Native LC-MS: From the Lab to the Manufacturing Floor

How is the characterization of non-denatured biomolecules driving scientific progress in industry and academia?

The use of native LC-MS has grown significantly in the last decade. But what continues to make this tool so useful for professionals in academia and industry? Heidi Vitrac, an Applications Scientist at Tosoh Bioscience, and Rob Haselberg, an Assistant Professor at the Vrije Universiteit Amsterdam, share their perspectives on the technique, and explain why it suits the needs of the curious minds behind blue skies research, as well as the practicalities of biopharmaceutical manufacturing.

What is "native" LC-MS?

Heidi Vitrac: Native MS refers to the process whereby large biomolecules and complexes can be transferred from a three-dimensional, functional existence in a condensed liquid phase to the gas phase via the process of electrospray ionization (ESI). Native MS allows solution state non-covalent protein interactions to be maintained in transmission into the gas phase of the mass spectrometer. When hyphenated with LC, native LC-MS refers to the analysis of biomolecules in their native, non-denatured state.

There has been a steady increase in the use of native LC-MS over the past 10 years, largely driven by the development and expansion of high-resolution mass spectrometers from various vendors. Setting up instruments and running native MS experiments is not as daunting a task as it was twenty years ago; back then, scientists had to build and customize their interfaces to perform fit-for-purpose analyses. We've also seen huge amounts of progress in the development of software and resources to support such analyses, significantly accelerating and simplifying data processing. Altogether, native LC-MS has become a much more approachable technique that even scientists with little MS training can use.

In which applications does the technique shine?

Rob Haselberg: In a biopharmaceutical context, native LC-MS helps provide a better understanding of proteins. Take monoclonal antibodies (mAbs), for example; their behavior can be analyzed on a molecular level – helping to determine how quickly they bind to receptors and providing insight into their physicochemical properties. There's a lot of information that can be extracted with the native LC-MS approach.

Vitrac: Thanks to various improvements in sensitivity, resolution, and mass accuracy of mass spectrometers, native LC-MS is now routinely used in many pharmaceutical and biotechnology laboratories for the analysis of mAbs and other protein therapeutics to assess purity, and profile antibody glycosylation. But the technique is not restricted to protein analysis. It can also be applied to macromolecular complexes, including drug-macromolecular complexes, lipid-protein interactions, and DNA-protein interactions. By preserving the native structure of the biomolecules of interest, native LC-MS allows us to determine the mass of intact assemblies, their precise stoichiometry, direct interactions between subunits, and the strength of inter-subunit interactions.

Can other technologies achieve the same goals?

Haselberg: Put simply, no! Of course, bioassays can be used to determine some of the functional characteristics of biomolecules, and surface plasmon resonance can help paint a picture of a molecule's binding and affinity kinetics, but native LC-MS provides additional insights – and much faster. Using this approach, you can quickly decipher the high-order structure of a given molecule as well as the covalent complexes it forms. And the basic architecture of a molecule can easily be revealed with native LC-MS.

What specific challenges does native LC-MS present?

Vitrac: The hyphenation of LC with MS is quite different from any other optical detector. The biggest difference pertains to the need to switch from the liquid phase to the gas phase, which is achieved in the ion source of the MS instrument. And that makes optimization of the mobile phase used in the LC separation a critical component to the method development process. And because native LC-MS often happens under physiological conditions (i.e., neutral pH), you cannot use organic solvent or acid in the mobile phase, which adds to method development challenges. Notably, native LC-MS is not restricted to size exclusion chromatography; indeed, several other modes of chromatography can be used in combination with native MS, including capillary iso-electrofocusing (cIEF), hydrophobic interaction chromatography (HIC), and anion exchange chromatography, as recently demonstrated for various mAbs.

Can native LC-MC be further improved?

Haselberg: Sometimes the practical questions we have are greater than the technology practically allows us to explore. So, compromises between separation and detection may occasionally be needed. From an academic point of view, flow rate and spectrometer compatibility also have to be considered. But working with a competent vendor can make a huge difference in how greatly these factors impact research.

Vitrac: From my perspective, the biggest needs are related to data interpretation. Biomolecules are complex and teasing apart the information on each component of the complex from a series of mass spectra remains challenging. Data processing software has improved enormously for biotherapeutics, but further development will help us better understand "out-of-the-ordinary" complexes.

Where does Tosoh Bioscience fit in?

Vitrac: We have accumulated a wealth of experience in the development of chromatographic methods – and we are happy to help optimize any separation. From academic environments to big pharma, our chromatography experts can support the growing needs of our customers. As proven specialists in the field, we work with biopharma partners at all scales – from UHPLC analysis to purification at the manufacturing scale. Any customer with a separation problem can simply contact us; we will take the time to listen and to understand the issue before finding the best solution.

Meet the Experts

Heidi Vitrac, Applications Scientist, Tosoh Bioscience.

"I obtained my PhD in Physical Chemistry at Paris Descartes University and quickly developed an enthusiasm for cross-disciplinary studies around analytical biochemistry. In 2008, I moved to the US and joined the University of Texas Health Science Center at Houston. I enjoyed taking on challenging projects and contributing to the advancement of collaborative work. And that's what led me to join Tosoh Bioscience in December 2020. With Tosoh Bioscience, I contribute to the development and optimization of analytical methods and processes for the separation, identification, and characterization of various biomolecules."

Rob Haselberg, Assistant Professor, Faculty of Science, BioAnalytical Chemistry, Vrije Universiteit Amsterdam

"I received a master's in analytical chemistry from the Vrije Universiteit Amsterdam in 2006 before moving on to my PhD, which focused on the characterization of biopharmaceuticals using a combination of capillary electrophoresis and MS. I've always been interested in biomolecular analysis and, drawing on that passion, I now work in the VU's BioAnalytical Chemistry group, where much of my research is geared towards understanding native protein characterization.

Haselberg: As a lead member of an analytical chemistry group, I work on improving hardware. For example, our group often requires columns that can manage low flow rates, so working with a company like Tosoh Bioscience – who truly understands our scientific needs – is great. They also host user meetings, where we can share our data and any challenges that we have encountered. These sessions are always useful as they share practical solutions with us too. We have had an enduring collaboration with the company – a testimony to the services it provides!

What's next for biomolecular characterization?

Haselberg: I predict that we'll see an increased use of affinity columns that incorporate the power of mass spectrometry in coming years. Why? Because it will enable us to purify and characterize molecules in a single system. I also think we will see more multidimensional chromatography, including hyphenation, that will help deepen our understanding of biomolecules. These techniques will help overcome some of the compatibility issues we face today.

Vitrac: Like Rob, I am interested in seeing how multidimensional chromatography can be used in native MS analysis. It has the potential to create powerful pipelines where the quality attributes of any given biomolecule can be assessed during the various stages of downstream processing. I am also curious about new applications beyond protein therapeutics and complexes. I look forward to seeing the next jump from academia to industry, especially regarding the analysis of DNA and RNA molecules inside complexes. One thing is certain: our team is truly invested in the success of our customers' work, so we will continue to find new ways to work together to take biopharmaceutical research to the next level.