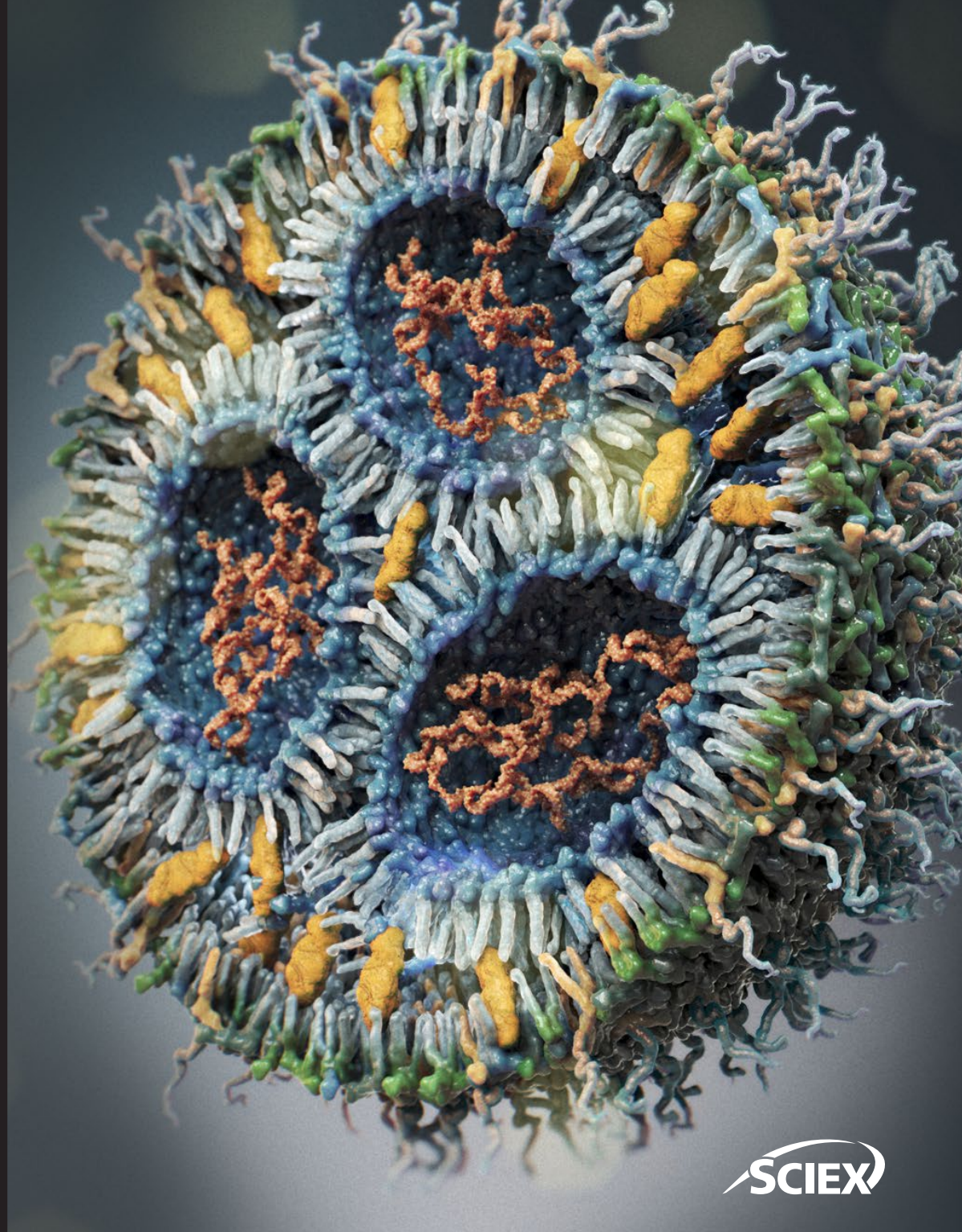

Let the future start today
Development of better LNP-based genetic medicines
SCIEX
The Power of Precision

Lipid nanoparticles (LNPs) are widely used for the delivery of vaccines and therapeutics such as in vitro transcribed (IVT) RNA, small interfering RNA (siRNA), antisense oligonucleotides (ASOs) and more.

The flexibility of LNPs regarding the type and size of the cargo, limited adverse effects and easier scale-up compared to viral vectors, are factors that contribute to the increased use of LNPs as delivery vehicles.

As a result, it is important to characterize these drugs to understand and limit LNP-related impurities to help mitigate risks to patient safety and product efficacy.

Discover how you can break through analytical boundaries with innovative and streamlined technology that provides relevant answers about your LNP-based drugs.



What the experts say

Jérémie Parot (PhD),

Research Scientist, SINTEF

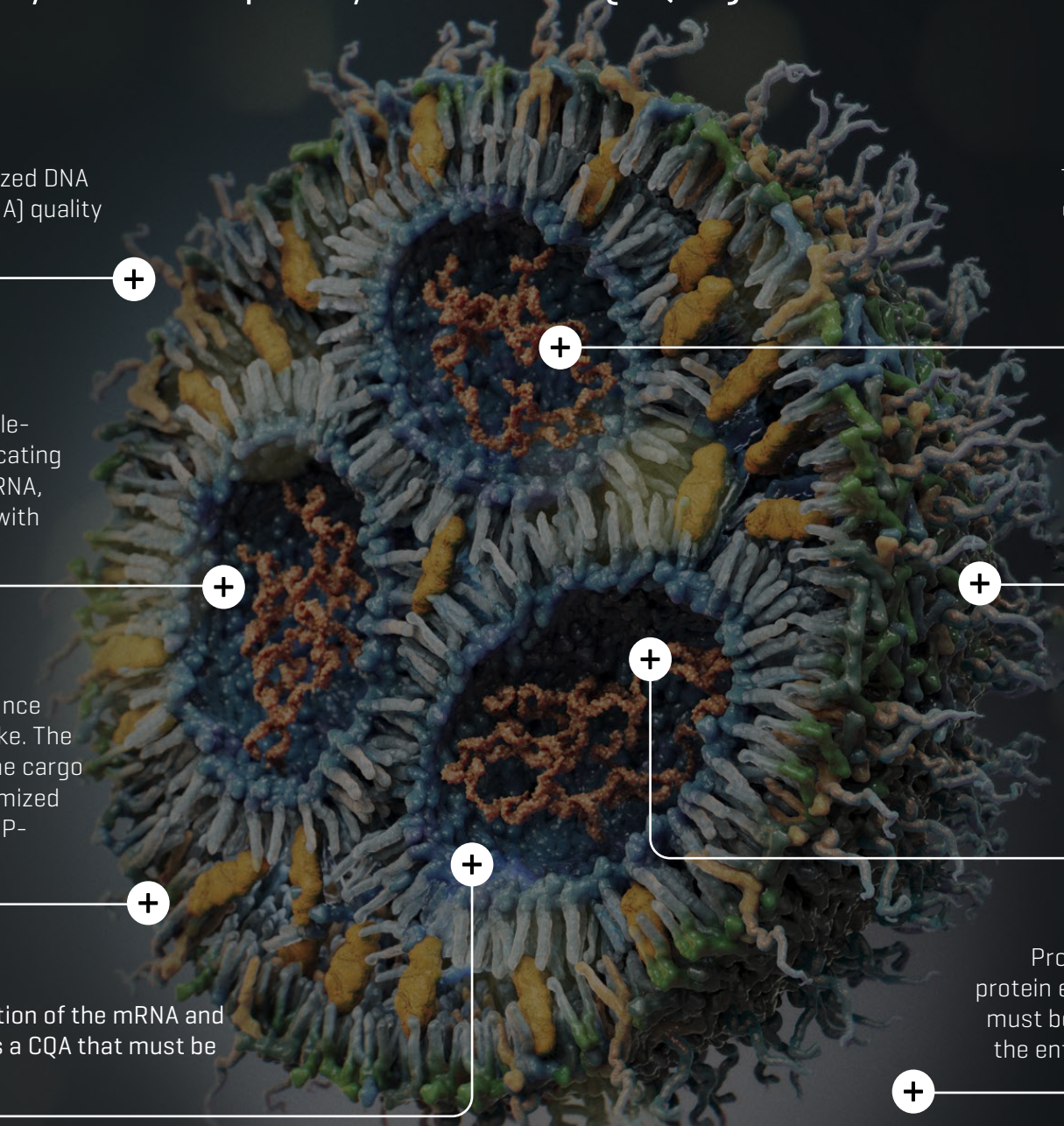
"Given the "one break no effect" with mRNA, the integrity of mRNA drugs must be assessed to ensure its quality. Capillary gel electrophoresis (CGE) is a key tool in this field. CGE systems, such as the BioPhase 8800 system from SCIEX, offering high resolution and efficiently characterizing RNA profiles, enable accurate quality control. Ensuring the integrity and purity of mRNA formulations is essential for the development of effective and safe therapeutics."

Adam Crowe (PhD),

Manager Analytical Development,
Precision NanoSystems ULC
(part of Cytiva)

"Maintaining mRNA integrity is essential for the manufacture of high-quality LNP vaccines. However, predicting LNP potency from legacy, low-resolution techniques remains challenging. In contrast, the SCIEX PA 800 Plus system equipped with the RNA 9000 Purity & Integrity kit enables robust, high-resolution integrity profiling of mRNA, even for larger constructs, removing a long-standing obstacle in mRNA LNP quality control."

Small but mighty: critical quality attributes (CQAs) of LNPs



Plasmid DNA

IVT RNAs are based on linearized DNA templates. Plasmid DNA (pDNA) quality is key for high product yields

Nucleic acid drugs

The genetic cargo—such as messenger RNA (mRNA), single-guide RNA (sgRNA), self-replicating RNA (srRNA), circular RNA, siRNA, and ASOs—must be handled with care to ensure its integrity.

Encapsulation

LNPs protect the drug substance and facilitate its cellular uptake. The encapsulation efficiency of the cargo must be understood and optimized during the development of LNP-based drugs.

mRNA 5’ cap

The 5’ cap prevents degradation of the mRNA and promotes its translation. It is a CQA that must be characterized.

mRNA 3’ poly-A tail

The tail of multiple adenosines at the 3’ end of mature mRNA is another CQA, protecting it from degradation and facilitating translation. Length and distribution are important factors.

Lipids

Keeping the fragile cargo safe and facilitating cellular update are important tasks of lipids used in LNPs. Their quality is essential to ensure effective drugs.

Structure

Nucleic acids can form secondary structures because of the complementary pairing of bases. These structures could affect the encapsulation or the stability of the LNPs.

Expression

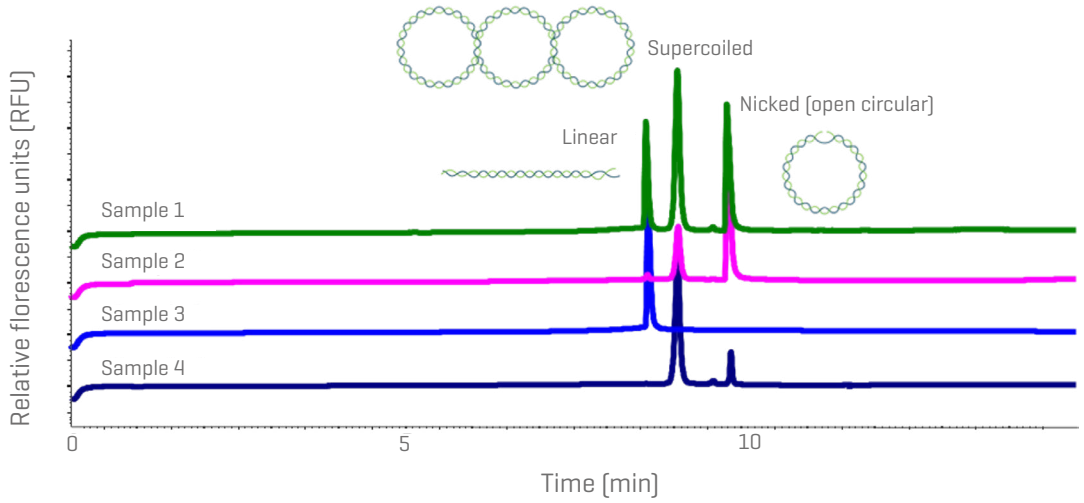
Proteins work in complex networks. Modulating protein expression through IVT RNAs or gene editing must be measured and can have various effects on the entire proteome, which need to be understood.

The circle of life
Plasmid DNA as critical starting material

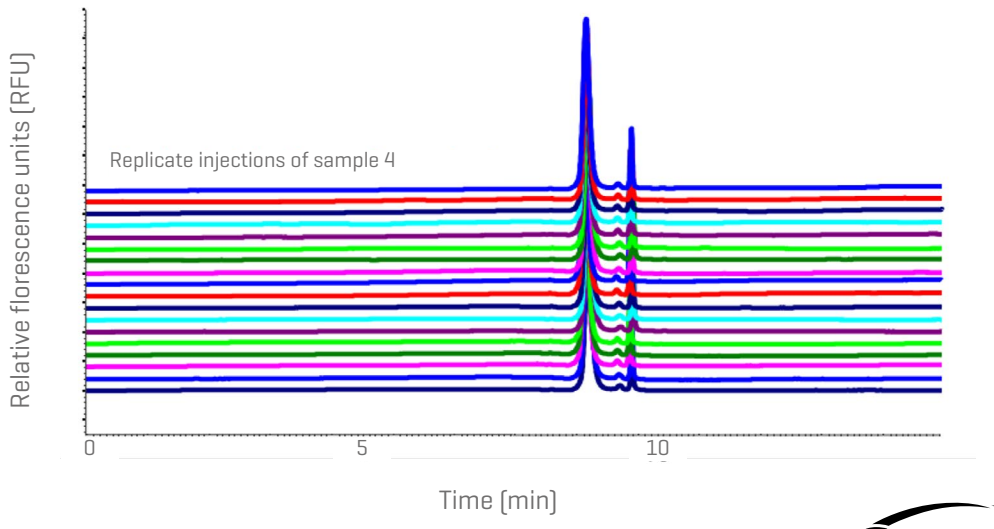
IVT RNA as a drug resulted in a paradigm shift with unprecedented potential. Since linearized pDNA serves as a template for mRNA, srRNA and other IVT RNAs, pDNA quality is crucial for IVT RNA production.
Apart from assessing the topology, the pDNA size and purity are factors that must be assessed.
Excellent resolution and sensitivity are needed to streamline differentiation between topological variants. For subsequent transfer of assays into quality control (QC), high reproducibility and robustness are key.

- Rely on excellent resolution of different topological variants of pDNA using the **PA 800 Plus system** and the dsDNA 1000 kit
- Achieve the highest sensitivity for early stage development samples with laser-induced fluorescence (LIF) detection
- Confidently transfer assays from development to QC with excellent precision using the **PA 800 Plus system**
- Streamline data management through compatibility with the Empower Chromatography Data System (CDS) from Waters and the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS)

Differentiate different topological variants with ease



Rely on a high-resolution method with optimal reproducibility



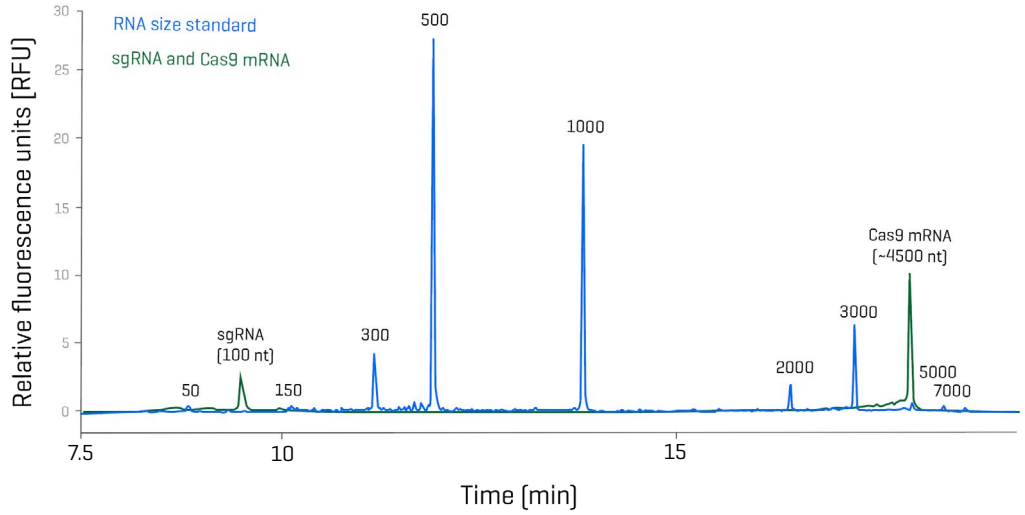
sciex.com/plasmid-DNA-analysis

How is your IVT RNA doing? mRNA integrity and purity

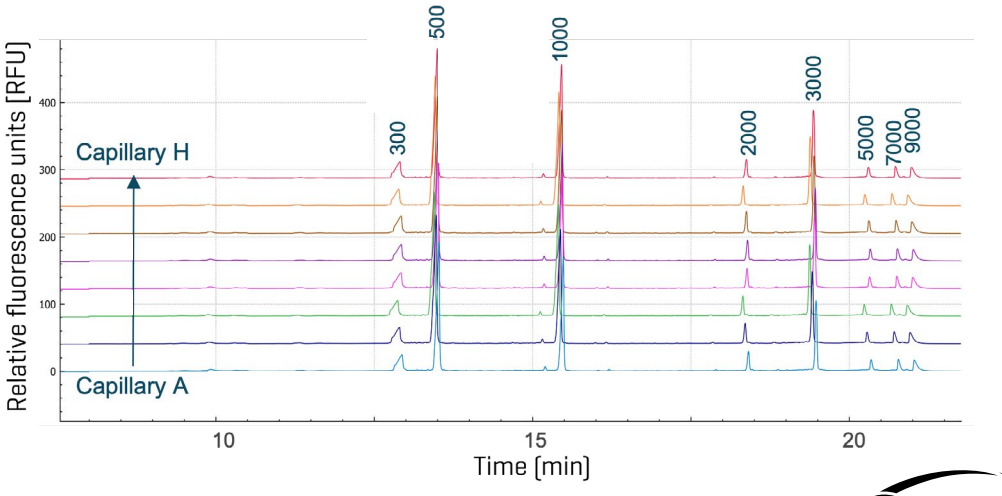
mRNA-based drugs can lose their efficacy when being truncated. In addition, process-related nucleic acid impurities may pose a safety concern. The integrity and purity of the construct are therefore important aspects for product quality. For gene editing applications, such as CRISPR/Cas9, different constructs sizes might need to be assessed. High resolution, excellent reproducibility and mitigating secondary structure formation are key for accurately assessing product quality.

- Determine the integrity and purity of your nucleic acid products from 50 up to 9,000 nucleotides (nt) and beyond with the **RNA 9000 Purity & Integrity kit**
- Break through boundaries with excellent resolution and reproducibility while minimizing secondary structures through temperature control using the **BioPhase 8800 system** and the **PA 800 Plus system**
- Streamline data management through compatibility with the Empower CDS and the Chromeleon CDS

Confirm integrity and monitor impurity profiles with excellent resolution



Rely on capillaries with highest reproducibility

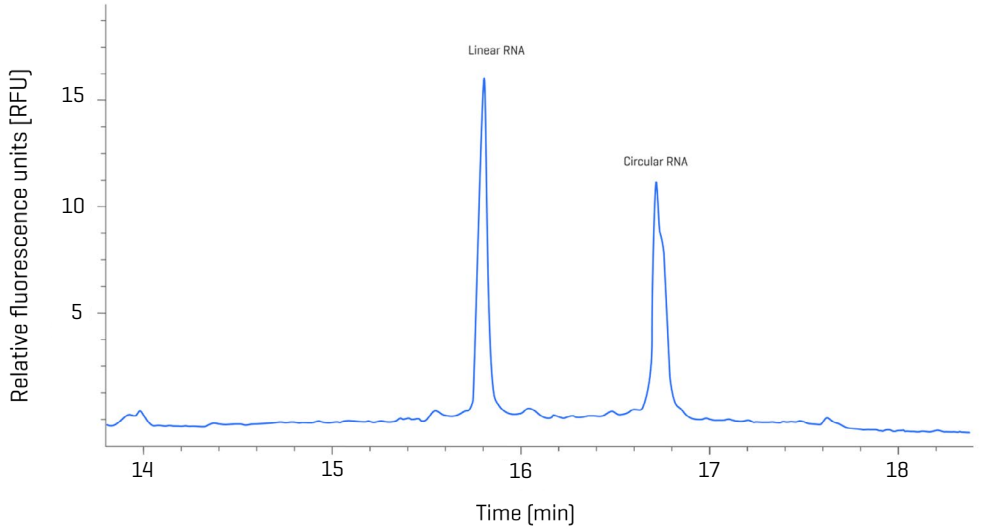


Are you still linear or already circular? Circular RNA assessment

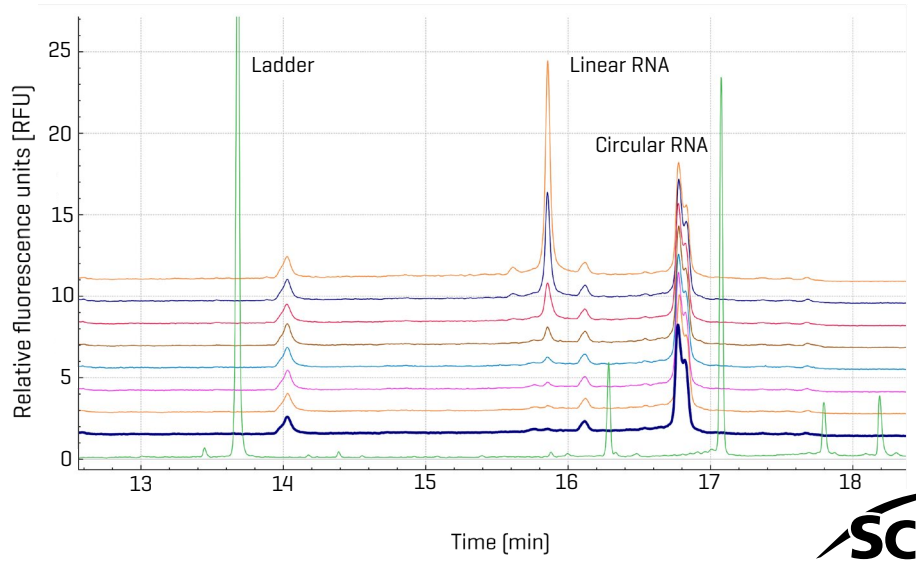
Circular RNAs are next-generation IVT RNAs that provide the benefit of high resistance toward exonucleases and functionality—without 5' cap or poly-A tail. To achieve circular RNAs, linear precursors are chemically or enzymatically ligated. Understanding the purity of the circular product requires a high-resolution separation workflow that can separate linear precursors, degradation and high molecular weight products from the desired circular RNA.

- Take charge of your product quality and determine the efficiency of your circulation processes with the **RNA 9000 Purity & Integrity kit**
- Leverage high resolution and reproducibility using the eight capillary **BioPhase 8800 system** and the single capillary **PA 800 Plus system**
- Confidently transfer assays from development to QC and streamline data management through compatibility with the Empower CDS and the Chromeleon CDS

Fully separate linear precursors from circular RNAs



Unlock the future with highest reproducibility

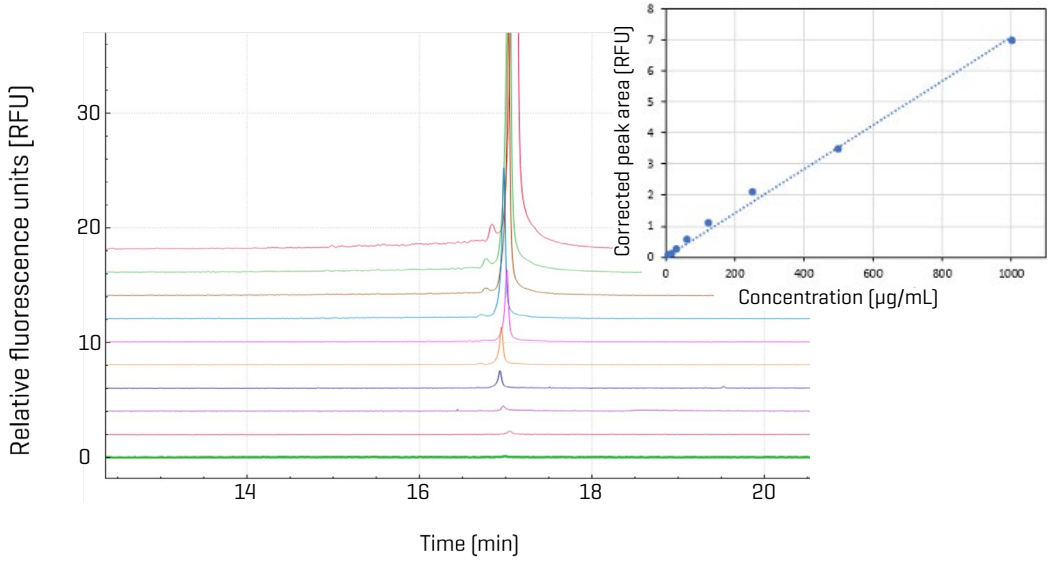


Know what is inside
Encapsulation efficiency of mRNA

LNPs are designed to keep the fragile genetic cargo safe. Degradation and loss of function of nucleic acids are accelerated if they are not sufficiently encapsulated. In addition, the cellular uptake of the drug can be impeded. Therefore, it is important to understand and optimize the encapsulation efficiency of the genetic cargo during the development of LNP-based drugs.

- Determine free and encapsulated mRNA amounts with excellent repeatability and sensitivity with the BioPhase 8800 system and the PA 800 Plus system
- Take charge of development decisions by understanding encapsulation efficiencies of drug substances with a reliable kit-based workflow using the RNA 9000 Purity & Integrity kit
- Simultaneously monitor degradation products in your samples by leveraging exceptional resolving power

Achieve it all—excellent resolution, sensitivity and linearity



Increase reliability and decrease your %CVs

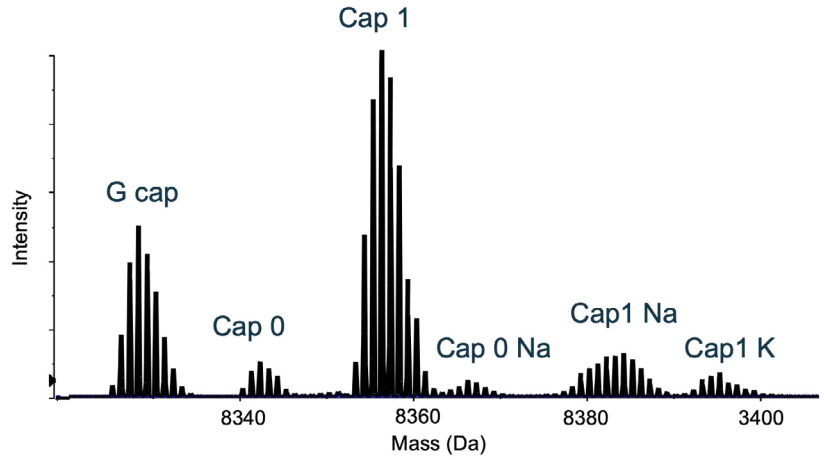
Nominal (µg/mL)	Measured (µg/mL)					Recovery
	#1	#2	#3	Mean	CV	
400	387.3	390.1	381.7	386.4	1.10%	97%
500	497.2	488.7	480.3	488.7	1.70%	98%
600	574.7	580.3	570.4	575.1	0.90%	96%

Caps are not just for the looks 5’ capping of mRNA

The 5’ cap of IVT mRNA has a direct impact on its stability and translation efficiency and is therefore considered a CQA. Since G cap, cap 0 and the mature cap 1 are linked to different efficiencies, detailed characterization and simultaneous relative quantitation are needed to ensure product quality. The difference between the capping structures is only 1–2 methyl groups, and this requires a high resolving power to be distinguished.

- Characterize your 5’ ends reliably with excellent data quality using the ZenoTOF 7600 system or the X500 system series
- Move past data processing challenges and readily identify 5’ caps and intermediate products with Molecule Profiler software
- Obtain relative quantitative information automatically in Molecule Profiler software, or tailor quantitation specifically to your needs within SCIEX OS software

Rely on accurate information using high-quality data



Understand your product quality with ease

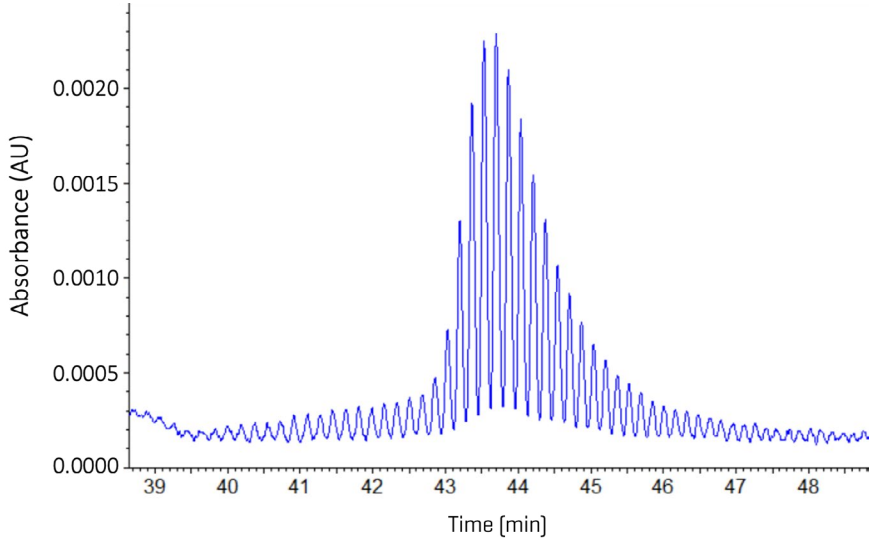
	Name	Neutral Mass	R.T. (min)	Peak Area	% Area
1	Capped - ppUncap	7980.17	9.61	8.84E+05	34.57
2	Gcap	8325.24	9.61	5.62E+05	21.96
3	Cap1	8353.27	9.61	4.02E+05	15.73
4	Capped - pUncap	7900.21	9.61	3.21E+05	12.53
5	ppp Uncap K+ adduct	8098.06	9.55	1.38E+05	5.41
6	ppp Uncap	8060.13	9.54	1.22E+05	4.79
7	Cap0	8339.27	9.65	7.12E+04	2.78
8	ppp Uncap Na+ adduct	8082.13	9.54	3.32E+04	1.30
9	Capped - ppUncap Na+ adduct	8002.17	9.61	2.12E+04	0.83
10	Capped - ppUncap K+ adduct	8018.13	9.61	2.24E+03	0.09

Get the length right 3’ end poly-A tail of mRNA

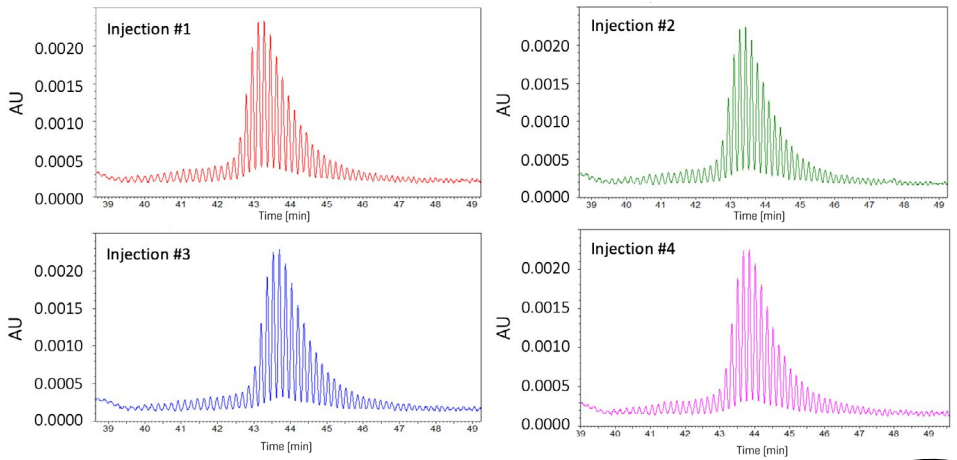
The 3’-end poly-A tail is a CQA affecting product stability and translation efficiency. Therefore, the question of its size and size distribution is of high relevance, regardless of whether the tail was template-encoded or enzymatically added, or a combination of these approaches was used. The determination of length and distribution profiles requires accurate assays with high resolving power.

- Take control of your IVT RNA quality with the ssDNA 100-R kit and the **PA 800 Plus system**
- Dig deeper than ever before into the dispersity of your 3’ poly-A tails with excellent resolution and sensitivity
- Confidently transfer assays from development to QC and streamline data management through compatibility with the Empower CDS and the Chromeleon CDS

Dig deeper with single-base resolution



Trust in excellent reproducibility



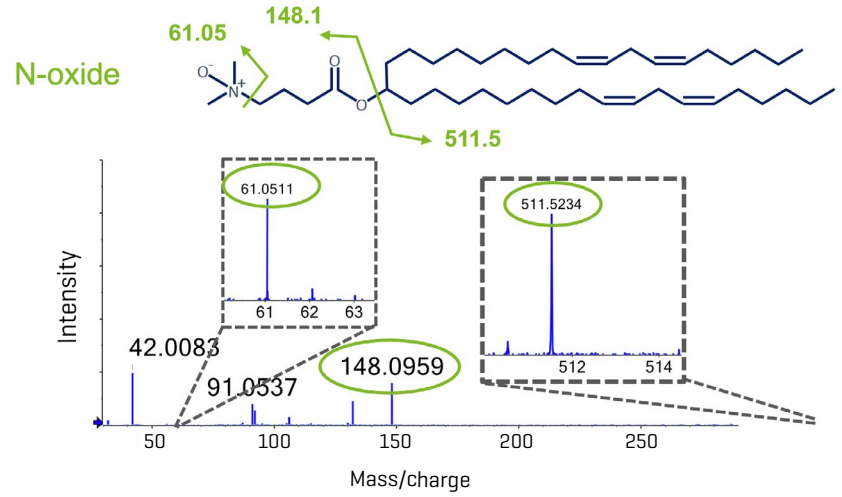
Eat, fragment, sleep, repeat

Characterization of lipids and related impurities

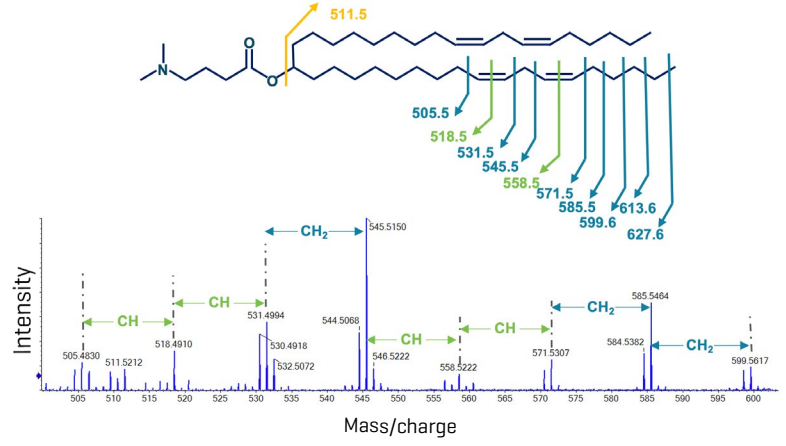
Ionizable lipids are key components of LNPs, complexing the negatively charged cargo and facilitating the cellular uptake. Their quality is critical for a stable and efficient product. Even very low abundance N-oxide impurities can lead to a loss of function. Structurally identifying N-oxides and differentiating them from other impurities are analytical challenges. Furthermore, saturation of double bonds of the lipids could impact the structure of LNPs and affect the final product.

- Fully understand the structures of your ionizable lipid components using electron activated dissociation (EAD) in the ZenoTOF 7600 system and Molecule Profiler software
- Differentiate between oxidated species and accurately localize double bonds or saturations with EAD
- Avoid missing relevant product excipients by leveraging a linear dynamic range >5 orders of magnitude and signal-to-noise enhancement with the Zeno trap

Stop guess work - Precisely localize oxygen additions



Determine locations of double bonds and saturations with confidence



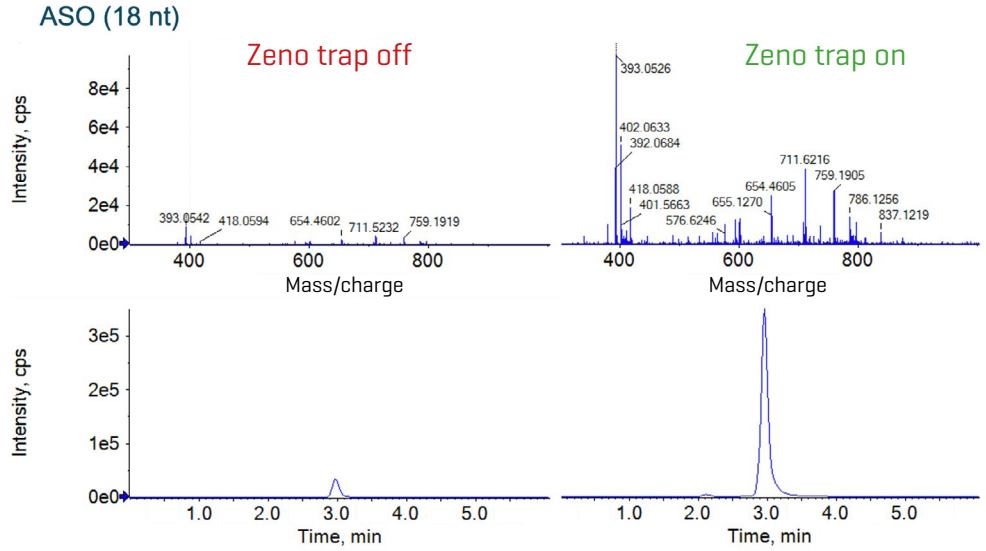
sciex.com/lipid-nanoparticles

Do you have your bases covered? Synthetic oligonucleotide therapeutics

Therapeutic oligonucleotides, such as ASOs, siRNA and aptamers, are approximately 18–30 nt in size. Because of their stepwise synthesis, shortmers with highly similar properties are common.
Frequently, ion-pairing reversed phase chromatography is used to separate full-length product (FLP) from impurities. This can negatively impact subsequent mass spectrometry analyses to determine sequences.

- Trust your results with excellent raw data quality based on exceptional negative ionization efficiency and declustering of adducts with the ZenoTOF 7600 system and the X500B QTOF system
- See more with less sample using the Zeno trap, available on the ZenoTOF 7600 system, to increase sensitivities across the entire mass range
- Confidently confirm the sequence of your oligonucleotides and obtain relative quantitative information of FLP and impurities with Molecule Profiler software

Level up the sensitivity for your oligonucleotides



Confidently confirm the sequences of your product and related impurities

ASO (18 nt)

moT* mo5meC* moA* mo5meC* moT* moT* moT* mo5meC* moA* moT* moA* moA* moT* moG* mo5meC* moT* moG* moG

Fragments: 60 of 60 Proposed Formulae

	Use	Mass (m/z)	Sequence	Ion	Charge	Error (ppm)	Intensity (cps)
60	✓	689.8801	moA*moT*moG*mo5meC*moT*moG*moG	y7	4	5.7	3479.1
59	✓	982.1989	moG*mo5meC*moT*moG*moG	y5	2	5.1	5596.4
58	✓	654.4621	moG*mo5meC*moT*moG*moG	y5	3	2.9	18943.8
57	✓	1546.3387	mo5meC*moT*moG*moG	y4	1	6.5	262.6
56	✓	772.6643	mo5meC*moT*moG*moG	y4	2	4.7	6839.2
55	✓	514.7749	mo5meC*moT*moG*moG	y4	3	6.9	1255.7

sciex.com/synthetic-oligonucleotides

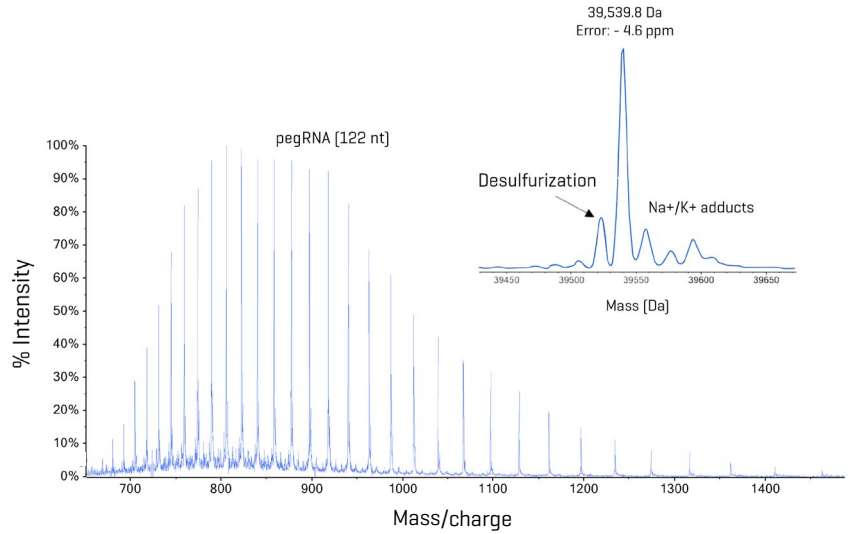
Heavy helpers

Intact mass determination of guide RNAs

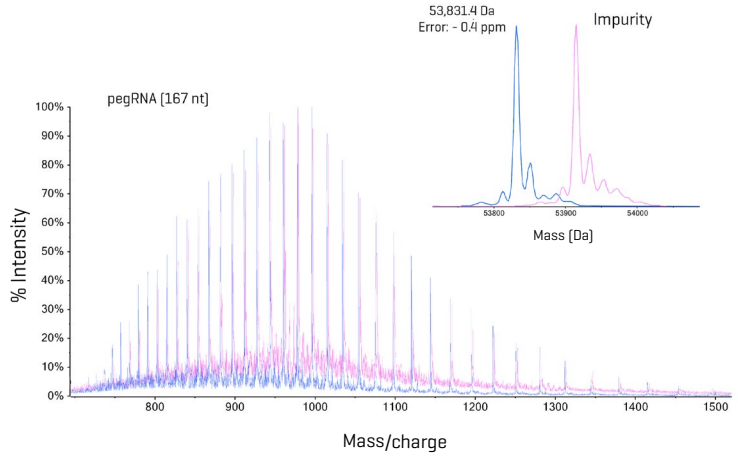
Gene editing approaches with CRISPR/Cas 9 rely on guide RNAs (gRNAs) recognizing a target DNA sequence. Latest approaches use sgRNAs or prime editing RNAs (pegRNAs) of ~100 nt and up to 250 nt in size, respectively. While the specificity and efficiency can be enhanced, the likelihood of introducing impurities increases during the stepwise synthesis of larger RNAs. The quality of sgRNAs is crucial for achieving high gene editing efficiencies. An error in one nucleotide can result in significantly reduced efficiency.

- Leverage excellent raw data quality through exceptional negative ionization efficiency and declustering of adducts with state-of-the-art source design
- Trust in your results with great mass accuracy using the **X500B QTOF system** and the **ZenoTOF 7600 system**
- Uncover relevant information on molecular weights and amounts of impurities
- Keep track of quantitative information about the main product and with **SCIEX OS software** and reconstructed quantitation

Assess the molecular weight of guide RNAs with ease



Take charge of the quality of your intermediate products



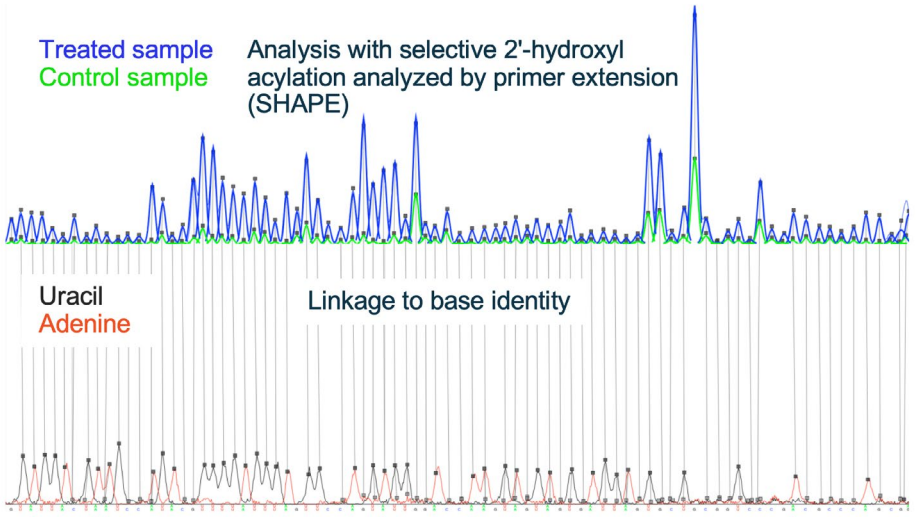
sciex.com/synthetic-oligonucleotides

How flexible are your oligonucleotides?
Determination of oligonucleotide structures

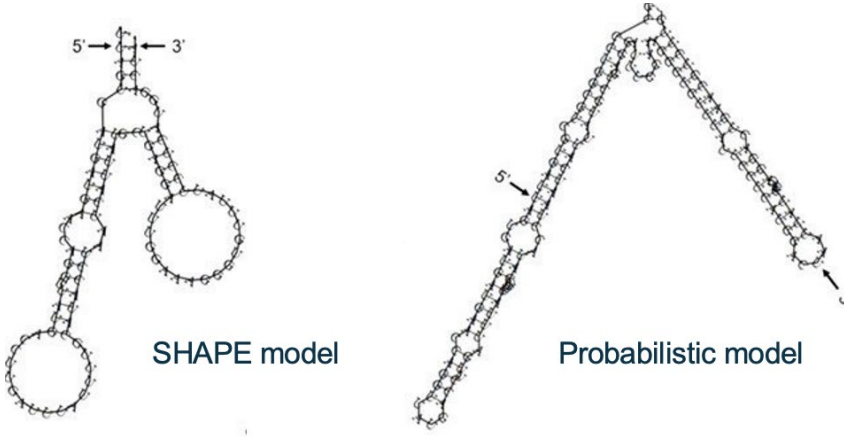
Nucleic acids are known to form secondary or tertiary structures, based on complementary base pairing of nucleic acids, which can impact their function. While software approaches for modeling exist, the prediction of tertiary RNA structures remains a challenge. Empirical approaches using chemical and enzymatic treatment often exhibit variation in selectivity regarding nucleotides and structures.

- Gain better insights into backbone flexibility by determining base-paired and unconstrained residues using the GenomeLab GeXP system
- Improve the structural prediction accuracy for your oligonucleotides of interest with empirical data free of nucleotide or structural bias
- Drive toward better understanding of how the formulation of LNPs might be affected by the structures or your genetic cargo

Find hotspots in RNA backbone flexibility



Increase your confidence in structural predictions



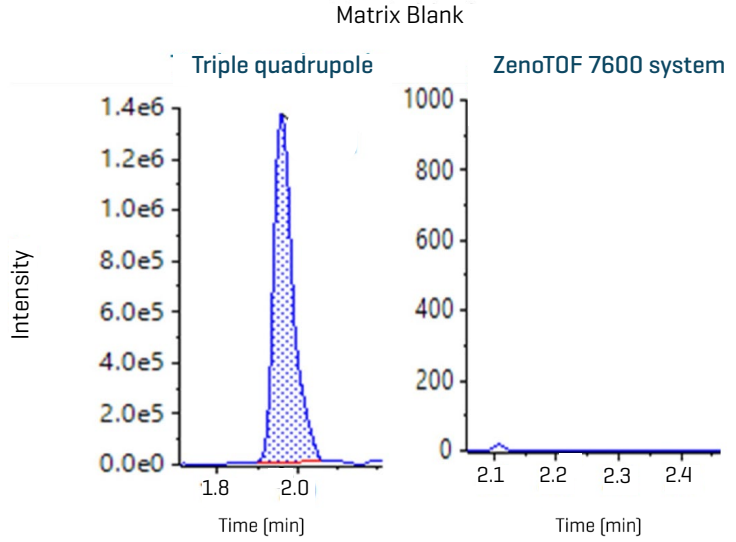
sciex.com/synthetic-oligonucleotides

Trust is good, but control is better
Protein expression analysis

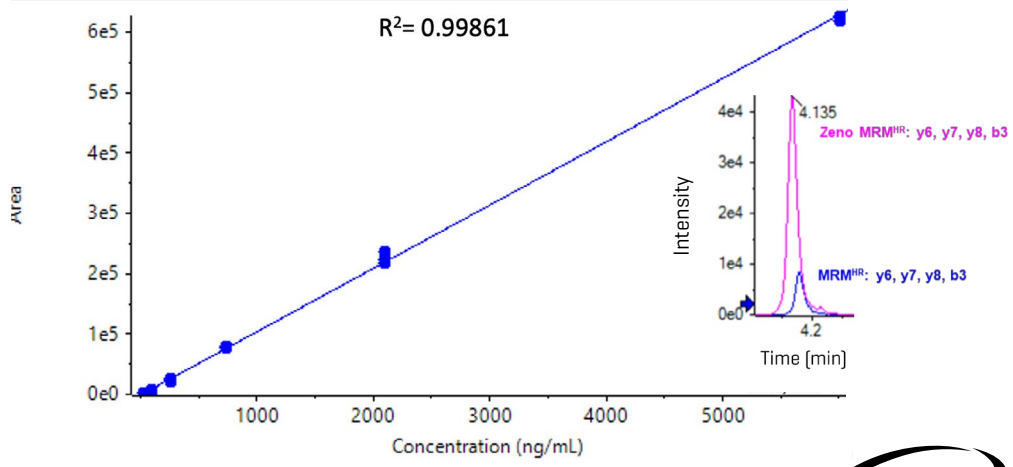
Upon successful delivery of the genetic cargo, drug substances such as IVT RNA are supposed to induce protein expression. While ELISAs and Western blots are widely used to determine functional potency, these assays are limited by the availability of antibodies with high specificity for the target protein. Flexible approaches that do not rely on antibodies can help with adhering to timelines and fast-paced changes in development pipelines.

- Break through the boundaries of complex matrices with accurate mass data using the ZenoTOF 7600 system
- Achieve reliable identification and excellent quantitation simultaneously with high data quality
- Rely on impeccable quantitative performance for decision making with high linear dynamic range and low limits of detection and quantitation using the Zeno trap
- Streamline your quantitative data processing with SCIEX OS software

Don't let matrix hold you back



Level up your MS/MS sensitivity with the Zeno trap



sciex.com/biomarker-quant

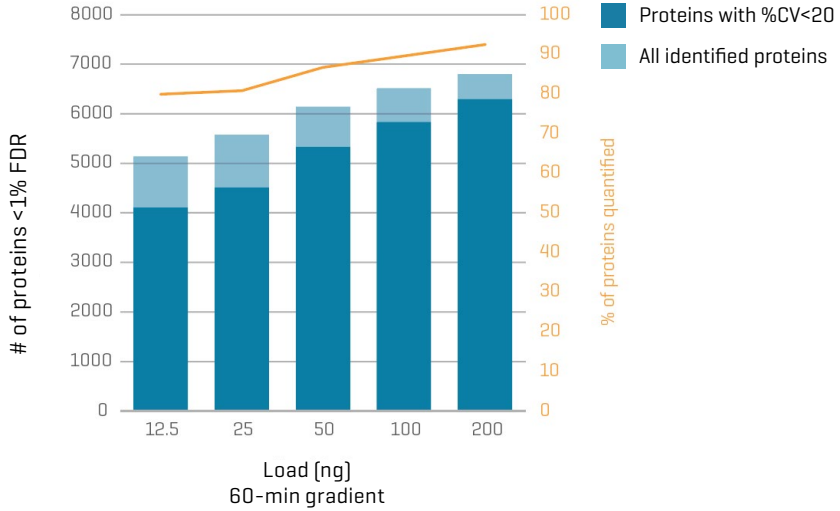
See the unforeseeable

Data-independent proteome analysis

Gene editing or inducing protein expression through IVT RNAs can affect the phenotype in various ways based on the complexity and interdependency of protein networks. Genomic readouts cannot provide sufficient insights into the potential disruption of gene regulators or detect changes to the proteome. On the other hand, protein assays, such as Western blots, are limited by antibody availability and cannot detect unexpected proteome-wide changes.

- Understand the effects of genetic modifications on the proteome level in an unbiased way with data-independent acquisition using Zeno SWATH DIA
- Dig deeper into changes using a limited sample amount with the highest level of sensitivity using the ZenoTOF 7600 system
- Determine the effects on certain proteins using simultaneous identification and relative quantitation of proteins

Dig deeper into the proteome



Understand changes to proteins with amino acid resolution

sciex.com/proteomics

Hardware to help answer your questions

ZenoTOF 7600 system

Discover an accurate mass spectrometry solution that combines powerful fragmentation technologies, excellent MS/MS sensitivity and a step-change in data-independent acquisition

BioPhase 8800 system

Unlock the future with highest data quality and the ability to run multiple samples in parallel

GenomeLab GeXP system

Take charge of your data and get the flexibility of a DNA sequencer and qPCR system—all in one

X500B QTOF system

Take control of biologics characterization with the accurate mass system purpose-built to simplify daily tasks.

PA 800 Plus system

Break through analytical boundaries with confidence using established technology with high data quality and kit-based workflows

Consumables and software to meet your needs

Streamline the processing of MS and MS/MS data of your synthetic oligonucleotides and lipids with **Molecule Profiler software**

Unleash the analytical power of a next-generation software platform for data acquisition and quantitative data processing with **SCIEX OS software**

Analyze double-stranded DNA fragments and plasmid isoforms with confidence using the dsDNA 1000 kit

Assess the purity and integrity of RNA therapeutics, vaccines and single-stranded oligonucleotides to help ensure the highest quality with the **RNA 9000 Purity & Integrity kit**

Perform effective quantitation and determination of protein purity and size with the CE-SDS Protein Analysis kit

Precision NanoSystems ULC (part of Cytiva)

Precision NanoSystems is a global leader in technologies, solutions and services for the development of LNP-delivered genomic medicines, including mRNA vaccines and therapeutics.

Our portfolio, combined with deep expertise in LNP formulations, accelerates drug programs to clinic and beyond.

- **NanoAssemblr® platform**
  Reproducible LNP production scalable from bench to clinic
- **Ionizable lipids**
  A library of LNP formulations available for all stages of drug development
- **Biopharma services**
  One-stop-shop solution for formulation, process, and analytical development with clinical LNP manufacturing

Precision NanoSystems. validated technologies increase stability, efficacy, yield, and quality of LNP delivered genomic medicines and lower the barrier to develop these important nanomedicines, accelerating timelines from concept to clinic.

Phenomenex

Phenomenex is a global technology leader committed to developing novel analytical chemistry solutions that solve the separation and purification challenges of labs that support the entire lifecycle of biotherapeutic modalities.

Incorporating expertise and knowledge through collaborations with our customers, we've put forth a solution portfolio designed specifically to address common challenges associated with modern biopharmaceutical analysis.

The Biozen™ Oligo LC column brings a unique combination of core-shell versatility and high pH ruggedness necessary for oligonucleotide separations. The Biozen Oligo column is packed in a unique bio-inert titanium hardware designed to minimize sample loss and adsorption issues typically seen with stainless steel hardware, demonstrating improved recovery and peak shape.

Learn more about Phenomenex biopharmaceutical product solutions and how we can help support your next project **here**.

SCIEX Now support network

SCIEX Now

- Manage your instruments
- Submit and manage support cases, track status and view history
- Access online training courses and articles
- Manage software licenses linked to your registered instruments
- View and report critical instrument statistics when connected to the StatusScope remote monitoring service
- Be a part of the SCIEX community by submitting questions and comments
- Receive notifications from SCIEX with content based on your preferences

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SCIEX Now Learning Hub

- SCIEX Now Learning Hub success programs provide LC-MS and CE training customized to meet your exact needs
- With a selection of training methods and certifications available, you can build a mass spectrometry learning program that is most suited to your lab and users
- By starting with a clear understanding of your desired learning outcomes, we help you improve lab productivity and consistency by designing and delivering a program that is focused on knowledge advancement and retention

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