SARTORIUS

Simplifying Progress

Orthogonal PAT analytics - essential to enable robust production of viral vectors and LNPs

Aleš Štrancar
Sartorius BIA Separations, Ajdovščina, Slovenia

Sartorius BIA Separations product and solutions portfolio

BIA's Proprietary Product and Services Offering

CIMmultus Monolith production-scale columns

PATfix systems & CIMac Monolith process control columns

Process and method development services

10 - 100x higher capacity	**up 3x** higher recovery	Higher integrity information at lower cost and better accuracy	Achieves rapid, high-resolution separations in minutes	The leading expert on large biomolecules and viral particles	Tailored services for each clinical phase up to and including production
up to 3x lower drug manufacturing cost	Column sizes **40L+**	Biocompatible	Fast and reproducible LC monitoring of large biomolecules	Drives long-term, embedded customer relationships	50+ Process and Method development experts

Serving Gene Therapy for > 25 years

Experts in fast bioproduct manufacturing process development – no royalties charged

>80 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, purity is THE key for better transcription and better transfection for purer mRNA**
- Minicircle DNA, ministering (shorten the pDNA), ssDNA
- **ssRNA and dsRNA, platform process from E.coli to mRNA and LNP**
- Adeno virus, more than 20 years experience
- **AAV (all serotypes, > 20 tested)**
- Life influenza virus (all serotypes)
- Vaccinia/MVA
- Exosome
- Bacteriophages (outstanding endotoxin removal)
- VLPs and inactivated vaccines
- **LNPs (single, multiple payloads, “decorated”)**
- IVIG
- IgM and more

Testimonials: need for speed

"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

https://www.zolgensma.com

"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 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

KEY MESSAGES

Do NOT trust analytics

Orthogonal analytical methods are mandatory to prevent wrong conclusions due to the analytical method misleading, and should be in place before you start process development

PATfix systems were developed to address these requirements

Process is as good as your analytics are
Product is as good as your analytics are

The more defined the process, the easier it's scale-up would be.

PATfix, all in one system, provides orthogonality to molecular biology methods

- 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 LC hardware
- **Selecting suitable column**
- Develop an internal standard
- **Develop/optimize the analytical method**
- Validate the method

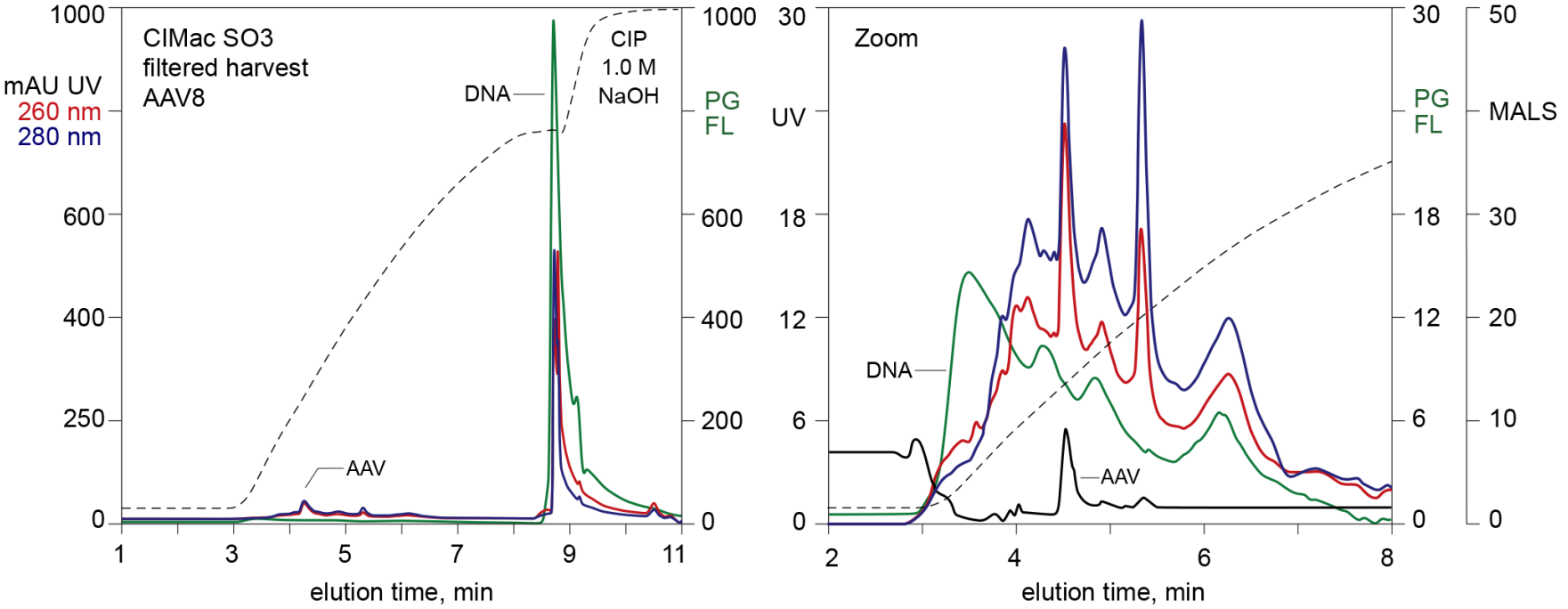
Qualified/validated methods include:

- AAV particle quantification
- AAV empty/partial/full determination
- Aggregate quantification
- Residual hc DNA and hc proteins
- Empty/full in the bioreactor and
- more

Fast process development enabled by multidetector PATfix

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

Novel, very reliable and affordable MLS detector, including the prep version.

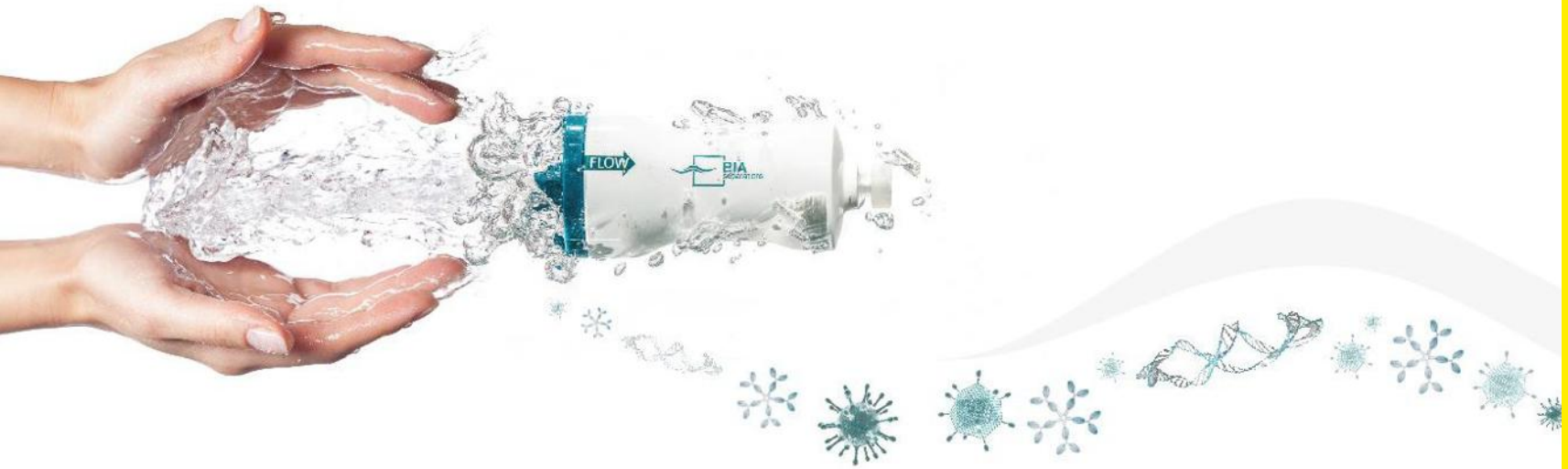


PATfix and CIMac ➔
Process understanding

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**.

BIA separations
Leaders in Monolith Chromatography

Simplifying Progress



From *E.coli* to LNP with one dimension PATfix system

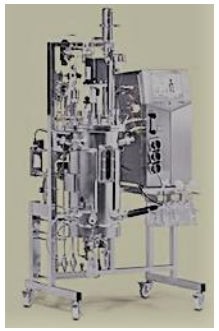
BIA separations

SARTORIUS

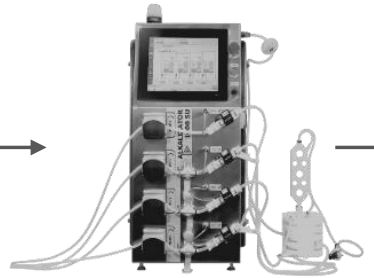
From *E. coli* to mRNA

Sartorius mRNA Drug Substance Production Workflow

Analytical workflow (PATfix pDNA; CIMac pDNA)



E.Coli Fermentation



Alkalizator $CaCl_2$ treatment

UF | DF

CIMmultus DEAE

Linearization

CIMmultus C4 HLD

UF | DF

Linear pDNA

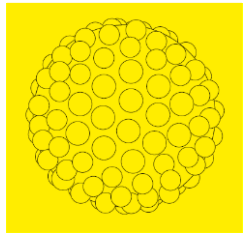
Linear pDNA

IVT in AMBR250 reactor

CIMmultus Toolbox Oligo dT, PrimaS, C4 HLD, SDVB

UF | DF

mRNA



LNP

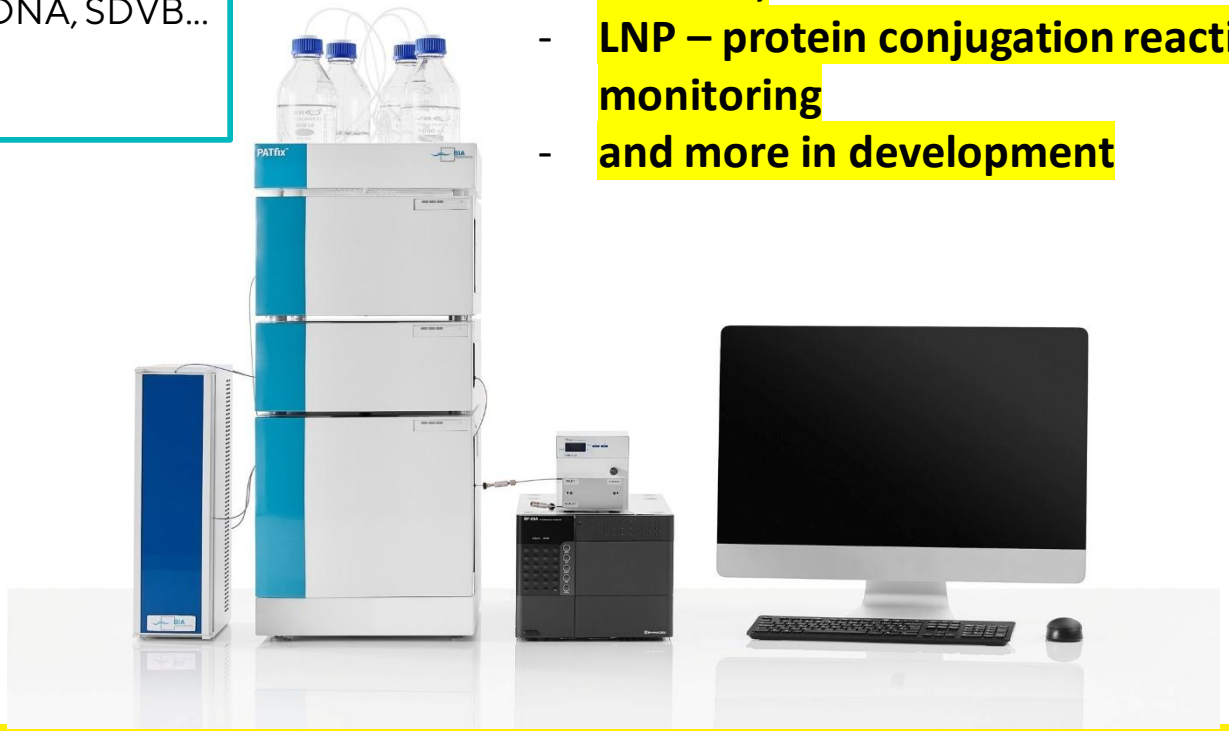
Analytical workflow (PATfix mRNA; CIMac PrimaS, CIMac Oligo dT, CIMac SDVB)

PATfix, all in one system, provides orthogonality to molecular biology methods

- 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 LC hardware
- Selecting suitable column
- Develop an internal standard
- Develop/optimize the analytical method
- Validate the method

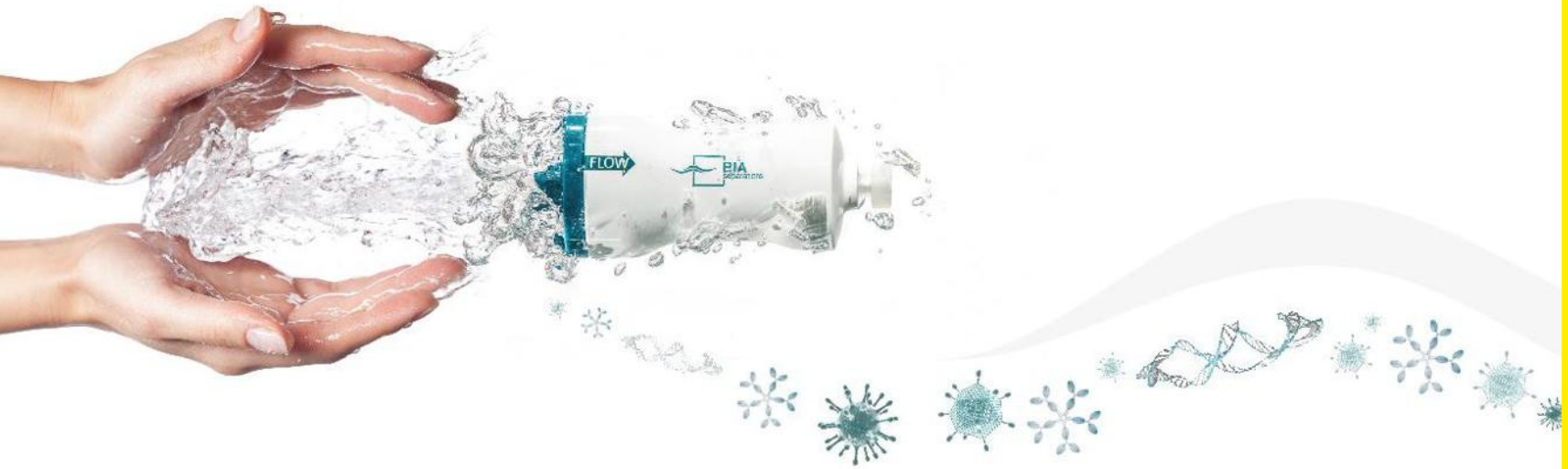


Qualified/validated methods include:

- pDNA purity and quantification
- mRNA IVT monitoring
- mRNA quantification and purity
- **LNP encapsulation, purity, size, heterogeneity, lapidated RNA amount,**
- **LNP – protein conjugation reaction monitoring**
- **and more in development**

BIA separations
Leaders in Monolith Chromatography

Simplifying Progress



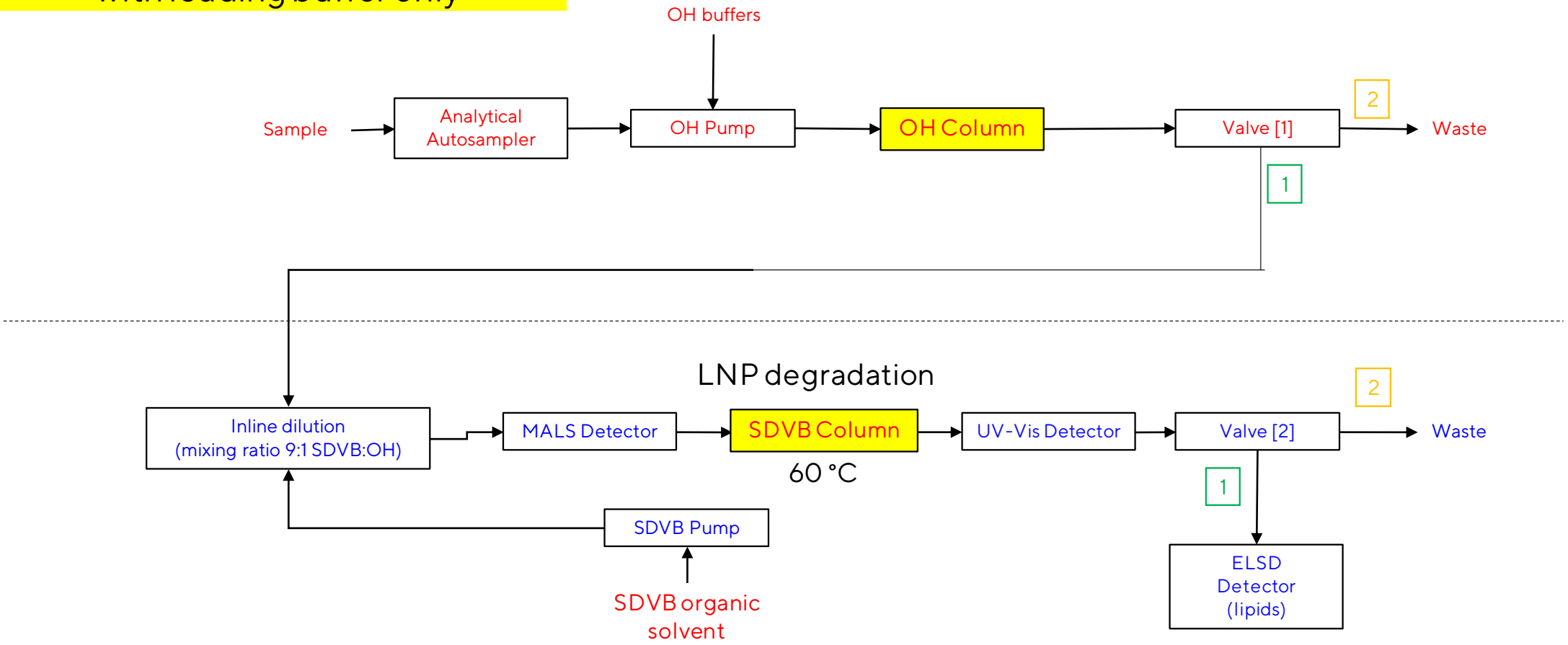
Two dimensions PATfix LNP Switcher for LNP process development and production

BIA separations

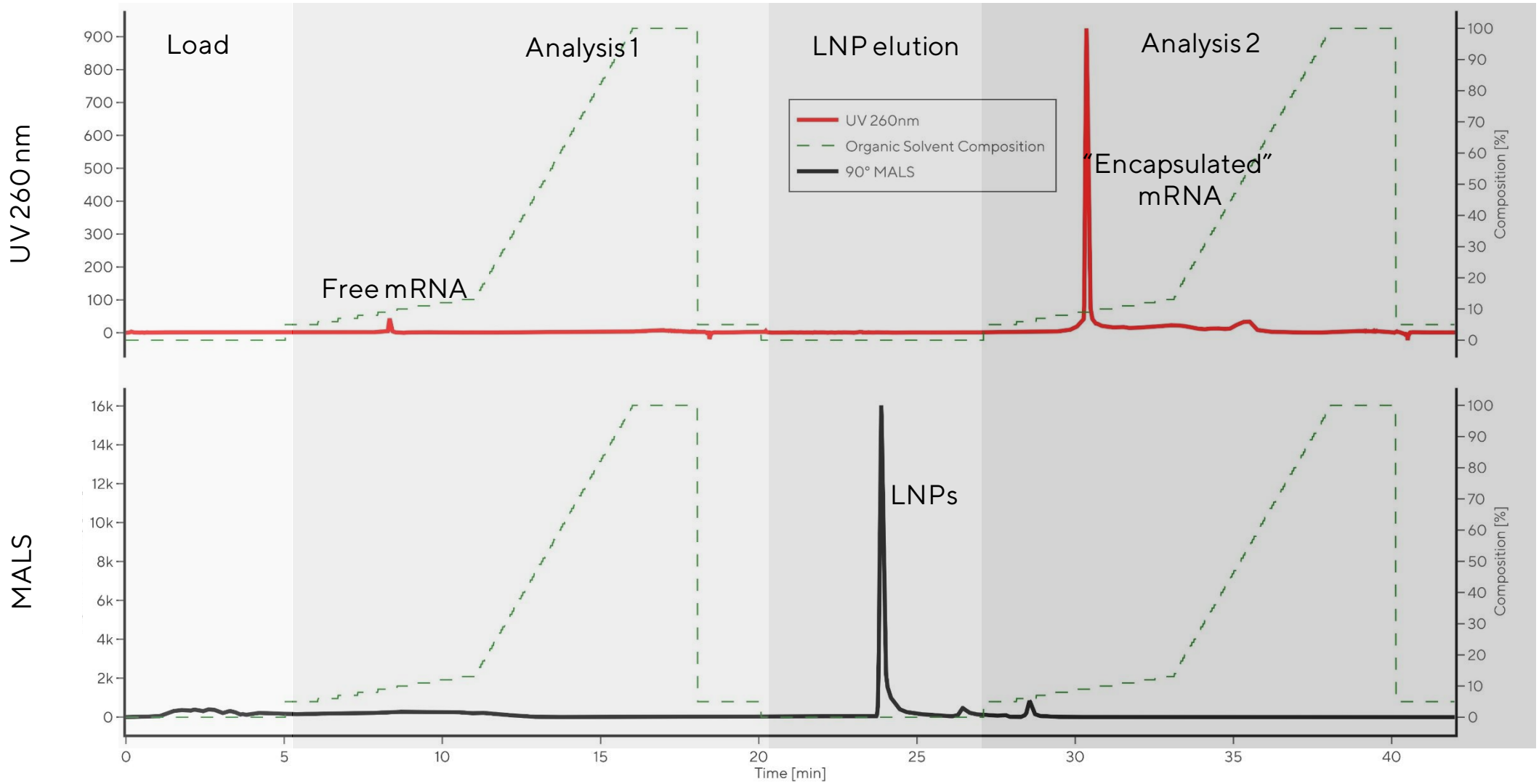
SARTORIUS

Encapsulation efficiency analysis using PATfix LNP Switcher (two dimensional LC)

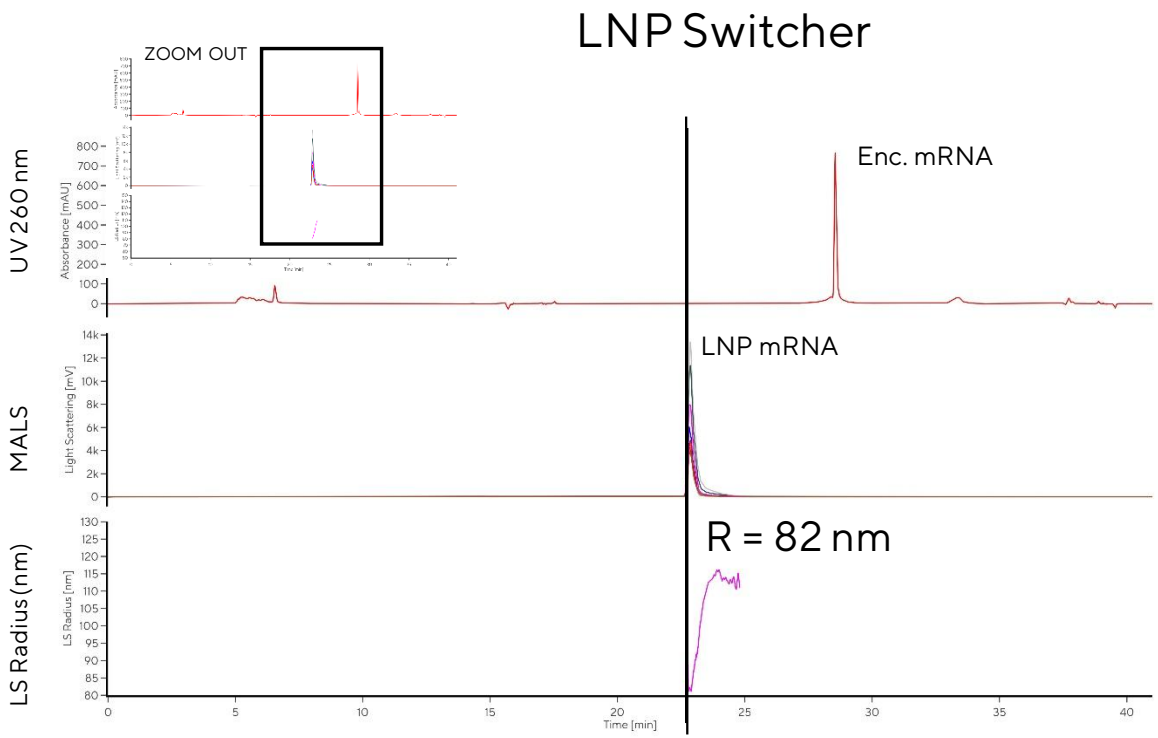
Direct injection of LNP formulations on LC Shearless monolithic column; no sample pre-treatment, dilution with loading buffer only

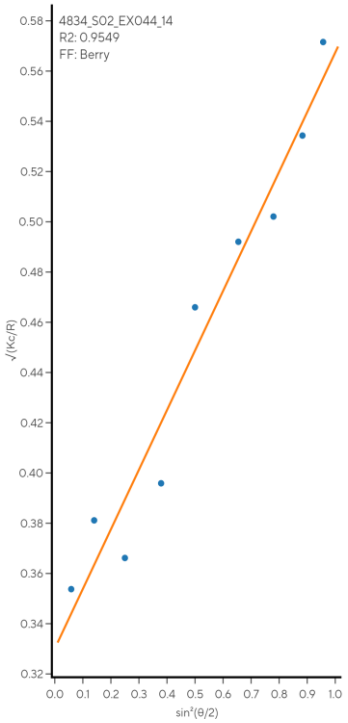


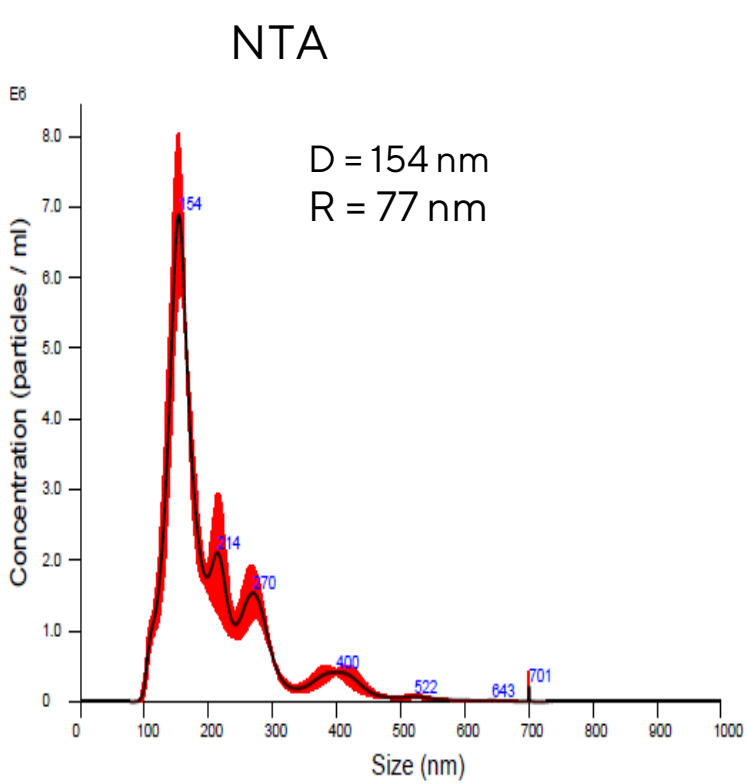
Encapsulation efficiency analysis using PATfix LNP Switcher – chromatogram



PATfix analysis orthogonal to LNP size & LNP size distribution measurements







- OH column separates particles into populations by size
- LC LNP Switcher enables determination of the size of lipid nanoparticles by utilising the MALS detector
- Radius was determined by modelling 9 scattering angle responses using the Berry model and a 40 nm PS standard
- LC method in agreement with Nano Tracking Analysis (NTA)

Encapsulation efficiency using PATfix LNP Switcher analysis – orthogonal to RiboGreen

Quant-it™ RiboGreen method

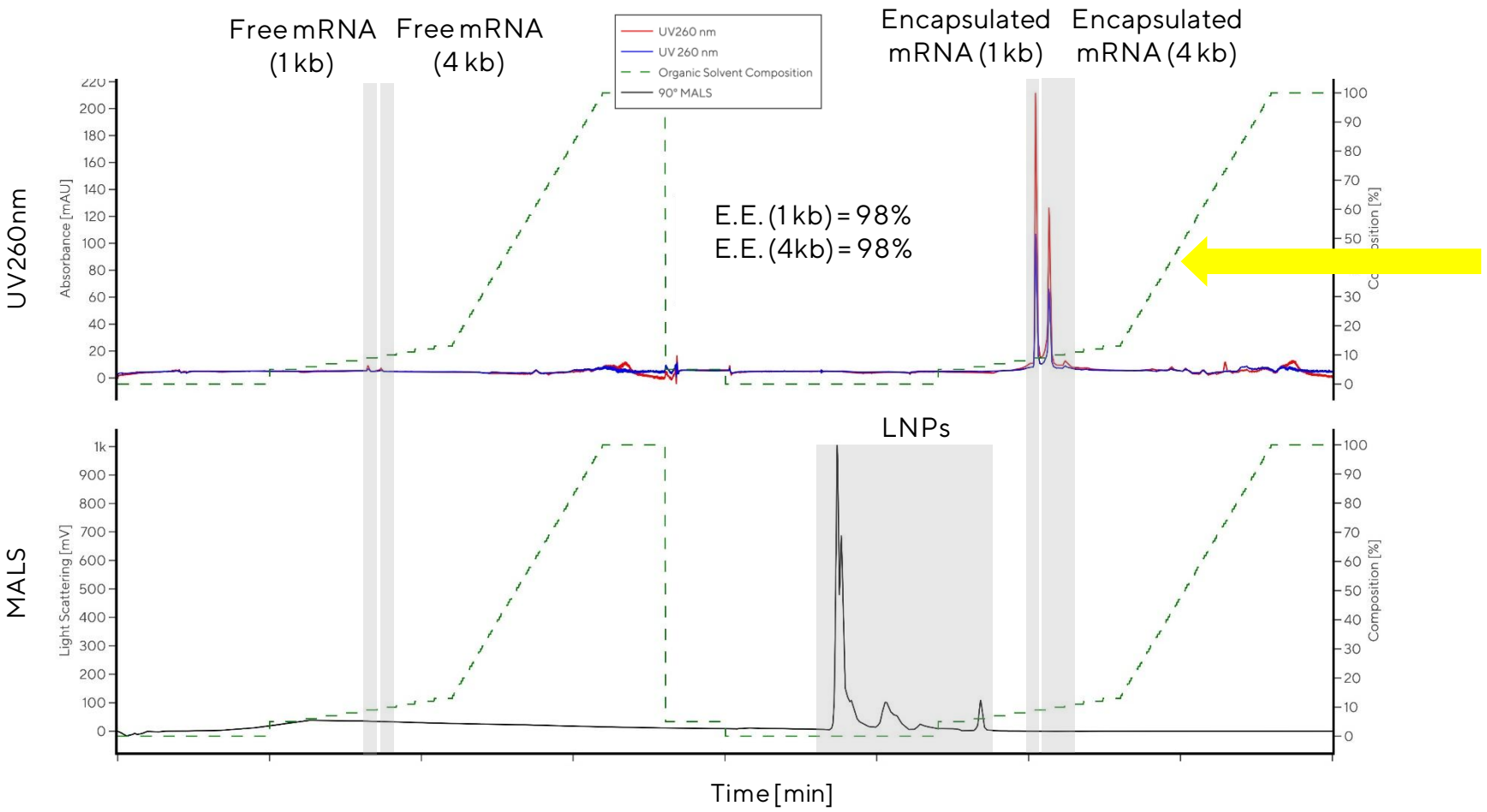
- RiboGreen dye is used for detection and quantification of nucleic acids
- Triton X-100 is applied for LNPs opening
- There is no significant difference in the results obtained with these two techniques

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

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

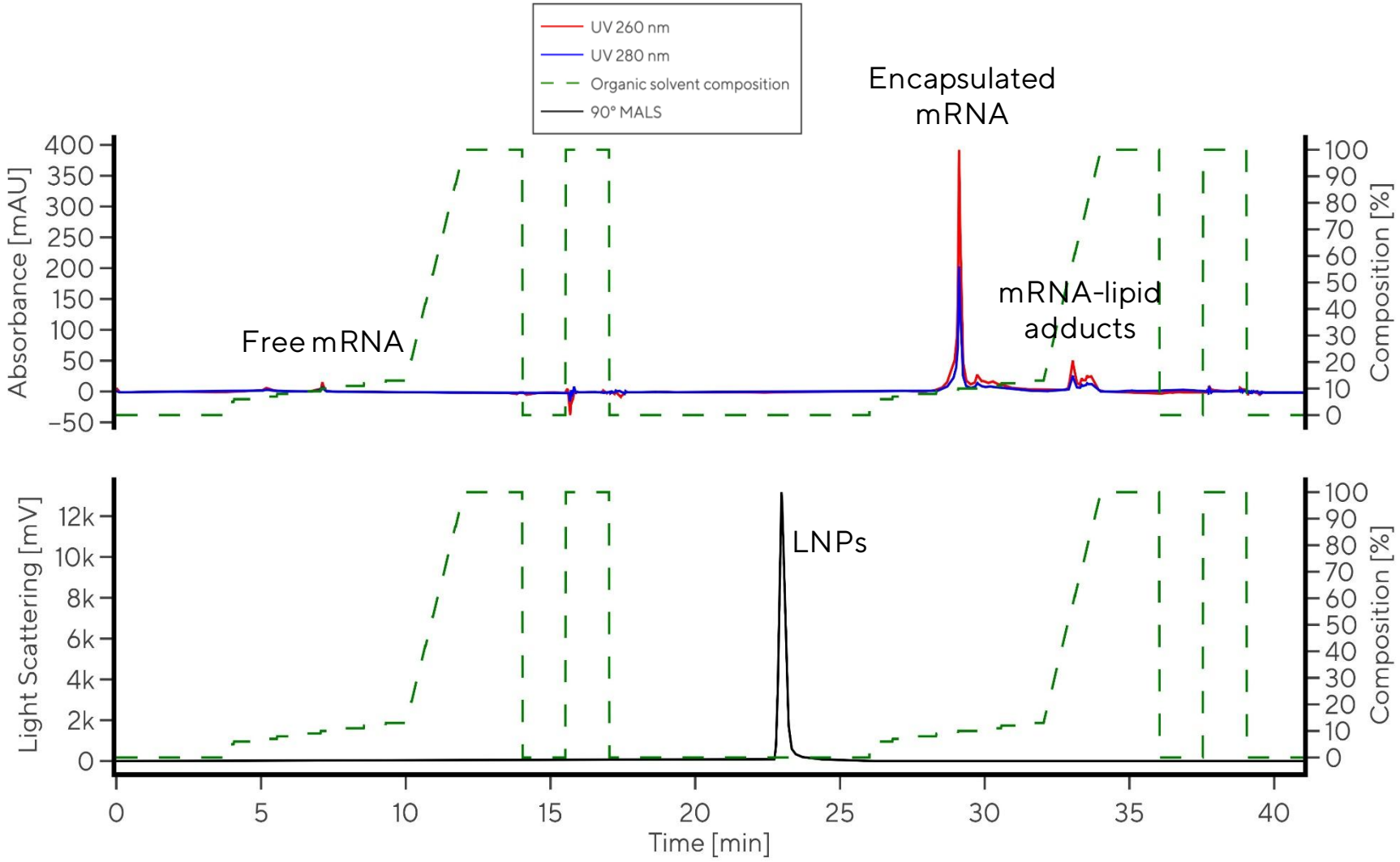
PATfix method can be used as orthogonal to RiboGreen method

PATfix LNP Switcher analysis of co-encapsulated cargo – LNP homogeneity analysis



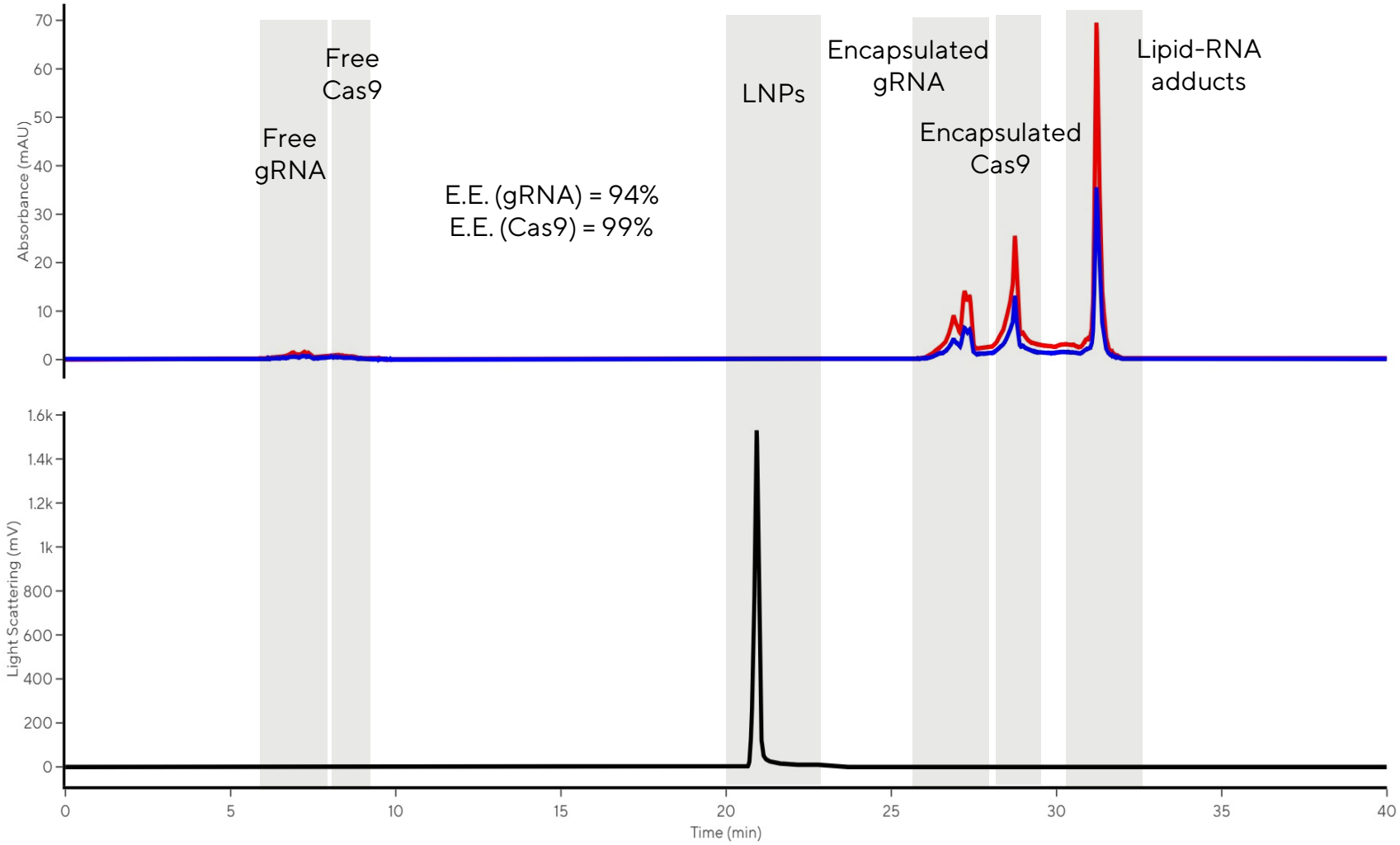
- LC switcher separates and quantifies co-encapsulated species, such as Cas9/sgRNA for CRISPR applications.
- Separate value of encapsulation efficiency for each mRNA can be assessed.
- LNP can have very high encapsulation efficiency, but can be very heterogeneous.

PATfix LNP Switcher allows for monitoring of lipidated mRNA impurities



- Enabled monitoring of mRNA-lipid covalent adducts formed from ionizable lipid impurities.
- Important for lipid screening and stability studies.
- Highly dependent on lipid choice, storage conditions, and raw material purity.
- LNP Switcher, unlike CE, does not need any sample pre-treatment!

PATfix LNP Switcher for CRISPR formulations

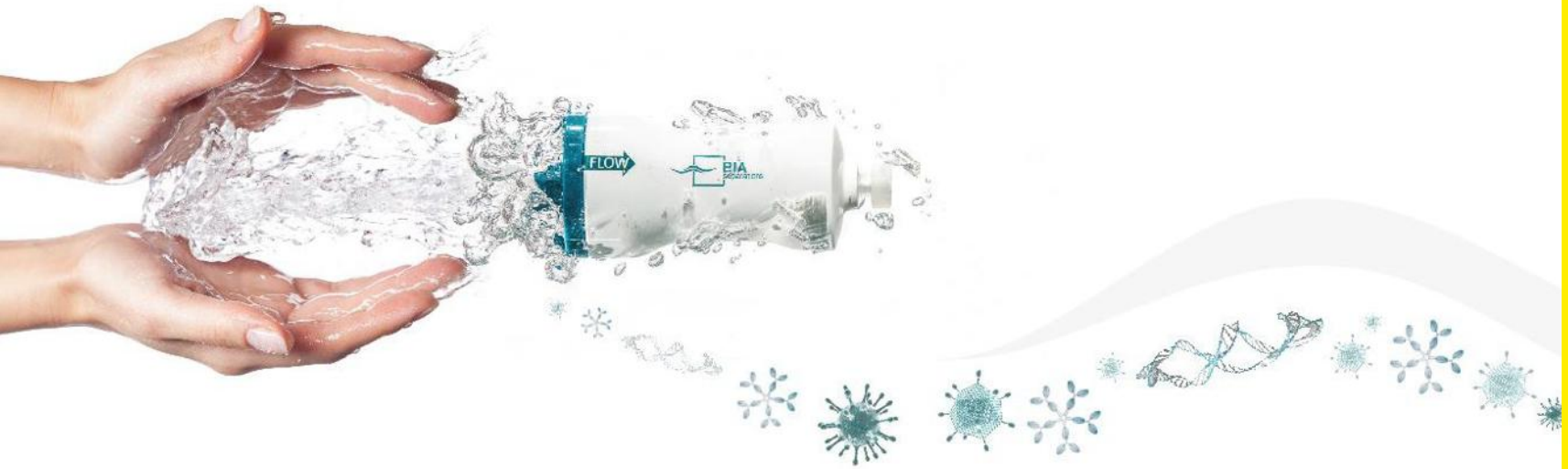


Just one sample injection allows for simultaneous:

- Separation and quantification of multiple-cargo formulations (e.g. CRISPR, CAR-T, combination vaccines)
- Accurate quantification of different nucleic acid species
- Encapsulation efficiency determination
- Heterogeneity assessment
- RNA-lipid adduct quantification
- RNA fragmentation assessment

BIA separations
Leaders in Monolith Chromatography

Simplifying Progress



Targeted LNP (tLNP) – conjugation monitoring

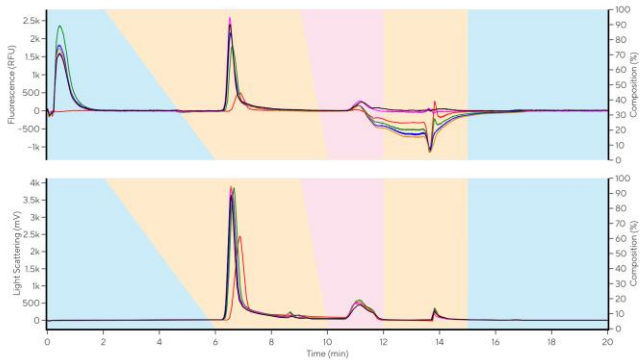
BIA separations

SARTORIUS

Conjugation reaction monitoring

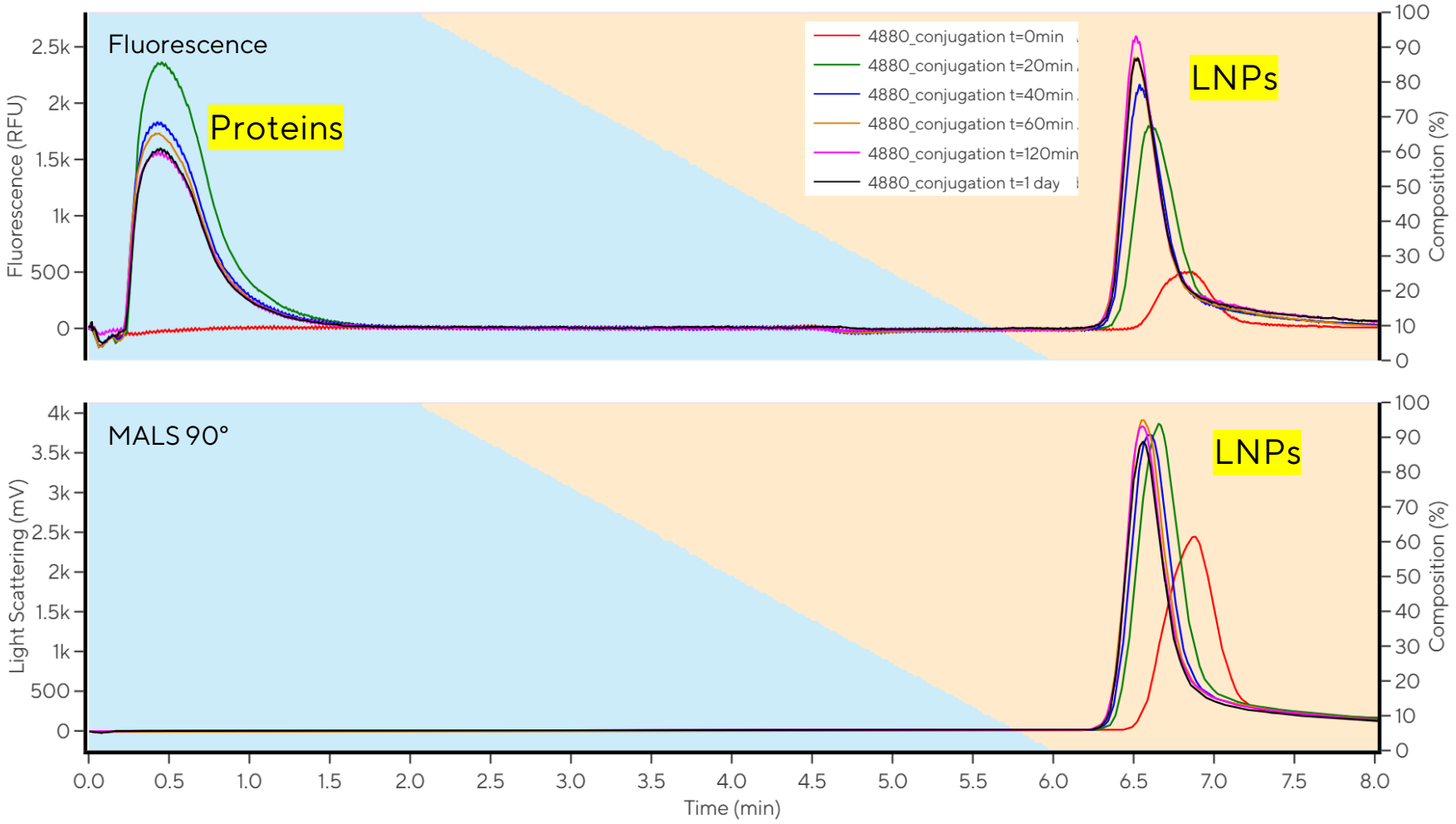
- On-particle conjugation reaction (incubation with protein for 2h)
- Two chromatographic methods were used to monitor conjugation reaction process: HIC (CIMac OH column - free protein monitoring) and AEX (CIMac QA column (targeted vs untargeted LNPs separation)
- Reaction progress tracked at different times after incubation starts:
  - t = 0 min
  - t = 20 min
  - t = 40 min
  - t = 60 min
  - t = 120 min
  - t = 1 day

Conjugation reaction monitoring – PATfix CIMmultus OH

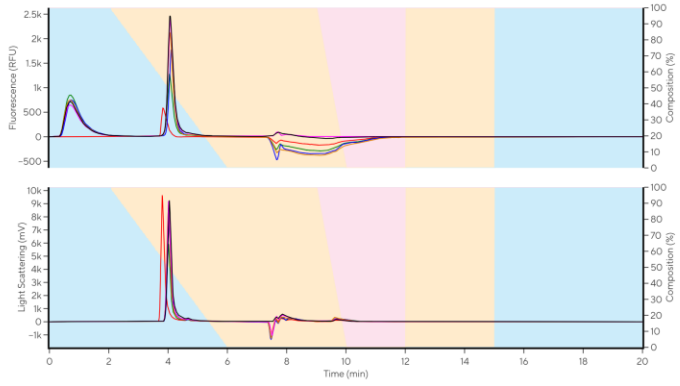


- t = 0 (red): untargeted particles, no proteins in flowthrough
- With progressing reaction, (t)LNP peak in gradient is shifting to the left, area is getting larger
- Fluorescence peak area is getting bigger as reaction progresses (more protein on the surface), but MALS peak stays roughly the same

Zoom-in

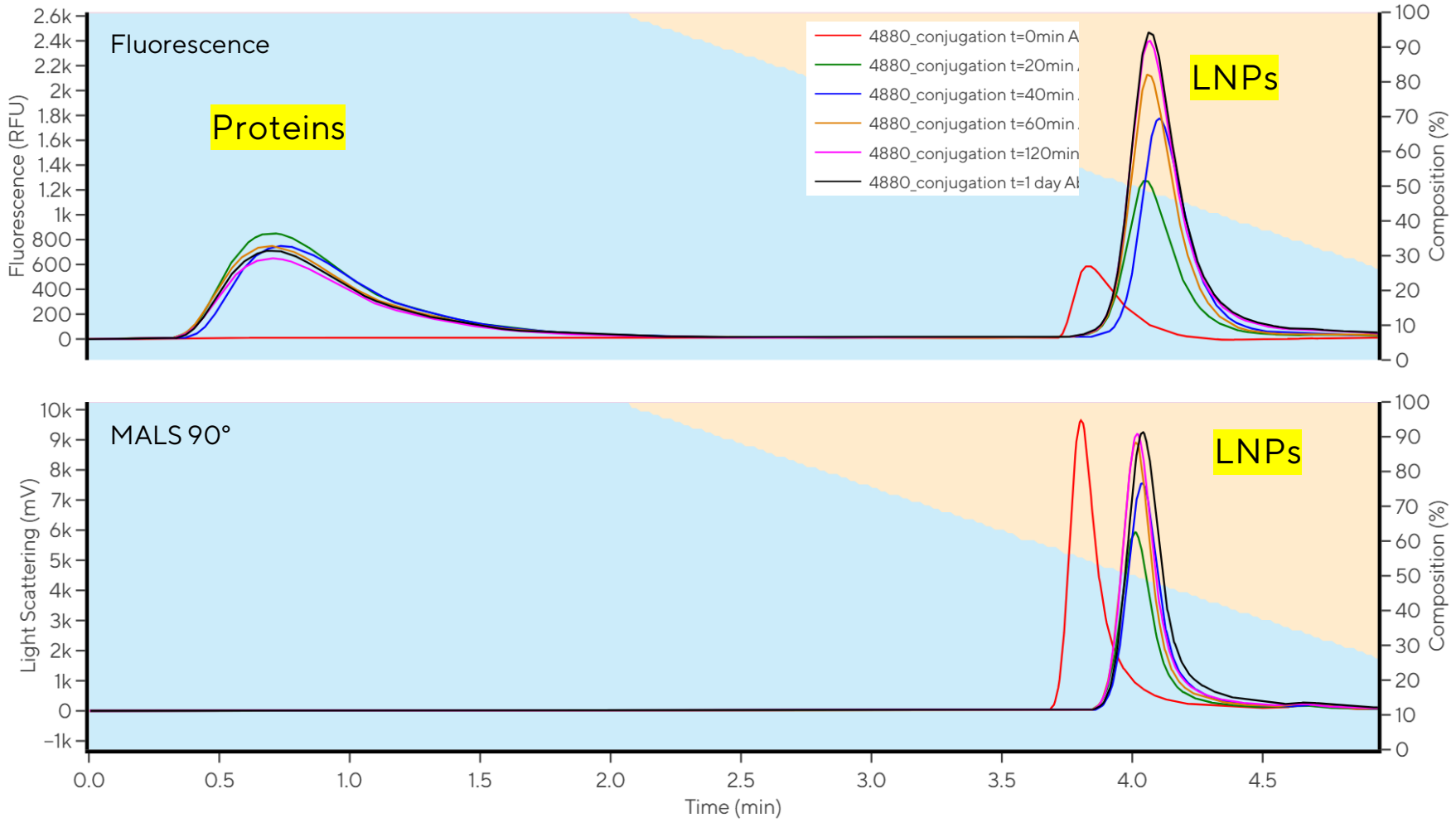


Orthogonal conjugation reaction monitoring – PATfix CIMmultus QA HR



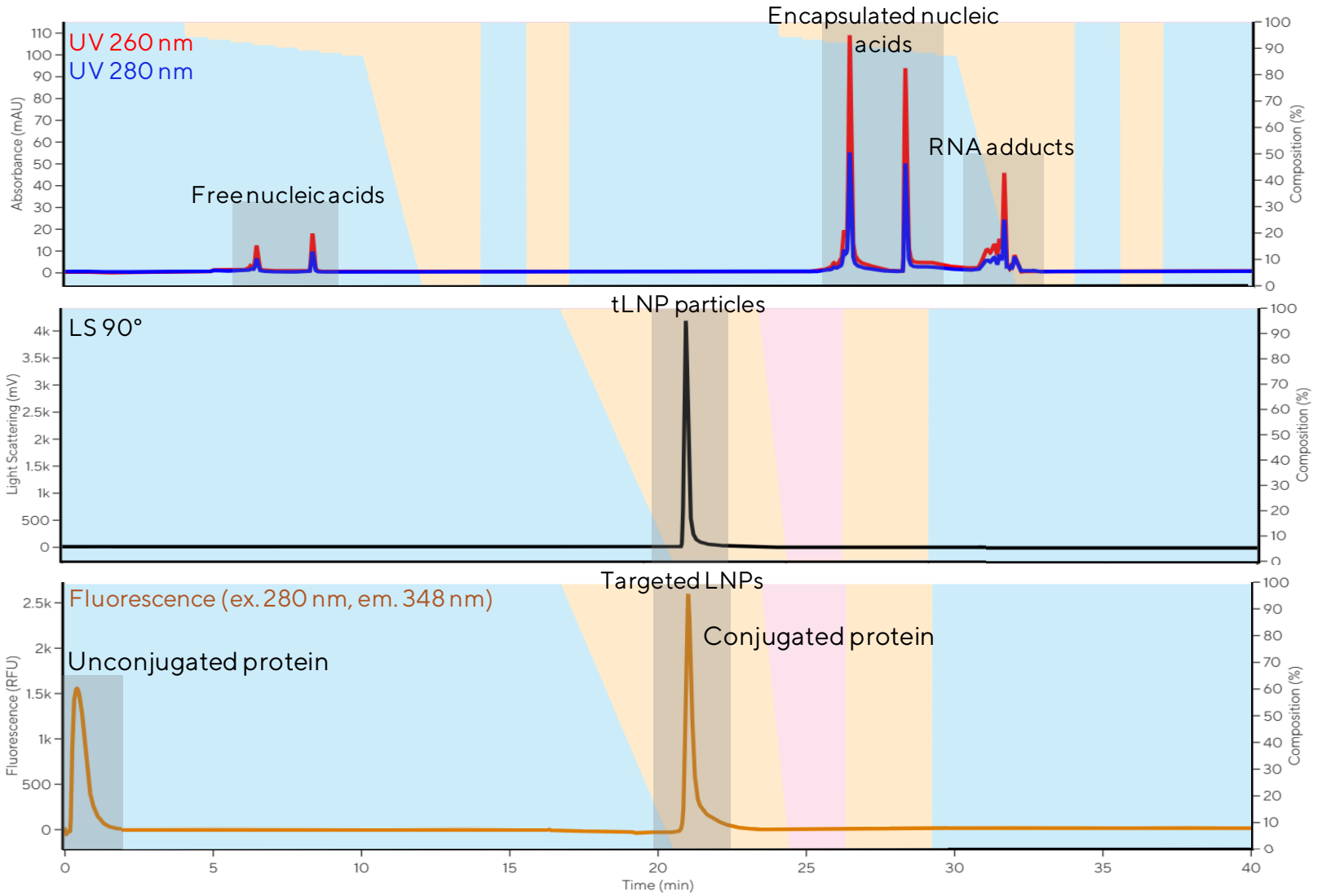
- t = 0 (red): untargeted particles, no FT
- Peak in gradient starts to shift to the right, fluorescence signal is getting bigger (more protein on particles), but MALS signal stays roughly the same

Zoom-in



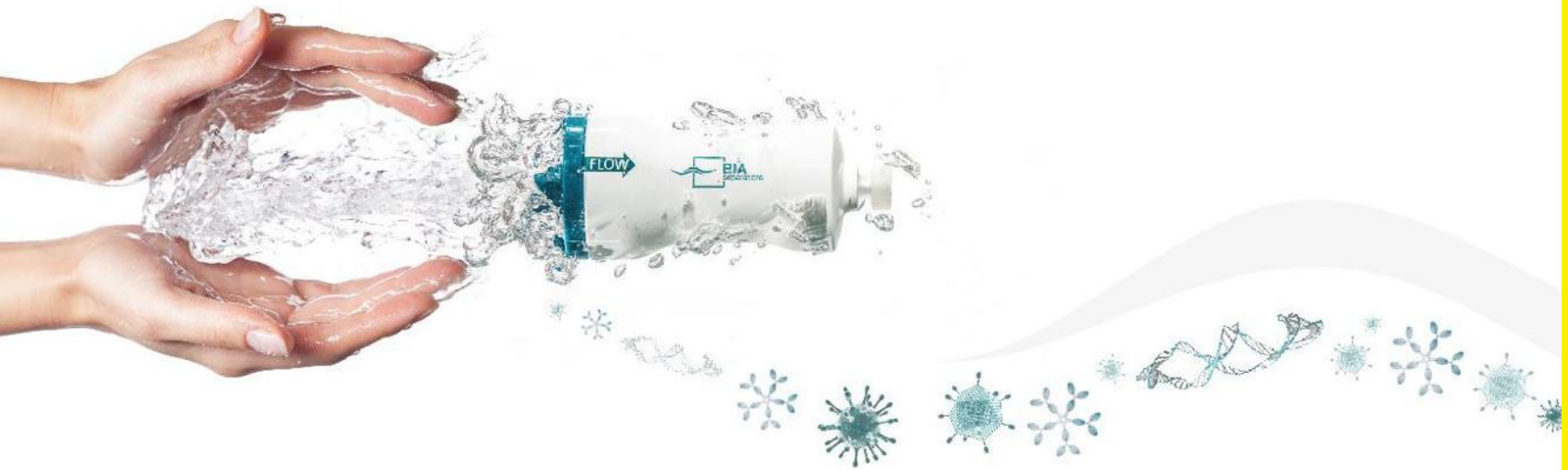
Conjugation reaction monitoring using PATfix LNP switcher

- LNP switcher used to <u>simultaneously</u> monitor:
  - the conjugation reaction between the protein and the LNP particle
  - nucleic acid content of LNPs
  - LNP encapsulation efficiency, concentration, lipidated RNA,...
- Monitoring the conjugation reaction is the key to optimize formulations performance and control the manufacturing process



BIA separations
Leaders in Monolith Chromatography

Simplifying Progress



Shearless LNP manufacturing

BIA separations

SARTORIUS

Shearless chromatography to replace TFF degrading LNP products

Challenge

Avoid scale-up issues and shear forces.

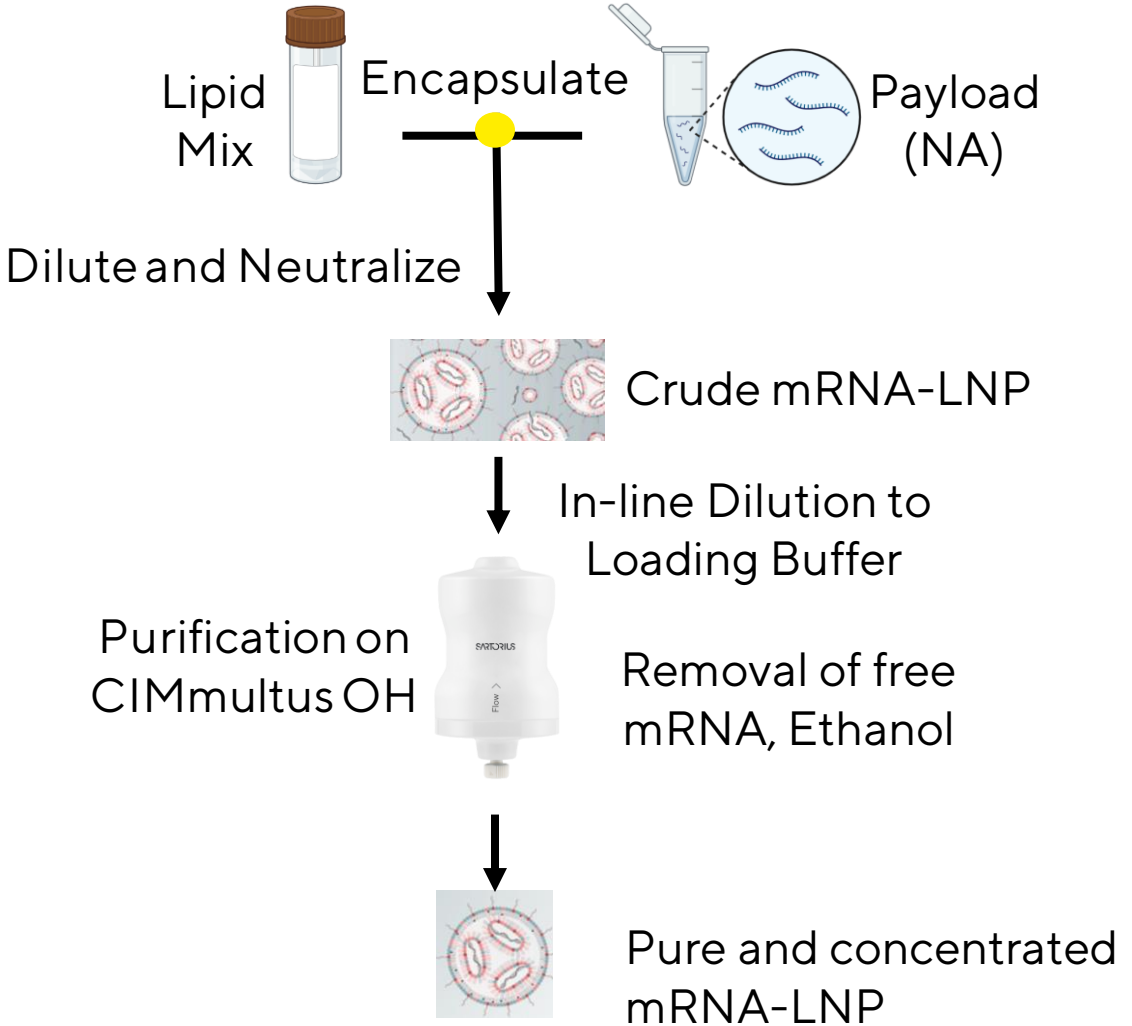
Concentrate LNPs and remove free mRNA.

Retain uniform and functional particles.

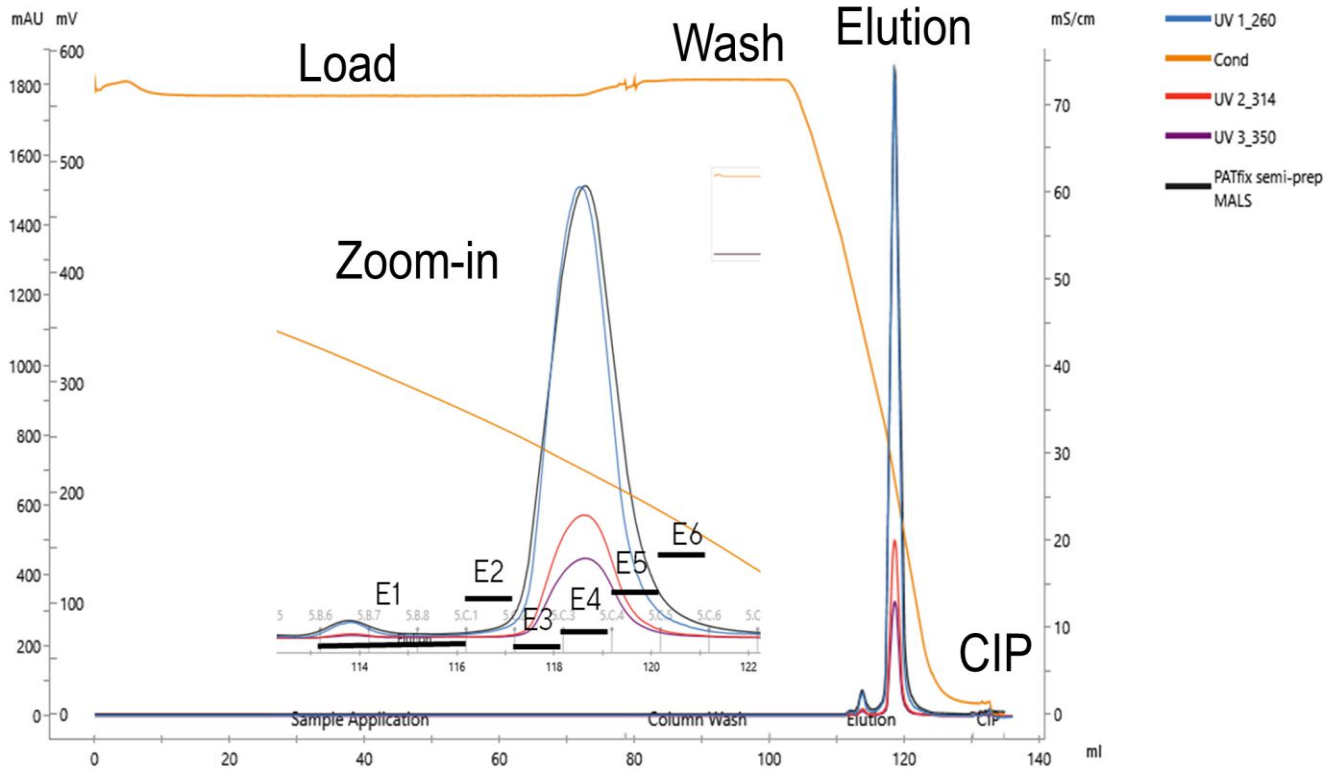
Remove EtOH

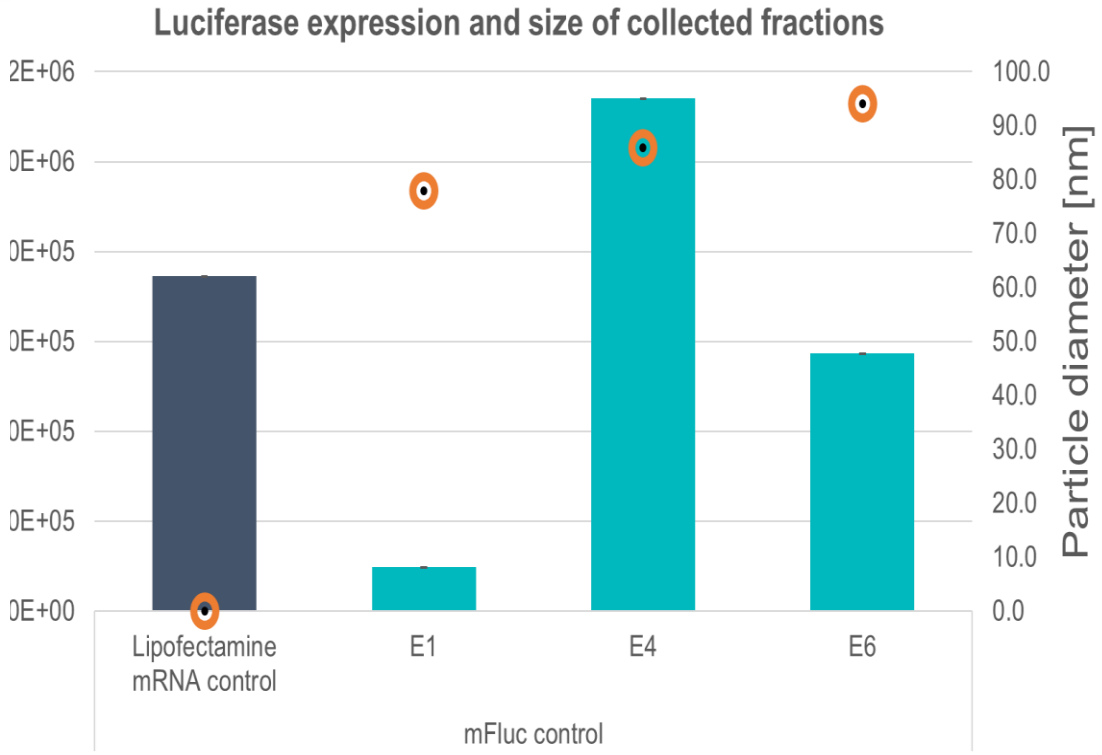
Solution

Low shear swift preparatory hydrophobic interaction chromatography on monolithic columns, CIMmultus OH



Shearless chromatography improves the potency / recovery





Purifying LNPs by chromatography using CIMmultus OH separates particles by size and can be used to remove subpopulations with lower potency.

Conclusions

- Analytical methods might be misleading. Orthogonal methods are needed for correct process understanding and its control.
- LNP PATfix Swither can be used as orthogonal to RiboGreen method and to DLS/NTA/Videodrop methods, and in addition allows for:
  - LNP encapsulation,
  - LNP purity,
  - LNP heterogeneity,
  - lapidated RNA amount,
  - LNP – protein conjugation reaction monitoring, and
  - LNP corona studies (coming).
- Purification using CIM OH monolithic columns gives recoveries >90%, even of more complex LNPs, and produces particles of higher uniformity and potency.
- Orthogonal in-process analytics, THE key pillar for the CMC.

KEY MESSAGES

Do NOT trust analytics

Orthogonal analytical methods are mandatory to prevent wrong conclusions due to the analytical method misleading, and should be in place before you start process development

PATfix systems were developed to address these requirements

Process is as good as your analytics are
Product is as good as your analytics are

The more defined the process, the easier it's scale-up would be.

Thank you for your attention!

Visit us at the Sartorius BIA Separations booth at exhibition hall

ales.strancar@biaseparations.com

SARTORIUS