Episode 3: Setting the Record in Deep Single-Shot Nano LC-MS Proteome Profiling

In episode 3 of this six-part series, Karl Mechtler, head of the protein chemistry facility at the Research Institute of Molecular Pathology, Vienna, Austria, discusses the critical role of nanoLC separation performance for reproducible and quantitative deep-dive proteomics analysis.

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LCGC: You provide services to multiple groups, and you have established strong collaborations worldwide with academia and industry partners. Looking back, what resulted in this success?
MECHTLER: We were one of the first who started to use nano high-performance liquid chromatography (HPLC) workflow with pre-concentration onto trap column. We had good cooperation with the world-leading company LC Packings. It was acquired by Dionex and around 10 years ago, Dionex was taken over by Thermo Fisher Scientific. The mass spectrometer used was the LCQ Classic ion-trap from Finnigan. The automatic evaluation of data was done with Sequest. With this, we had a super sensitive setup for that time.

LCGC: Do you consider LC-MS to be the primary tool in proteomics research?
MECHTLER: Yes, absolutely. LC-MS will play a very important role for the next 20 years.

LCGC: What are the main factors you optimize to reach maximum performance in single-shot deep-dive LC-MS proteomics?
MECHTLER: The most important factors to increase performance are better sensitivity, better sample preparation, a perfect LC setup, and a super-sensitive mass spectrometer. In sample preparation, we work together with the leading company Cellenlon. In chromatography, we have a very fruitful cooperation with PharmaFluidics, which recently launched a column for single-cell proteomics. In mass spectrometry, we cooperate with Thermo Fisher and use their latest MS technology in combination with FAIMS pro-ion mobility device. This has allowed us to increase the sensitivity by a factor of 10.

LCGC: Do improvements in liquid chromatography make a difference for LC-MS proteomics?
MECHTLER: Yes, absolutely. The role of liquid chromatography in proteomics is very underestimated. New technology developments such as the chip-LC in combination with new, innovative column material will play a much bigger role in the future. Above all, the field of proteomics should focus more on chromatography.

LCGC: Are we approaching a fundamental limit in the number of proteins identifiable in a single-shot experiment? What is the next step to push the results even further?
MECHTLER: The sensitivity in chromatography through new column materials and also new mass spectrometers, which are being developed for single-cell proteomics, are the next steps.

LCGC: What are the attributes of an ideal LC-MS system from your perspective?
MECHTLER: Robustness is the most important factor in the daily handling of LC-MS. Many people

forget the interface between nano-HPLC and MS—the nano-spray source. Easy-to-handle, dead-volume-free components and a robust ionization with needles are the key factors for the next developments.

LCGC: What makes LC-MS measurements from single cells or small tissues such a promising area of research when considering the associated technical challenges?
MECHTLER: The biggest technical challenge in single-cell proteomics is working with small sample volumes such as 20 nanoliters. It is impossible to pipette 20 nanoliters by hand—it requires very special equipment. However, in cooperation with Cellenlon, we have found a reasonable solution.

LCGC: What advances are necessary to continue to push the future of low-input proteomics even further? What is required from LC separation performance and throughput?
MECHTLER: The current big question is whether it is necessary to reduce the diameter of the HPLC columns. This would, again, bring a dramatic gain in sensitivity; however, it should be also noted that the dead volume problem is much greater for columns with a 20-micrometer diameter. The dead volume problem can be avoided by filling the column directly into the nanospray needle. The second disadvantage of ultra-low nano-flow is the slow flow rate, which, in turn, decreases sample throughput.

LCGC: How will LC-MS proteomics develop further?
MECHTLER: LC-MS will become even more robust and easier to use in the future. In the future, gradients will become shorter, new acquisition methods, such as data-independent acquisition (DIA), will soon become standard. This will lead to many more medical projects where many samples are needed for statistical operation.

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LCGC: Imagine you have a nano LC-MS system that can provide confident and quantitative results with 10,000 proteins for one sample with the ability to analyze 1,000 samples per day. What would be your first research target?
MECHTLER: This would open many new opportunities in precision medicine to find biomarkers for different diseases. For example, we are working on kidney disease in conjunction with hemodialysis.