Episode 2: LC-MS Proteomics: Will Micro-Flow LC Transform it into a Routine Tool?

In part 2 of this six-part series, Bernhard Küster from the Technical University of Munich discusses how micro-flow LC changed the way large-scale LC-MS proteomics projects are executed in his laboratory.

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LCGC: Your group at the Technical University of Munich became one of the key centers of proteomics research worldwide. What are the main contributors to this success?
KÜSTER: First, you have to have a good idea of what you want to do in research. Then you need to find people to help you pursue that idea. With proteomics, you have to put the technology platform in place, get it up and running, and make sure it performs to the level that you need it to—the hope is you find results worth sharing with the community. We've been fortunate that some of our research ideas have turned out to be relevant.

LCGC: How would you describe the role of technologies that allow for versatile research areas in proteomics?
KÜSTER: Proteomics is a technology, and as such, it is a tool that can be applied to a range of scientific questions. Therefore, technology is of absolute paramount importance and that includes the typical three things of getting a sample, getting it ready for the proteomic measurement, and data analysis that hopefully tells you something about that biological system you've looked at.

LCGC: With a lot of focus on LC and MS proteomics, you continue to develop new methods and optimize existing methods. What is driving this process, and what are the main requirements for the ideal LC-MS technique?
KÜSTER: Proteomics, as we know it today, has LC-MS/MS as a central analytical component. As everyone is trying to push the boundaries of being able to analyze proteomes faster, in more depth, and in more detail, both the LC and MS sides have to be improved. After that, it's a ping-pong game between the two: when mass spectrometers became more sensitive, the LC systems had to catch up in terms of robustness, which may then challenge the mass spectrometer again. They could, ideally, come together in a way in which they provide great separation power, great sensitivity, great speed, and great depth, all at the same time.

LCGC: In recent years, your group actively developed and adopted day-to-day use of micro-flow LC-MS analytical methods. What are the main reasons for this move?
KÜSTER: This started with work by Juraj Lenčo from Charles University in Prague—he showed that micro-flow LC-MS could work for proteomics, and I was stunned by how good the data looked. So, we continued to develop this further for the reasons I listed before: to be faster, more comprehensive in less time, and more in-depth. Micro-flow LC-MS seemed to be a sweet spot for many applications.

LCGC: Is there controversy with LC regimes for MS proteomics? What are the main discussion topics?
KÜSTER: I wouldn't say there's a controversy. For historical reasons, nano-LCs were the drivers of success in proteomics because small amounts of starting material were used and processed

efficiently. But that has changed as mass spectrometers have become more sensitive that downsides the benefits of nano-LC separations. Today, people take a more balanced view in that you use either technique at low- or medium-flow rates, depending on the application.

LCGC: Having both the nano- and micro-flow LC-MS setups in the lab, how are you defining which projects should be executed on which instruments?
KÜSTER: For us, that is quite simple. Whenever sample quantities are small, we use the nano-LC systems; when sample quantities are larger, we use the micro-LC setup. Another area the nano-LC systems are advantageous is the analysis of post-translational protein modifications because they are usually low abundance, and therefore, you need all the sensitivity from the complete system that you can get both LC and MS.

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LCGC: What are the main advantages and limitations of micro-flow LC-MS, and will micro-flow transform proteomics into a routine tool?
KÜSTER: Micro-flow LC-MS has transformed the way we do proteomics in my laboratory. Half of our systems run micro-flow. One of its main advantages is that you get very good chromatographic separation, so your quantitative data is great along with robust operation. You don't have to tend to your instruments as much, which means you can take on larger projects than are usually possible using nano-LC systems. However, there is one clear downside of micro-flow LC-MS: sensitivity is about three to 10-fold less than the nanosystems. As long as you can work around that limitation, the advantages outweigh the limitations for many applications.

LCGC: Are there any areas where nanoflow LC-MS will be irreplaceable in proteomics?
KÜSTER: For the time being, these will be applications where we are struggling to get enough material, and the outcome of that may be single cell or even sub-cellular proteomics. There the nano-LC flows will still be important for a long time.

LCGC: With a lot of progress in tools and instrumentation for proteomics research, how will the field develop further? What are the next big trends in proteomics?
KÜSTER: That's a difficult question because as we are developing the methodology further, more people are seeing that they can be applied to many areas of science. We will see a great diversification of the scientific fields in which proteomics will be used. For example, in plant science—it isn't established yet, and it is a big area.

Another big trend is moving proteomics into actual clinical applications either by looking at body fluids, which would make it routine or by looking at individuals and the tissues resected by surgeons to see if the proteomic profiles tell us something in addition to the genomic and the transcriptomic profiles that are often applied already in cancer research.

There is a trend moving proteomics closer to structural biology where the sensitivity of proteomic methodologies is much higher than typical structural-biology techniques such as X-ray, crystallography, etc. It's a nice marriage also to cryo-EM and methodologies like that.

And lastly, another area that is growing is drug discovery, especially with covalent drugs being so popular right now. The only readout that can be used for assessing covalent drugs is proteomics, which is why this will be a big trend over the next five years.