Technology Developments in Functional Proteomics

A primary goal of biomedical research is to figure out what the thousands of proteins and their hundreds of thousands of variants do in each cell; recent advances in functional proteomics technologies are bringing the goal of understanding and harnessing the human proteome into the realm of the possible.

By Katherine Austin at Frost & Sullivan

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Proteins are the workhorses of the cell, playing a part in everything from energy transport and metabolism to cellular communication. We now understand the role of defective proteins in causing disease; however, although the human genome has been sequenced, only a fraction of the proteins implicated in disease have actually been identified. Less than 2% of all clinical diseases have been attributed to a single protein defect.

Proteomics research is undergoing explosive growth. Nearly every major biotech and pharmaceutical company has set up a proteomics programme. Functional proteomics is playing a major role in drug discovery, biomarkers, molecular diagnostics and antibody therapies.

As its name implies, ‘functional proteomics’ research is focused on the determination of protein function, both in terms of individual proteins and in terms of how they interact with one another. Functional proteomic technologies enable the identification of functions for previously uncharacterised human proteins, the discovery of other proteins with which they interact and the development of an understanding of their involvement in important disease pathways.

CELL SIGNALLING PATHWAYS

Each protein and its interacting partners form a network which reads like a map. Understanding cell signalling pathways and the manner in which cells communicate will provide greater understanding of disease mechanisms, revealing potential drug targets that are more likely to be successful. Once drugs are developed, pathway knowledge is critical in understanding the downstream effects of drug treatment. Better knowledge of pathways will lead to fewer adverse side effects.

Researchers previously assumed that proteins were isolated entities, acting independently of surrounding proteins. Today, we know that numerous cellular processes – while controlled and carried out by proteins – are not the result of individual protein actions. Rather, they are carried out by protein ‘machines’, or aggregates of 10 or more proteins. Protein-protein interactions are thus not only one-to-one, or pair-wise; in some instances, as many as 50 proteins can interact to form one large complex. These types of protein-protein interactions make attractive drug targets for the pharmaceutical and biotechnology industries.

Protein-protein interactions are also crucial in investigating intercellular and intracellular signalling pathways. For example, signals from the exterior of a cell are transmitted to the inside of that cell by protein-protein interactions of the signalling molecules via receptors, protein-kinase cascades, and so on. Signalling pathways regulate cellular characteristics and processes such as physiology; proliferation, changes in shape and motility; differentiation, adhesion and intercellular interactions.

Protein-protein interactions are considered to be responsible for the development of pathological processes such as prion diseases and Alzheimer’s disease. These interactions are demonstrating great potential as new targets for novel drug.

Recent advances in functional proteomics technologies are making the aim of understanding and harnessing the human proteome possible. Several examples are discussed below, including: a new sample-preparation technique that reduces protein preparation time from 18 hours down to 15 minutes; a protein microarray fabrication technique that will soon allow as
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many as 10,000 spots to be arrayed on a single chip; and a platform for high-throughput mass spectrometry that will allow the analysis of complex protein mixtures to be miniaturised and accelerated.

If we could monitor all of the proteins in a cell simultaneously, it would enable us to understand the pathways that the cell uses to maintain homeostasis or growth under different physiological conditions. However, this is currently a task beyond the capabilities of current technologies. Functional proteomics makes the scope of the problem more manageable, by narrowing the field to specific proteins or pathways.

**DRUG DISCOVERY AND DEVELOPMENT**

Aside from basic research, the primary – and certainly most potentially lucrative – application for functional proteomics research is drug discovery and development. Functional proteomics can help in target identification and validation; the hope is that this will ultimately lead to new drugs that target specific diseases.

Functional proteomics is playing a major role in current drug discovery efforts. Essentially, all of the processes a cell carries out are linked in a complex network of interactions between proteins, DNA, carbohydrates, and other molecules and atoms. Thus a protein’s ultimate function is not necessarily a direct effect upon another molecule; it can be to regulate or otherwise influence a process several steps removed. This web of interactions is important in drug discovery because, among other things, understanding it may help researchers find specific points in a given biochemical process that can be influenced by a drug, while producing minimal side effects.

Leads for pharmaceutical compounds can be identified and optimised by studying their effect on levels of protein production and activity. These drug leads include not only therapeutic proteins, but also small-molecule drugs that bind to target proteins. These proteins are often regulatory mediators of complex cellular pathways, such as the signal transduction pathways responsible for diseases such as allergies and asthma, immune disorders and cancer. For example, protein kinases are a major category of potential drug target currently under intense scrutiny.

The market is desperately in need of new, less invasive screening and diagnostic tests. Screening tests based on protein biomarkers that have been identified through functional proteomics can detect cancer and other disorders at an early stage – improving the chances of patient survival.

Monoclonal antibodies are another major category of therapeutic molecules under intensive development; these can have a shorter time-to-market and higher success rate compared with traditional pharmaceuticals. Monoclonal antibodies represent one of the strongest growth areas of the therapeutic proteins market sector. (There are over 100 antibodies in clinical trials, approximately 50 of which are human monoclonal antibodies.) Functional proteomics is aiding researchers in identifying therapeutic targets against which monoclonal antibodies may be directed.

In another example, researchers at the University of Texas Southwestern Medical Center have used functional proteomics to discover that mitochondria contain a protein that triggers the immune system to attack viral invaders. Understanding how boosting mitochondrial antiviral signaling protein (MAVS) function causes cells to resist viral infection could have important clinical implications. Treatments that enhance the activity of MAVS may prove to be useful in boosting immunity against viruses.

As an example of functional proteomics in toxicity screening, technology developed by Serenex Inc was used to rescue a stalled Phase I drug candidate called Geldanamycin – a cancer therapeutic that was found to inhibit the growth of transformed cells in culture but also exhibited severe toxicity. While the therapeutic target was known, the protein responsible for the toxicity was not. In addition to confirming the efficacy target, Serenex was also able to determine that the inhibition of ADE2 (a protein involved in purine biosynthesis) was responsible for Geldanamycin’s toxicity. Based on these findings, new compounds were selected for development, and this programme was successfully outlicensed and is in Phase II development.

**MARKET DRIVERS**

With the development of a single drug costing an average of $500 million, and only 30% of approved drugs even recovering these costs, pharma is under growing pressure to streamline and optimise the identification of novel therapeutics. To address this need, pharmaceutical companies are seeking methods for collecting large amounts of data in order to dissect complex, disease-causing interactions between proteins within the cell, and between proteins and the extracellular environment.

Factors that are driving the growth in both genomics and proteomics include the following:

1. Pressure on Big Pharma to accelerate drug discovery
2. Structure-based design is proving successful in drug discovery
3. An increasing demand for molecular diagnostics and personalised medicine
4. Robust drug pipelines increase company valuation

**ENABLING TECHNOLOGIES**

Enabling technologies for proteomic studies in general include the following:
2D gel electrophoresis, Spectrometry, NMR, Surface Plasmon Resonance and Phage display
- Immunohistochemistry and Affinity chromatography
- Activity-based probes
- Sample preparation

Technologies that are especially useful for functional proteomics include phage display, protein inactivation (either by genetic techniques or the use of inhibitory compounds), surface plasmon resonance and yeast 2-hybrid analysis.

Specific advances in enabling technologies include the ‘Proteomics Grade Trypsin Spin Column’ from Sigma Aldrich. With this product, the company says, waiting on trypsin to digest samples is no longer a major bottleneck prior to mass spectrometry analysis. The Trypsin Spin Column reduces the time to digest complex protein mixtures from as much as 18 hours with conventional methods to only 15 minutes.

A device developed at the Georgia Institute of Technology will reduce the time needed to analyse proteins, including their structure and interactions with drugs and medical devices. The researchers have developed a nanospray platform for high-throughput mass spectrometry that will allow the analysis of complex protein mixtures – such as those found in human serum – to be miniaturised and accelerated. The platform is called an ‘array of micromachined ultrasonic electrospray’ or ‘AMUSE’, and it is possible that AMUSE devices could be made cheaply enough – a few dollars apiece – to be disposable.

Using current research methods and materials, it has been possible to attain protein microarray densities of up to about 800 spots. Harvard Medical School (HMS) and the Harvard Institute of Proteomics have entered into a collaborative agreement with nanotech company Lumera. Under the terms of the agreement, Lumera and HMS will develop a next-generation silicon chip substrate that combines Lumera’s ‘NanoCapture’ technology with HMS’s ‘NAPPA’ methodology (for ‘nucleic acid programmable protein arrays’). The resultant 10,000-spot very high-density protein arrays are expected to significantly increase the speed of proteomic analysis.

The NAPPA technology starts with a printed cDNA array and generates a self-assembled protein array using a combination of chemistries and biological methods. A cell-free expression mix produces proteins from the printed genes; the resulting expression product is immobilised on a surface capture system providing for fresh, easily definable, protein arrays made directly from cDNA libraries, and easily printed with commonly available equipment and methods. The arrays can be stored and easily handled, very much like today’s commonly used expression arrays. Protein is produced and captured only when the user is ready to use the array.

TECHNICAL CHALLENGES

Unfortunately, proteins are far more complicated than DNA. Technical problems have plagued functional proteomic R&D on every front. Thus there are a number of challenges that technology developers will need to address before functional proteomics is accepted as a standard, high-throughput approach.

Current technical restraints in functional proteomics include:

1. Limited sample quantity for analysis (blood, tissue, and so on)
2. Throughput – while called ‘high’ – is still too slow
3. Assay sensitivity (detecting low-abundance proteins is still a challenge)
4. Validation and reproducibility (lack of accuracy, lack of standardisation)
5. Complexity (the proteome, and all of its interactions, are extraordinarily complex)

The development of various proteomic kits and targeted solutions is fraught with pitfalls, many of which are associated with the vast range of chemical and physical properties associated with different proteins. Some of these include: the complexity of the protein-interaction map; a lack of standardisation, which makes it difficult to compare or validate results from different labs; and a lack of protein-specific capture agents, such as antibodies, which are essential for techniques such as ‘protein chip’ microarray analysis.

New tools and research strategies are needed on all fronts for protein expression, purification, screening and measuring protein interactions. Standard methods have been too slow and labour-intensive, and it is essential that automation and throughput be increased. Assay sensitivity – the ability to detect low-abundance proteins – must also be increased. In addition, standardisation of the various assay technologies is paramount, so that results can be accepted as reliable for pharmaceutical applications.

CONCLUSION

The Human Genome Project has provided the genetic sequence for all of the proteins in the human body. A primary goal of biomedical research is to figure out what these thousands of proteins and their hundreds of thousands of variants do in each cell. Enter functional proteomics.

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