Accessing Greater Dimensions of Biological Understanding Through LC-MS Proteomics

Proteomics has the potential to answer vital biological questions, optimise drug development and improve clinical care. Seeing these benefits, more researchers are embracing proteomic profiling, driving demand for ever-larger proteomics studies and the high-throughput techniques needed to deliver them. But, while available affinity-based methods can provide high-throughput proteomic analysis, their specificity may be low. Luckily, liquid chromatography-mass spectrometry (LC-MS) can effectively address the shortcomings of affinity-based approaches, offering both depth and throughput of data. This article discusses the evolution of high-throughput proteomics, the opportunities presented by LC-MS, and how companies are helping researchers to use this technology to drive the advancement of clinical research and care.

The rich information contained in biopsy samples can offer crucial insights into many different diseases. To access this information through biopsy sample profiling, next-generation sequencing (NGS) has traditionally been the method of choice, as it can pick up somatic mutations, helping to identify cancer-related genomic alterations. However, much of the data gathered from mutations is inconclusive, meaning it is not always possible to link and correlate genomic data to direct the best cancer treatment. Now, biopsy profiling can be performed using deep proteomics capable of near-proteome-wide coverage, providing a whole new dimension of understanding regarding the impact of genomic changes. This is because, while genomics can identify genomic variants, proteomic profiling elucidates phenotypic information, revealing more detail about biologically meaningful changes that result from these genomic variants. With the ability to characterise drug action and diseases in a way that can be used to better direct treatment options, deep profiling using a proteomic approach represents a powerful tool to improve healthcare.

The Benefits of Proteomic Profiling

Within the past decade, proteomic profiling has rapidly expanded due to methodological advances and application to exciting new research areas. But what exactly is proteomic profiling?

Proteomics refers to the in-depth study of the protein complement of a genome. Typically, proteomic profiling takes one of two forms – unbiased or targeted – with each providing different insights invaluable for drug discovery and clinical care. Unbiased proteomics – also known as discovery proteomics – explores all proteins detectable in a sample, without predefining specific proteins of interest. This makes unbiased proteome profiling exceptionally useful for supporting drug discovery through identification of new drug targets, exploring modes of action (MOA) and unveiling novel biomarkers. Targeted proteomic profiling, on the other hand, identifies and quantifies ions of specific mass at a specific time, and effectively filters out background noise. This makes the method highly useful for measuring predefined proteins and proteoforms, even in complex samples. Importantly, the ability of targeted proteomics to quantify a predefined panel of proteins makes the method useful in clinical care. Targeted proteomic profiling is already being used in clinical trials for pharmacodynamic biomarkers to inform dosing decisions.[1]

With such benefits, it is clear that proteomic assays will be increasingly used to support and enhance future clinical trials to provide a deeper understanding of how the full proteome changes in disease. Demand for proteomic profiling is therefore increasing, with a major focus on larger studies that can tackle the dynamic nature of the proteome. This means new advances and technologies – particularly high-throughput methods – will be required.

Challenges in Proteomic Profiling: Depth, Throughput and Dynamic Range

Should you choose depth or sample throughput? This was traditionally one of the biggest challenges researchers faced when taking a proteomic approach, because conventional approaches are generally able to provide only one or the other. However, both factors are vital to truly understand biology. As an additional challenge, the dynamic range problem in proteomics needs to be addressed – a single cell can have six orders of magnitude of dynamic range from the lowest to the highest copy number protein (Figure 1), yet current techniques are not sensitive enough to accurately quantify miniscule amounts of protein. In other omics-based approaches, such as genomics, this problem can be overcome by using an amplification technique, but equivalent solutions are lacking in proteomics.

Solutions for High-throughput Proteomic Profiling

Several companies have addressed the high-throughput problem by using affinity-based panels. These panels use aptamers or highly optimised antibodies to bind epitopes in the target tissue. In more standardised approaches, these assays can be combined with a readout using NGS devices. In some cases, such methods offer high throughput that enables studies of over 10,000 samples. Despite this, affinity-based methods encounter a wealth of significant drawbacks. First, because they don't provide peptide-level readouts, but only measure the presence of a short epitope on a protein, they can suffer from low specificity. In the event these short target epitopes become inaccessible due to conformational changes or other molecular interactions, further specificity and reproducibility issues emerge. Second, because these methods depend on antibodies or aptamers binding to known protein domains for detection and quantification, they can only offer a targeted, panel-based approach – unbiased exploration is simply not possible. And, without the ability to uncover unknown or less well-studied proteins, their value in discovery applications is severely restricted. Such assays are also confined by the breadth and specificity of the panels and reagents that are available.

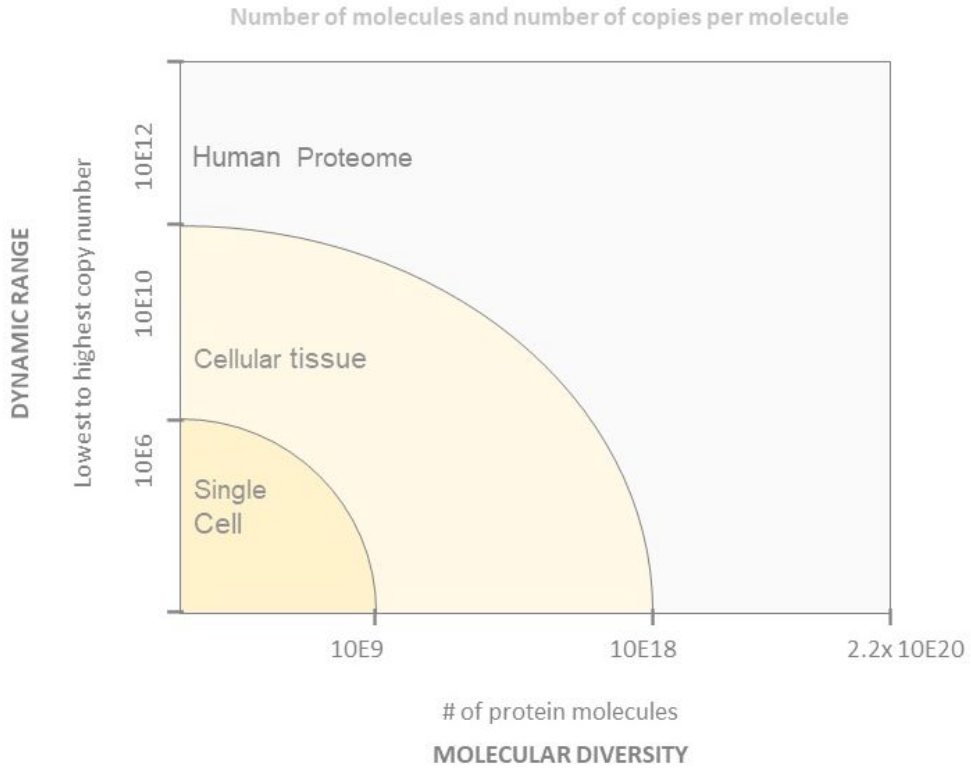

Figure 1: Graph highlighting two critical analytical challenges in proteomics – the broad molecular diversity and large dynamic range of protein molecules and proteoforms at the single cell, cellular tissue and human proteome levels.

Additionally, affinity-based approaches are only applicable to blood-based samples, and no multiplexes or affinity-based methods of a reasonable size for solid tissues are readily available for these yet. This is because non-blood tissue samples are normally preserved as formalin-fixed, paraffin-embedded FFPE tissues, which are notoriously difficult to analyse since formalin can induce protein structural changes and cross-link modifications.[2]

Companies have tried to develop protein sequencing technologies to realise high-throughput proteomics. For a tissue of interest, the proteins or peptides are initially immobilised on a surface, and a degradation approach is then taken to enable the read-off of a sequence of this immobilised target. While this technique shows some promise, the technology is still in its infancy – only fundamental proof-of-principal data is available to-date. It is, therefore, difficult to assess the impact of this approach, and the method is far from ready to be applied to complex proteomes.

LC-MS: Delivering Deep Profiling and High Throughput

A technique that is showing great promise for high-throughput proteomic profiling is liquid chromatography-mass spectrometry (LC-MS). Today, LC-MS is routinely applied to high-throughput analysis of small molecules and is now increasingly being deployed for deep proteomics. With more recent advances in LC-MS instrumentation, the throughput and scale are changing.

LC-MS methods can address and explore almost all the important dimensions of a protein – from functional aspects, such as post-translational modification (PTM), to structural elements, such as cross-linking, and this can be done at the peptide level across the entire proteome. Since LC-MS is tissue-agnostic, any type of tissue or body fluid from any species can be investigated. And, vitally for proteomics, LC-MS is sensitive enough to detect both lower-abundance and higher-abundance proteins, solving the dynamic range challenges that other techniques face.

In stark contrast to affinity-based methods, LC-MS proteomics lends itself well to both unbiased discovery research and pre-defined targeted protein panels. The clinical transferability of the technology ensures that the insights generated in early-stage, unbiased discovery research can be applied in the development of targeted panels for absolute protein quantification in clinical settings.

Despite the benefits, there are key challenges to accessing high-throughput deep proteomics with LC-MS – most notably, the need for a wide skill set that encompasses sample preparation, chromatography, instrument handling and maintenance. On top of this, LC-MS provides multi-dimensional (and often convoluted) data that can be tricky to understand, so extracting useful information from LC-MS requires expert analysis.

It is also challenging to accurately design LC-MS-based studies to obtain the relevant outcome, as proteomics results in finding unexpected and dynamic changes – not just in detecting the presence or absence of a protein. For many laboratories a related hurdle is applying the correct experimental method to the sample, although standardisation of methods can address this shortcoming.

Several other complicating factors are often faced, particularly when scaling high-throughput LC-MS workflows from hundreds to thousands of samples. For example, one

of the main bottlenecks is implementing a reproducible, high-performing and robust method for separations. Additionally, as a single instrument does not have the capability to run the many thousands of samples needed, multiple instruments must be carefully maintained and controlled to ensure the results are comparable.

Simplifying LC-MS-based Proteomics to Broaden Accessibility

In the face of these challenges, several companies are working hard to support researchers to adopt high-throughput LC-MS proteomics. For example, some companies are strongly focusing on automating sample preparation – something which is common for small molecule analyses, but has rarely been used in proteomics until now. Additionally, scalable software has been developed to handle large datasets, and experiments that include randomisation and quality control are being carefully designed – something that has been a roadblock for many non-specialist companies trying to scale their deep proteomics workflows.

Biognosys: Attaining meaningful biological insights through LC-MS proteomics

One company that has made significant steps towards LC-MS-based high-throughput proteomics is Biognosys, a global organisation specialising in large-scale proteomics solutions. In a recent pan-cancer clinical study performed on plasma samples from patients affected by lung, prostate, breast, colorectal and pancreatic cancer, the team at Biognosys reported the highest performance to date for single injection methods across hundreds of samples.[3] Here, novel biomarker candidates in colorectal and pancreatic cancer were identified, known biomarkers were identified and validated, and new models were developed to classify the disease state.

In a separate study, Biognosys showed MS-based proteomics to be excellent at analysing complex samples preserved using FFPE – the study used more than 1,000 FFPE tissue samples and demonstrated performance rivalling fresh frozen tissue analysis.[4] Furthermore, in a joint study with Indivumed,[5] Biognosys used an optimised, semi-automated workflow to deeply characterise the proteome and the phosphoproteome of matching normal and tumor samples, obtaining a great depth of analysis. This was the largest protein expression and phosphorylation study ever performed, using thousands of clinical samples. Application of deep tissue-based proteomics in clinical samples was demonstrated in one of the company's pilot studies, where an unprecedented depth of 13,000 quantified proteins was reached in lung tissue.[6]

Building on this is Biognosys' pioneering work in the newly emerging field of immunopeptidomics. To date, the team has analysed lung cancer needle size biopsies, recovering thousands of immunopeptides of excellent quality and reproducibility,[7] and through collaboration with Johns Hopkins University School of Medicine, opened a window into the aging brain, showing the potential of MS-based proteomics in analysis of cerebro-spinal fluid.[8]

Specialist companies such as Biognosys are showing the unmatched precision and depth of LC-MS-based methods in deep proteomic profiling and highlighting its potential to revolutionise the development of personalised clinical diagnostics. Many of the biomarkers identified from studies such as those above came from low abundance regions, and so would have been missed by shallow profiling. Furthermore, such studies have rendered scaled-up deep plasma studies that include a protein depletion step accessible, where they had previously been limited to low throughput.

Maximising Proteomic Impact

LC-MS proteomics closes the chasm between depth and throughput, with the capability to analyse more than 10,000 proteins deeply in a single run. However, this doesn't mean affinity-based methods should be ruled out. For example, affinity methods can be more sensitive in materials such as plasma, and can measure some cytokines that aren't currently within reach of LC-MS. Because both methods have their own strengths and can offer insight into different types of protein, they can be thought of as complementary: initial screens can provide first-level insight, and LC-MS can play an important role in answering more targeted questions.

At its core, proteomics is the study of proteomes. Often, it is approached purely from the perspective of quantifying proteins, but in reality, it can provide so much more. Proteomics, when its full potential is unlocked, provides rich insights into proteins across multiple dimensions – from levels of expression to functional and structural diversity in different biological contexts. In light of this, and to accommodate its true scope, proteomics should be redefined as the multi-dimensional, at-large characterisation of proteomes. Ultimately, it is the understanding of how proteomes function that is essential to understanding biology – not just their presence and abundance.

LC-MS in Proteomics: A Bright Future

The proteomics journey is just beginning. Proteomics data obtained through LC-MS gives phenotypic functional insights far beyond the depth of information that genomics can offer – and the throughput of samples may outpace what's possible with genomics sequencing runs in the future. We anticipate the volume of proteomics data will continue to grow tremendously, opening many exciting opportunities to accelerate drug development and better enable precision medicine.

Nevertheless, it is vital that the data obtained is made useful. As methods to access high-throughput information are developed, new ways to understand and analyse the data from LC-MS at scale will be needed. Whichever way this is achieved, addressing biological questions from a proteomic perspective will give deep and meaningful answers to solve biology's most challenging problems.

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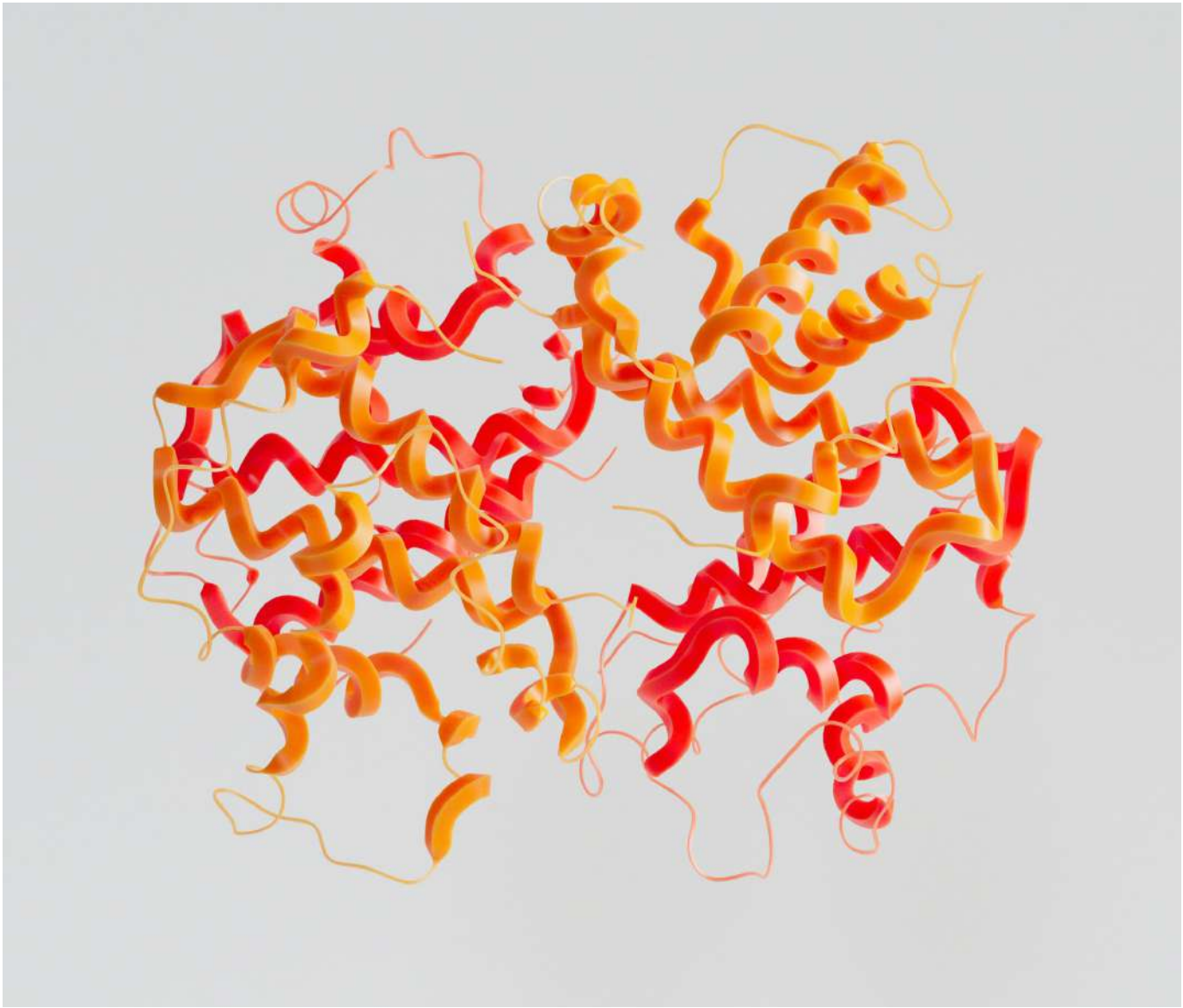

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