Advance Laboratory Techniques

Lecture 5

Protein Microarrays

A protein microarray (or protein chip) is a high-throughput method used to track the interactions and activities of proteins, and to determine their function, and determining function on a large scale. Its main advantage lies in the fact that large numbers of proteins can be tracked in parallel. The chip consists of a support surface such as a glass slide, nitrocellulose membrane or microtitre plate, to which an array of capture proteins is bound. Probe molecules, typically labeled with a fluorescent dye, are added to the array. Any reaction between the probe and the immobilised protein emits a fluorescent signal that is read by a laser scanner. Protein microarrays are rapid, automated, economical, and highly sensitive, consuming small quantities of samples and reagents. The concept and methodology of protein microarrays was first introduced and illustrated in antibody microarrays (also referred to as antibody matrix) in 1983 in a scientific publication and a series of patents. The high-throughput technology behind the protein microarray was relatively easy to develop since it is based on the technology developed for DNA microarrays, which have become the most widely used microarrays.The typical image of protein microarrays is shown as Figure 1.

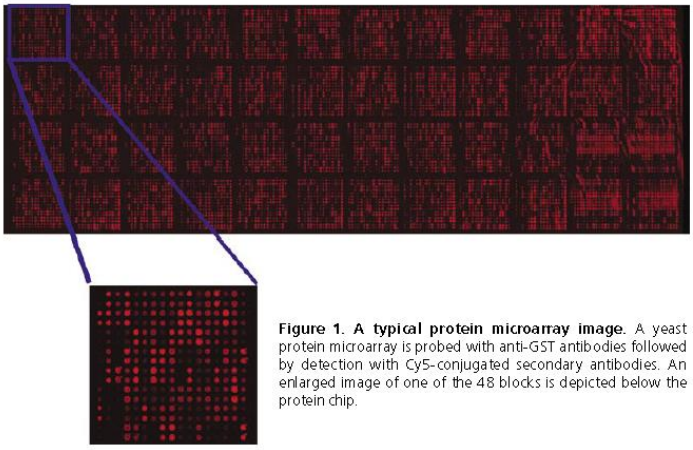


Figure 1. A typical protein microarray image. A yeast protein microarray is probed with anti-GST antibodies followed by detection with Cy5-conjugated secondary antibodies. An enlarged image of one of the 48 blocks is depicted below the protein chip.

Types of arrays

There are three types of protein microarrays that are currently used to study the biochemical activities of proteins.

Analytical microarrays : are also known as capture arrays. In this technique, a library of antibodies, aptamers or affibodies is arrayed on the support surface. These are used as capture molecules since each binds specifically to a particular protein. The

array is probed with a complex protein solution such as a cell lysate. Analysis of the resulting binding reactions using various detection systems can provide information about expression levels of particular proteins in the sample as well as measurements of binding affinities and specificities. This type of microarray can be used to monitor protein expression levels or for biomarker identification, clinical diagnosis, or environmental/ food safety analysis. In order to increase affinity and specificity, analytical microarrays usually employ a signal amplification system and sandwich assay format, in which the first antibody is spotted on the array and then a captured antigen on the chip is detected with a second antibody that recognizes a different part of the antigen (Figure 3).

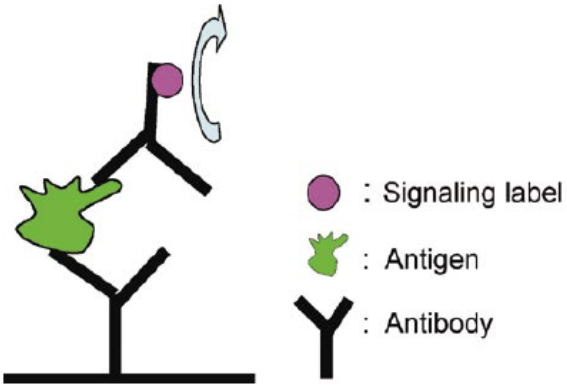


Figure 3. A sandwich assay format. A multivalent antigen is first caught by a capture antibody immobilized on the surface and then detected by a detection antibody. The label is usually tagged on the detection antibody and can be further amplified.

Functional protein microarrays : (also known as target protein arrays) are constructed by immobilising large numbers of purified proteins and are used to identify protein-protein, protein-DNA, protein-RNA, protein-phospholipid, and protein-small molecule interactions, to assay enzymatic activity and to detect antibodies and demonstrate their specificity. They differ from analytical arrays in that functional protein arrays are composed of arrays containing full-length functional proteins or protein domains. These protein chips are used to study the biochemical activities of the entire proteome in a single experiment.

Reverse phase protein microarray : (RPPA) involve complex samples, such as tissue lysates. Cells are isolated from various tissues of interest and are lysed. The lysate is arrayed onto the microarray and probed with antibodies against the target protein of interest. These antibodies are typically detected with chemiluminescent, fluorescent or colorimetric assays.

Applications

There are five major areas where protein arrays are being applied:

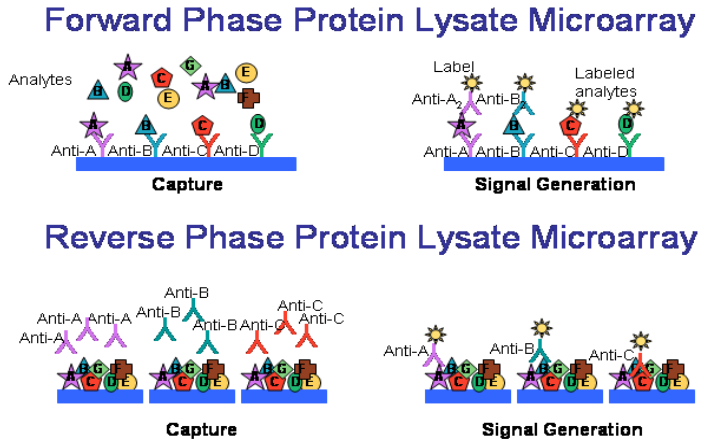
Diagnostics : involves the detection of antigens and antibodies in blood samples; the profiling of sera to discover new disease biomarkers; the monitoring of disease states and responses to therapy in personalized medicine; the monitoring of environment and food.

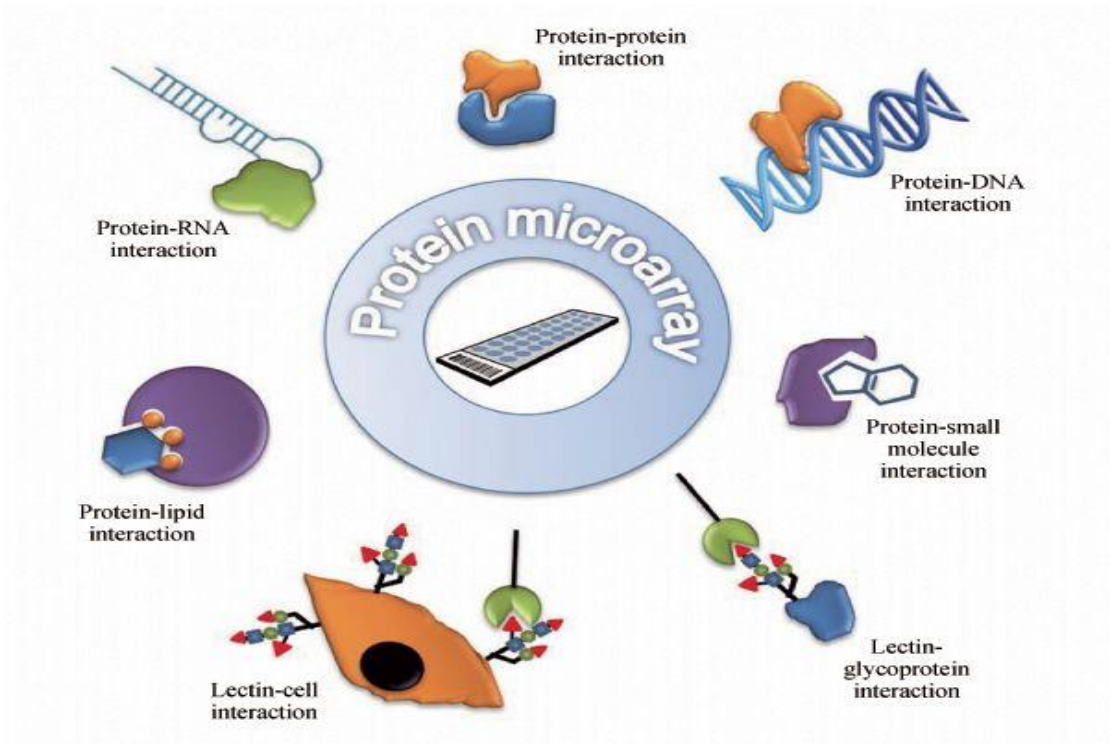
Proteomics : pertains to protein expression profiling i.e. which proteins are expressed in the lysate of a particular cell.

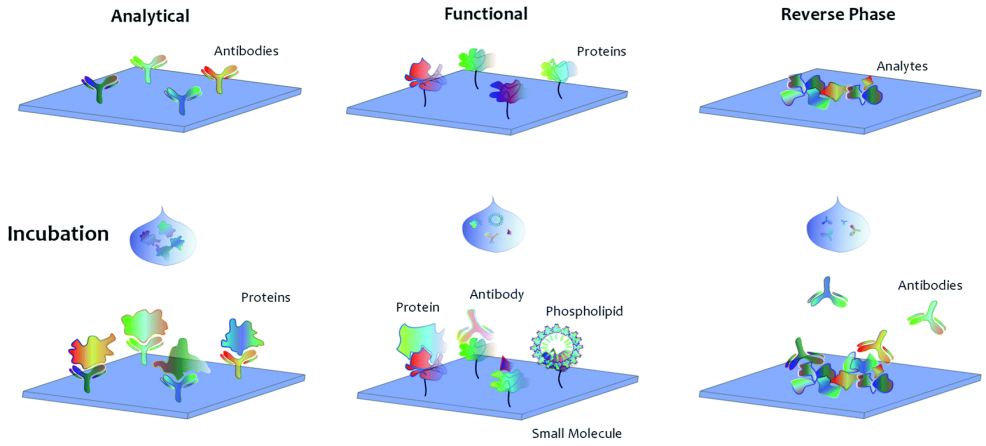
Protein functional analysis : is the identification of protein-protein interactions (e.g. identification of members of a protein complex), protein-phospholipid interactions, small molecule targets, enzymatic substrates.

Antibody characterization : is characterizing cross-reactivity, specificity and mapping epitopes.

Treatment development : involves the development of antigen-specific therapies for autoimmunity, cancer and allergies; the identification of small molecule targets that could potentially be used as new drugs.






Analytical
Antibodies
Functional
Proteins
Reverse Phase
Analytes
Incubation
Proteins
Protein
Antibody
Phospholipid
Small Molecule
Antibodies