Protein Progression

Peptide and protein microarrays have advanced significantly in quality, paving the way for innovations in bioanalytics. However, microarrays are not without their complications

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Microarrays offer a well-established bioanalytical format for high-throughput studies of many different biomolecules. In the field of peptide and protein microarrays, great progress has been achieved in immobilising large numbers of native target molecules, such as recombinant human proteins or peptide libraries to microarray surfaces. With an increased content at high quality, protein and peptide microarrays offer new possibilities in proteomics and pharmaceutical R&D. Simultaneously, label-free sensing has evolved from highly sophisticated, but low-throughput, to powerful multiplexed screening tools. The combination of these advancements opens a new field of bioanalytics.

Analysis at a Glance

Proteins are involved in almost any process of a living cell. Therefore, proteomics is a key discipline of modern life sciences today. Furthermore, protein-protein interactions of human cells (the so-called human interactome, see Figure 1) and the interactions of proteins with other biomolecules are key for understanding any physiological process and manipulating it (eg, by a pharmaceutical or biopharmaceutical drug).

Protein scientists have developed different bioanalytical approaches based on proteins immobilised in a microarray format. Here, the focus lies on protein microarrays representing a set of species-specific (in most cases, human) proteins to study the binding of a specific molecule or sample to these target proteins in a single assay. The trend is towards a fraction up to the complete genetically encoded proteome.

Simply, protein microarrays can be created in two different ways. The first is to immobilise the offline-produced protein species using a robot/printer to disable the different proteins spatially resolved. The second is based on technologies which synthesise the proteins directly from its DNA information in close proximity to the subsequent protein spot by *in vitro* expression. These proteins are then captured onto the surface to form spots of a protein microarray. The bottleneck of protein microarrays is the availability of native and functional immobilised proteins, ideally representing one-to-one copies of the functional protein species of a cell. Human proteins expressed (eg, those in *Escherichia coli*) do not meet this ideal situation, but this method allows for the expression of many thousands of proteins at reasonable effort. Protein production in eukaryotic cells is much more sophisticated, and can provide the highest quality of proteins in terms of correct folding and post-translational modifications. A drawback for both offline protein production strategies is that they suffer during the mentioned immobilisation process to the surface, which can disturb the structure and functionality of the proteins.

As a result of these drawbacks, other technologies, such as the nucleic acid programmable protein array (NAPPA) approach, have been developed. This method tackles these problems by capturing proteins from *in vitro* translation in spot resolution without the need for a protein printing process. This is made possible by the DNA arrays, which are used as the information carrier during the expression step. Furthermore, NAPPA opens the door to a much higher degree of customisable protein content. The same underlying idea is also available, as the so-called microarray copying technology, with the additional advantage that multiple identical microarrays can be produced in more efficient workflow from one template (1).

Recently, Sengenics came up with a new variation of the classical protein array approach based on offline-produced molecules (2). Here, the functionality of the proteins, which is directly linked to its correct folding, is verified by a reporter affinity tag, which itself only can serve as an

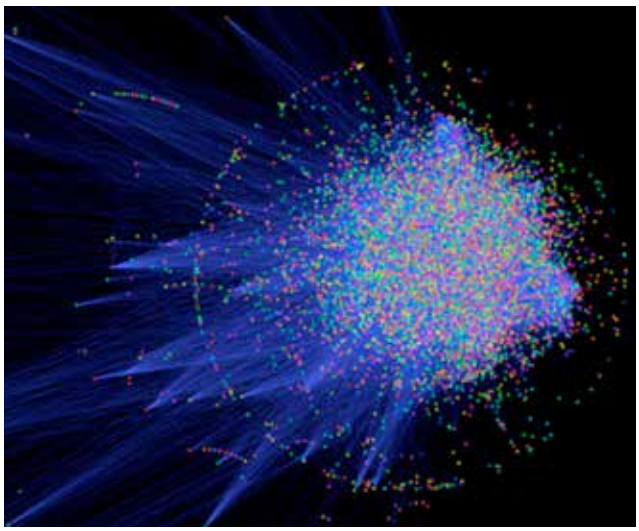

Figure 1: Picture visualises the human protein 'interactome', which is the total of all protein interactions in a human (human interactome network visualised by Cytoscape 2.5 by Keinono is licenced under CC-BY-SA-3.0)

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immobilisation tag when the folding is correct. Another approach recently published describes the production of a G protein-coupled receptor (GPCR) array by a technology called virion display, which offers a new bioanalytical platform for the development of GPCR-targeted drug candidates (3). This race of technologies provide high-quality protein microarrays, which represent a majority of the human proteome in a single microarray, and enable proteome scientists to trial new applications, like studying the mode of action of a drug or potential cross reactivities of a lead biopharma candidate due to off-target binding. Furthermore, diagnostic applications and other proteomic studies involving, for example, serum samples will contribute a valuable piece of knowledge to our understanding of diseases such as cancer.

Label-Free Technologies

Originating from the most frequently used optical principles in label-free detection, several approaches could be transferred to multiplex-capable imaging methods. The dominating approaches can be classified into refractometry, reflectometry, and scattering. In the latter case, Raman and surface-enhanced Raman scattering offer the advantage of structural information, which can be gained from a given area (or spot) on a transducer surface. This approach can be used to identify label-free microorganisms in a microarray lay-out, for example (4).

Most commercially available label-free technologies offering imaging capability make use of refractometry approaches. These are based on the detection of refractive index changes due to binding of molecules onto a transducer surface. The dominating technology is surface plasmon resonance imaging (SPRi). Here, a planar transducer system is coated with a thin metal layer (typically gold). This enables the detection of refractive index changes caused by a molecular binding event, such as a change in the resonance frequency of the plasmonic vibrations of the electrons in the metal layer. The advantage of SPRi, compared to scattering methods, is the easier realisation of imaging readers at higher resolution, offering a higher degree of necessary multiplexing for many microarray applications. However, besides the drawback of the essential metal layer, which makes the surface properties of a transducer much different from the situation on a microarray surface, multiplexing still comes with technical limitations. Nonetheless, SPRi systems have demonstrated their great potential in different application areas, such as antibody detection in complex matrices (5). Although GE Healthcare, with its brand Biacore, discontinued its SPRi product line quite a while ago, other companies, such as Carterra, IBIS, Plexera, or Horiba, are still active in this market (6-10).

Reflectometry-based methods offer the advantage that they do not need a metal layer on the transducing layer system. The signal formation just employs the reflection of light at interfaces between materials with different refractive indices. Ellipsometry is a version of reflectometry, which was also transferred into an imaging method. The set-ups typically make use of the phenomenon of total reflection of light at the interface between a transducer and a tethered biolayer. The read-out is similar to the situation of reflectometric interference spectroscopy (RIfS), with the difference that the RIfS signal, as a measure of the optical thickness (product of refractive index and physical thickness), is much more stable towards temperature changes. The realisation of a RIfS set-up with imaging capabilities is technically challenging due to the needed spectral information from each point from a transducer surface. The recently proposed single colour reflectometry (SCORE) approach circumvents this drawback and, therefore, combines the advantage of robustness towards temperature changes and simplicity of transducers without metal layers (11). Furthermore, SCORE can easily be scaled to high density microarray layouts, resulting in a much higher degree of multiplexing compared to SPRi.

However, why should a user change from the well-established and comparatively cheap fluorescence-based read-out to a label-free technology? The answer is rather simple. One big advantage is the fact that no label is necessary, which opens the door to much easier test formats (eg, direct test format with no need for washing or staining steps). The binding of molecules from a sample to ligands immobilised in an array format can be followed in real time, without a subsequent incubation with staining reagents being necessary. Furthermore, the limitations in multi-colour staining do not apply. The second – and perhaps, more important – advantage is the possibility of performing kinetic assays to extract information on kinetics and affinities for all reactions of a microarray experiment.

Combining Imaging and Analysis

When label-free technologies started evolving towards imaging-based systems, not enough applications demanded the label-free multiplex approach – at least, not for the described protein microarrays. Today, scientists have started to realise the high synergistic potential of this method

combination. Major expectations of this fruitful technology combination are, for example, a new powerful toolbox in basic proteomics research and an accelerated lead characterisation process of biologics. Both application areas will benefit from a much higher data quality and depth of information in a test environment, which is much closer to native conditions compared to other state of the art labelled technologies. The key of success is now combining the most suitable and powerful variants of protein arrays and imaging-based label-free detection technologies into new system solutions.

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