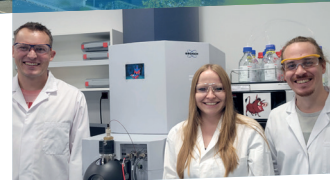




The Axolabs Team



Axolabs GmbH
Kulmbach, Germany

Customer Insights

Bridging the MS/MS data gap in the field of nucleic acid and oligonucleotide-based therapeutics

Scientists at Axolabs employ OligoQuest's powerful tools for the interpretation of RNA and oligonucleotide data to improve preclinical and clinical development of DNA- and RNA-based therapeutics and nucleic acid medicines.





Working with Bruker

Axolabs has worked closely with Bruker since 2007. The team at Axolabs describes why this relationship has flourished over the years.

"Our company is driven by science. We need methods and techniques that meet our customers' advanced requirements in this rapidly expanding field. Bruker is easy to work with, and always provides helpful advice if we have technical issues or problems with methods. That's something that's continued over the years. For example, we recently had some issues with MS/MS fragmentation, and the Bruker team jumped in to help us. We rely on each other, and it's been a great relationship. We haven't had the same experience with other vendors."

Axolabs GmbH

As a leading custom research organization (CRO) in the field of preclinical and clinical development of DNA- and RNA-based therapeutics and nucleic acid medicines, Axolabs GmbH, a member of the LGC Group, has provided high-end solutions and consultancy services for the development of innovative drugs for more than 22 years. The company's 200 employees provide a broad range of services for therapeutic oligonucleotides and mRNAs that includes bioinformatics, chemical synthesis of oligonucleotides, oligonucleotide conjugates and formulations, analytics, bioanalytics, in vitro and in vivo testing.

Located in Kulmbach, Berlin (Germany) and Petaluma (US), customers turn to the Axolabs team for profound knowledge, high quality standards and excellent research methodology. The company's client list includes most of the leading research groups in the field of nucleic acid and oligonucleotide-based therapeutics worldwide, including more than 20 Big Pharma enterprises, 250 biotech companies, and 30 academic research institutes and venture capital firms. The team at Axolabs describes the company's work:

"We believe in science for a safer world. Our strengths lie in our ability to support our customers in that process of getting new drugs to market that can improve the way the medical community treats disease."

Sequence accuracy of RNA and oligonucleotides is critical to the company's work to ensure the correct activity and expression as well as avoiding off-target effects or toxicity to patients. To support these efforts, Axolabs has been working with Bruker's OligoQuest™ software to offer enhanced RNA and oligonucleotide characterization for the company's clients.

Research Interests

Oligonucleotide characterization by mass spectrometry (MS) has gained significant interest recently with the increased use of DNA and RNA as research reagents, as well as pharmaceutical drug molecules. The growing importance of oligonucleotides in research, diagnostics and gene therapy has also increased regulatory interest in tools to verify their sequence, as well as identify and quantify related impurities.

With its extensive experience in the field, Axolabs has developed a unique and proprietary assay system for the sensitive detection of oligonucleotides from biological matrices for the analysis and characterization of drug metabolism and pharmacokinetics (DMPK) and absorption, distribution, metabolism and excretion (ADME) properties of oligonucleotide-based therapeutics. That provides the basis for the company's related services, which include:

- Sensitive detection of single- and double-stranded oligonucleotides
- Extraction-free and robust procedures with the option of parallel quantification of oligonucleotide and target mRNA from a single sample
- Assays compatible with conjugates and different oligonucleotide chemistries
- Simultaneous detection of metabolites
- Downstream MS identification of oligonucleotide metabolites
- Assays established in regulated environment (GLP/GCP)
- Detection of 5'-phosphorylation of siRNAs as a marker for cytoplasmic delivery and a unique tool to determine pharmacokinetic/pharmacodynamic modeling correlation

Additionally, the company provides services for the quantitative detection of mRNA therapeutics including pharmacokinetic/toxicokinetic analysis of mRNA therapeutics from various biological matrices by:

- qRT-PCR using SYBR Green or TaqMan
- Quantigene® branched DNA assays
- Sensitive methods that do not require mRNA extraction
- Parallel quantitative detection of oligonucleotide and target mRNA from a single sample
- Assays established in a regulated environment (GLP/GCP)

The team at Axolabs describes the company's work:

"We help our customers with the characterization of their molecules of interest, including purity, identification, and other necessary assays; we can do all types of stability analysis. Eventually, if a customer has a molecule they want to bring to market, we help them develop all the data necessary for an investigational new drug (IND) filing."

"Our bioinformatics and biology groups also help clients screen from a large pool of molecules to narrow down the candidate of interest and which ones might be successful."



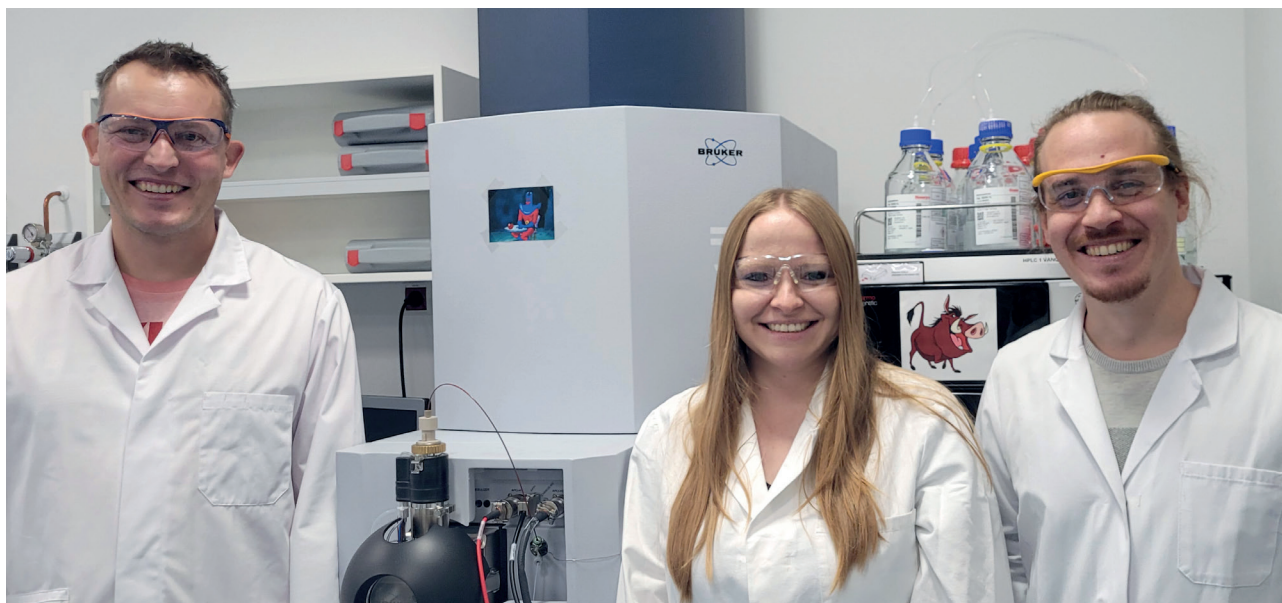
Interpretation of RNA and oligonucleotide data

RNA and oligonucleotide sequence accuracy is a critical attribute impacting molecular safety profiles to avoid off-target effects or toxicity, and compound efficacy such as correct activity and expression. For Axolabs customers, characterization of production related impurities or degradation products is of key importance during the approval process of biopharmaceutical products.

Liquid chromatography coupled with MS (LC-MS) methods are established tools for this characterization process. However, intact mass confirmation of impurities and by-products of oligonucleotides is not always sufficient and information at the nucleotide sequence level is required. Tandem MS (MS/MS) experiments coupled with enzymatic digests for longer RNA molecules are needed to fully establish the molecular identity of an LC-MS peak. Additionally, because oligonucleotide MS/MS spectra are complex, their manual interpretation is time-consuming and requires in-depth analysis skills, rendering the analysis of more than a couple samples a day difficult and expensive, as well as often resulting in bottlenecks.

Companies like Axolabs benefit from the combination of isotopic fidelity with high resolution in instruments like the Bruker maXis II and timsTOF Pro 2 to enable measurement of biopolymers in the 30-50 kDa range with sub-ppm mass accuracy. Robustness, sensitivity and, most importantly, high dynamic range in negative mode allow the detection and identification of impurities based on intact mass. These characteristics have benefited the characterization of analytes ranging from siRNA APIs, single guide RNA for gene editing (sgRNA) and mRNA specific assays (5' capping, polyA tail).

Axolabs has invested in Bruker maXis II quadrupole time-of-flight MS (QTOF-MS) systems to characterize highly modified oligonucleotide sequences because of the instruments' ability to determine mono-isotopic masses of intact oligonucleotides and their fragment ions with high mass accuracy. The team at Axolabs explains the importance of this instrumentation in their laboratory.



The Axolabs team participating in this co-operation with Bruker (from left to right): Stephan Seiffert, Teamleader Analytics & Principal Scientist, Julia Schneider, Principal Research Associate, Timo Schierling, Scientist.

“The QTOF-MS instruments are extremely advantageous for our oligo work. We have 12 Bruker QTOF-MS instruments currently, as well as the corresponding software package. They are vital to our work. Even more than the instrumentation, however, we rely on the support team from Bruker. Their customer service is excellent.”

OligoQuest

In January 2022 Axolabs began testing the beta version of Bruker's OligoQuest software, which offers enhanced RNA and oligonucleotide characterization for antisense research, RNA therapeutics, confirmation of guide RNA sequences for CRISPR, and other gene editing techniques. Isotopic fidelity combined with ultra-high-resolution MS has shown tremendous promise for these types of analyses. By exploiting the high isotopic fidelity and mass accuracy of Bruker's maXis II and timsTOF platforms, OligoQuest enables identity confirmation by intact mass and MS/MS analysis. Customers like Axolabs are using OligoQuest for confirmation of modified RNA sequences and base exchanges in isomeric oligonucleotides.

OligoQuest's flexible sequence editor supports natural nucleic acid building blocks as well as custom backbone, base and sugar modifications. Proven algorithms for peak detection and deconvolution of MS and MS/MS data obtained with high isotopic fidelity and ultra-high-resolution ensure correct mass assignments even in high dynamic range samples. The team at Axolabs has been involved in testing the beta version of OligoQuest, as well as providing feedback to the Bruker development team. It was an opportunity they were eager to pursue. They explain:

“Because we have a lot of Bruker instrumentation, and we focus on oligo analyses, it was a natural progression to try the beta version of OligoQuest. It's a win-win for both companies, as we get to make suggestions for the software, and Bruker learns from our experience with the software in a laboratory setting. We're still using the beta version, and now we're testing its integration with Bruker BioPharma Compass software.”



OligoQuest bridges the MS/MS data gap by adding powerful tools for the interpretation of RNA and oligonucleotide data to Bruker's BioPharma Compass® suite. A flexible sequence editor supports a wide range of nucleic acid building blocks, both natural and synthetic. The OligoQuest™ workflow in BioPharma Compass is wizard driven to simplify the method setup and accommodates customer-defined sequence definitions to easily include the wide range of modified nucleotides used by the pharmaceutical industry.

The Axolabs team has particularly benefited from the OligoQuest SNAP algorithm with top-down MS for the interpretation of spectra with large numbers of overlapping multiply charged ions. This powerful MS/MS annotation engine automatically computes and identifies terminal and internal fragments, allowing rapid analysis of complex MS/MS data in a straightforward, easy-to-interpret fashion. OligoQuest offers algorithms and workflows to annotate tandem MS data from oligonucleotide sequences longer than 100 nucleotides. The Axolabs team describes how that capability streamlines their workflows.

"The SNAP algorithm in OligoQuest helps us with selecting the mono-isotopic signal of one fragment. Because you only reduce it to the mono-isotopic signal and you don't have the isotopic ones, you don't have many mismatches. In our former analysis we didn't have the SNAP algorithm, so there was a higher probability of mistakes. Additionally, OligoQuest's improved usability makes it much more efficient. We get an overview of complex data with one graphic. This data visualization brings the results to life."

That capability holds the potential to greatly benefit Axolabs' customers, particularly during the approval process of therapeutic oligonucleotides and mRNAs. The team at Axolabs explains: *"We always need to make sure our customers have the product they expect to have. Of course, there's always the chance that by a synthesis, you get a base switch in the sequence. So, the sequences will not be the same anymore, but the mass will be the same."*



"The only way to find out if that happened is to do full sequencing on your oligos. OligoQuest helps us to get this data easier in an efficient way."



Future Developments

The team at Axolabs sees its relationship with Bruker as a key element to success as they chart new territory in DNA- and RNA-based therapeutics and nucleic acid medicines. That includes expanding the use of OligoQuest in the company's other operations, like its quality control department. Additionally, the team sees a role for OligoQuest in its recently founded Axolabs Berlin GmbH, a newly established GMP contract manufacturing facility for chemical DNA and RNA therapeutics. The team at Axolabs describes these plans.

"Our goal is to have these analyses run under GMP conditions. Our move towards becoming a GMP producer would benefit from software like OligoQuest as we validate new methods."

Additionally, Axolabs plans to continue to improve its services for therapeutic oligonucleotides and mRNAs, helped by its ongoing partnership with Bruker. The team at Axolabs describes these plans.

"We're testing out the timsTOF Pro 2 now, and we have other plans to work with Bruker as we expand our service offerings. We're always looking for new, cutting-edge methods and techniques that provide benefits for our customers."

And, as always, Axolabs appreciates the support they get from Bruker. The team believes the freedom to share knowledge and ideas serves both companies well, and the ability to collaborate contributes to Axolabs' long-term objective of using science to create a safer world. The team at Axolabs explains.

"We have a good partnership with Bruker. If we have a technical question, they always answer quickly. That's not what we've experienced with other companies."

We've had questions that take other vendors a month to respond. With Bruker, we always get immediate responses, and that's important in a fast-moving field like ours."

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About Axolabs

Axolabs is the world's leading custom research organization (CRO) in the field of preclinical and clinical development of DNA- and RNA-based therapeutics and nucleic acid medicines. The Axolabs team is committed to providing high-end solutions and consultancy services for the development of innovative drugs. The growing company, located in Kulmbach, Berlin (Germany) and Petaluma (California, US), is a member of the globally active LGC Group. The laboratory's expert team has 22+ years of experience in the development of oligonucleotide therapeutics and has access to excellent infrastructure. Axolabs is well known, from big pharma and biotech to academia and venture capital, for its profound knowledge, high quality standards and excellent research. Its broad range of services for therapeutic oligonucleotides and mRNAs covers bioinformatics, chemical synthesis of oligonucleotides, oligonucleotide conjugates and formulations, analytics, bioanalytics, and in vitro and in vivo testing.

For more information, please visit: www.axolabs.com

About Bruker Corporation

Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels. In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research and clinical microbiology.

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