Episode 4: Making Ultra-Sensitive Analysis of Limited Samples and Single Cells a Reality

In the fourth episode of this six-part podcast, David Perlman, senior principal scientist and director of ultrasensitive proteomics at the Merck Exploratory Sciences Center, discusses where LC-MS proteomics has the largest impact, the need for single-cell proteomics, the promise single cells have on our day-to-day lives, and more.

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LCGC: Why did you decide to work in the field of proteomics, particularly using LC-MS technologies?
PERLMAN: I have a diverse background across multiple fields in biological, physical, and chemical sciences, but I find these areas are all mutually beneficial and mutually inform and enhance one another. They come together to help me better understand complex biological systems and build better working models.

LC-MS-based proteomics is the way to physically interrogate the chemical makeup of cells and tissues at a meaningful resolution. There's no better way to get at the protein composition of biological systems and examine their dynamics in normal, healthy states as well as disease states. Other analytical techniques measure proteins or other components within the cell, but most of these involve antibodies, which themselves have huge caveats and provide a very narrow tunnel vision of the epitopes they were raised to detect, or the techniques involved in direct measurements or measurements of species within the cells like RNA transcripts are often widely divergent from cellular phenotype. These other techniques can provide a lot of data, but these data can be biologically muddled or misleading. So, there is no other way for an unbiased quantitative measure of protein levels within cells and no better way to follow their dynamics across space, time, or stimuli than LC-MS-based proteomics. For me, it's the means to understand the mechanisms of life and roots of disease—that's why I'm drawn to it.

LCGC: Considering that LC-MS proteomics is just a tool, where is its largest impact?
PERLMAN: First, I disagree with the assertion that LC-MS-based proteomics is just a tool. I describe it instead as a rapidly developing transformative-enabling technology. It's a field or several fields, and it's improving rapidly year after year, which ultimately provides greater delivery of quantitative information about more species within cells but also greater impact on the way we develop drugs, understand disease, identify and vet new smarter drug targets, etc. It's an ever-increasingly powerful, fundamental technique with no less of an impact on biology and medicine than microscopy. It has already become a central component of biomedical research, and its use is only going to intensify over the coming years.

LCGC: What are the main technological challenges with moving cutting-edge proteomics research into real-life applications?
PERLMAN: This is a great question because the technology could be more widely used than it is. A huge barrier historically has been the difficulty in setting up and maintaining a state-of-the-art LC-MS-based proteomics platform, especially the finickiness and lack of robustness of the highest

Regarding throughput and proteomic depth, it depends on the type of experiment. As a rule of thumb, we shoot for 2,000 to 5,000 proteins quantified per single cell and do it at a rate of 1,000 or more cells a day to make it through meaningful experiments in a reasonable amount of time.

end LC-MS technologies. However, this has been changing rapidly: the more the technologies are made user-friendly and robust, the better. This gets them into more hands of creative experimentalists and biologists and not solely operated by specialized instrumentalists. Other than that, the impact of current proteomics on real-life applications is limited mainly by the inspiration and educated engagement of the researchers themselves, which I hope is growing year by year.

LCGC: You are one of the first who started to work on single-cell proteomics. Can you briefly explain the need for this type of application?
PERLMAN: For decades, we've been performing bulk proteomics experiments on biological systems or human tissues that don't behave like bulk uniform material on the cellular level. These have all been so-called "wearing-blender-type" experiments where you grind everything up into a murky stew and try to make some sense out of this sludge. But real biology is defined by cellular heterogeneity that is meaningful, functional heterogeneity between cells, both across space or location within a tissue, and across time and as a result of a response to stimuli, pathogen, or something internal to the cells such as cell-cycle control, etc.

Cellular heterogeneity is a feature of biology, a feature of disease, and a feature of the response to therapeutics, so it really matters. With single-cell proteomics, we aim to characterize cellular heterogeneity, so we can understand how real biological multicellular systems work. More specifically, single-cell proteomics can be used to help better understand the mechanisms of resistance to chemotherapy drugs so that smarter treatments or combinations can be developed. It can also be used to help define antigens that are co-expressed on the surface of cells, such as cancerous or other disease cells, which could be exploited for targeting these cells with something like a therapeutic antibody or drug antibody conjugate. Single-cell proteomics can be also used to understand how certain types of immune cells like macrophages change their phenotype from pro-inflammatory to anti-inflammatory states or vice versa during the course of disease and how this change could be reversed by therapeutics.

LCGC: Single-cell LC-MS proteomics relies on recent advances in LC, MS, labeling reagents, and smart data acquisition and data processing methods. How important are each of these contributors?
PERLMAN: Each of these components plays a role, as do innovations in sample handling and sample prep prior to the LC-MS. It's only been through putting all these components together that we've been able to see the first exciting results in the field. When you're grasping for every bit of material you can get out of a single cell and skirting the lower limits of detection, an increase in performance in any of these components translates into tangible gains in results.

I find having a reliable nano-flow LC that is capable of consistent performance at the ultra-low flow rates, which deliver the highest sensitivity into the instrument, is a critical factor. This has been a weak link in the past and a source of a lot of frustration, but everything plays a role in the end. All these factors are intensified by the single-cell proteomics application because of the large numbers of uniform high-sensitivity LC-MS runs that we have to acquire in each experiment just to make it through enough cells to get the answers we are looking for.

LCGC: Nano-LC coupled with MS is established as the gold standard in bulk proteomics. What are specific requirements for ultra-sensitive analysis specific to LC instruments?
PERLMAN: For ultrasensitive proteomics, we need ultra-low flow rates, so we need LC systems that reliably deliver run-after-run at these flow rates with smooth, uniform gradients. We need the highest performance columns as well for low-abundant samples that concentrate the analytes into the sharpest possible peaks. We also need autosampler features such as reliable pickup of small volumes and vial bottom-sensing so that the entirety of samples can be injected. It's also critical that the system fittings and liquid junctions are configured to produce true zero dead volumes and that the internal fluid paths are minimized. All this is necessary to reduce losses that otherwise bring down the experiment in a proverbial death by a thousand cuts.

LCGC: What can make LC-MS proteomics of single-cells a widely adopted technique? What are the requirements for analytical throughput and proteome depth?
PERLMAN: So far, it's been the domain of a few brave souls, and this is largely because of the artisanal nature of some of the initial sample preparation techniques and the extreme difficulty of setting up and maintaining an ultrasensitive proteomics platform at the utmost levels of performance. This has been a big barrier, even a psychological barrier for some, but this is changing. As the sample-prep techniques become well adapted for miniaturization and automation, and as the technologies for LC-MS become more robust, easier to use, and faster, this field will open up to more practitioners. I expect explosive growth in the next few years.

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quantified per single cell and do it at a rate of 1,000 or more cells a day to make it through meaningful experiments in a reasonable amount of time. We're currently approaching this depth but not yet at this throughput. We'd love to see numbers in the tens to hundreds of thousands of cells per day, but a thousand cells per day is likely attainable with existing or slightly improved technologies.

LCGC: LC-MS sensitivity is one of the key requirements for single-cell and limited sample amounts analysis. What are the other technical requirements?

PERLMAN: Injection carryover must be minimized otherwise every cell looks the same. Sample injections, LC gradients, and column performance have to be optimized and entirely uniform run-to-run so that the data align across large data sets. One key feature of single-cell proteomics is you have to have all your eggs in one basket. Every bit of every sample must be injected—you only have one shot to make the most of that sample. All your instrument parameters must be optimized for low-abundance materials to be able to extract as much data as possible out of this fleeting sample as it rushes by. For the same reason, the more onboard and on-the-fly instrument intelligence—which will greatly improve the quality of both shotgun and targeted single-cell LC-MS experiments in the future—that can be leveraged, the better to enhance the overall quality and depth of the data in the end.

LCGC: Analysis of single cells has a lot of promise that might directly affect each of us. How long do you think it will take to see this happen?

PERLMAN: Other single-cell analytical methods such as microscopy or flow cytometry have been a central component of biomedicine for decades and are impossible to divorce from the development of the medicines and vaccines that are currently on the market. My assertion is that because of its additional power and depth of molecular insight that is so closely tied with cellular phenotype, single-cell proteomics will be integrated rapidly into discovery and developmental-phase research for the next generation of medicines. I expect this transformation to occur within the next three to five years.