Simplifying Progress

Optimize Your Process From pDNA Template to LNP Encapsulation With the Help of a Single Analytical Platform

The Winding Road from E.coli to LNPs, February 15th, Boston, MA

SARTORIUS

Agenda

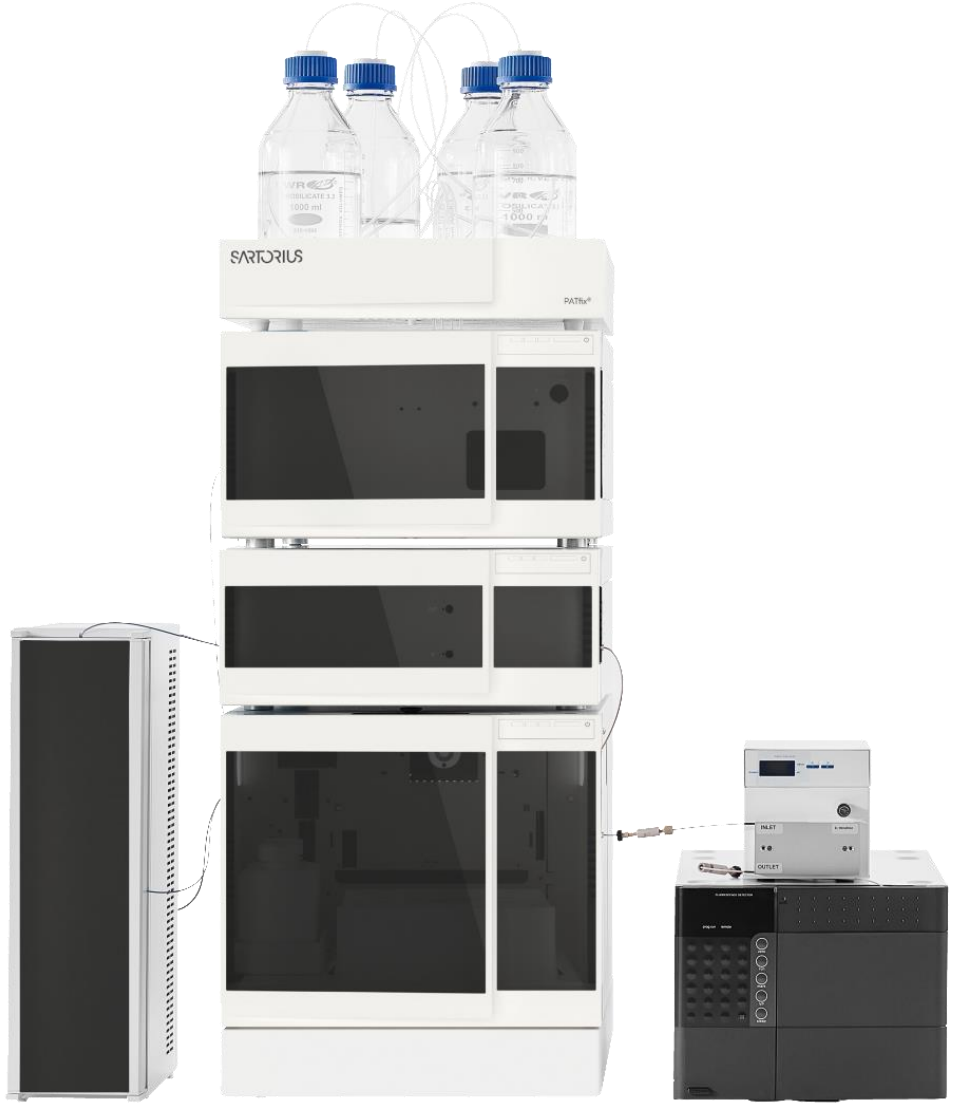
About mRNA and nucleic acids

pDNA linearization monitoring using PATfix

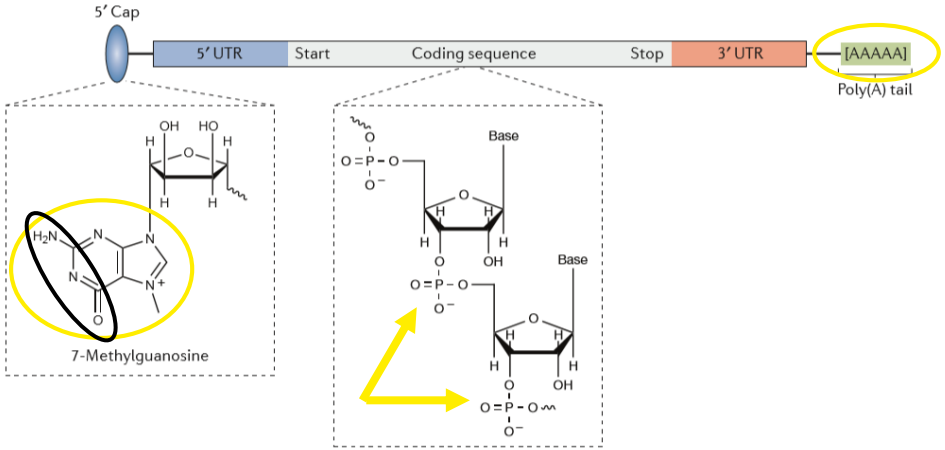
In vitro transcription yield and productivity optimisation using PATfix

Monitoring capping efficiency

New method for encapsulation efficiency



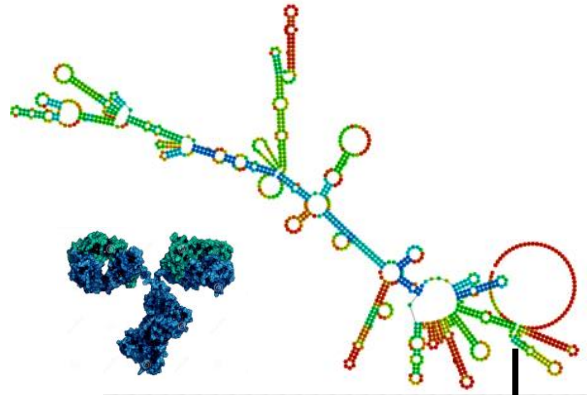
Structure and Properties of mRNA



mRNA structure: Hajj, Khalid A., and Kathryn A. Whitehead. "Tools for translation: non-viral materials for therapeutic mRNA delivery." *Nature Reviews Materials* 2.10 (2017): 17056.

- Negative charge of the phosphate backbone
- Base hydrophobicity/aromatic character
- Poly-A tail
- Base hydrogen bonding

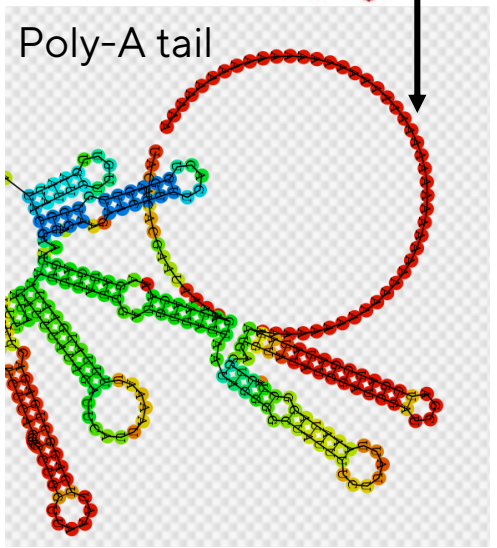
- mRNA much bigger than proteins (e.g. IgG)



- mRNA is base-paired

BUT

- The structure is dynamic

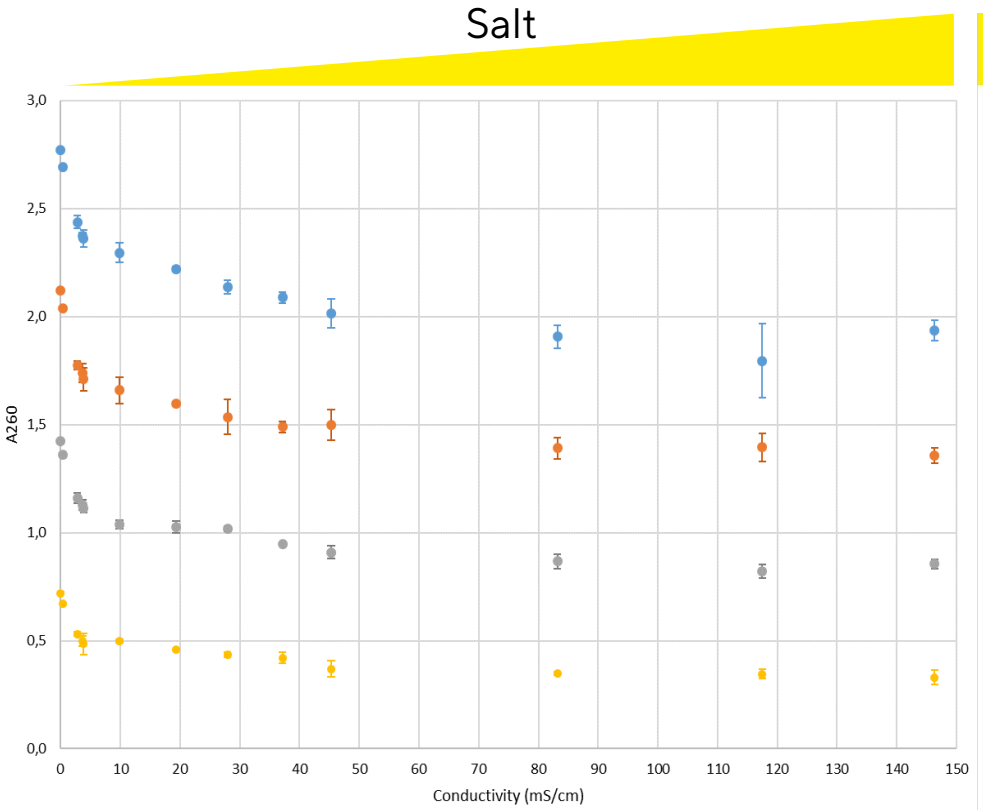


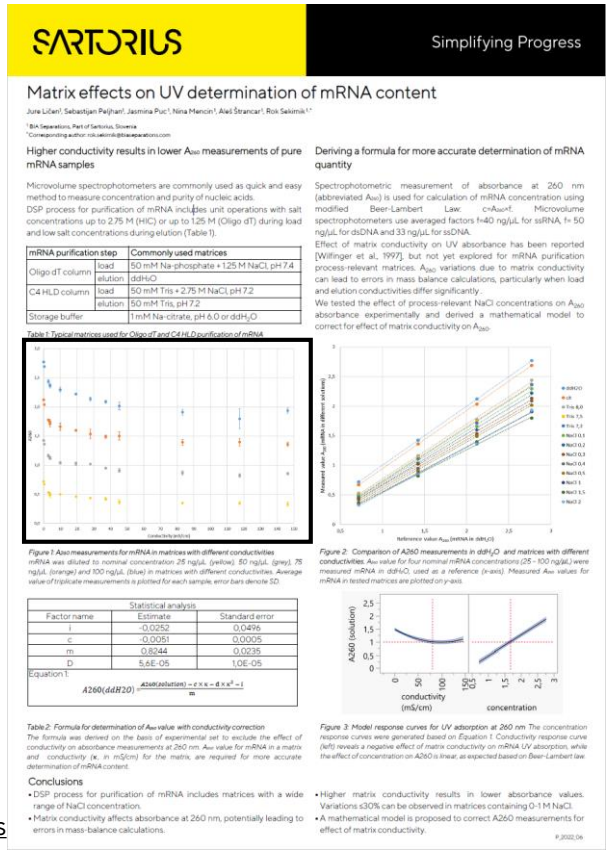
eGFP construct with 45 nt poly-A tail, minimum free energy prediction, poorly understood

http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi; Jure Ličen, Anže Martinčič Celjar

Quantification is Affected by Matrix and Other Nucleic Acid Species

- UV absorbance of nucleic acids is affected by the buffer (salt concentration and pH)
- Dyes have off-target binding that skew results
- Both contribute to erroneous readouts that can lead to wrong conclusions
- Biochromatography – separation before quantification




SARTORIUS Simplifying Progress

Matrix effects on UV determination of mRNA content

Higher conductivity results in lower A_{260} measurements of pure mRNA samples

Deriving a formula for more accurate determination of mRNA quantity

Conclusions

Matrix effects on UV determination of mRNA content - Sartorius BIA Separations

mRNA Drug Substance Production Workflow

Analytical workflow (PATfix pDNA; CIMac pDNA)

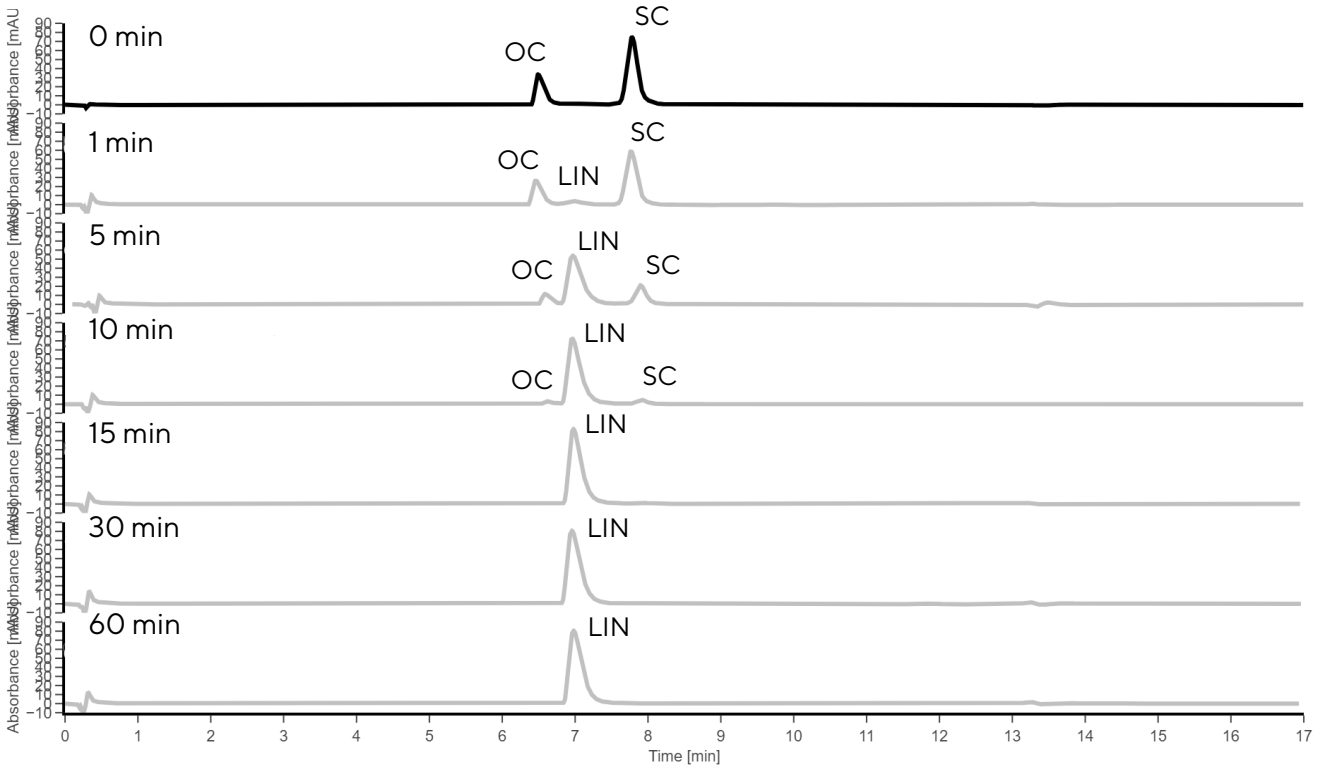
Cell harvest → Alkaline lysis $CaCl_2$ treatment → UF | DF → CIMmultus DEAE → Linearization → CIMmultus C4 HLD → UF | DF → Linear pDNA

Linear pDNA → IVT reaction → CIMmultus Toolbox Oligo dT, PrimaS, C4 HLD, SDVB → UF | DF → Filtration → mRNA

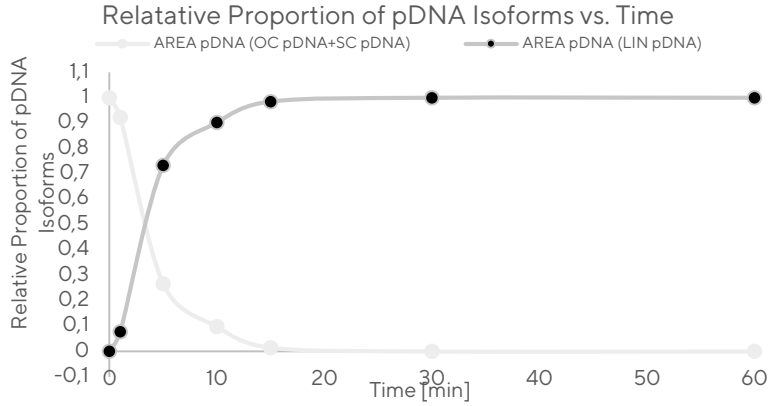
Analytical workflow (PATfix mRNA; CIMac PrimaS, CIMac Oligo dT, CIMac SDVB)

PATfix™ pDNA Platform For pDNA Linearization Control

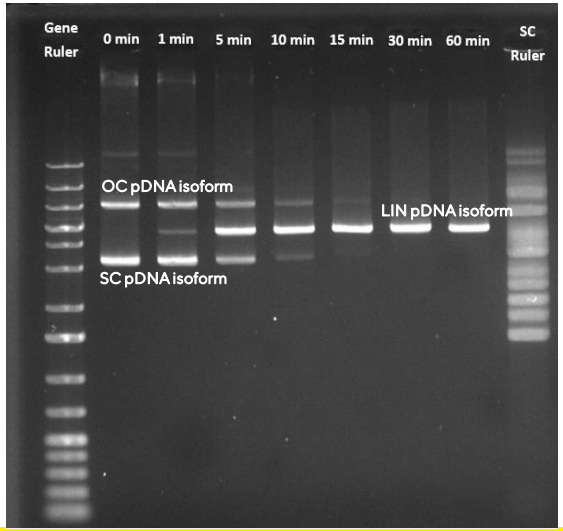
PATfix pDNA monitoring of linearization reaction



Kinetics of reaction



Agarose gel electrophoresis

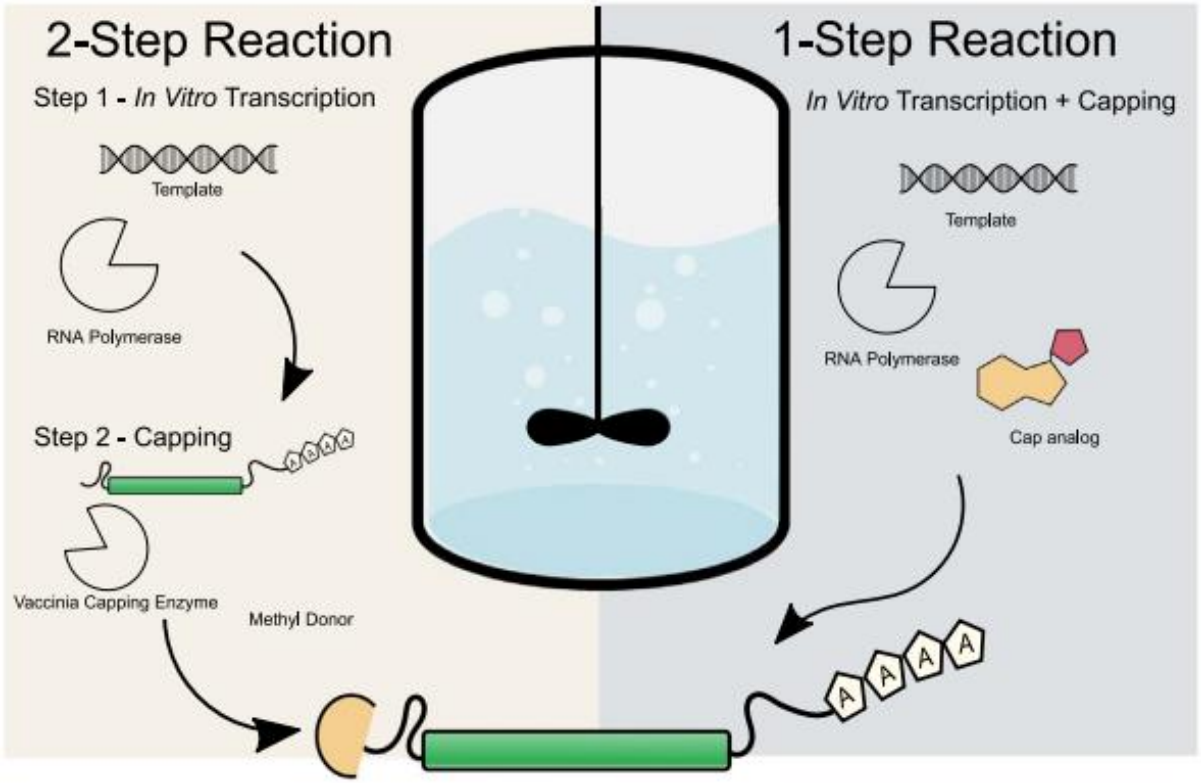


- PATfix™ pDNA platform enables us to study kinetics of pDNA linearization reaction
- Information of remaining OC & SC isoform and possible other contaminants

In Vitro Transcription Reaction – Very Fast Production of mRNA From DNA Template

- Reaction time is typically 2-3 hours for batch processes
- High yield (low reaction volumes)
- IVT is a multi-component reaction :

  - Plasmid (dsDNA)
  - RNA polymerase (e.g. T7)
  - NTPs (optional modified NTPs)
  - Capping reagent (optional)
  - $MgCl_2$
  - Pyrophosphatase (optional)
  - RNAse inhibitor
  - Spermidine
  - DTT
  - Buffer

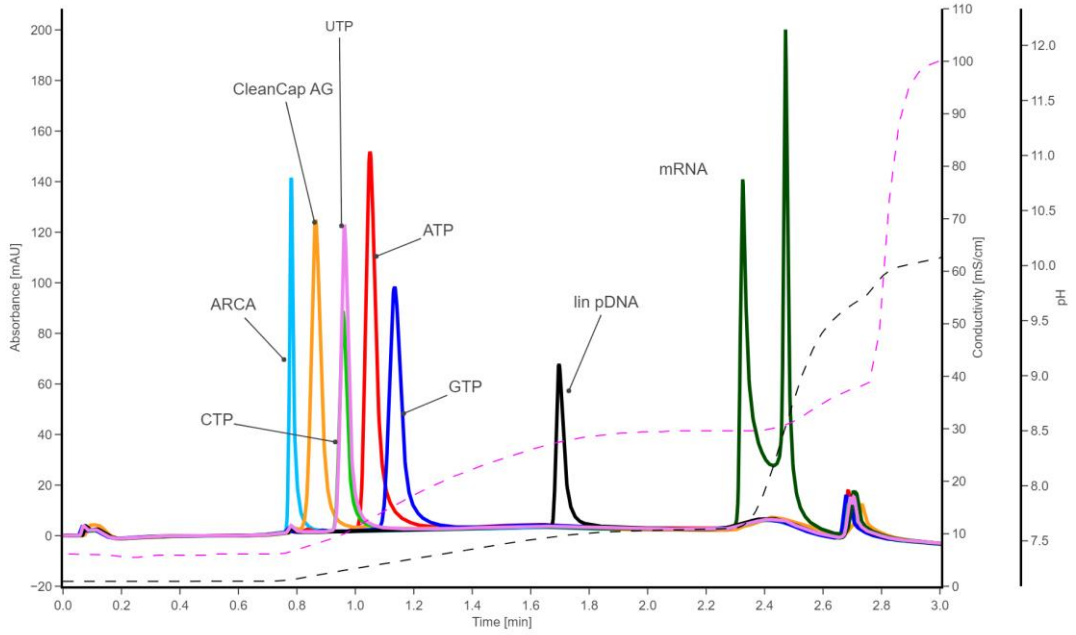


Rosa, S. S., et al., Vaccine. 2021 Apr 15; 39(16): 2190–2200.

mRNA Analytics: Monitoring IVT - Paradigms For Rapid At-Line Analytics

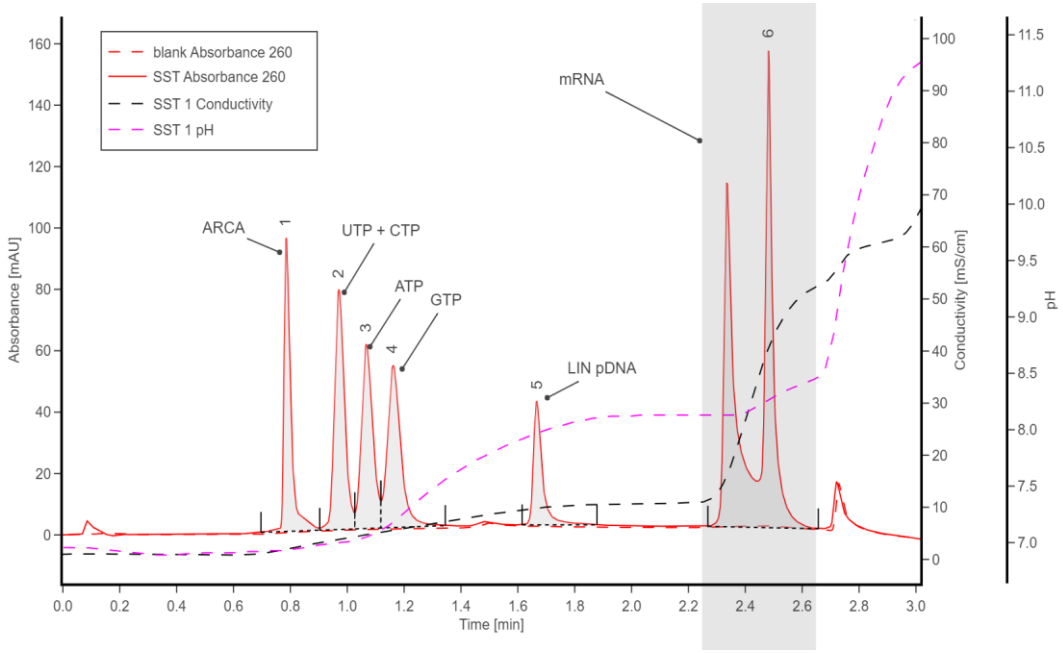
CIMac PrimaS - multimodal

- Multi-parameter method
- New paradigm for mRNA
- NTP, capping, RNA content. Applies to all RNAs

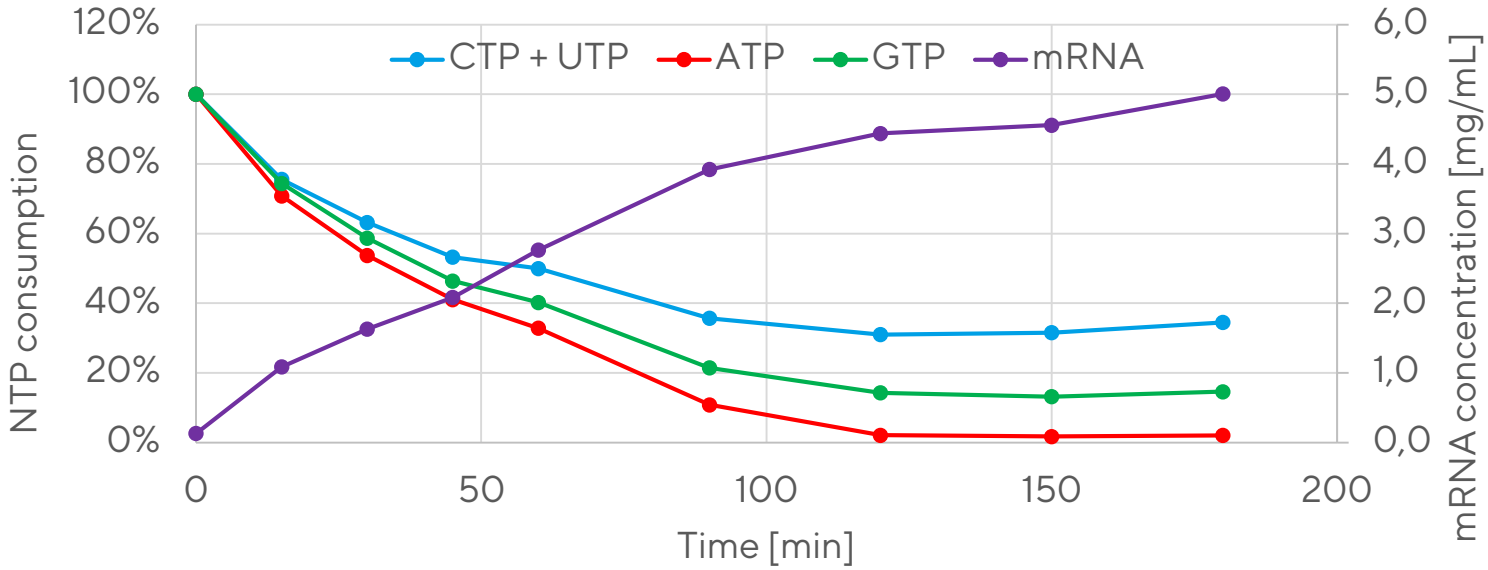


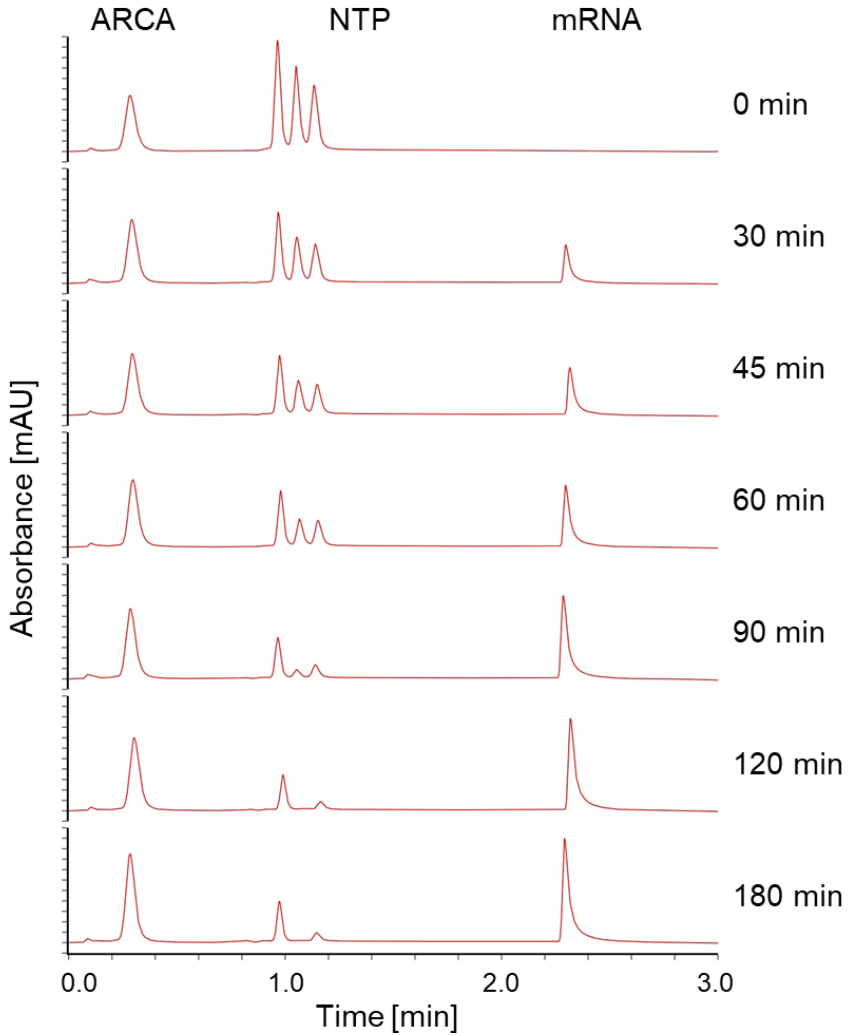
PATfix mRNA platform

- 2.5 min for results, 8 min method
- SST-validated
- Includes additional methods for orthogonal insight

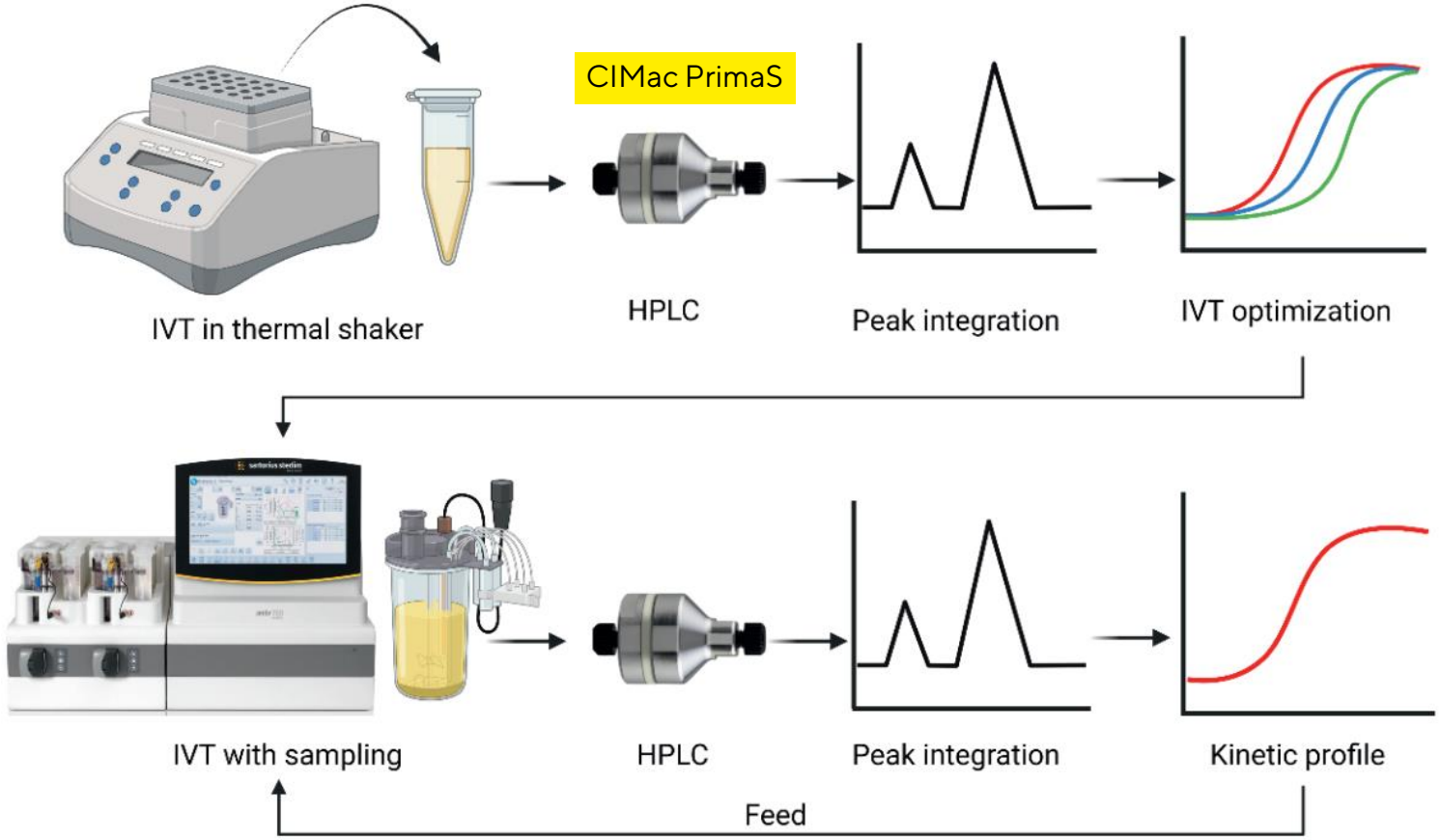


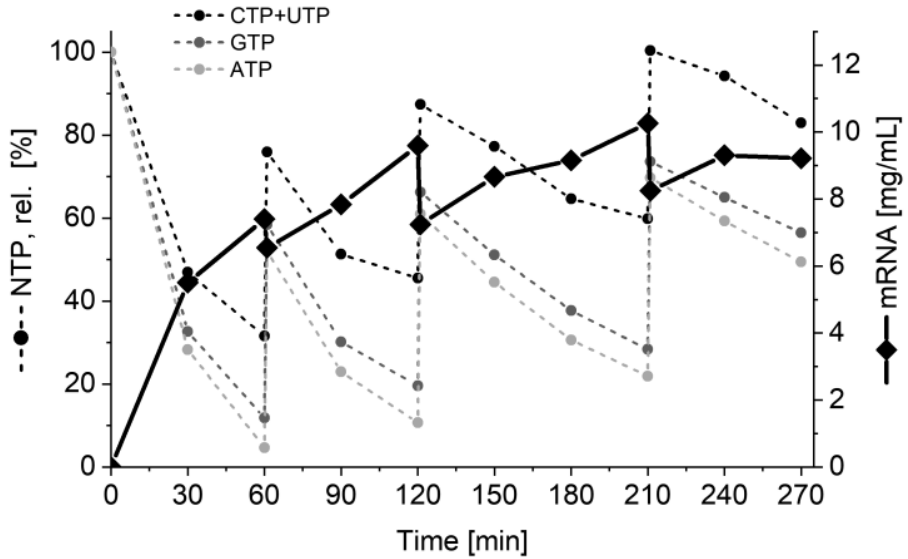
- The IVT reaction can be monitored at-line **by CIMac PrimaS**
- mRNA production kinetics is monitored. Productivity maximum can be identified, to prevent degradation.
- Consumption of nucleotides and concentration of capping reagent can simultaneously be studied
- Effects of feed addition can be studied





CIMac PrimaS Provides Tight Control Over IVT Reaction

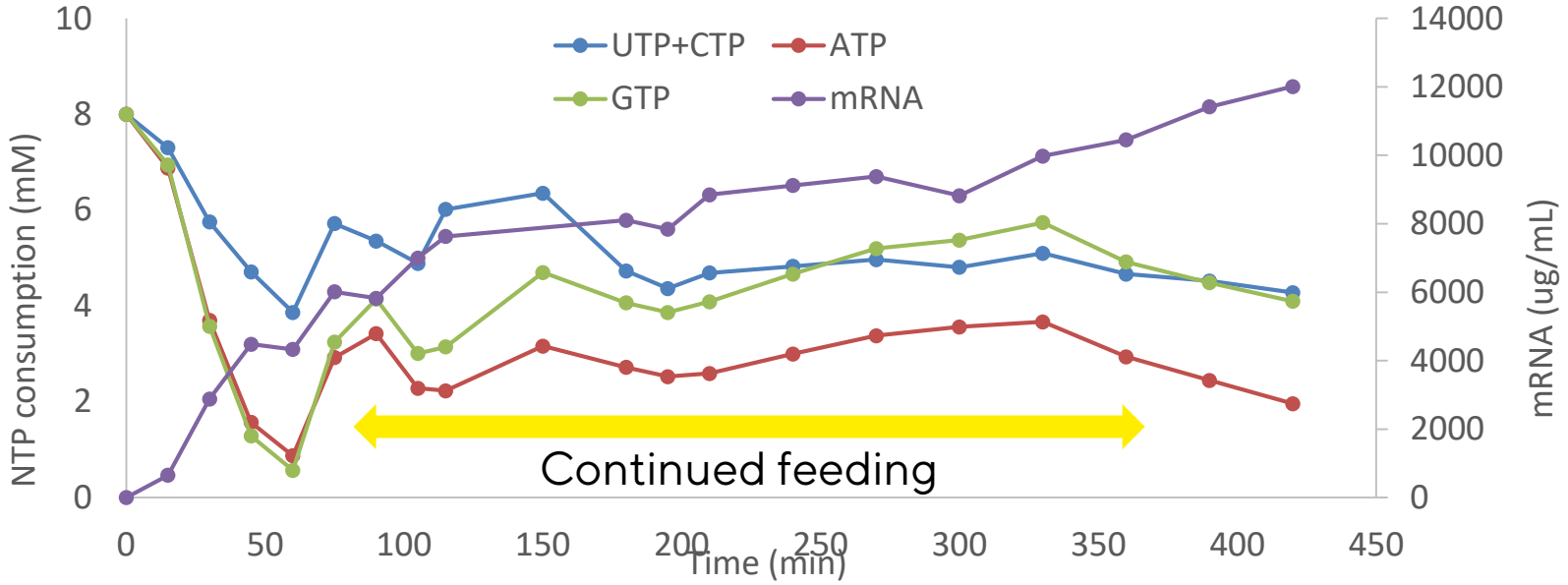




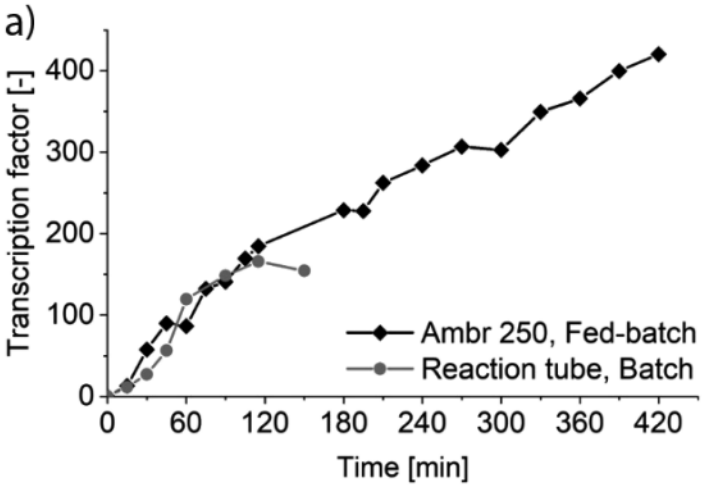
- Determination of optimal IVT conditions and characterisation of reaction kinetics in thermal shaker.
- NTP feeding strategy designed and tested in thermal shaker and transferred to automated scale-up Ambr 250 system.

Skok, Janja, et al. "Gram-Scale mRNA Production Using a 250-mL Single-Use Bioreactor." *Chemie Ingenieur Technik* 94.12 (2022): 1928-1935.

A Step Towards Continuous Manufacturing: Fed-Batch



PATfix mRNA



- Real-time monitoring of NTP in reaction container.
- Reaction kinetics in scale-up comparable to thermal shaker (linear increase in transcription factor)

Skok, Janja, et al. "Gram-Scale mRNA Production Using a 250-mL Single-Use Bioreactor." *Chemie Ingenieur Technik* 94.12 (2022): 1928-1935.

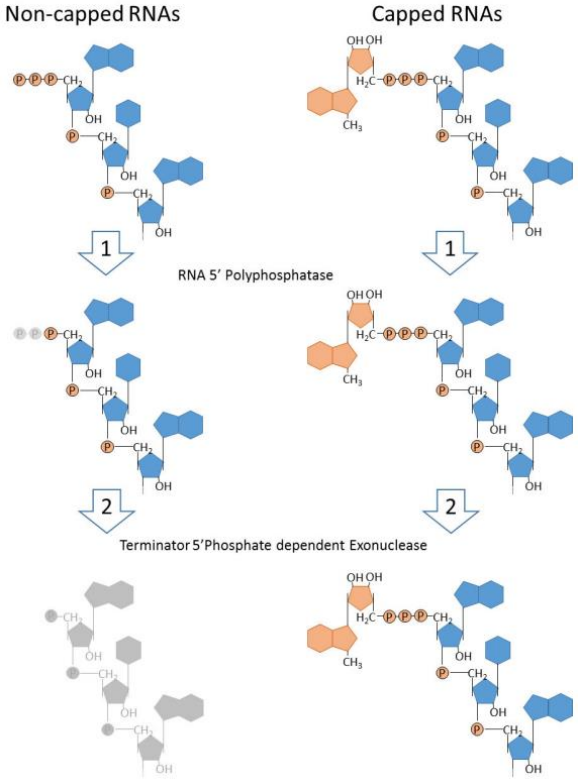
CIMac PrimaS: Capping Efficiency (with Enzymatic Digestion)

IVT reaction

IVT with capping reagent, e.g. ARCA or CleanCap

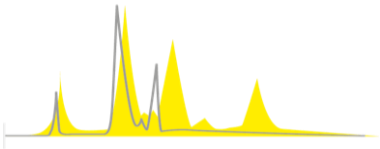
Systematic study of variables, e.g. GTP:ARCA ratio, addition of NTPs

5′ polyphosphatase + 5′ terminator exonuclease



Enzymatic treatment selectively degrades uncapped mRNA

CIMac PrimaS



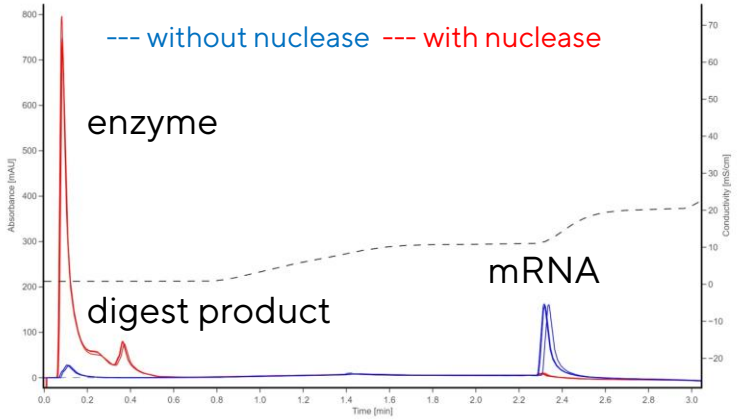
Quantification of undigested mRNA by PATfix mRNA analytics

Calculation of %undigested mRNA by comparison to no-enzyme control

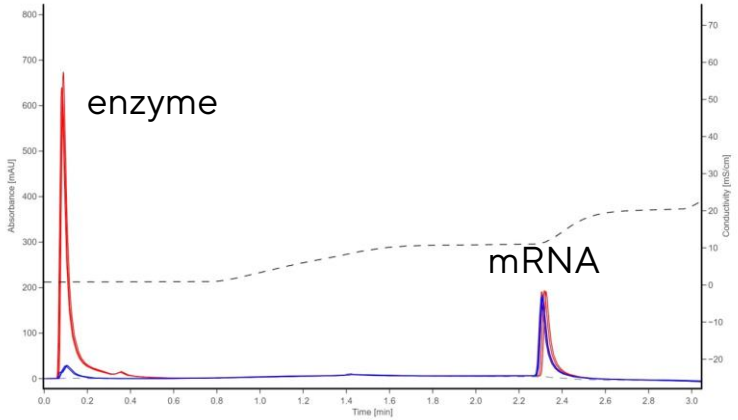
Chiron, Stéphane, and Philippe H. Jais. "Non-radioactive monitoring assay for capping of messenger RNA." *Transl Genet Genom* 1 (2017): 46-49.

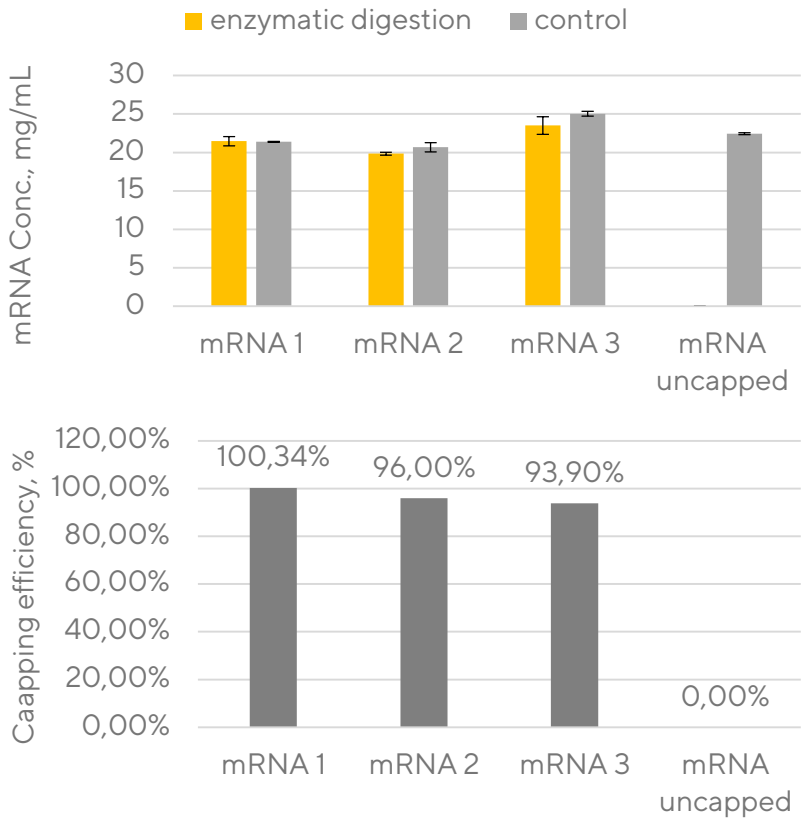
Capping Analysis of mRNA

Uncapped mRNA



Capped mRNA





Raw material control

- pDNA content/purity
- NTP
- Capping reagent
- Enzyme purity

1

IVT reaction monitoring

- mRNA content
- NTP content
- Capping reagent content
- dsRNA content

2

You have mRNA

But the story is not over

Drug substance stability

- Stability studies
- Content

6

mRNA purification

- Capture & polish
- Optimization workflow

3

5

Drug substance analytics

- Critical quality attributes (purity, content)

4

DSP In-Process analytics

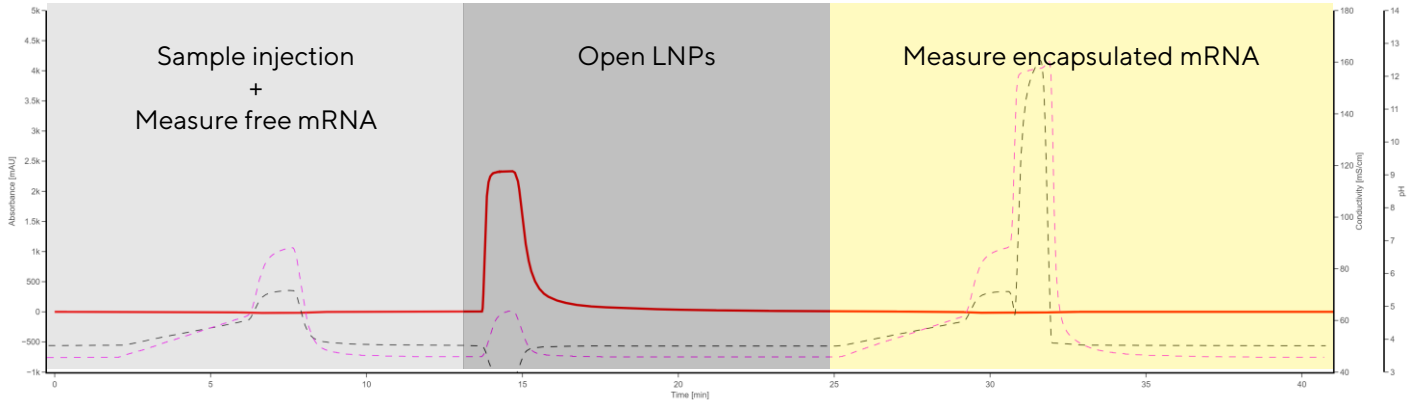
- Process performance
- Purity, content of mRNA

Sekirnik, Kostelec, Chromatography in mRNA Production Workflows, BPI December 2021, Chromatography in mRNA Production Workflows - BIA Separations

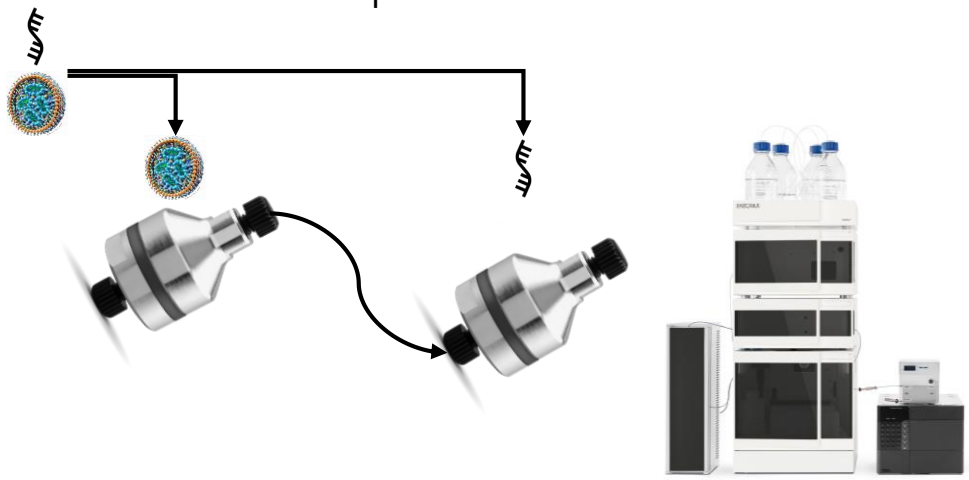
Monitoring Encapsulation Efficiency

- No dyes required
- Double column setup
- At-line quantification with immediate feedback

- Method steps overview on a blank sample:

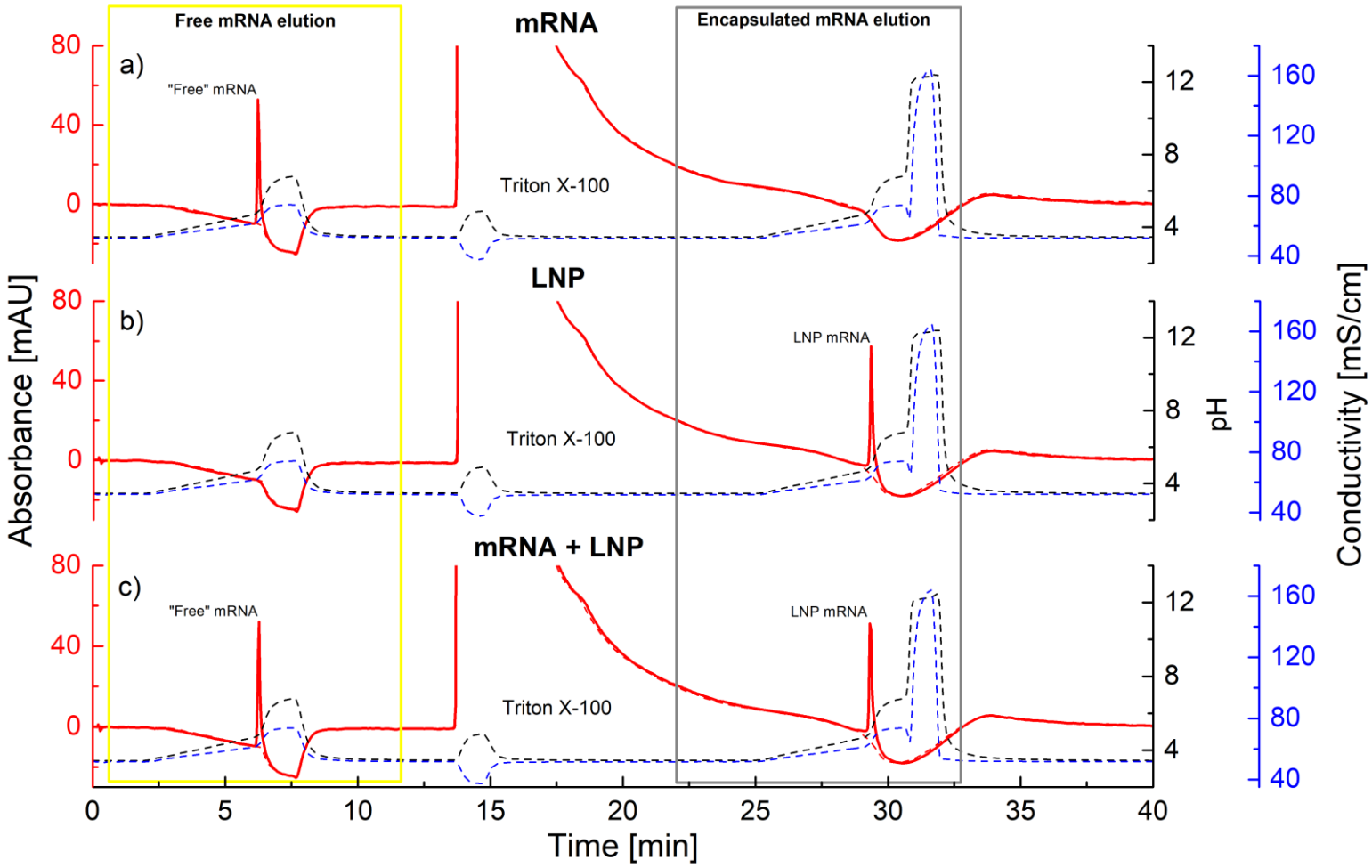


Each column binds
one of the two species



Monitoring Encapsulation Efficiency

- Comparison chromatograms of free vs encapsulated mRNA
  - mRNA sample is mFix4
  - LNP sample is encapsulated mFix4
  - 50/50 mixture of the two
- Triton X-100 is used for opening the LNP particles
- mRNA from LNPs is then captured on the second column
- A gradient is used for elution of mRNA from the second column



*this method is still under development

Take home messages

General

- pDNA has trackable isoforms
- mRNA has a dynamic structure
- **Separation before quantification**
- Absorbance is matrix dependent
- Dyes can have off-target effects

pDNA a critical raw material

- pDNA purification
  - Monitor pDNA purification
  - Track sc/oc content and ratio
- pDNA linearization
  - Monitor sc/oc/lin
  - Determine & optimize kinetics

mRNA process development

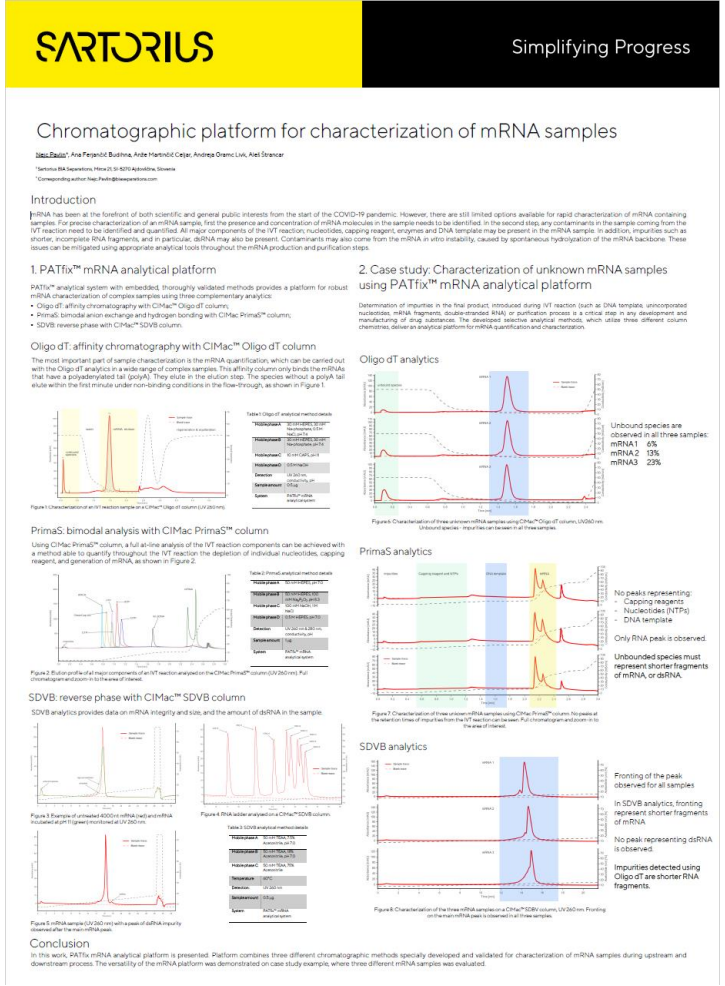
- mRNA synthesis
  - Track all key components
  - Determine & optimize synthesis kintetics – **double yields**
- mRNA purification
- Capping analytics
- **mRNA encapsulation analytics**
  - **What would you want to see?**

Stop by our poster

PC4 team: Andreja Gramc Livk, Nejc Pavlin, Ana Ferjančič Budihna, Anže Martinčič Celjar

Aleš Štrancar

Blaž Bakalar
Product Manager, PATfix platform
Blaz.Bakalar@biaseparations.com


SARTORIUS

Simplifying Progress

Chromatographic platform for characterization of mRNA samples

Introduction

1. PATfix™ mRNA analytical platform

2. Case study: Characterization of unknown mRNA samples using PATfix™ mRNA analytical platform

Oligo dT: affinity chromatography with CIMac™ Oligo dT column

Oligo dT analytics

PrimaS: bimodal analysis with CIMac PrimaS™ column

PrimaS analytics

SDVB: reverse phase with CIMac™ SDVB column

SDVB analytics

Conclusion

SARTORIUS