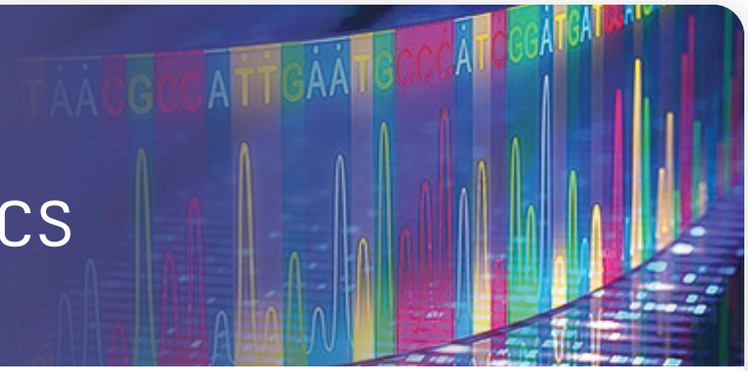
MARKET ANALYSIS:
SINGLE CELL PROTEOMICS



The Market Drivers For Single Cell Proteomics

The genetic, functional, or compositional heterogeneity of healthy and diseased tissues presents major challenges in drug discovery and development. Recent advances in high throughput technology-driven approaches allowed the global characterization of small tissue samples or organoids down to a single cell. These pioneering developments are already able to provide essential information about encompassing cellular heterogeneity and the complexity of cellular interactions. Single cell technology is one of the most attractive areas to invest in the life science industry, continuing to draw funds from venture capitalists. The impetus for improvements in single cell analysis is driven by the fact that interpreting population-average protein levels is fundamentally confounded when samples consist of heterogeneous cells.

Recent advances in Single Cell Analysis have elucidated substantial heterogeneity among seemingly identical cells at genomic and transcriptomic levels. These discoveries have spurred scientists to develop new tools that can investigate single cells from a broader set of 'Omics'. Proteomics and metabolomics, for example, are particularly interesting as they are closely correlated with a dynamic picture of cellular behaviours and phenotypic identities. Besides, proteomics uniquely addresses not only the expression side of the protein abundances, but also the degradation side, which contributes equally in steady state and which is dominant in certain processes, such as programmed cell death. Thus, looking at proteomics at the level of individual cells is important.

As such, there is a constant goal of delivering the deeper analysis and lowest cost per cell, which is keeping the innovation curve steep. Furthermore, the rate of improvement in Single Cell analysis by the 'Omics' field is expected to accelerate due to emerging technologies such as micro/nanofluidics and microfabricated interfaces for mass spectrometry.

Notable solution providers in the area of Single Cell Proteomics are Thermo Fisher and Bruker, who offer analysis tools that can be used throughout various Single Cell workflows for a variety of basic, translational, and clinical research projects.

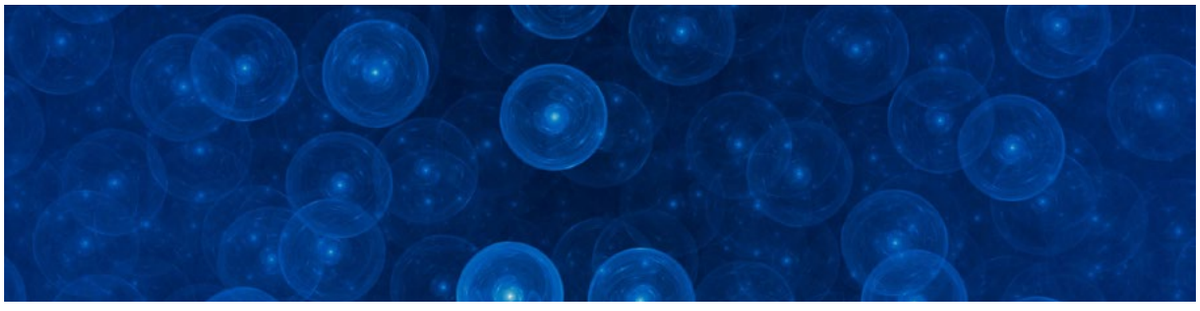
There is development work ahead for computational tools in Single Cell Proteomics, as well as the need for standards and benchmarking to make sure quantification is well-performed. Overall though, until labs can accurately analyse large numbers of single cells quickly and to a significant proteome depth, the resulting data will not be biologically interesting. Thus, there is an intermediate goal of analysing small numbers of cells.

What Is Single Cell Proteomics, And What Can It Do?

Proteomics is the large-scale study of proteomes in a biological context; in this case, a single cell. It was once assumed that cell populations were homogeneous, but the latest evidence shows that heterogeneity does in fact exist even within small cell populations. Interest in studying the assemblages of proteins at the single cell level arises from the fact that cellular heterogeneity within an isogenic cell population is common, due to stochastic gene and protein expression, and also due to the fact that at the moment of intervention (e.g., drug treatment) cells find themselves in different phases of cell cycle. The fate and physiology of individual cells is controlled by networks of proteins, yet our ability to quantitatively analyse protein networks in single cells has remained limited. Using Single Cell Analysis means that cell-to-cell variations can be represented more accurately, instead of the stochastic average. In addition, this level of analysis can also help to elucidate the interactions between individual cells, which has important implications for disease.

Individual cells have huge numbers of proteins, an estimated 10 billion copies of about <10,000 different gene products in mammalian cells, and being able to study even a small fraction of this total has helped scientists to make important biological discoveries. In addition, this kind of analysis is important as although Single Cell Genomics and Transcriptomics are at a more advanced stage of development, benefitting handsomely from polymerase chain reactions, they are not completely sufficient. For example, measuring transcript levels alone is insufficient for studying and understating many physiological and pathological processes, not least because the changes of protein levels human across tissues and cell differentiation are poorly predicted by the corresponding changes in mRNA levels. The usefulness of mRNA levels as surrogate for signalling activated by post-translational modifications is even more limited.

Also, as proteins are the functional molecules, their study provides a more accurate depiction of cellular conditions. Consequently, Single Cell Proteomics is needed to build a more complete picture of the assemblages of single cells, which can be summarised as helping to describe cellular heterogeneity and cellular interactions.

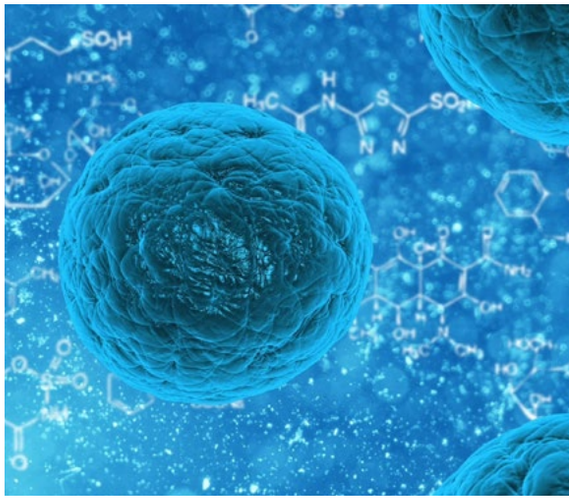


Potential And Challenges

Single Cell Proteomics is highly desirable but was considered impossible with the current level of mass spectrometry beyond special cases of very large cells. However, the approach using carrier proteome and tandem mass tag (TMT) multiplexing made it practical to analyse mammalian cells. Professor Nikolai Slavov and proteomics facility head Bogdan Budnik developed Single Cell ProtEomics by Mass Spectrometry (SCoPE-MS), and validated its ability to identify distinct human cancer cell types based on their proteomes. The second generation of this method, SCoPE2, has increased quantitative accuracy and the number of quantified proteins and cells over 3-fold while lowering cost over 3-fold. Mass spectrometry (MS) is poised to surpass the current limitations of Single Cell Proteomics by developing powerful methods to routinely quantify thousands of proteins and proteoforms across many thousands of single cells.

The greater understanding of cellular processes that Single Cell Proteomics can provide has been suggested as bringing many potential benefits and therapeutic opportunities, particularly in the area of stem cells. For example, Single Cell Analysis can target specific populations and therefore cell signalling pathways and networks for self-renewal and for differentiation. Single Cell Analysis also has the potential to more accurately identify cancer cells and their unique susceptibilities and drug resistivity, and could elucidate neural communication in unprecedented detail, potentially yielding new strategies to understand and treat neurological disorders.

However, Single Cell Proteomics is still in its infancy compared to other Single Cell omics technologies, and one major way Single Cell Proteomics lags behind other forms of Single Cell Analysis is the lack of high-throughput methods. Missing values in the detection of hundreds of proteins in Single Cell Proteomics represent a great challenge. Strategies for improvement include noise reduction, exploration of stochastic resonance as well as data-independent acquisition. In addition, this means that many challenges persist for computational analysis, and it remains challenging in computational proteomics to interpret the multiplexed quantification channels. There are also other challenges such as the low abundance of proteins in individual cells and the inability to amplify proteins. Additionally, the low multiplexing capacity of conventional assays, such as immunofluorescence and immunohistochemistry, presents another challenge.



Single Cell Proteomics, widely considered to be at least a decade away just a couple of years ago, has already made tremendous impact on the proteomics field, opening eyes to new possibilities and greatly improving the sample handling culture, as well as highlighting the limitations of existing approaches. It is estimated that hundreds of laboratories worldwide are now engaged in trying to conquer this area, with many starting their efforts with the SCoPE-MS approach. The latter is however unlikely the last word in the field. The race is on, and there is no doubt that someone exceptionally good – or lucky – will bring about the technology that would finally break through the critical benchmark. Indeed, Bogdan Budnik is currently working on exactly this, and hopes to put out an article this coming January that maps out the path to deep single cell coverage. Exciting times lie ahead.

A THANK YOU TO OUR EXPERT SPEAKERS, CONVERSATIONS WITH WHOM INSPIRED THIS PIECE AND HELP US CONTINUE TO PROVIDE YOU WITH HIGH-QUALITY, INDUSTRY-LEADING EVENTS

Bogdan Budnik, Proteomics Facility Head, Harvard Center for Mass Spectrometry

Dr. Budnik is Director of the Mass Spectrometry and Proteomics Resource Laboratory at Harvard University and has extensive experience with the characterization of proteins and peptides via mass spectrometry. His facility has in depth expertise with quantitative proteomics approaches. In his laboratory a new technique called SCoPE MS was developed for analysis of single cell proteomic samples.

Bogdan received a PhD in Analytical Chemistry from Southern Denmark University, before completing a post doc in Cardiovascular Proteomics at Boston University. Later, he gained experience as an Instructor within the Proteomics Center at Harvard Medical School, before in 2019 he took role of Principal Scientist at the Harvard Center of Mass Spectrometry. Here, he oversees all advanced projects in the fields of proteomics and metabolomics.

Nikolai Slavov, Professor, Northeastern University

Nikolai received his BS from MIT in 2004 and then pursued doctoral research in the Botstein laboratory at Princeton University, aiming to understand how cells coordinate their growth, gene expression, and metabolism. As a postdoc in the van Oudenaarden laboratory at MIT, Nikolai characterized trade-offs of aerobic glycolysis (also known as Warburg effect) and obtained direct evidence for differential stoichiometry among core ribosomal proteins, suggesting that specialized ribosomes regulate protein synthesis. His laboratory developed Single Cell ProtEomics by Mass Spectrometry (SCoPE-MS), and validated its ability to quantify thousands of proteins in single cells and classify them based on their proteomes.