Breaking through boundaries with CRISPR gene editing

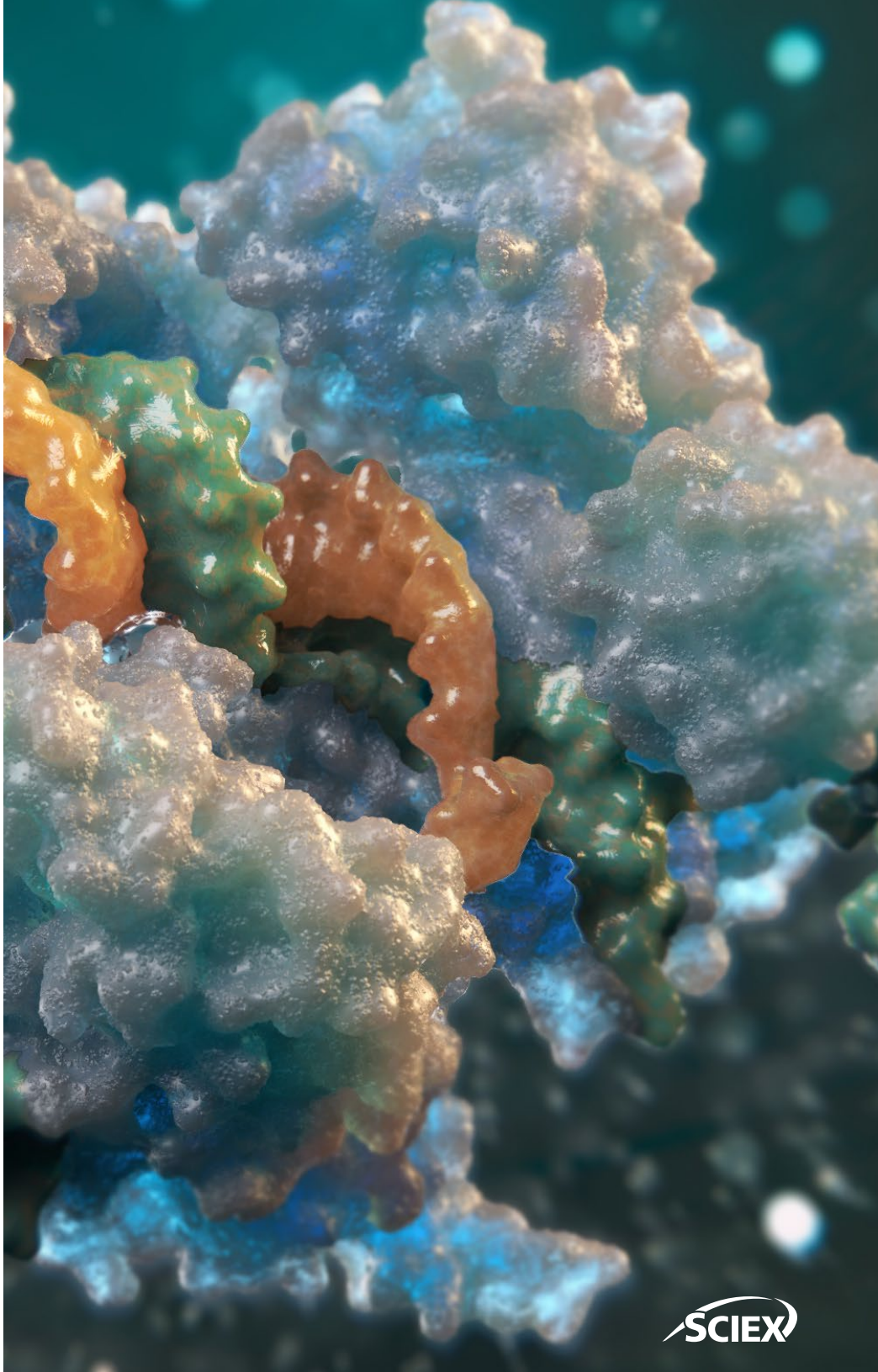
Analytical solutions for the development of CRISPR gene editing approaches

SCIEX

The Power of Precision

The discovery of the CRISPR/Cas9 mechanism opened doors for new, viable ways of treating severe diseases. Intensive research on guide RNAs (gRNAs), Cas mRNA and proteins, and their delivery mechanisms, has increased specificity, safety and efficiency tremendously in the last several years. These research efforts have also led to an increase in the variety and complexity of CRISPR-based therapies.

Have a strong partner by your side to streamline analytics and realize the full potential of your life-changing therapies. Explore how intuitive, informative and flexible analytical solutions let you break through characterization boundaries of CRISPR/Cas9 therapies.



What the experts say

Hugo Gagnon, PhD

Chief Scientific Officer, Allumiqs

"What's the outcome in the cell? Is it on target? Is it off target? What are those undesired effects? Is there some percentage of misintegration? That's what we focus on with our ZenoTOF 7600 system."

Tingting Li, PhD

Manager, Cell and Gene Therapy Applications, SCIEX

"Analyzing the purity and integrity of critical molecules, such as sgRNA (including pegRNA) and Cas9 mRNA, demands a level of scrutiny that surpasses conventional methods. The capillary electrophoresis systems and kits from SCIEX stand for pivotal platforms, providing unparalleled resolution and sensitivity for development and quality control. Thus, they help ensure the fidelity and success of genome editing endeavors."

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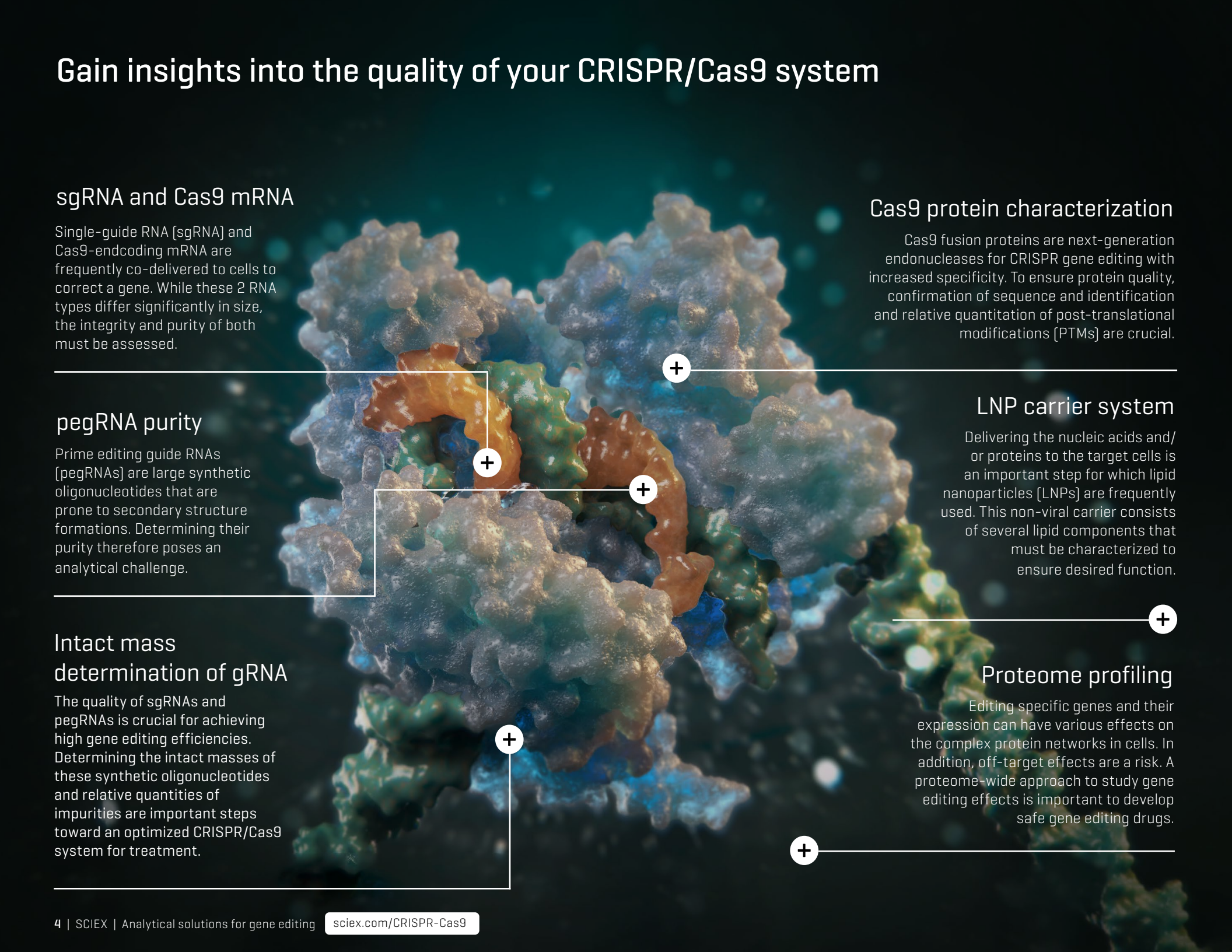
Gain insights into the quality of your CRISPR/Cas9 system



sgRNA and Cas9 mRNA

Single-guide RNA (sgRNA) and Cas9-endcoding mRNA are frequently co-delivered to cells to correct a gene. While these 2 RNA types differ significantly in size, the integrity and purity of both must be assessed.

pegRNA purity

Prime editing guide RNAs (pegRNAs) are large synthetic oligonucleotides that are prone to secondary structure formations. Determining their purity therefore poses an analytical challenge.

Intact mass determination of gRNA

The quality of sgRNAs and pegRNAs is crucial for achieving high gene editing efficiencies. Determining the intact masses of these synthetic oligonucleotides and relative quantities of impurities are important steps toward an optimized CRISPR/Cas9 system for treatment.

Cas9 protein characterization

Cas9 fusion proteins are next-generation endonucleases for CRISPR gene editing with increased specificity. To ensure protein quality, confirmation of sequence and identification and relative quantitation of post-translational modifications (PTMs) are crucial.

LNP carrier system

Delivering the nucleic acids and/or proteins to the target cells is an important step for which lipid nanoparticles (LNPs) are frequently used. This non-viral carrier consists of several lipid components that must be characterized to ensure desired function.

Proteome profiling

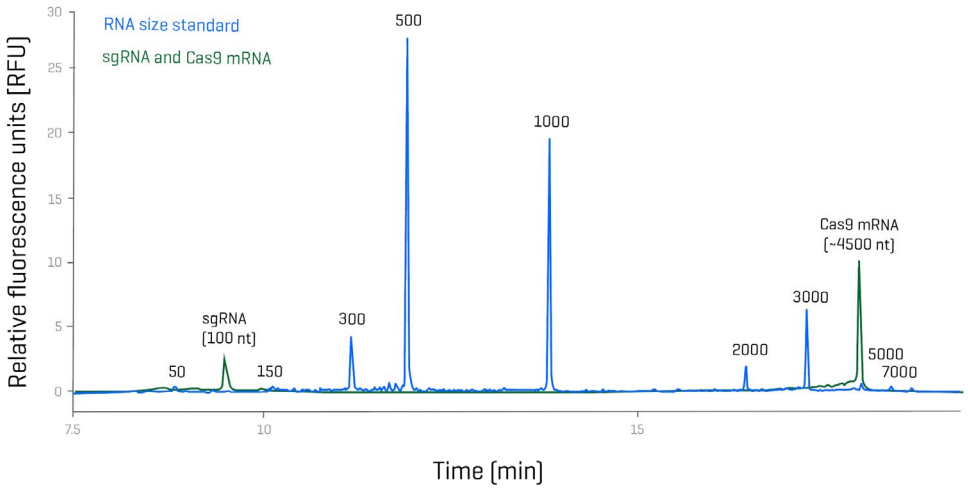
Editing specific genes and their expression can have various effects on the complex protein networks in cells. In addition, off-target effects are a risk. A proteome-wide approach to study gene editing effects is important to develop safe gene editing drugs.

sgRNA and Cas9 mRNA characterization

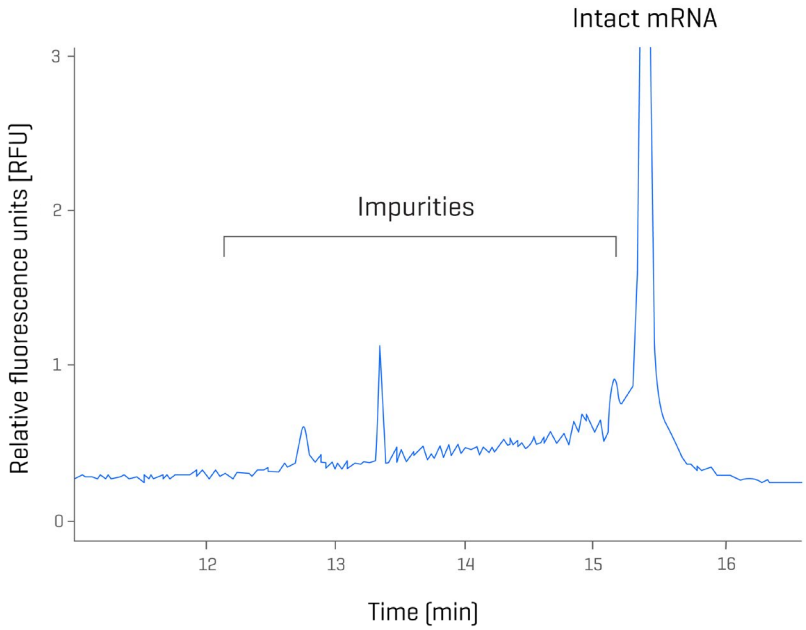
For therapeutic purposes, the naturally occurring CRISPR RNA (crRNA) and the trans-activating CRISPR RNA (tracrRNA) are commonly engineered into a sgRNA. While sgRNAs consist of approximately 100 nt, Cas9-encoding mRNAs are >4000 nt in size. Assessing the purity, confirming the integrity and quantifying both RNAs—sgRNA and Cas9 mRNA—are key for successful gene editing.

- Be confident in your intermediate products' quality within the same analysis without compromising on data quality with the **RNA 9000 Purity & Integrity kit**
- Understand related nucleic acid impurities and determine sizes and quantities, simultaneously using the **BioPhase 8800 system** and 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)

Confirm sgRNA and mRNA integrity in 1 assay



Confidently monitor impurity profiles with excellent resolution



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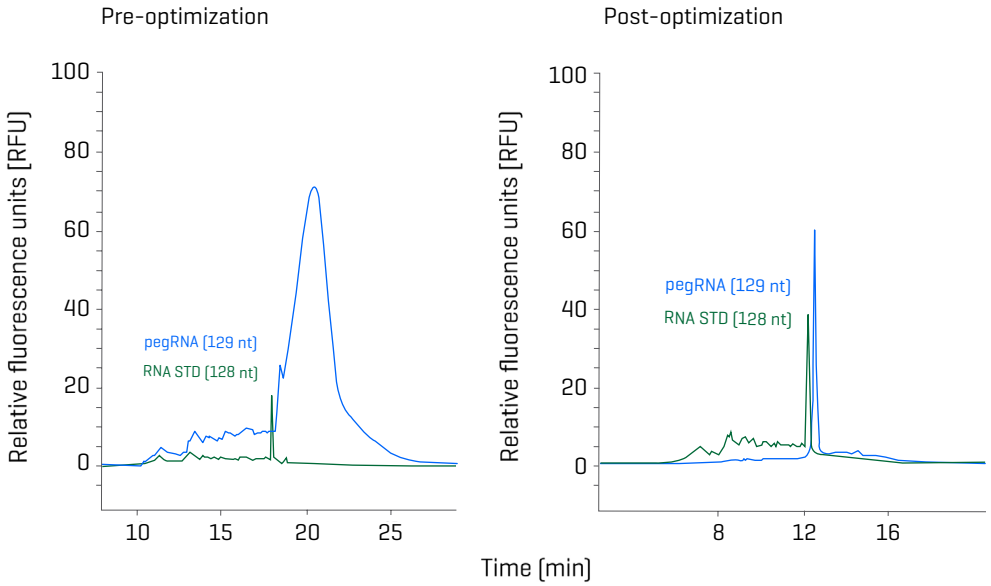
Prime editing: pegRNA purity

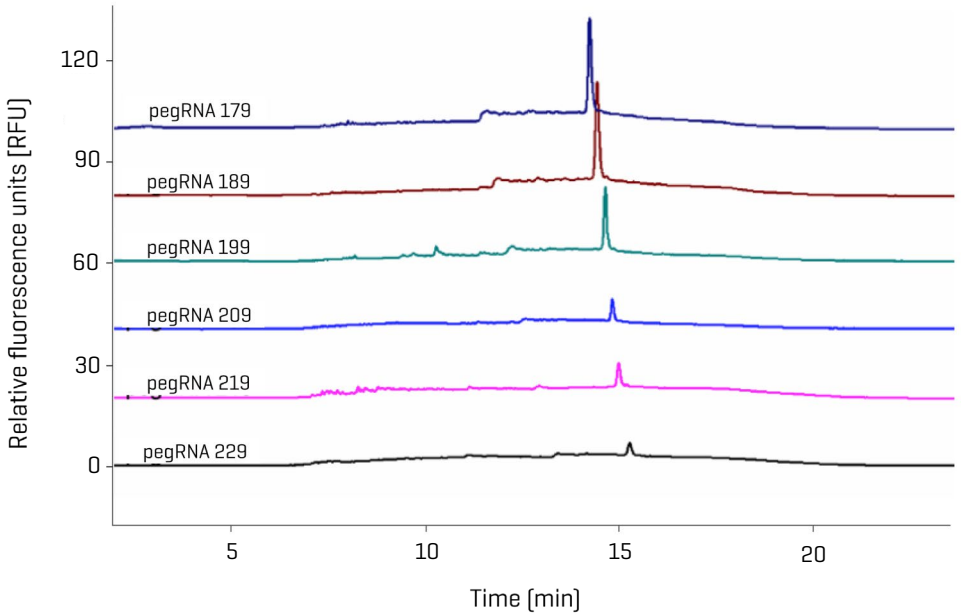
Prime editing (PE) is a promising approach with increased specificity and efficiency. It consists of an sgRNA with a reverse transcriptase template sequence and a primer binding site. With a length of approximately 120-250 nt, synthetic pegRNAs are prone to many impurities derived from their stepwise synthesis. Their complementary bases can lead to secondary structure formation that is resistant to common denaturating strategies, posing an additional analytical challenge to overcome.

- Achieve superior resolution of gRNA, sgRNA, pegRNAs and related impurities with the ssDNA 100-R kit and the **PA 800 Plus system**
- Break through the boundaries of secondary structure formation and determine the purity of challenging, intermediate products with reliable temperature control
- Streamline data management through compatibility with the Empower CDS and the Chromeleon CDS

Break through limitations linked to secondary structures



Rely on a superior resolution



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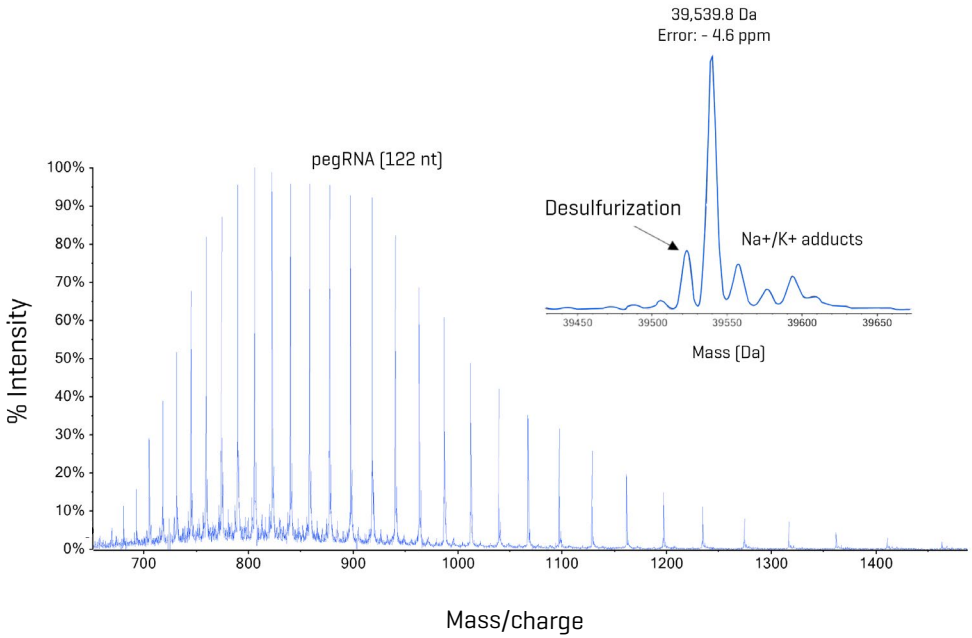
Intact mass determination of guide RNAs

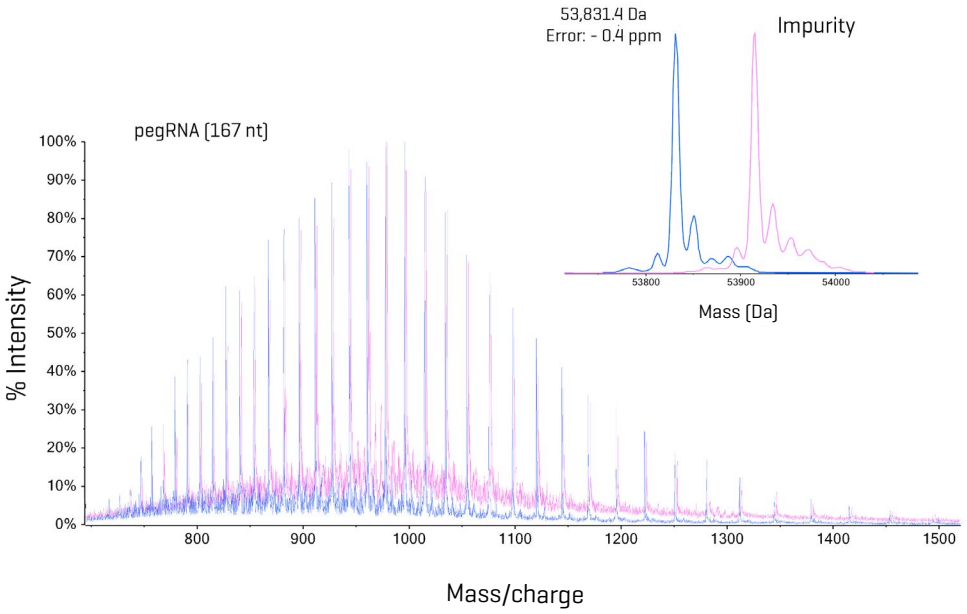
Guide RNAs can vary in size from ~17 nt and ~65 nt in length for crRNA and tracrRNA, respectively, to ~100 nt for sgRNA and up to 250 nt for pegRNA. While the specificity and efficiency can be increased with the latest sgRNAs, the likelihood of introducing impurities increases during the stepwise synthesis of larger RNAs. The quality of sgRNAs is crucial for achieving high gene editing efficiencies. An error in 1 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 impurities and with **SCIEX OS software** and reconstructed quantitation

Assess the mass information of guide RNAs with ease



Take charge of the quality of your intermediate products



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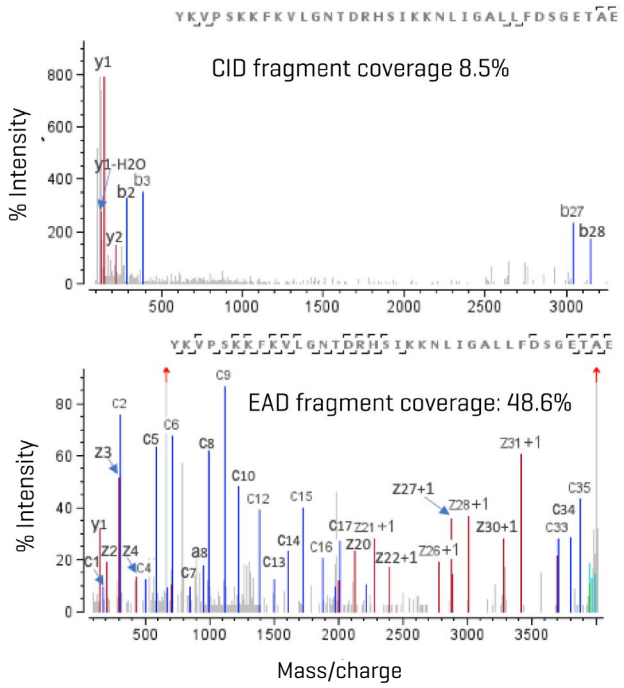
Amino acid sequence and PTMs of Cas proteins

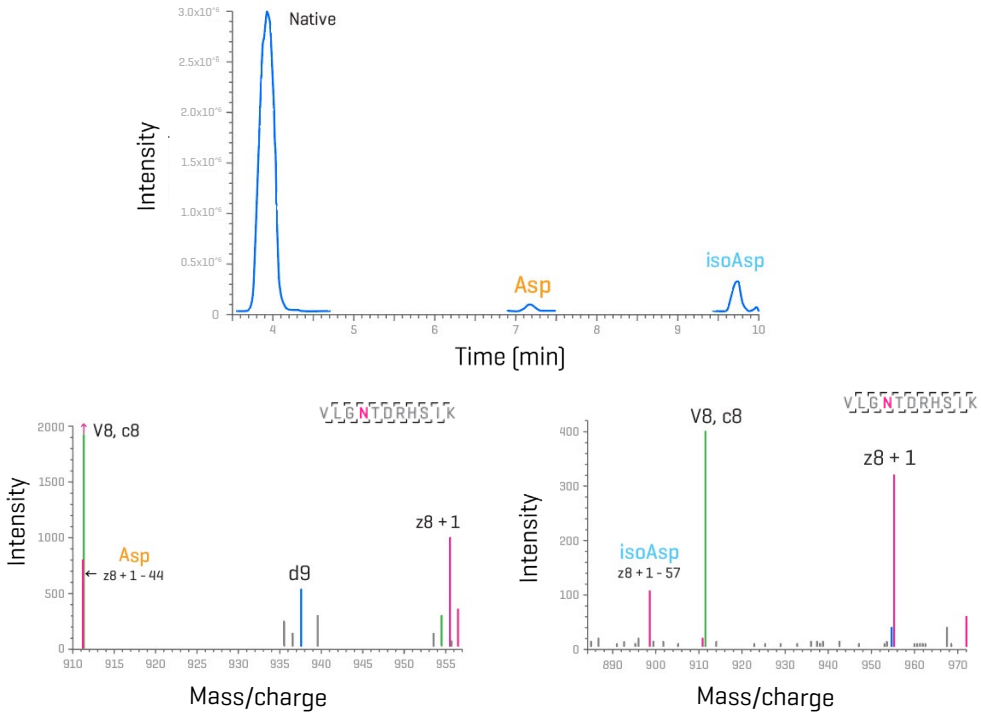
To increase target specificity further, Cas9 fusion proteins are being studied. The confirmation of the target amino acid sequence and the identification of low-abundance PTMs to ensure product quality require a deeper look into these engineered proteins. While a peptide-mapping approach can provide relevant information, it can be challenging to achieve sequence coverage that is high enough and full elucidation of PTMs to characterize product quality attributes (PQAs) and critical quality attributes (CQAs).

- Obtain high protein sequence and fragment coverage despite limited sample quantities with highly sensitive accurate mass data acquisition
- Identify PTMs, their locations and relative quantities with the **X500B QTOF system** and the **ZenoTOF 7600 system**
- Differentiate amino acid isomers and determine exact locations of fragile PTMs with electron activated dissociation (EAD), an intuitive alternative fragmentation technique available on the **ZenoTOF 7600 system**
- Take back your time with accurate and streamlined data processing using **Biologics Explorer software**

Know your sequence inside and out with EAD MS/MS



Fully understand and localize challenging PTMs



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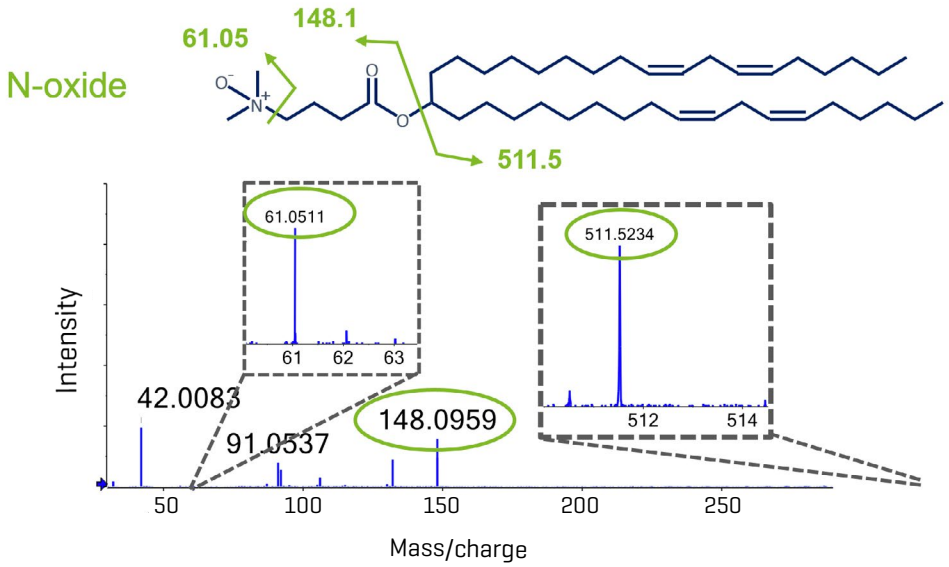
Characterizing the carrier: Lipids and related impurities

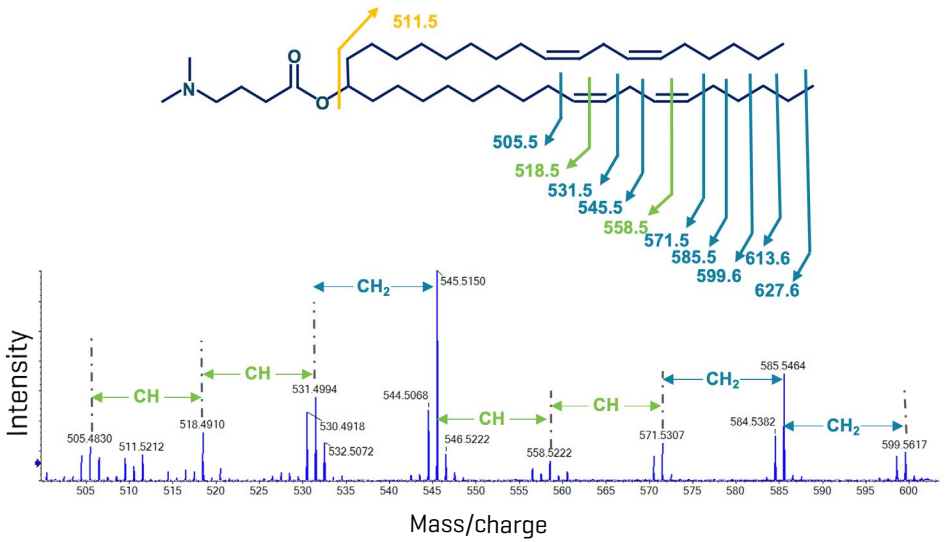
The flexibility of LNPs with regards to the type and size of the cargo, limited adverse effects, and easier scale-up compared to viral vectors, are factors that contribute to the increased use of LNPs as delivery vehicles. Ionizable lipids are a key component of LNPs and their quality is critical for a stable and efficient product. Even very low abundance N-oxide impurities, which are difficult to be fully structurally elucidated, can lead to a loss of function of genetic cargos. In addition, other lipid-derived impurities can impact the quality of the final product and therefore must be characterized.

- Confidently determine product quality by understanding the structures of your ionizable lipids with electron activated dissociation (EAD) in the **ZenoTOF 7600 system**
- Differentiate between oxidated species, localize double bonds and saturations accurately and structurally elucidate relevant lipid-derived impurities to determine product quality
- Take back your time by streamlining EAD data processing with **Molecule Profiler software**
- Be confident in detecting relevant product excipients by leveraging a linear dynamic range >5 orders of magnitude and signal-to-noise enhancement with the Zeno trap

Exactly localize oxygen incorporations



Determine locations of double bonds and saturations with confidence



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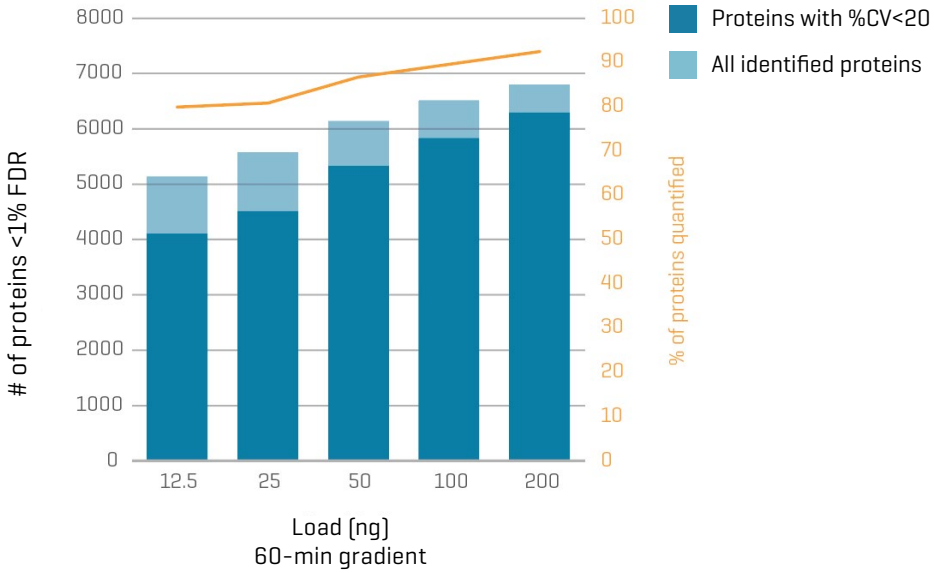
Off-target effects: Proteome profiling for gene editing

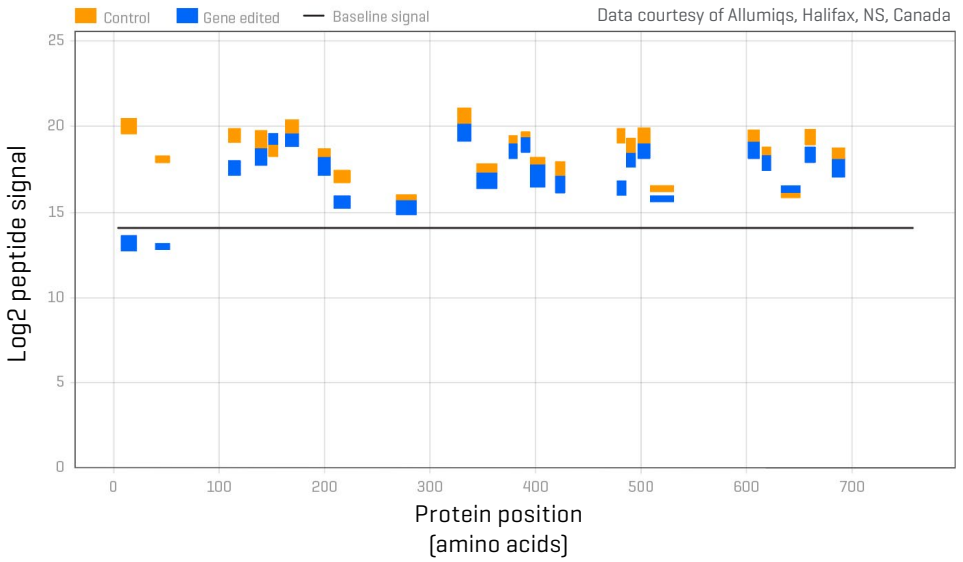
In addition to specificity challenges, gene editing can affect the phenotype in various ways based on the complexity and interdependency of protein networks. Genomic readouts cannot provide sufficient insights into the potential disruption of gene regulators or detect changes to the proteome. Protein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

- Break through the boundaries of gene editing by seeing and identifying the unexpected
- Understand the effects of gene editing on the proteome level in an unbiased way with data-independent acquisition (DIA) using **Zeno SWATH DIA**
- Dig deeper into changes despite limited sample amounts with increased sensitivity using the Zeno trap on the **ZenoTOF 7600 system**
- Achieve confident identification and simultaneous quantitation with excellent MS/MS data quality

Dig deeper into the proteome



Understand changes to proteins with amino acid resolution



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Hardware to provide answers to your questions

ZenoTOF 7600 system

A high-resolution mass spectrometry solution that combines powerful MS/MS sensitivity, fragmentation technology and a step-change in data-independent acquisition

X500B QTOF system

A QTOF system purpose-built to simplify everyday biologics characterization

BioPhase 8800 system

Built for biopharmaceutical scientists who need the highest data quality and the ability to run multiple samples in parallel

PA 800 Plus system

Designed to characterize therapeutic molecules with confidence using high data quality and kit-based workflows

Software and consumables to meet your needs

Streamline the processing of MS and MS/MS data from lipids with **Molecule Profiler software**

Enable highly accurate and informative workflows for full characterization of proteins with **Biologics Explorer software**

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

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**

Be covered for the assessment of single-stranded nucleic acids with coated capillaries, gel and standards in the ssDNA 100-R kit compatible with the PA 800 Plus system

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- 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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