Episode 5: Accelerating Productivity of Nano LC-MS Analysis with Advanced LC Setups

In the fifth episode of this six-part series, Bogdan Budnik, principal scientist at Harvard Center for Mass Spectrometry, discusses common challenges related to nano LC-MS proteomics analysis of very diverse sample types, improvements in proteomics analysis, and how single-cell proteomics will impact the medical field.

Bogdan Budnik
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LCGC: What are the reasons engaging you to continue working in nano LC-MS proteomics?
BUDNIK: The field of proteomics is a broad definition of our ability to look at nature and its biology, which is what we've been doing for the last 20 years. We try to improve our tools to examine the complexity of biology—how we can learn what it is, what we can do if it's broken, etc.

LCGC: What are the most common challenges related to nano LC-MS proteomics analysis of diverse sample types?
BUDNIK: We have a very diverse community of biologists around us who brought their samples into our lab over the years. For the LC-MS unit, it's a challenge to ensure that each group or individual scientist gets their answer, no matter how complex or not complex their samples are and how much material they have. That's why when scientists come to us with both large and/or small amounts of samples, they need to be properly assigned to an LC-MS system that can deal with such complexity or with a little amount of sample.

LCGC: While working with multiple scientific teams, you also improve existing LC-MS methods and setups. What are the drivers for these activities?
BUDNIK: Within recent years, proteomics shifted toward a quantitative approach. Instead of asking what is there, the main biological question now is how much is there? Quantitative approaches are mainstream right now, and the improvements over the years have focused on analyzing smaller and smaller amounts of samples. That's the number one priority for our community.

Number two, since there is a large number of samples being analyzed by proteomics, we want to make sure our instruments are working more efficiently, so we can increase the number of samples we can analyze per day.

LCGC: How would you describe the role of LC technologies in the overall improvements of LC-MS workflows?
BUDNIK: There are different types of approaches. Many use micro-flow rates, but now, everything is done using nano-flow rates. There are two streams of people going in two different directions, answering two different questions: with microflow LCs and clinical proteomics, there will be a lot of improvements with the data-independent acquisition (DIA) approaches via faster turnaround times of large sample amounts, while low nanoflow systems are moving toward single-cell analysis.

This is where productivity and sensitivity meet the demand of reproducible runs on an LC-MS system.

LCGC: Can you describe the benefits that bring a tandem nano-LC setup?

BUDNIK: In my lab at Harvard, we've had Tandem Mass Tag (TMT)-labeled breakthroughs for the single-cell analysis. Since then, there are a lot of projects done in this area. We usually analyze eight cells per sample, and the biological questions are in hundreds of cells. This is why you need a lot of LC-MS runs. This is where productivity and sensitivity meet the demand of reproducible runs on an LC-MS system. This is also where a tandem system comes into play. With the help of Thermo Fisher, we've set up a system that is much faster than one-by-one simple analysis with a single pump. Since the implementation of the double system, our instruments work in tandem goes much faster than the conventional approach of the single pump.

LCGC: LC-MS proteomics is moving from identification and semi-quantitative measurements of protein abundance changes to quantitative analysis. What is required from LC technology to support this movement?

BUDNIK: Yes, as I mentioned before, the majority of biologists come to our labs asking how much is there. The quantitative analysis of current samples is an important piece, and not only just quantitative, but the quality of the quantitation is playing a very key role in many biological questions that scientists are trying to answer, which is what we're doing with LC-MS.

The liquid chromatography part in this question usually comes in the reproducibility of the sample analysis, meaning all the chromatographic peaks need to come at the same retention time. As such, the reproducibility of the analysis comes from the reproducibility of the columns and the LC system. This is our future focus: reproducibility sample-to-sample on an LC system.

LCGC: Has LC-MS technology significantly improved over the last several years?

BUDNIK: Yes, there has been a lot of progress in this area. The column is the biggest breakthrough. The new columns use a chip technology instead of packed material, which makes them very reproducible. The packed columns have also improved in quality and reproducibility.
Both improvements enable us to analyze more samples per day and week.

LCGC: How would you describe a day in the lab if you had the ideal LC-MS technology for proteomics?

BUDNIK: That would be a very relaxed day. I'd put the samples into the autosampler to start injecting and go home because I could do the analysis online ... the ideal day in the lab would be no day in the lab. All LC and MS systems would be working, running my samples, and we wouldn't have to spend any time on repairs. We'd only need to be in the lab for sample preparation. (How we prepare samples needs improvement.)

LCGC: Where do you see the next big step in the proteomics field? What's the next breakthrough?

BUDNIK: Single-cell proteomics will be the standard type of analysis. Another area we'll see improvement in is a large dataset that will run not only at specialized labs but near hospitals where the analysis for RNA or DNA sequencing is done—this could confirm or defer ideas about the progress of the disease or treatment of each patient. Single cell will be a key area where we can see different types of cells and clinical proteomics. Samples from the patient will be analyzed on the fly in real-time so doctors have answers on how the treatment is or isn't working. These are the two areas I see proteomics playing a key role.