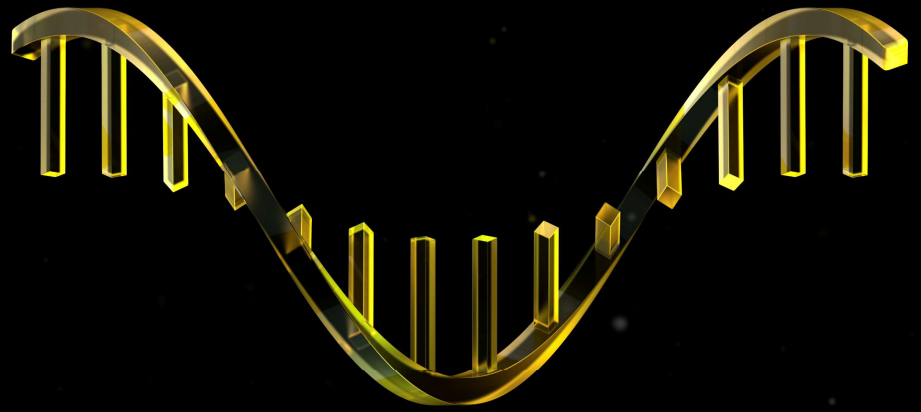
Simplifying Progress



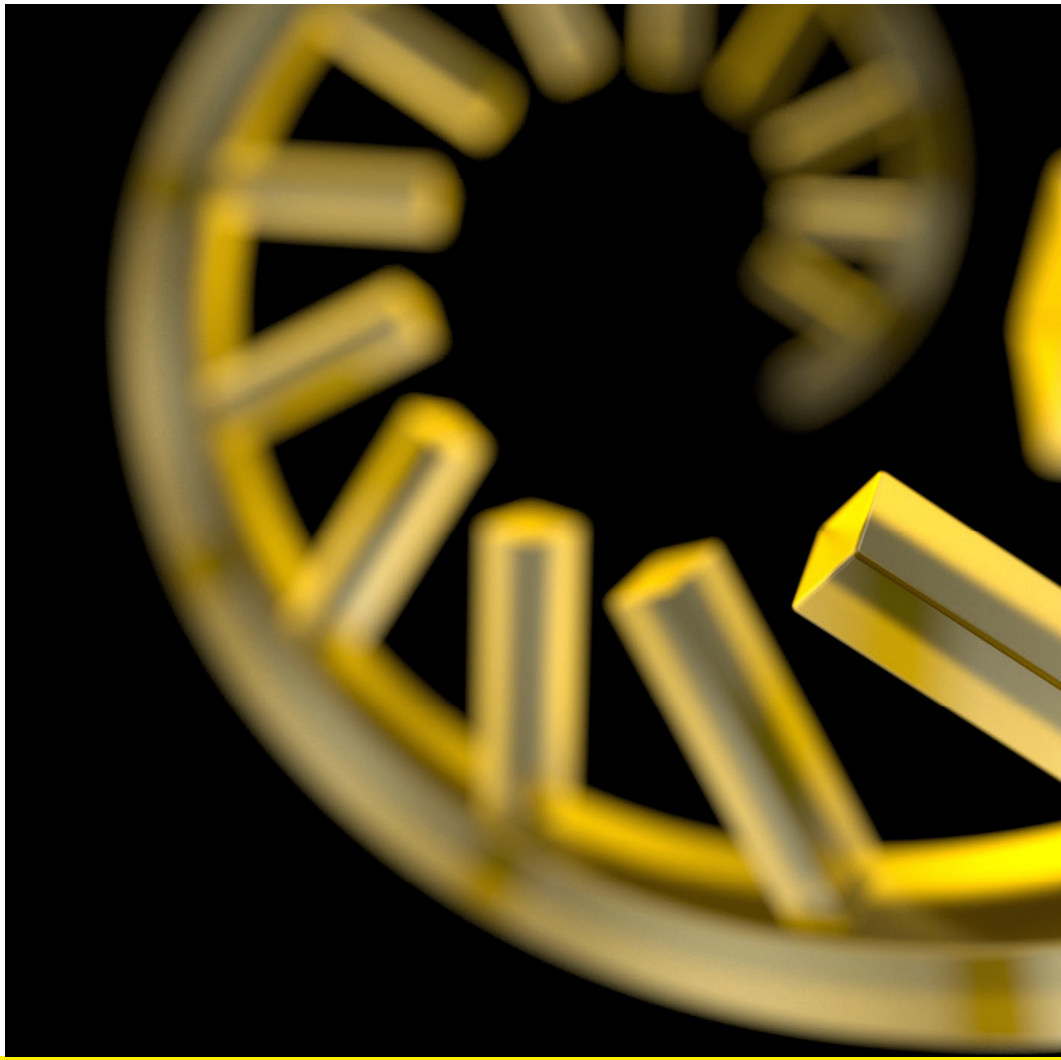
Scalable, Flexible and Reliable Solutions to Simplify Your m-RNA Production

DCVMN, Dec 14th 2022

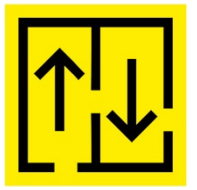
SARTORIUS

Agenda

- Introduction
- Upstream – *in vitro t*ranscription
- Downstream Solutions
- Formulation Development & Storage
- Analytical Solutions



Lessons Learned from the Race to CoViD- 19 vaccine

	Speed	Process	Facility
			
Traditional approach	▪ Long development timeline – 10 years average	▪ One bug, one drug ▪ Inactivated, attenuated, recombinant proteins	▪ Dedicated facilities ▪ Large capital investment
The new way forward	▪ Shorter process development timelines ▪ Decrease time from pre-clinical to first in human	▪ Closed processes , Single-use processes ▪ Platform-able processes to gain speed and agility (mRNA, viral vectors)	▪ Increase use of CMOs and technology transfer ▪ Flexible ballroom for rapid changeover

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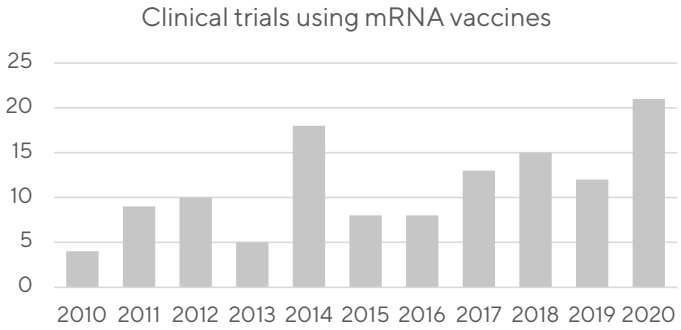
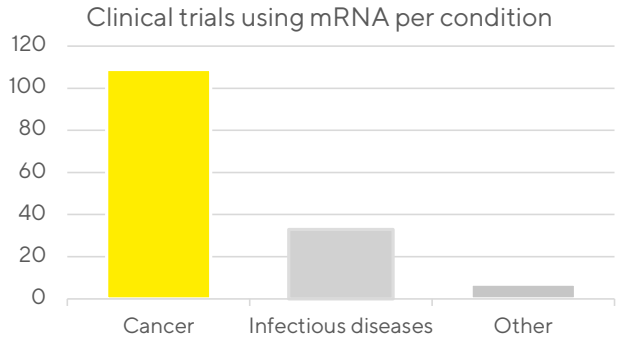
Importance of mRNA Beyond CoViD- 19





- Current demand for mRNA is generated primarily from CoViD-19. Approval of Pfizer|BioNTech and Moderna vaccines brings closer to reality using mRNA in other therapeutic areas, such as cancer and infectious diseases.
- Scalable purification methods using chromatography will replace laboratory scale methods such as precipitation. Early implementation can lead to faster development and shorter time to clinic.

Adapted from Rosa, Sara Sousa, et al. "MRNA Vaccines Manufacturing: Challenges and Bottlenecks." *Vaccine,* vol. 39, no. 16, 2021, pp. 2190-2200.

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Key Drivers for mRNA Manufacturing

Current Technology

- Use plasmid for DNA template
- In vitro transcription
  - Enzymatic capping
  - Co-transcriptional capping
- LNP-Based Formulation
- Downstream needs to be adjusted to fit different capping methods

Pain Point

- High-cost raw materials (especially enzymes).
- No standardized platform approach
- DNase/RNAase contamination

Speed to Clinic | Market

- Robust scalable processes
- Standardization of the process
- Process simplification
- Skilled professionals

Process Improvement

- Increase manufacturing yield
- Reduce cost per dose
- CQA & Analytical tools
- In house capacity expansion

Flexible Processes

- Multiple product options
- Modular and mobile
- Single use technology
- CDMO service

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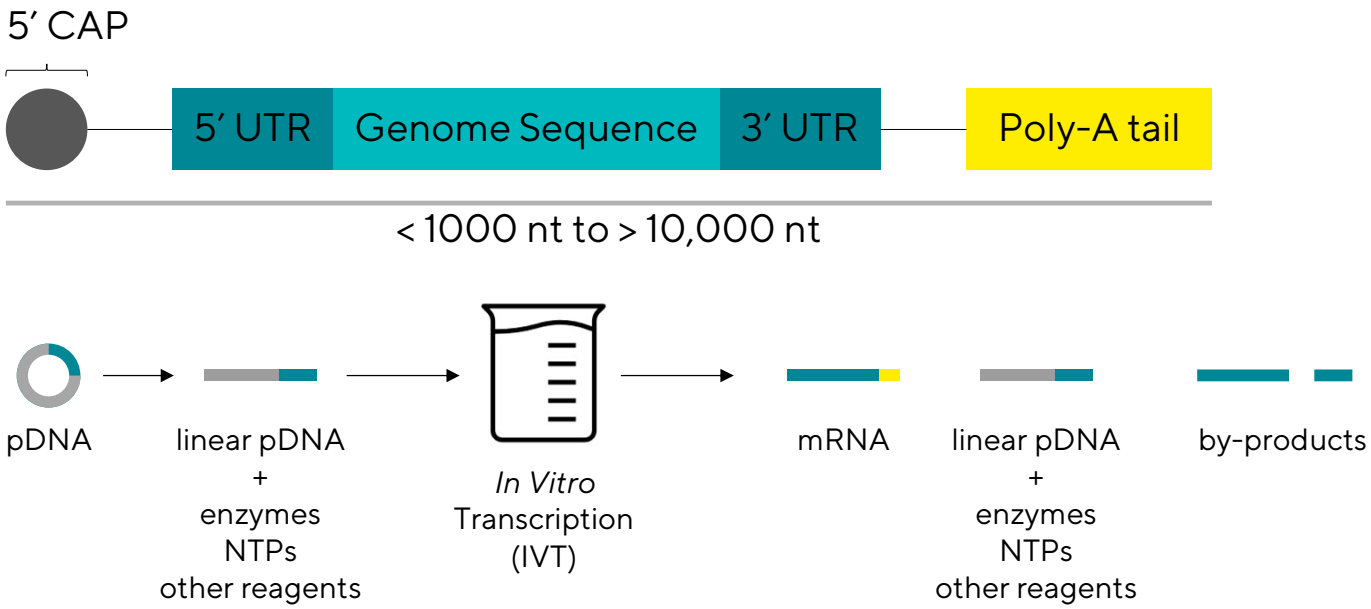
Mature mRNA Structure and Production



- Single-stranded RNA
- 5′ Cap and poly-A tail are required for successful protein expression in cells.
- *In Vitro* transcription produces RNA from a DNA template, often a plasmid DNA
- 5′ Cap can be added co-transcriptionally (during IVT), or post-transcriptionally
- Poly-A tail can be encoded in the DNA template, or added enzymatically after IVT

Good understanding of IVT reactions is fundamental to maximise productivity and document the purity.

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mRNA Workflow and Sartorius Solution

Synthesis

Pro Mixer & BIOSTAT®

Commercial phase (50 - 200 L)

BIOSTAT® B

ambr®250 or 15

PD & Clinical phase (< 10L)

Sartorius Plasmid solutions

* LNP: lipid nanoparticle

IVT | UF/DF | Capture | Polish | Buffer Exchange after LNP* | Sterile Filtration

TFF system: Hydrosart® 30-300KD | Resolute Flowdrive/BioSMB | Oligo dT/ Prima S | C4 – HLD/SDVB | Hollow Fiber or Hydrosart ® | Sartopore ® 2 XLG

HPLC and analytical column is also available for mRNA production. PD services can also be provided.

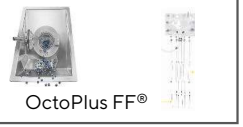
Pro Mixer

Flexsafe® Bags

Palletank® for Storage

Buffer Preparation for LNP

Final Filling



OctoPlus FF®

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Agenda

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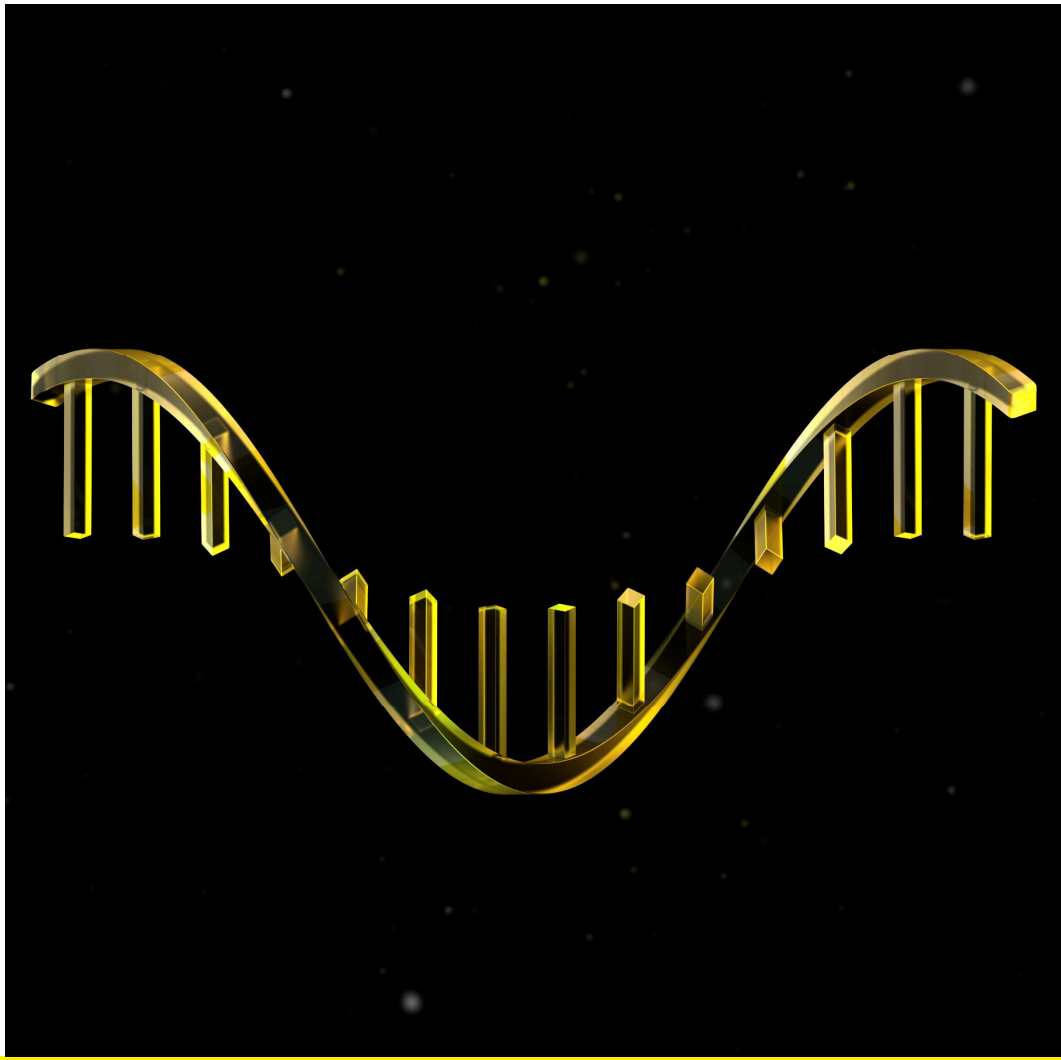

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Unique Challenges of mRNA IVT

Pain points	No standard efficient IVT reaction protocol available	High cost and weak supply chain of raw materials	Lack of dedicated tools supporting PD of mRNA	DNAse and RNAse contamination / instability of mRNA	No dedicated analytical methods for this new modality
Needs	High-throughput	mRNA expertise	Single-use technology	Analytical solutions	

*LNP: Lipid nano particle

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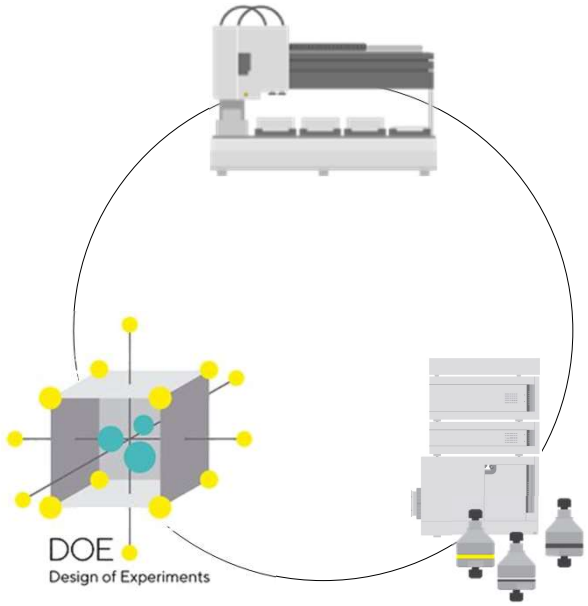
Simplify IVT Process Development for mRNA

You can get quick access to IVT process understanding in a simple way with High throughput PD and analytical tools

- ☑ Screening of mRNA candidates
- ☑ Screening and identification of CPPs and CQAs
- ☑ Set Design Space



mRNA IVT PD package

Bioreactors:
- Ambr® 15 Multi-Parallel Bioreactors
- Univessel® SU
- Biostat® B-DCU

Software:
- MODDE® DOE Software

Analytical solutions:
- PATfix™ HPLC
- CIMac™ PrimaS Analytical Columns
- Cornerstone® Biomanufacturing Development Services

Plasmid:
- pDNA production solution

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Building up IVT Understanding with Analytics

Analytical method	Parameter
AGE	RNA, DNA size and nucleic acid contamination
Capillary electrophoresis (CE)	RNA size, purity
SDS PAGE	Protein contamination
HPLC	Purity

Common laboratory methods for characterisation of IVT products.

- Optimisation of reaction conditions for different RNA constructs can lead to improved process performance, notably for longer RNA molecules, such as saRNA
- IVT contains a large number of variables to measure and adjust: ratio of individual reagents (nucleotides, capping reagent, polymerase, pyrophosphatase, etc), buffer conditions (Mg2+ ion, DTT, etc), reaction time, temperature
- Modelling of reaction kinetics in relation to the interaction between reagents can further improve our process understanding.
- Most methods are time consuming, lack automation, and most of all, measure one parameter at a time.

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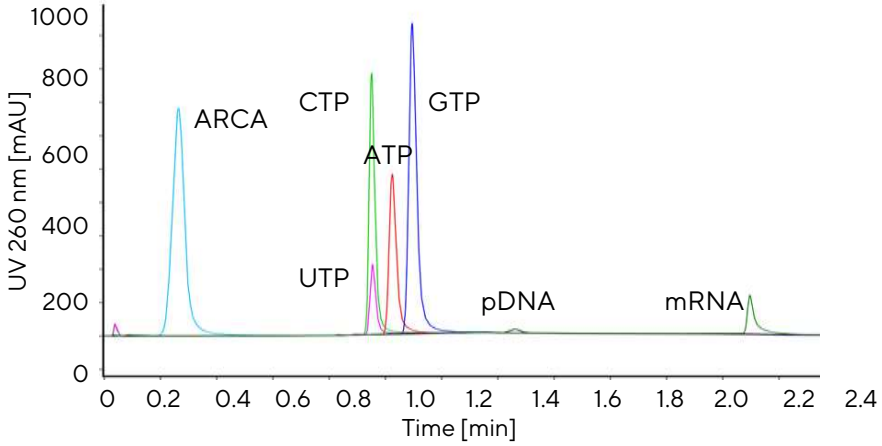
Building up IVT Understanding with Analytics

- New analytical column chemistries, HPLC can provide valuable more information on the IVT reaction.
- CIMac PrimaS can separate and quantify multiple individual IVT reaction components in a single, rapid assay



CIMac PrimaS (P.N. 110.5118-2), Buffers: MPA (50 mM HEPES pH 7), MPB (50 mM HEPES, 200 mM sodium pyrophosphate, pH 8.5), Method: 0–1 min (100 % MPA), 1–1.8 min (gradient to 20 % MPB), 1.8–2.5 min (gradient to 80 % MPB). Full method not shown. Flow rate 2 mL/min, PATfix™ HPLC system, UV absorbance at 280 nm, injection volume 25 µL.

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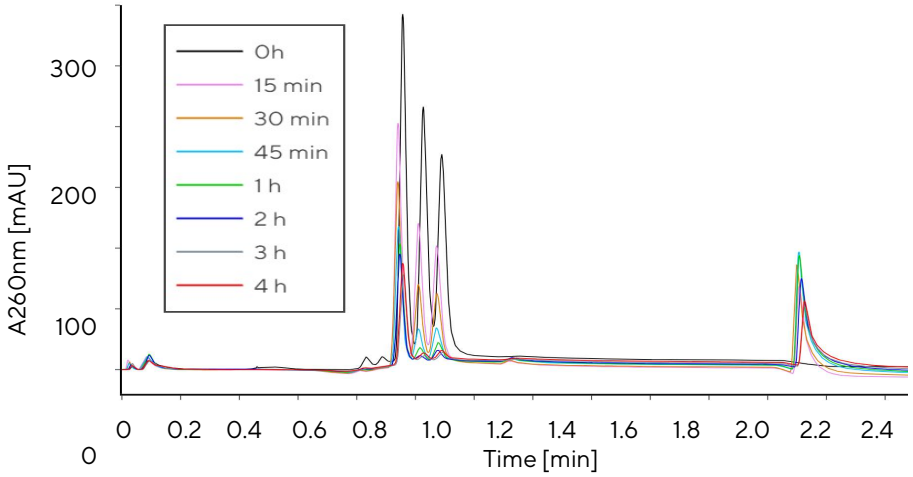
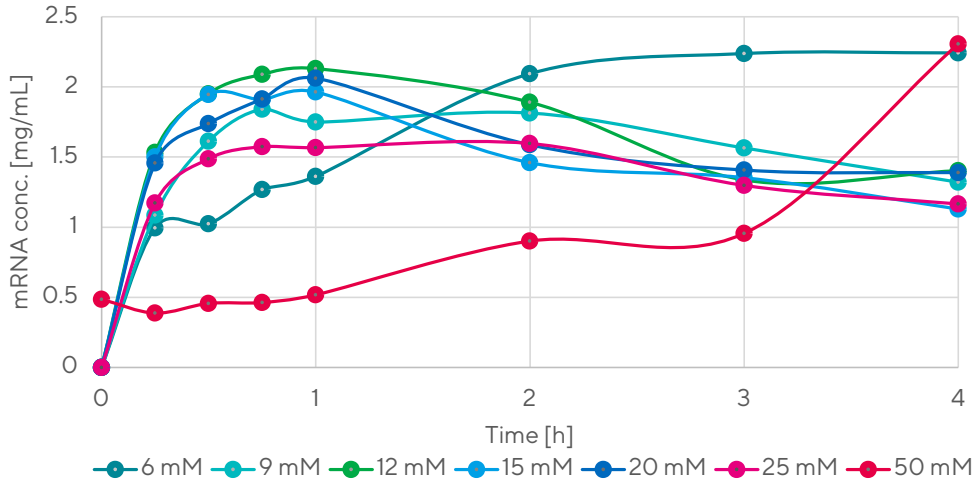
Building up IVT Understanding with Analytics



Sample: IVT reaction inactivated with EDTA and diluted in MPA (200-fold dilution). Conditions: 20 µg/mL linear pDNA, 500 U RNA polymerase per µg pDNA, 4 mM ATP, CTP, UTP and GTP each, 1U/ µL RNAse inhibitor, 1U/ mL pyrophosphatase, X mM Mg^{2+}

Reaction kinetics with relation to concentration of Mg^{2+} ions in the reaction



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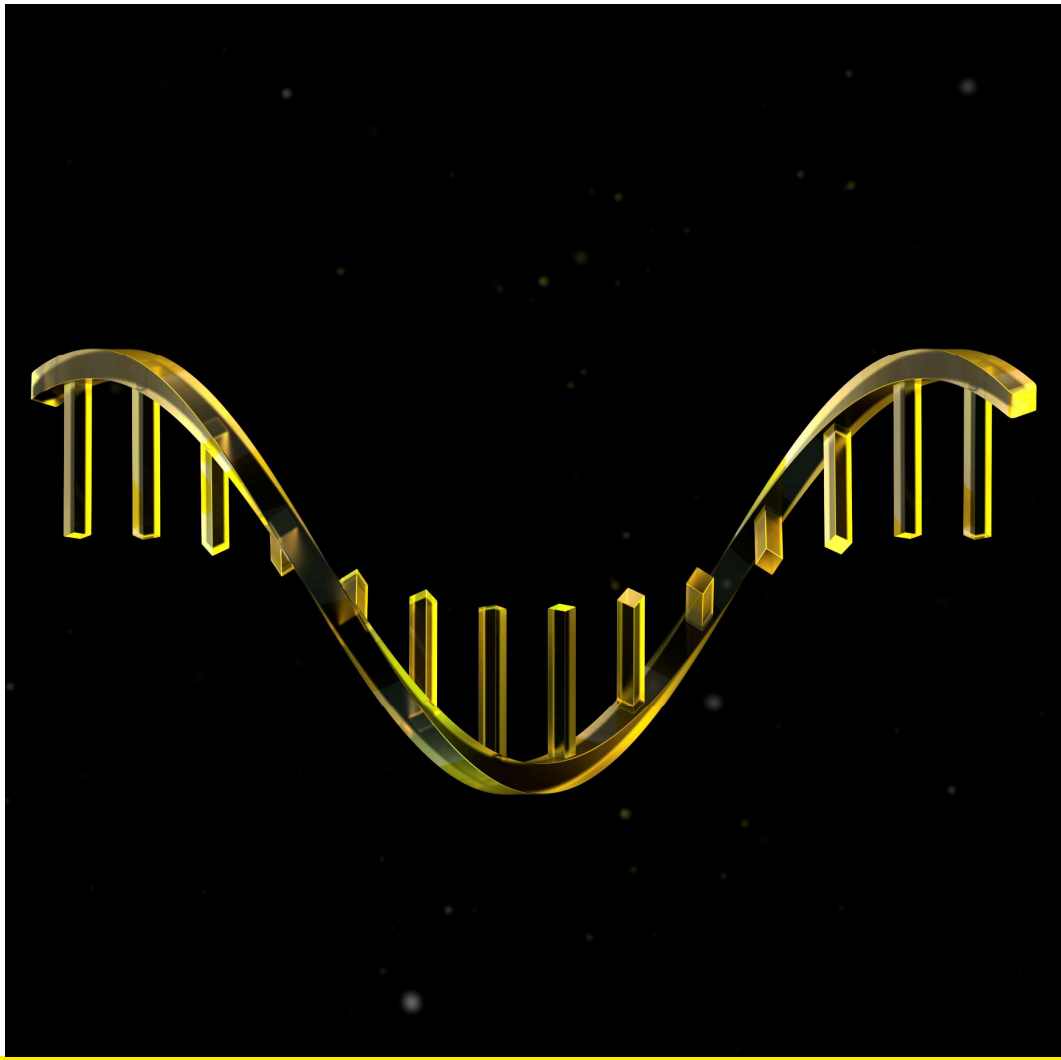

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Unique Challenges of mRNA Downstream Processing

Pain points	IVT protocol is still evolving, impacting the DSP	There is no established DSP platform that can be applied to all mRNA	Reagents and raw materials are poorly defined and bring contaminants	Impurities are very similar to the target and may interact with it	Many technologies used in labs are not scalable	mRNA are large molecules, shear sensitive and relatively unstable
Needs	Monolithic technology	mRNA expertise	Single-use technology	Proven Scalability	Toolbox of filters	

*LNP: Lipid nano particle

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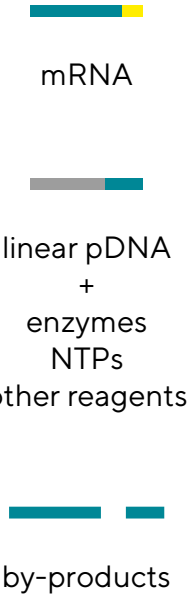
What are we Separating mRNA from?

- IVT reaction components (DNA template, enzymes, NTPs, capping and/or other reagents)
- Contaminants in the raw materials:
  - In the plasmid: E.coli proteins, DNA, and RNA.
  - In the enzymes: fragments, host DNA, RNA, proteins.
- Endotoxins, if introduced during processing
- Reagents used for any additional processing, such as plasmid digestion post-synthesis or capping enzymes
- RNA variants: dsRNA, truncations, fragments, aggregates.

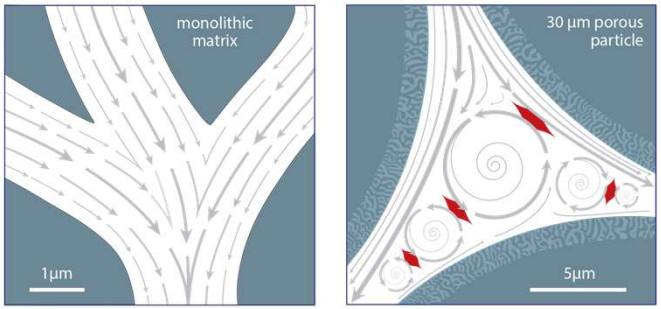


Chromatography

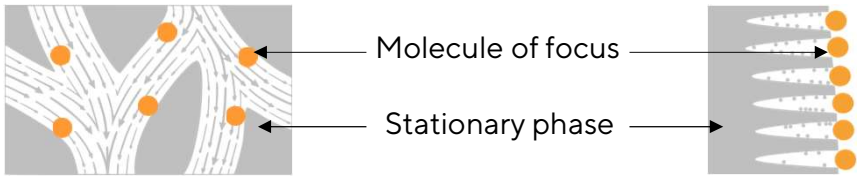
DCVMN

Chromatography Media Selection for Capture from IVT

Accessible surface area for large mRNA molecules ⟩ Low shear environment ⟩ Convective mass transport ⟩ High capacity and easy scalability ⟩ Suitable chemistry selection



Laminar flow in monoliths produces very low shear. Flow in voids of resin packed column is turbulent and produces zones of countercurrent flow that create high shear stress (red).



Large flow through channels provide complete surface accessibility to large mRNA molecules. This enables high capacity. Lack of dead-end pores (no diffusion) provides flow-independent performance.

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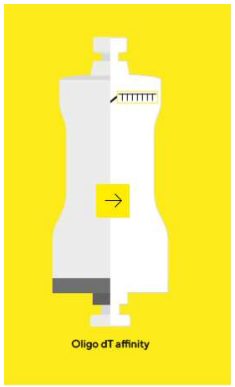
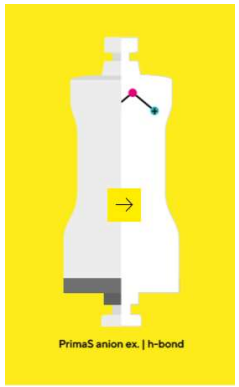
Selecting the Optimal Ligand Chemistry

- Currently there is no single IVT platform for mRNA production
- Key elements of mRNA (5'-cap and poly-A tail) can be added in a IVT reaction, or post-transcriptionally
- Purification solutions are needed to accommodate different processes



Oligo DT18

- Capture of polyadenylated mRNA directly from IVT Mixture
- Affinity purification



PrimaS

- Capture of all mRNA directly from IVT mixture
- Anion-exchange & Hydrogen-Bonding

Sartorius mRNA toolbox

Two capture options cover different production scenarios – affinity, anion exchange

Multiple polishing options – targeted removal of residual impurities, can be selected as needed

Can be adapted to existing production processes, and accommodate manufacturing requirements (e.g. high temperature, constraint on organic solvents, etc.)

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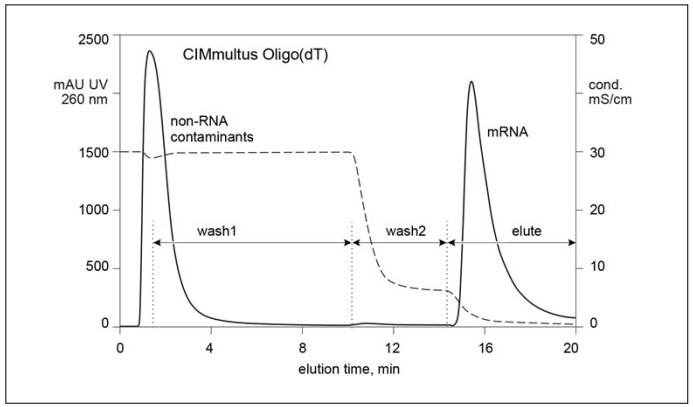
Highly Efficient Affinity Capture of mRNA with Poly-A



- Binding capacity up to 6 mg/mL
- Elution recovery > 90%
- Caustic stable (0.5 M NaOH)
- Elution in weak buffer or water

CIMmultus® Oligo dT can be used as :

- One-step purification of research grade ssRNA
- High-resolution capture method in a multi-step purification process,
- A polishing method
- An analytical method for estimating quantity and purity of mRNA in a sample.
- mRNA up to 10 kb or more

Chromatography conditions*:

Equilibrate/wash1: 50 mM sodium phosphate, 500 mM NaCl, 5 mM EDTA, pH 7.0. Wash2: 50 mM sodium phosphate, 5 mM EDTA, pH 7.0. Elute: 10 mM Tris, pH 7.5. Flow rate: 5 column volumes/minute.

Data source: Technical Note: Purification of mRNA with CIMmultus® Oligo dT

*Conditions should be optimized for specific application, NaCl concentration range 250 mM to 1.5 M

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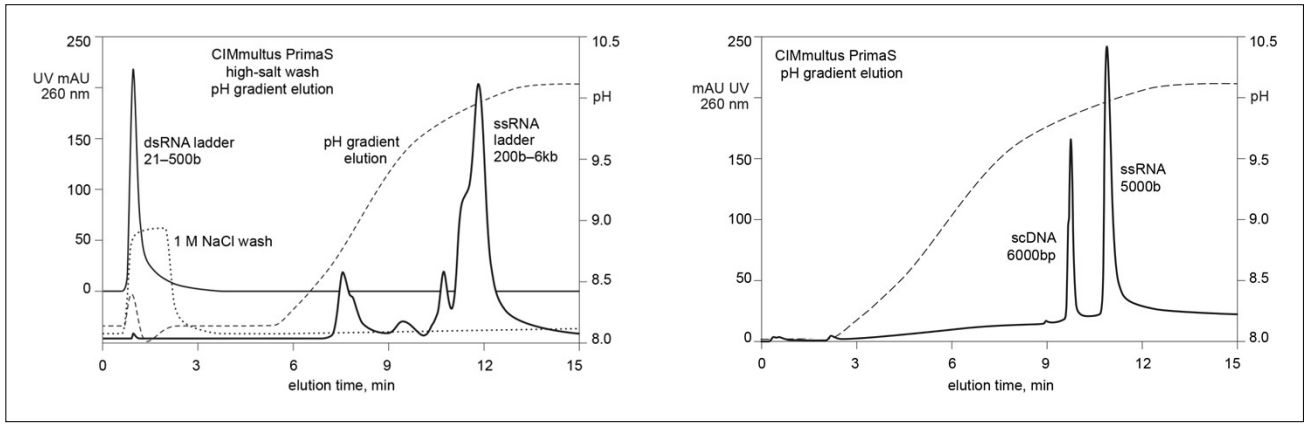
High-resolution Multimodal Anion Exchange Chromatography to Capture ssRNA

Removal of dsRNA from ssRNA and size fractionation of ssRNA



CIMmultus® PrimaS can be used as:

- One-step purification of research grade ssRNA
- A high-resolution capture step in a multi-step purification process

- Ambient temperature, aqueous conditions for removal of DNA, ds species, and size fractionation.
- Non-affinity capture, **directly from IVT reaction mixture.**

Data source: Instructions: Purification of mRNA with CIMmultus® PrimaS

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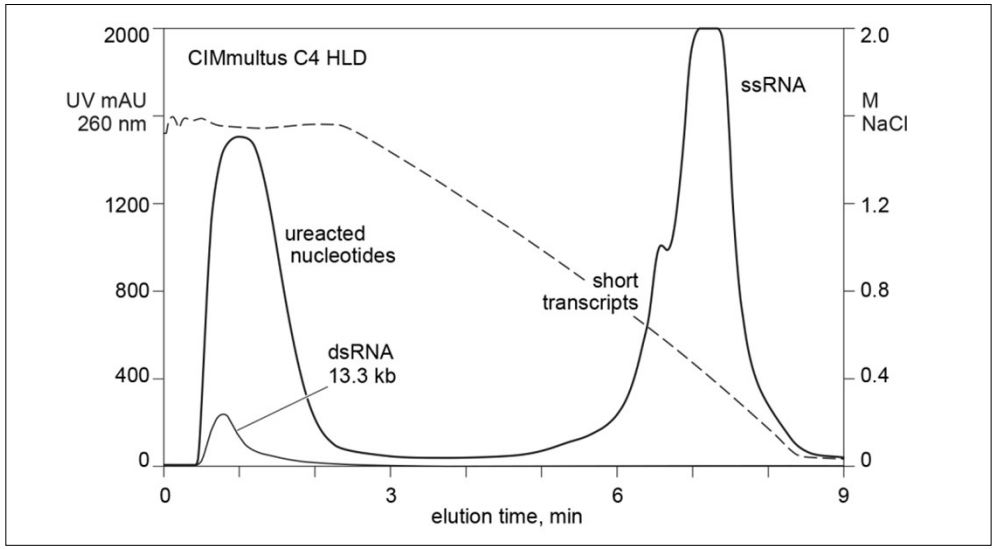
Excellent Protein Removal by Hydrophobic Interaction Chromatography

Separation of in vitro transcription mixture on CIMmultus® C4 HLD



CIMmultus® C4 HLD gives its best results as a polishing method :

- It can be used to produce research grade ssRNA from in vitro transcription (IVT) mixtures but
- Binding can be performed with any salt that precipitates ssRNA.
- Sample loading at industrial scale can be performed by in-line dilution.
- It may also separate single stranded ssRNA from truncated forms, double-stranded dsRNA, and DNA.

Data source: Technical Note: Purification of mRNA with CIMmultus® C4 HLD

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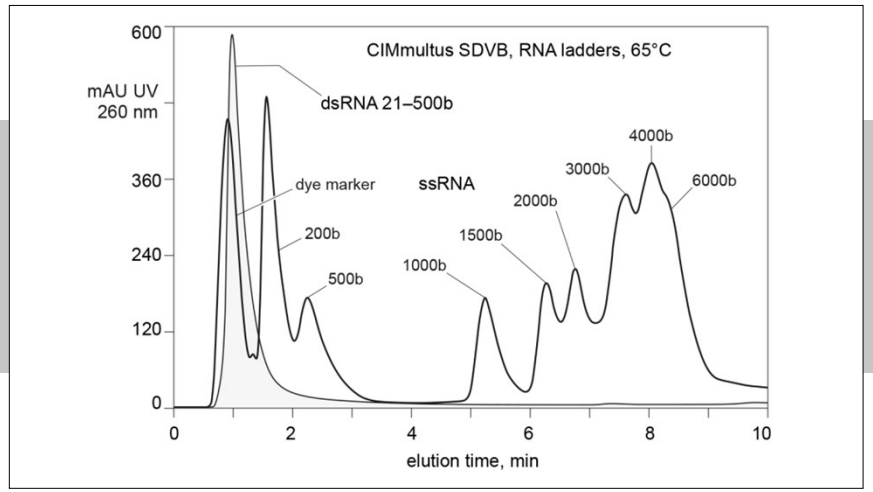
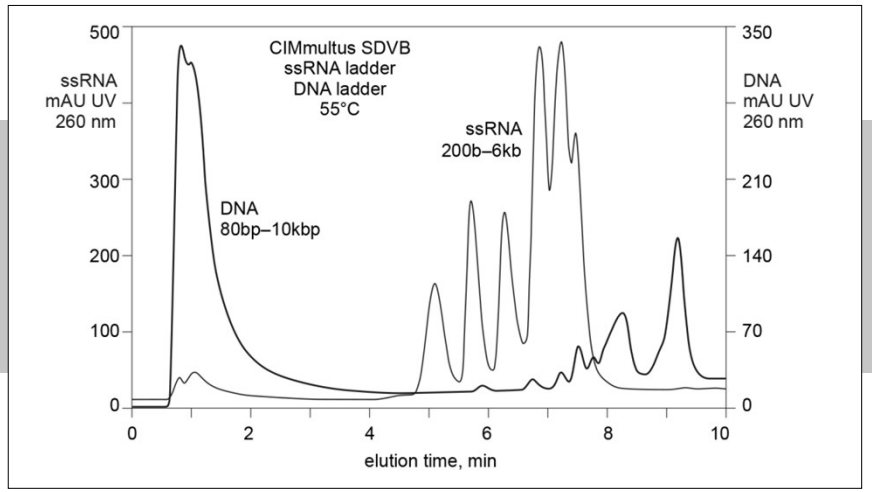
Proven Polishing Method - Reverse Phase Chromatography

Proven polishing method for separation of ssRNA from truncated forms, dsRNA, and DNA.

Also proven in literature as analytical column by CIMmultus® SDVB





Data source: Nwokeoji et al, J. Chrom. A 1484 (2017) 14–25 and J. Chrom. B 1104 (2019) 212–219.

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Services Offered by Sartorius BIA Separations

CORNERSTONE Process Solutions



Process Development Services

- Single methods or start-to-finish procedures
  - Including SOPs, training, transfer, scale-up, on-site support.
- Enveloped viruses (influenza) and non-enveloped viruses (AAV, phages)
- Exosomes, pDNA, mRNA, proteins.

Analytics Development Services

- Single methods or full process monitoring
  - Product quantity
  - Product quality
  - Process and impurity monitoring
- Methods for viruses, exosomes, pDNA, mRNA, impurities, other biomolecules

Custom Monolith Development and Manufacture

- Development of immobilization protocols for any ligand:
  - Affinity, ion exchange, HIC, RPC, and more.
- Preparation of custom monoliths at any scale.
- Provision of protocols to customers who prefer to do their own immobilization.

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mRNA Downstream Solutions

PD & Commercial Phase

Resolute Flowdrive

- SU system designed for pilot-scale, clinical and commercial production
- Flexible to use resins and membrane adsorbers with Resolute or other columns

Resolute BioSMB

- For next generation processes and ability to reduce cost of affinity capture step
- Seamless scalability from PD to commercial manufacturing with two systems: BioSMB PD and BioSMB Process
- Fully single-use and gamma irradiated

Concentration | Diafiltration | Sterile Filtration

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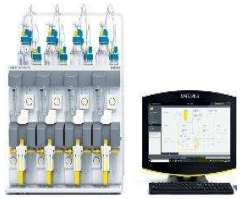
Screening: Toolbox of Filters for Low Adsorption and High mRNA Recovery

Sartoflow® Smart TFF System



Ambr® Crossflow
(5 mL – 500 mL screening)

Hydrosart® 100/300 KD Sartocon Slice

Hollow Fiber TTF modules (up to 750kD) Green line

SartoScale 25 filters for screening

Select the filters and crossflow devices to get the best recovery from a comprehensive toolbox of scalable solutions:

- Purification to get rid of DNA fragments, residual enzymes such as Polymerase (99 KDa), Dnase (74 kDa), Pyrophosphatase (19 KDa) and other impurities.
- Hydrosart, a state-of-the-art crossflow membrane designed for low adsorption of mRNA and LNP, available in a 50cm² and scalable format
- Hollow fiber modules for gentle TFF and maximized mRNA yield in a 52cm² scalable format
- Run your experiments using the Sartoflow® Smart benchtop system designed for process development, including a low shear membrane pump for highest mRNA recovery

- The broadest range of PES membrane combinations that adapt to all process steps, for high mRNA and LNP recovery, available in Sartoscale 25 format for screening with lowest product volume

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Manufacturing: Mitigate Risk of RNAse Contamination

Single-Use TFF Systems

Multi-Use TFF Systems

Sartocon Self-contained modules and assemblies

Single-use Hollow Fiber Green line

Filter transfer sets

Ensure successful scale-up and mitigate the risk of RNAse contamination by using single-use solutions:

- Mitigate risk of contamination and RNAse degradation with Sartocon® Self Contained Units, hollow fibers and Maxicaps filters which can be delivered gamma sterile, facilitating closed processing
- Facilitate closed processing by using pre-assembled transfer sets including filters, tubes and connectors
- Simplify your supply chain by using off-the-shelf standardized pre-designed solutions
- Ensure process automation and control by using single-use systems designed for downstream or liquid processing
- Rely on the long history of being filtration champion, with quality products and an extended validation guide

DCVMN

Sterile Filtration for mRNA and LNPs

PES

Sartopore ® 2/XLG/Platinum

Screening

Sartoscale 25 device

SU Assemblies

Filter transfer sets & Maxicap MR

A toolbox of filters to find the best balance between filtration capacity and recovery

- Low non-specific binding which results in high recovery
- A wide range of sterile filters and pore size combinations to meet all needs
- All filters available in Sartoscale 25 format for screening with lowest product volume*
- Simplify process development with the use of scalable options

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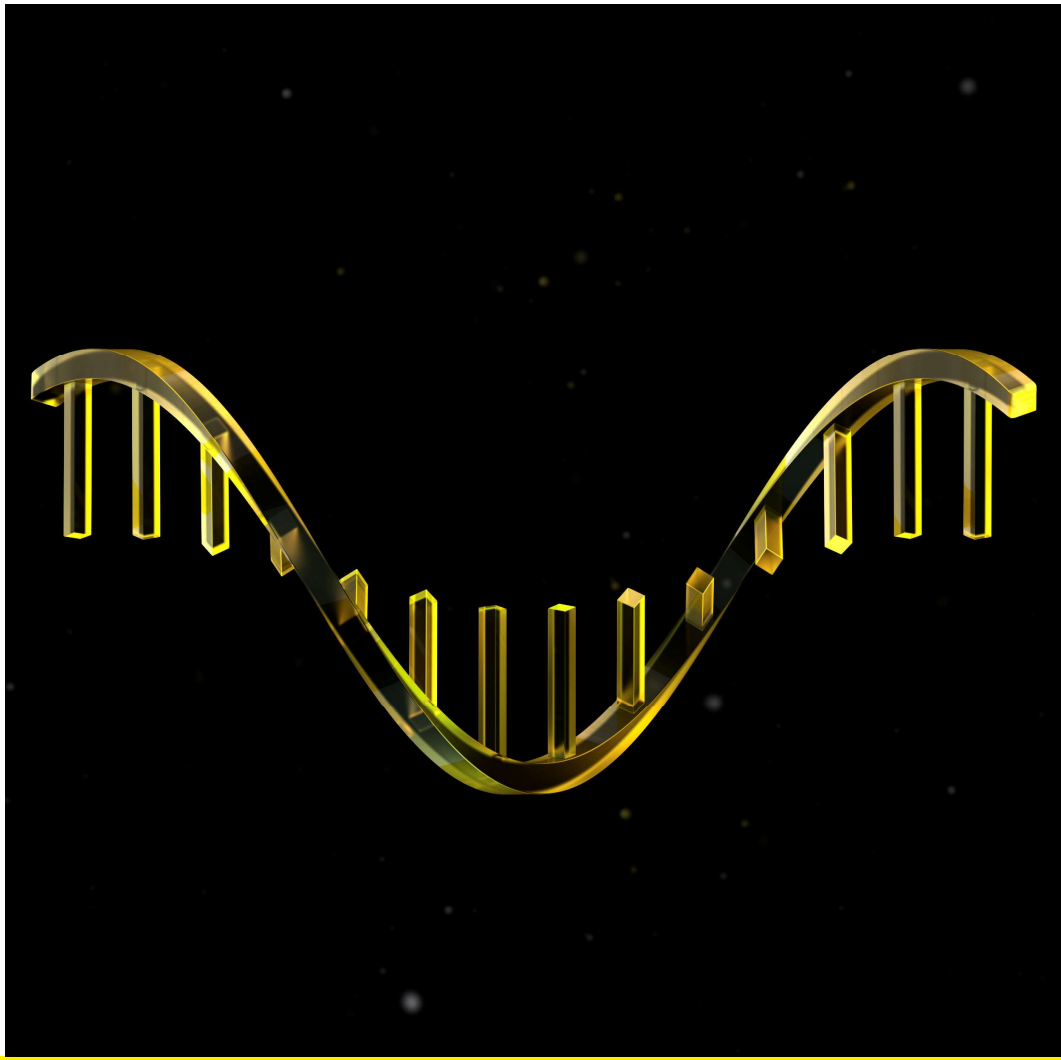

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Unique Challenges of LNP Development, Storage and Shipping of mRNA

Pain points	LNPs are fragile and shear sensitive	mRNA is relatively unstable and easily degraded by RNAse	Performance differs from small-scale to late-stage	Current LNPs are stored at extremely low temperatures
Needs	Toolbox of filters	Single-use technology	Proven Scalability	Controlled Freeze|Thaw

*LNP: Lipid nano particle

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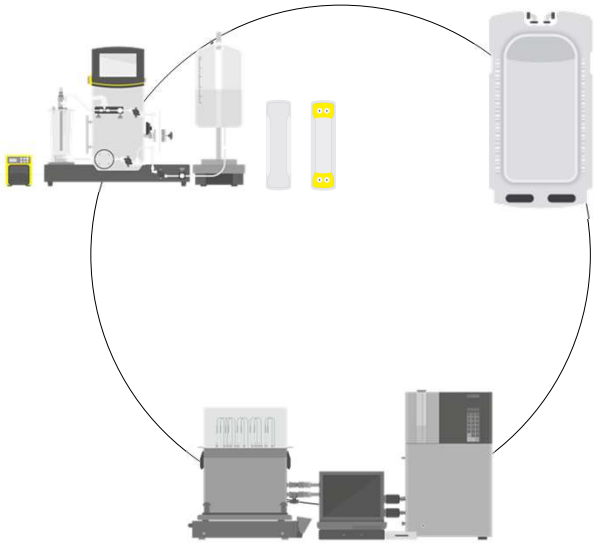
Simplify Formulation and Filling Development of mRNA

Speed up your LNP development with high throughput screening TFF and controlled Freeze/Thaw system

☑ Screening and identification of CPPs and CQAs

☑ Set Design Space



LNP formulation and filling development mRNA package

TFF systems & consumables:
- Sartoflow® Smart TFF System
- Hydrosart® 100/300KD Sartocon® slice 50 TFF Filters
- Hollow Fiber TFF (100KD/300KD)

Filters:
- Sartopore® 2/XLG Sterile Filters

Software:
- MODDE® DOE Software

Freeze/Thaw System:
- Celsius® S^3 Benchtop Freezer

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Controlled Freeze/Thaw Characterization for mRNA Formulation

Celsius® S³ Benchtop Freezer

Celsius® Pak Bags

Ensure stability of drug product and drug substance during storage by optimizing the freeze & thaw steps:

- Benefit from an end-to-end integrated approach for faster and more efficient testing and validation of freeze & thaw, assuring quality at scale
- Investigate optimal process conditions using the Celsius S3, generate a consistent samples library to investigate product stability, storage and shipping process steps
- Enables freeze/thaw characterization in 30–100 mL single-use bags with the same product contact material from lab-scale to commercial-scale Celsius® platforms
- Automated operation and data collection, combined with accurate temperature monitoring, at well-defined last-point-to-freeze location

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Late-Stage mRNA Storage and Shipment with Controlled Freeze|Thaw

Celsius® CFT Freeze and Thaw Solution

Celsius® FT Plate Freezer

Celsius® Pak Bags

Celsius® FS Filling Station

Celsius® SSM Shipper

Celsius® FFTp SU Container

Biosealer® TC Sterile Disconnection

Biowelder® TC Sterile Connection

Ensure safe and simple preservation and transfer of frozen mRNA and LNPs :

- Control your process to minimize the adverse effects of the cryoconcentration in your LNP with a controlled-rate freeze and thaw system with Celsius® CFT
- Ensure consistent freeze|thaw performances in single-use bags with same product contact material from 1L up to 100L. One film for all to facilitate process validation with Celsius® CFT
- Celsius® FFTp - pre-assembled and ready to fill single-use containers to leverage existing infrastructure, fully qualified down to -80°C
- Mitigate the risk of contamination and RNase degradation with gamma-irradiated single-use bags
- Sterility Assurance during connection and disconnection, thanks to effective solutions adapted to your process requirements
- Confidence ® Validation Services to support your DNAse|RNAse mitigation strategy

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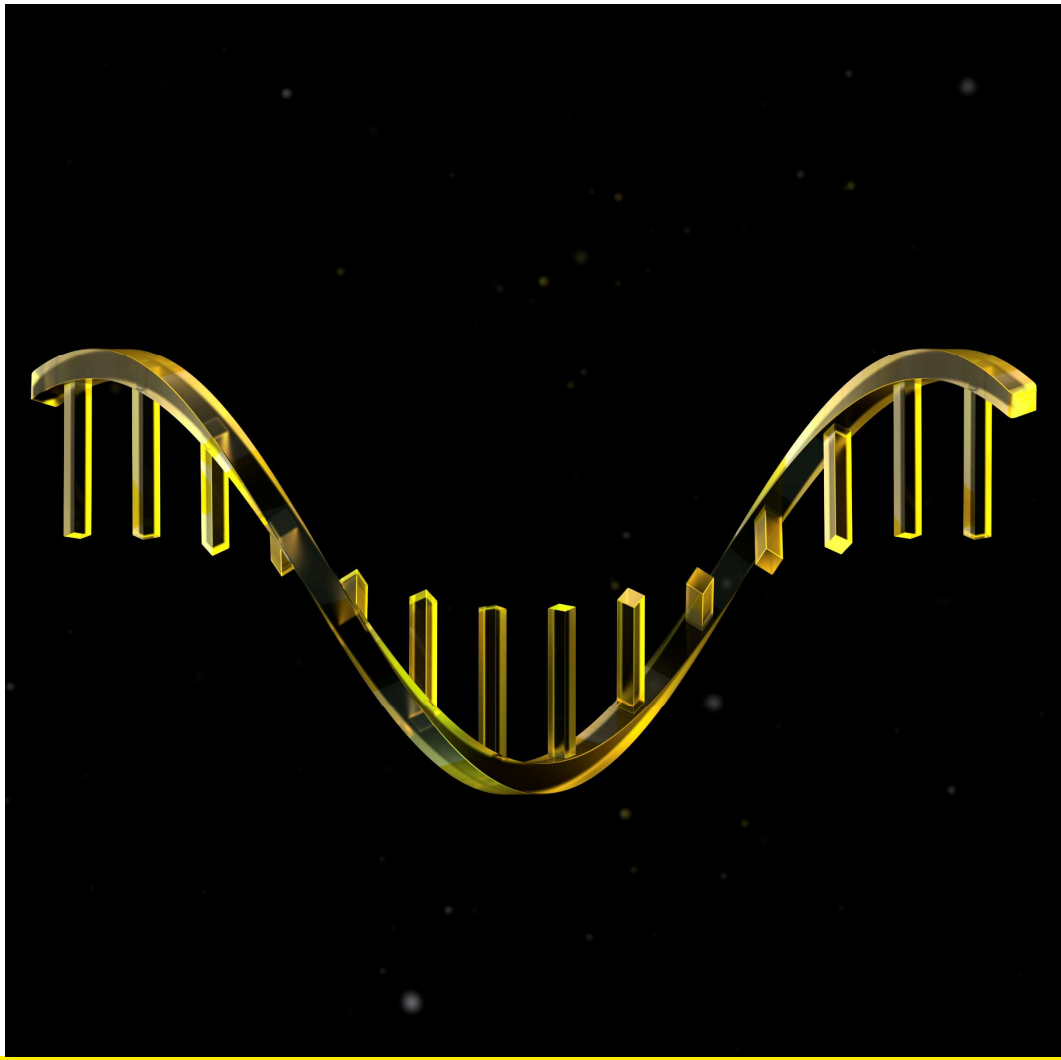
Agenda

- Introduction
- Upstream – *in vitro t*ranscription
- Downstream Solutions
- Formulation Development & Storage
- **Analytical Solutions**



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Process Characterization using mRNA PATFix Platform

PATfix™ HPLC

CIMac™ PrimaS Analytical Monolith

- Three distinct analytical approaches:
  - IVT reaction monitoring
  - mRNA quantification
  - Quality control for mRNA size, integrity, and detection of dsRNA
- Sensitive detector suite for low sample consumption
- Server-client based approach - access your data securely from anywhere (office, home)
- Part 11 compliance allows easy implementation of PATfix analytics in FDA approval process

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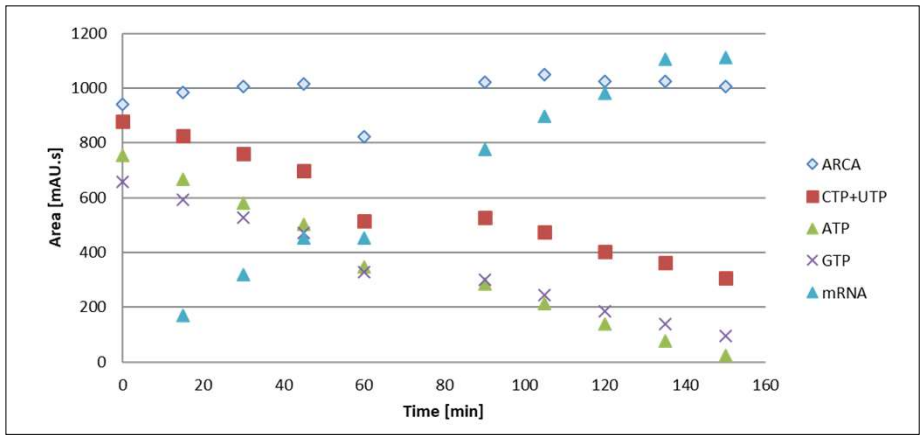
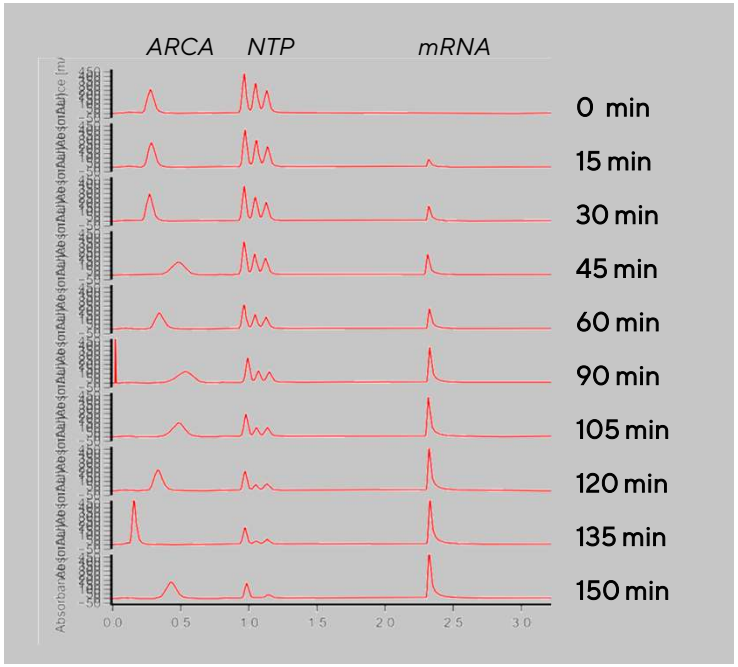
Building up IVT Understanding with Analytics





- IVT reaction monitored at-line by CIMacTM 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
- Effects of feed addition can be studied

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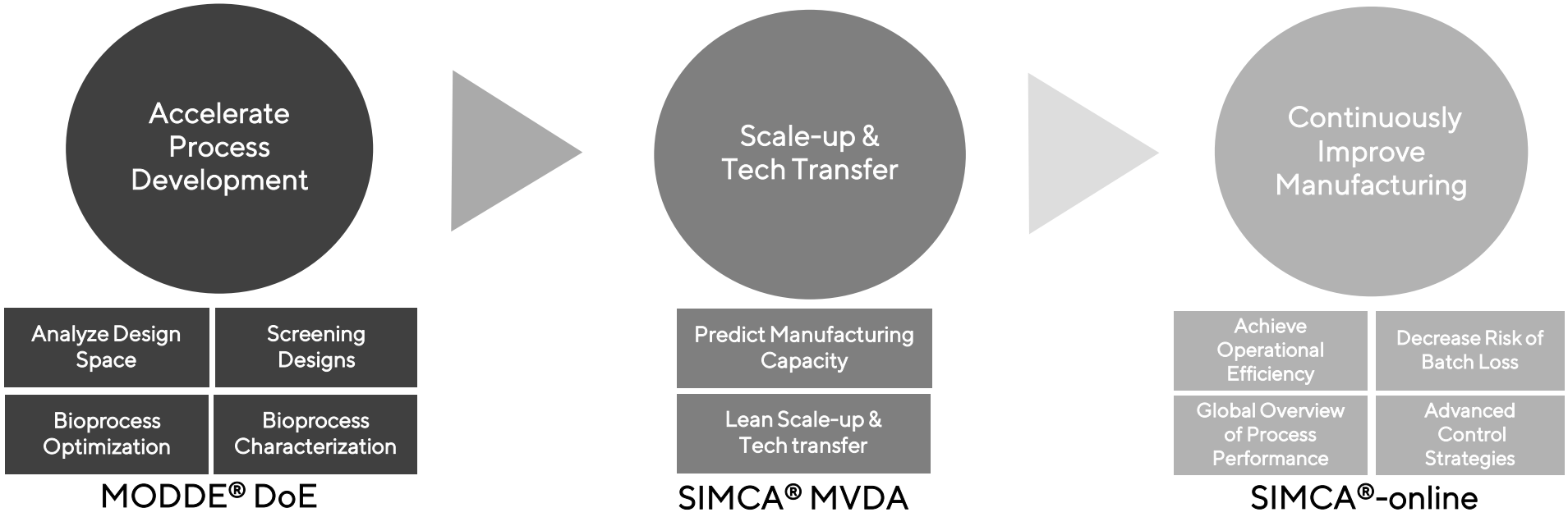
Maximize Process Understanding & Control Using Data Analytics

Systematically approach process development studies and reduce the numbers of experiments run

Accelerate scale-up and de-risk tech transfer while ensuring process robustness

Monitor and control manufacturing processes to deliver high quality product



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Sartorius Solutions Simplify Progress And Fast-Track Time-to-Market

Expertise

Combining high-throughput with innovative analytics and supportive experts enable you to quickly develop in-house expertise

Innovation

Only innovative solutions dedicated to mRNA applications can eliminate some of your challenges and support your next generation process

Scalability

Develop the process with the end in mind to ensure successful implementation at late-stage from a cost, process and supply perspective

Thank you!

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