

# PERFECTING PROTEIN ANALYSIS WITH MASS SPEC



*Rachel Rowlinson,  
Specialist in Protein  
Mass Spectrometry  
at Peak Proteins*

**Proteins are valuable molecules for a broad range of applications, but their expression and purification can often require extensive engineering, analysis and troubleshooting in order to obtain a correctly folded protein with the desired therapeutic activity. Peak Proteins provides a full service for protein manufacture and structure determination, and uses mass spectrometry as an essential part of its toolkit to deliver high quality products to its customers.**

Peak Proteins, based in Cheshire, UK, is a company with decades of experience in protein science, using its extensive knowledge to support a wide range of customer projects. Rachel Rowlinson, Specialist in Protein Mass Spectrometry at Peak Proteins, discussed how mass spectrometry assists the company's work and enables it to offer a precise and reliable means of investigating proteomics and protein characteristics. She explained: "Peak Proteins was founded five years ago, and our main focus is on the manufacture of proteins as tools to support small molecule and biologics drug discovery. We perform anything from large-scale protein expression to protein crystallography and structure determination. Anyone can contact us at any point during their project, from construct design through to expression and purification, and we can engineer the protein sequence according to each customer's individual requirements."

The team performs mass spec analysis to look for intact protein mass, as well as more in-depth peptide mapping. "I joined the Peak Proteins team around a year ago to form the protein mass spec analysis side of the business, where we routinely use this technique to assist our team workflow. Protein expression can be a difficult art to master, as not all proteins fold themselves as beautifully as others, and this affects their activity – a lot of our work is troubleshooting, and this is where mass spec really helps us make key decisions about the proteins we work with. I've been working in protein analysis for over 25 years now, so I've seen many of the evolutions in this field, including

the advancement of mass spectrometers, which are now essential tools for this work."

The scientists at Peak Proteins use mass spectrometry as a regular part of their day-to-day work, and have chosen SCIEX systems due to their reliability and ease of use. Rachel explained: "We have a SCIEX X500B QTOF System that we regularly use to confirm protein identity, as well as for sequence and mass determination, and to look for post-translational modifications. It's fantastic for this kind of routine analysis and it's great for QC; it switches between different analyses – such as intact mass analysis or peptide mapping – seamlessly. This thorough testing gives additional information to the customer, an extra degree of confirmation that we are supplying the correct protein, and that it's 'good to go' for their needs. We also provide a stand-alone service to external customers, using the X500B. Anybody can contact us and ask for mass spec analysis – there are a number of ways we can help with customers' projects, including looking at binding partner interactions and antibody characterizations. We're always happy to discuss customer requirements and see if the X500B might suit their needs."

"We have a SCIEX ExionLC on the front end of the X500B that uses a very simple formic acid-based buffer system. The instrument has two different columns, one C4 column for intact mass, and a C18 column for peptide mapping. I only use one set of buffers for reverse phase, so it's a simple valve switch depending on what kind of analysis I want. Having a



QTOF system is great too, as you really get the resolution, which is fantastic for what we do. It's such a simple process, when I want to use the system, it's just a case of walking up to the instrument and deciding which analysis I want to do; at the click of a button, everything else is done for you, including the calibration."

Rachel continued: "Our throughput varies depending on what projects we have on at any time; some weeks we can perform 10 to 20 intact masses, other weeks it can be more. We probably run peptide mapping analysis around 20 to 30 times, but there is the capability to run more. I regularly queue up runs overnight for both of these workflows, which means that the next day I can come in and start the analysis of all the samples – it's an ideal tool for protein work. I've previously set up a 36-hour run for intact masses and peptide mapping, and our data was ready and waiting for us afterwards with great results. The sample turnaround is very efficient; I can run intact mass analysis in five minutes, and peptide mapping is routinely only a 10-minute run."

"The X500B is the perfect fit for our lab space; it's great having such a compact instrument as it means that you don't need to have a specific mass spec lab. The X500B sits at one end of our protein purification lab, and the beauty of this is that the instrument is open access for a lot of our scientists. It only took me about 20 minutes to show everyone how to use it, and now they run their own intact mass samples without needing supervision. It's so simple, quicker than the alternative of running a gel, and gives us a lot more information. I've worked on many mass specs

in the past, and this is by far the most reliable and intuitive instrument I've used; it's an extremely accurate machine giving us great confidence in the results we can provide to our customers."

"We use the SCIEX Bio Tool Kit routinely for intact mass analysis, as we can quickly deconvolute the data to get results of the mass of our proteins. It's a really informative tool that we wouldn't be without. I also routinely use BioPharmaView Software, as we are manufacturing bespoke proteins with unique sequences or tags that are quite different to what's in the public domain. BioPharmaView is key to our data analysis, allowing us to search all of the peptide mapping data against that bespoke sequence. We can instantly tell the customer if there's an issue, for example, if we can't see the mutation or if it looks like some of the protein has degraded. There's no end to what you can do with both BioPharmaView and the Bio Tool Kit, which dovetail perfectly with the reliability of the X500B instrument," Rachel concluded.

To find out more about Peak Proteins, visit [www.peakproteins.com](http://www.peakproteins.com)

To find out more about the SCIEX X500B QTOF System, visit [www.sciex.com/products/mass-spectrometers/qtof-systems/x-series-qtof-systems/x500b-qtof-system](http://www.sciex.com/products/mass-spectrometers/qtof-systems/x-series-qtof-systems/x500b-qtof-system)

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