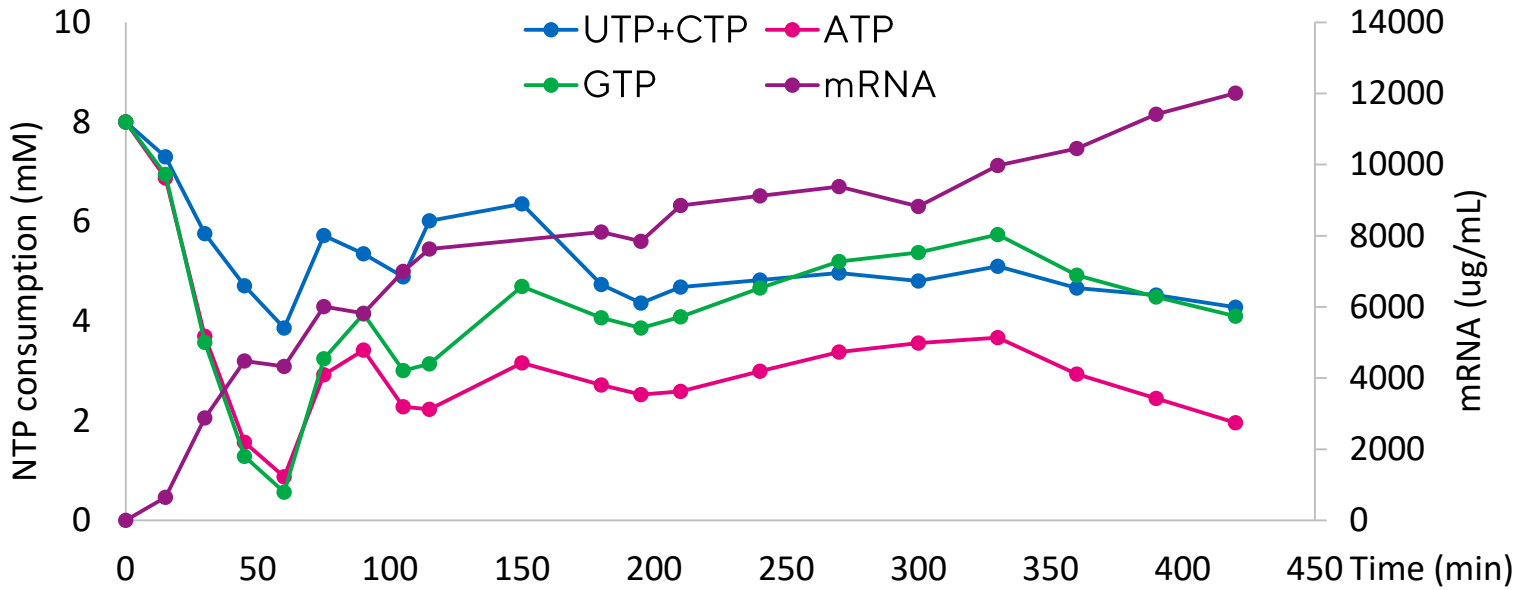
Building Up IVT Understanding by Using PATfix Analytics

- 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 Optimization Using AMBR Reactor and PATfix



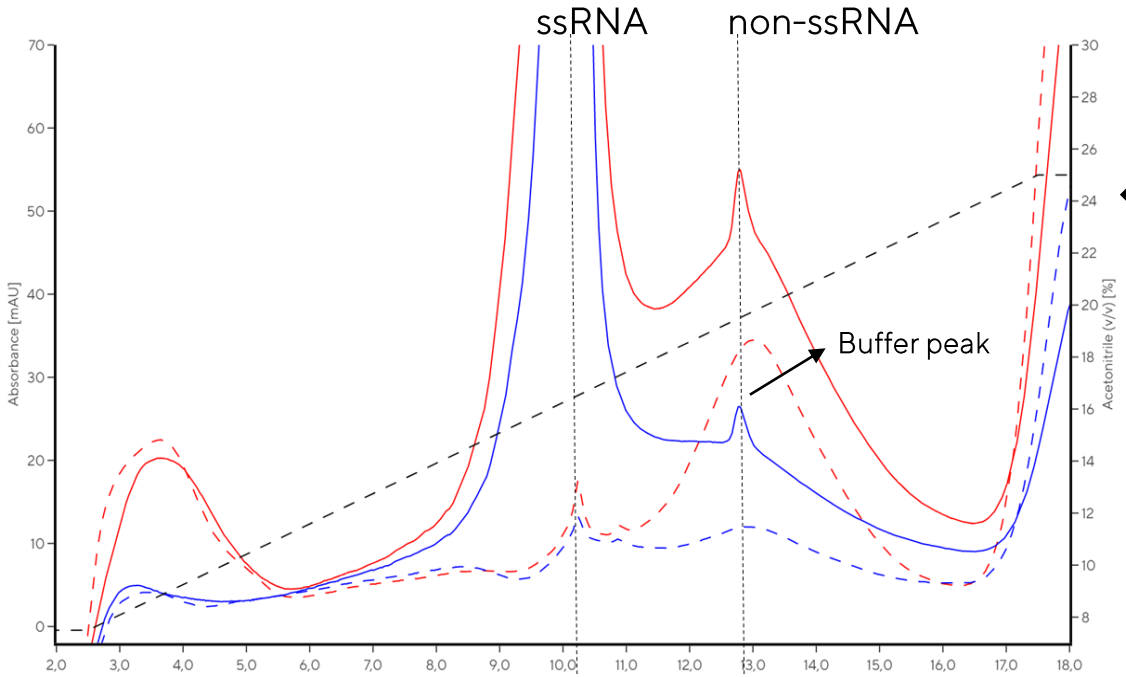
AMBR250 Reactor

PATfix mRNA System

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

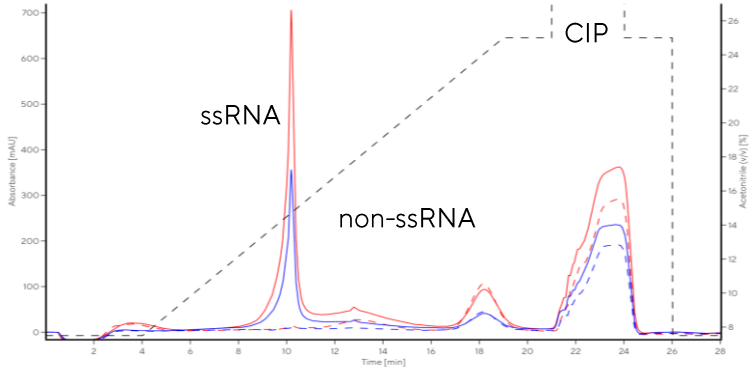
CIMac SDVB Column – Orthogonal Determination of the ds mRNA

Zoom-in view

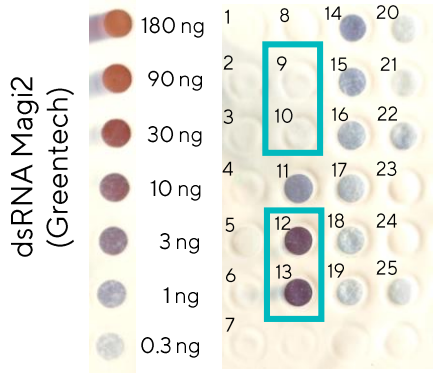


- Buffer A: 100 mM TEAA; pH 7.0; Buffer B: 100 mM TEAA, **25% ACN**; pH 7.0; Buffer C: 100 mM TEAA, **90% ACN**; pH ~7.0
- Thermostat: 60°C

Zoom-out view



Dotblot from SDVB fractions



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

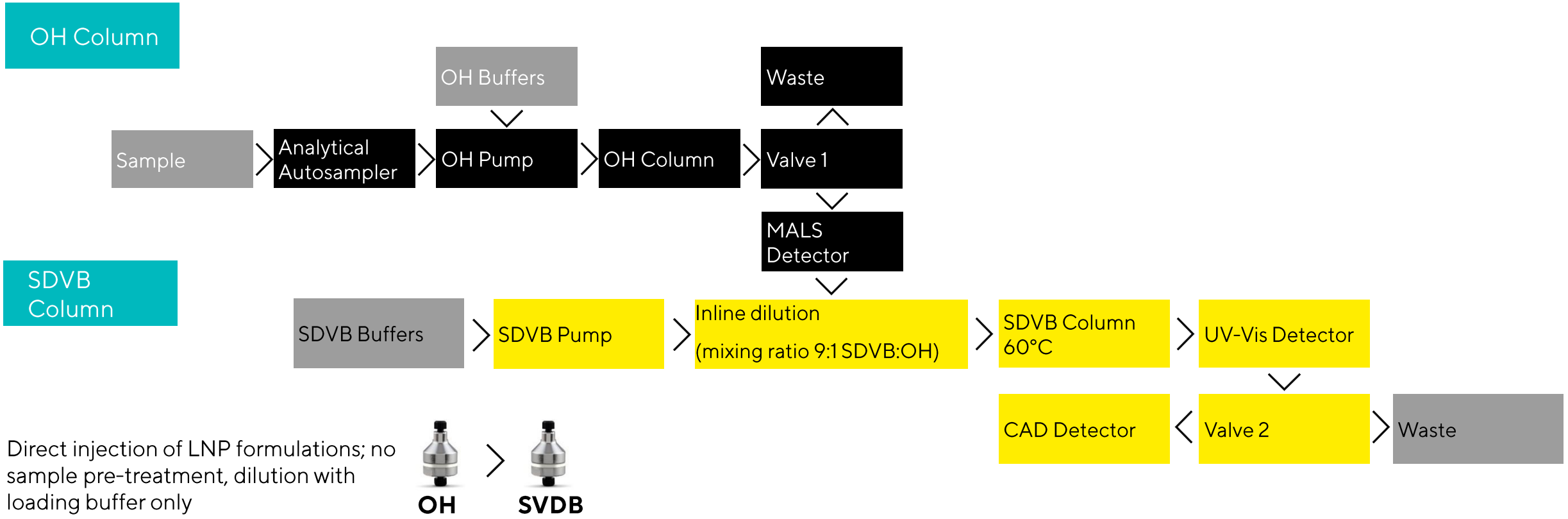
PATfix In-Process Determination of Encapsulation Efficiency

State of the Art LNP Synthesis

LNPs were produced on NanoAssemblr Ignite at Precision NanoSystems

- mRNA: 4.000 nt mRNA (0.118 mg/mL) in PNI formulation buffer (Precision NanoSystems)
- Lipid mix: 12.5 mM Genvoy ILM in ethanol
- Cartridges: Ignite NxGen cartridge
- N/P ratio: 4
- Flow rate ratio : 3
- Total flow rate: 12 mL/min

Encapsulation Efficiency Analysis Using PATfix Switching System – Flow Chart



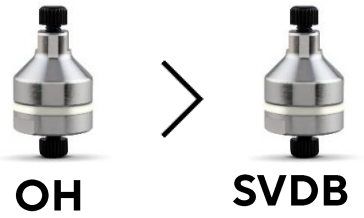
Direct injection of LNP formulations; no sample pre-treatment, dilution with loading buffer only

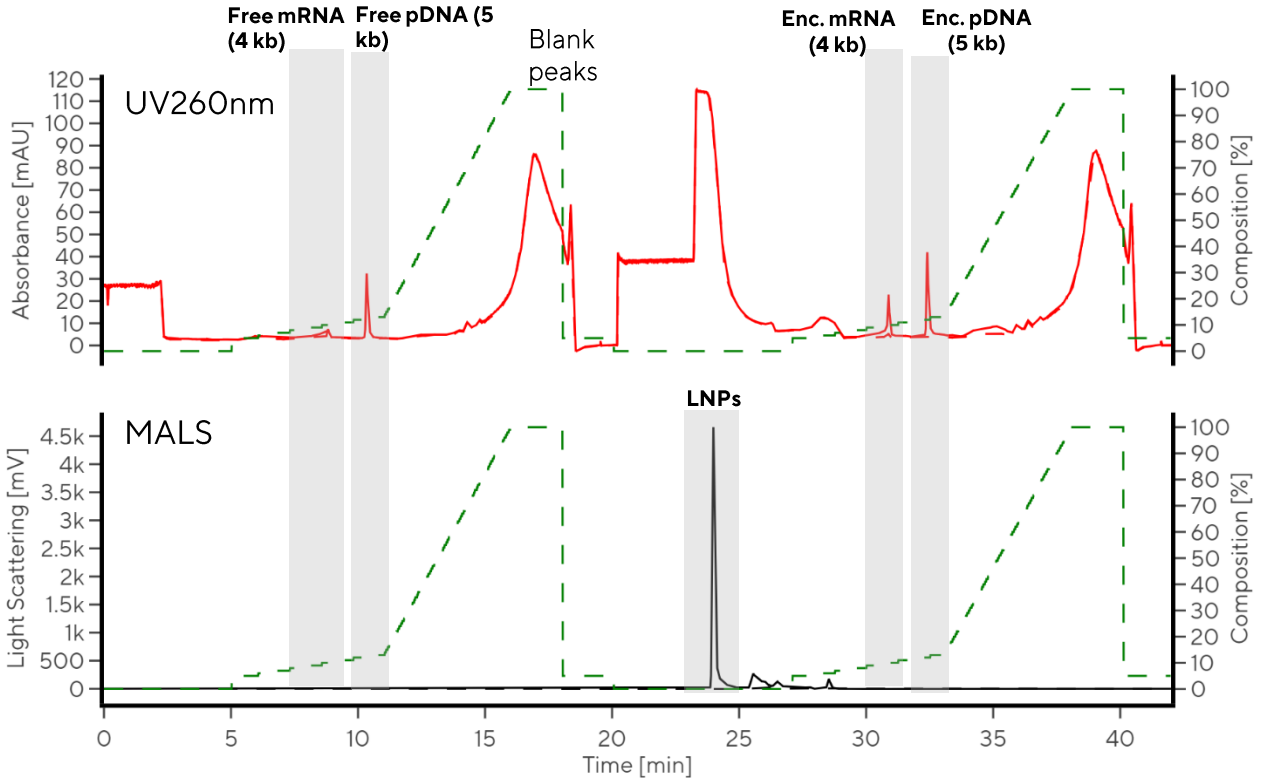
OH **SVDB**

Encapsulation Efficiency Analysis Using PATfix Switcher

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

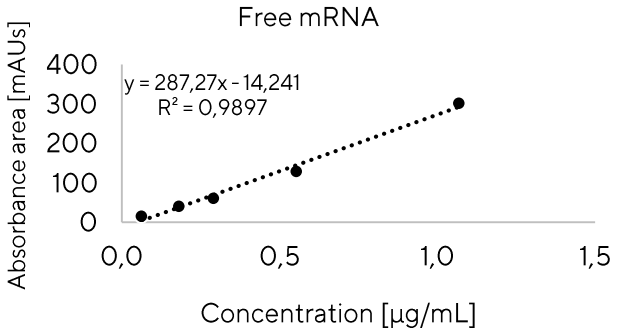


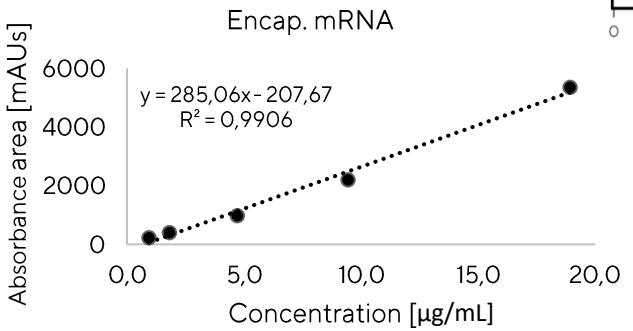


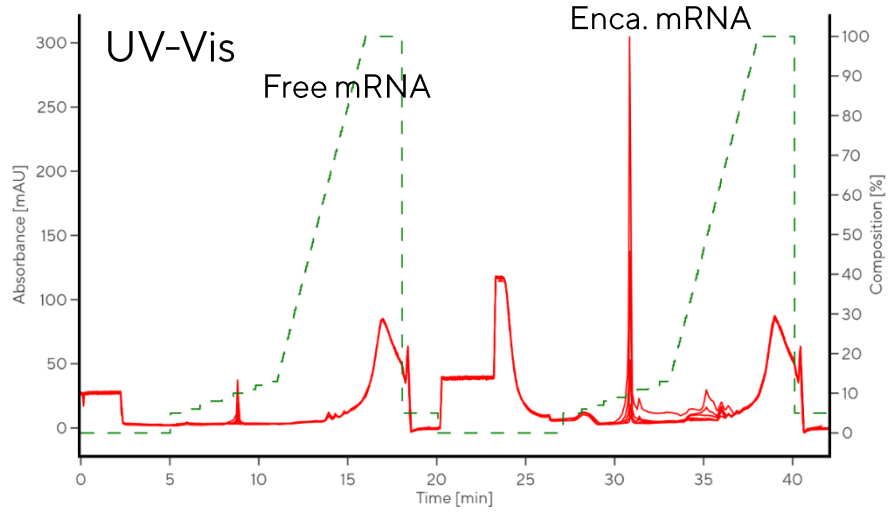
LNP PATfix Switcher – LOD/LOQ & Linearity

- 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



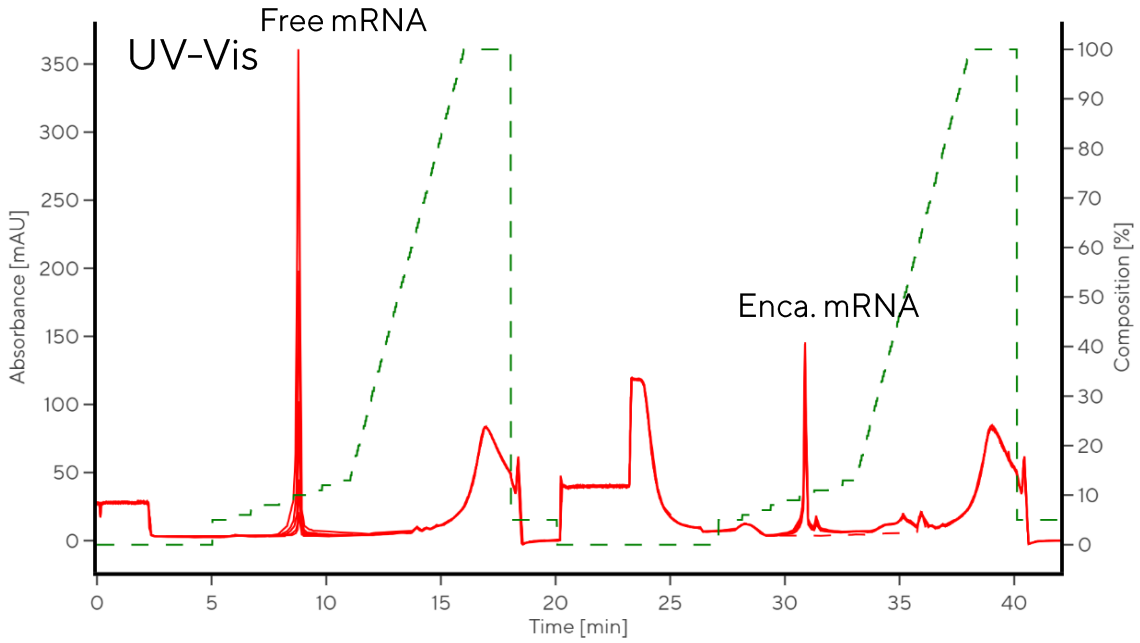




LNP PATfix Switcher – Recovery

- Concentrations of spiked free mRNA in LNP mRNA with concentration of 10 µg/mL: 1, 2, 5, 10, 20 µg/mL
- Recovery of free and encapsulated mRNA calculated from calibration curves.

Spiked concentration of free mRNA (µg/mL)	Recovery of Free mRNA	Recovery of encapsulated mRNA
1	86%	94%
2	101%	95%
5	94%	101%
10	99%	99%
20	101%	99%



LNP PATfix Switcher – Good Comparison With Quant-it™ RiboGreen Assay

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:

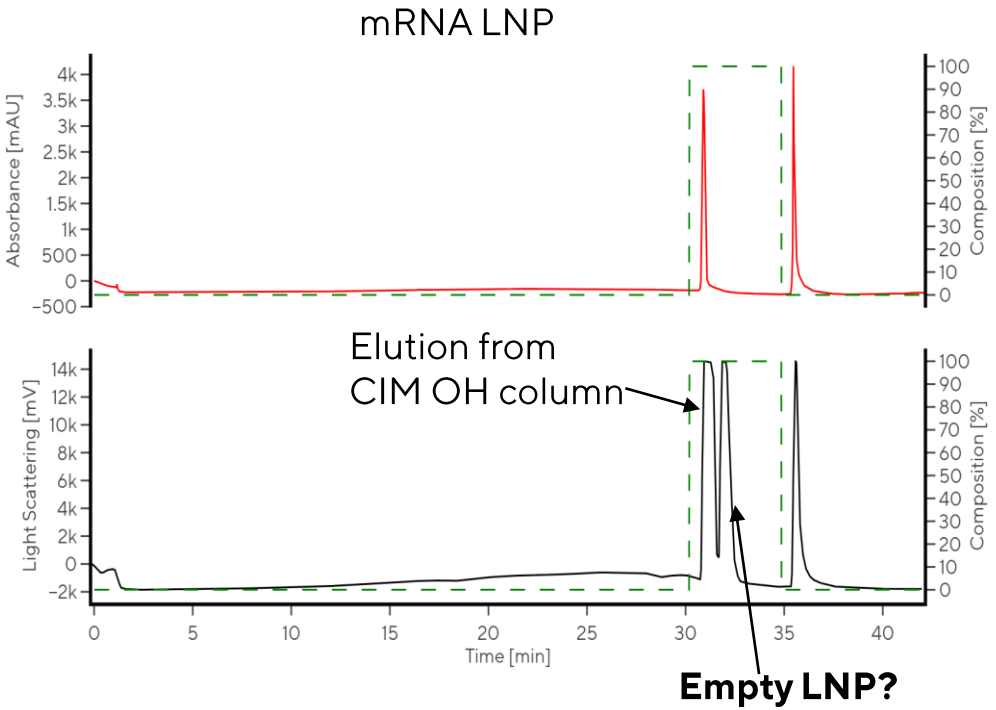
Sample	LNP Switcher % encapsulation	Quant-it™ RiboGreen % encapsulation
LNP mRNA	72	71

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

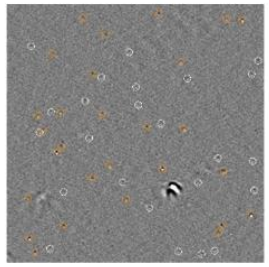
PATfix In-Process Analytics of LNP Size Distribution

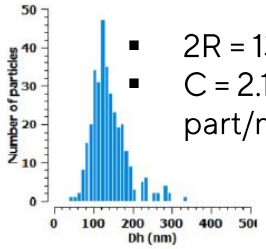
LNP Is Stable When Purified Using Buffers Suitable for CIM OH Column Separation

- 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



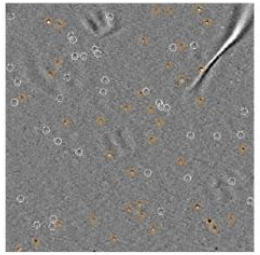
Crude sample

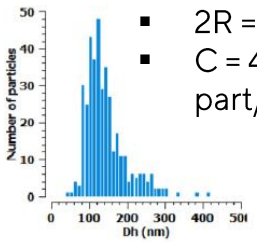




- 2R = 132 nm
- C = 2.15e+9 part/mL

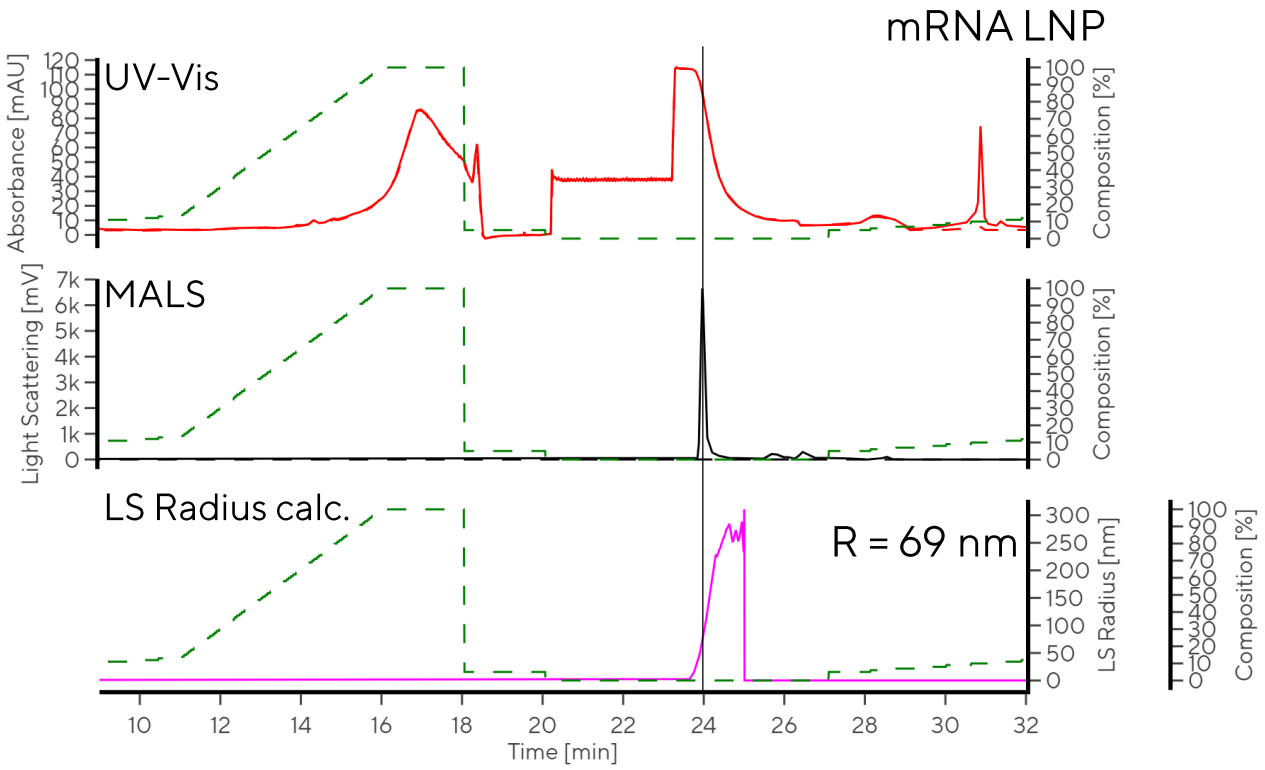
Elution from OH column

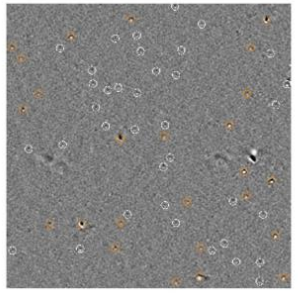


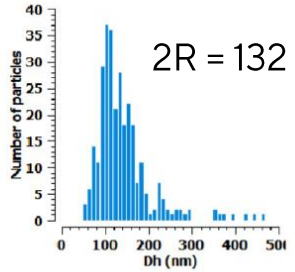


- 2R = 130 nm
- C = 4.90e+9 part/mL

LNP PATfix Switcher – mRNA|LNP Size Distribution Analytics

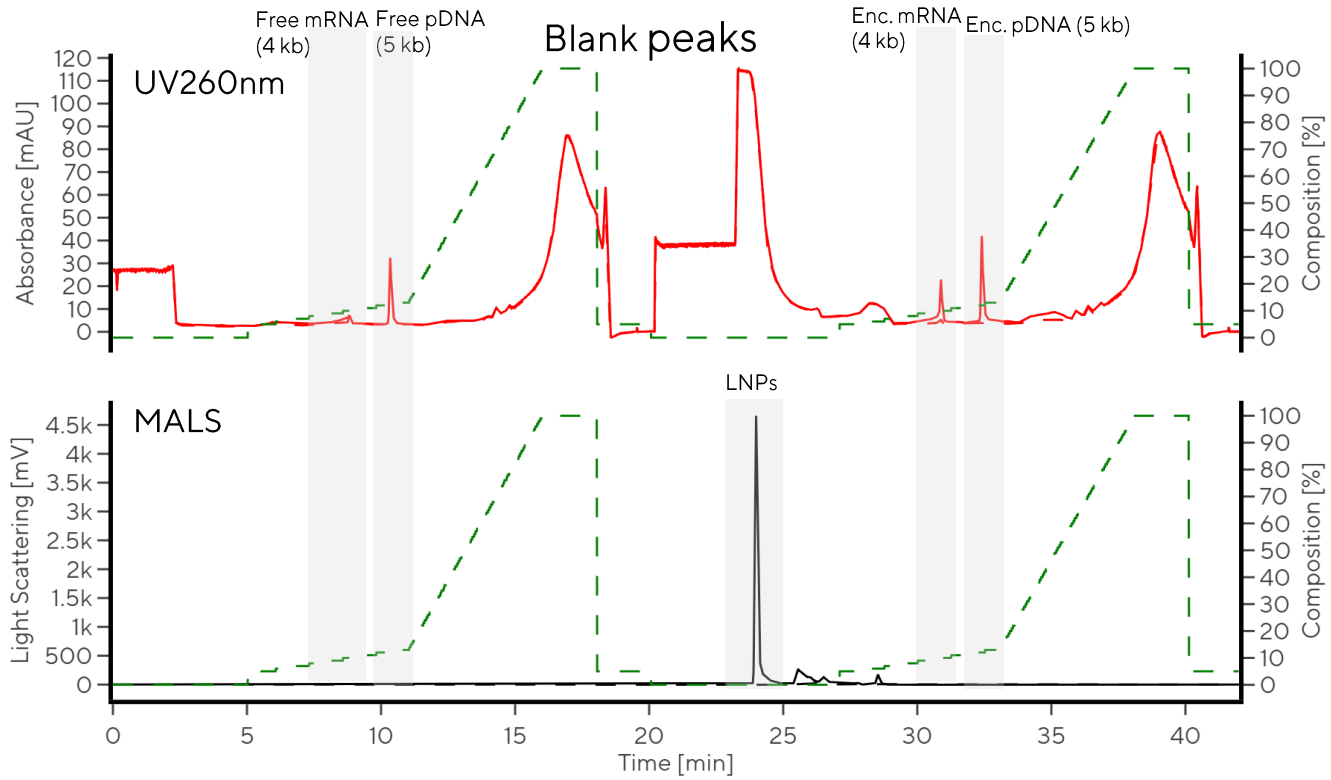






- LNP Switcher was used for size determination of LNP mRNA particles
- Radius was determined by modelling different scattering angles using Berry model
- In agreement with Videodrop (similar to NTA method) measurement

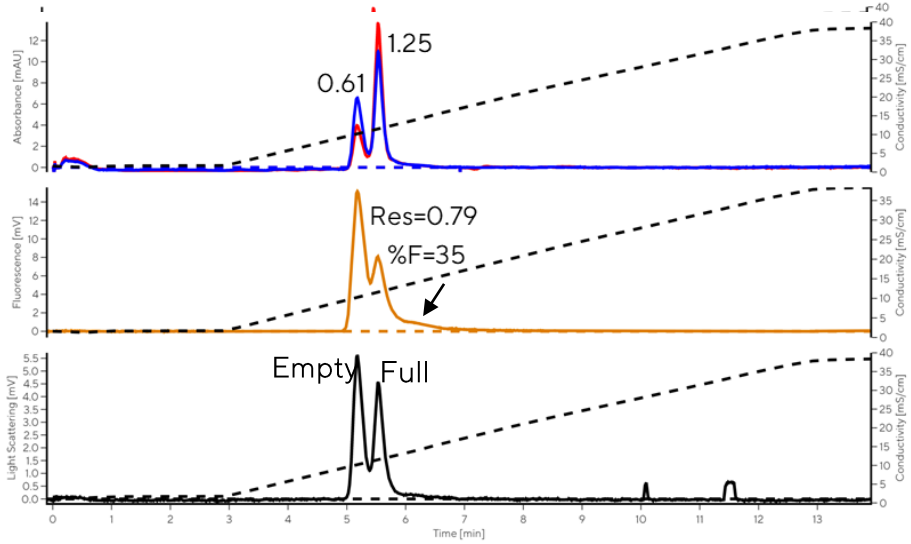
PATfix Switcher – Same Method Used for mRNA Encapsulation Efficiency, LNP Amount and LNP Size Distribution Analytics



Work In Progress – Fractionation of Different LNP Species

Experience With Separation of AAV Capsids Helps To Better Fractionate LNPs

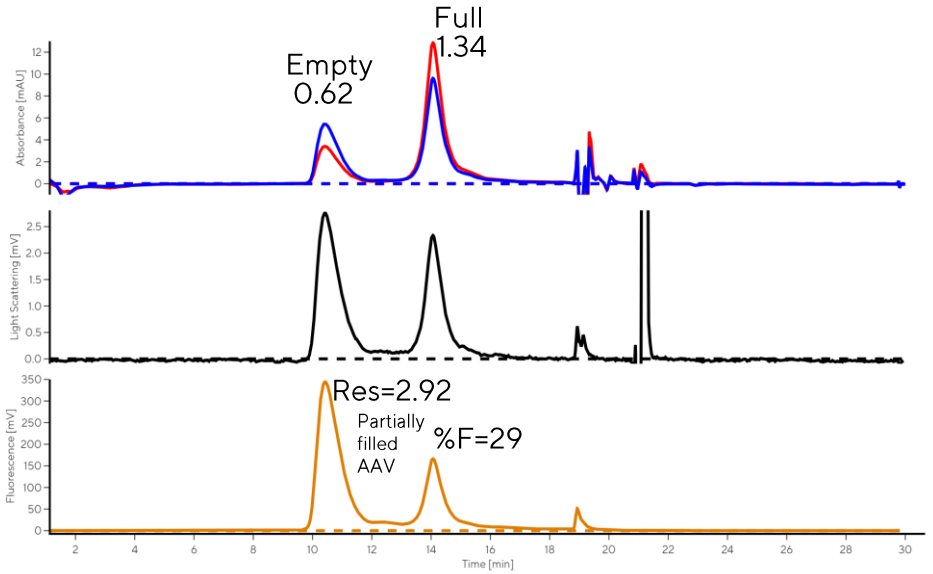
Standard QA method
(0-400 mM linear NaCl 100 CVs gradient; pH 9.0)



Detectors:
- UV 260 nm
- UV 280 nm
- FLD Trp
- MALS 90°

Method optimization

Novel QA method*



Standard Method:

- Low resolution between full AAV and other inactive capsids
- 3 species observed
- Need metod for better characterization of AAV sample heterogeneity

Novel Method:

- Significantly enhanced Empty/Full resolution
- At least 5 species observed
- Fractionation of AAV capsids

***Patent pending**.

Conclusions

- **High purity of raw materials** and PATfix 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 NTA|videodrop method.**
- PATfix, purpose designed LC system is an **excellent tool for mRNA|LNP process development and fast in-process control** at any point of the manufacturing process.
- Most important, for PATfix system enables **safer product and cheaper mRNA|LNP manufacturing costs.**

Thank you.

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