Protein Microarrays

The first question to ask is what are we going to array?

Two answers exist

a. The protein(s) of interest
b. The substrate(s) of the protein(s) of interest.

Depending on what is attached we may be looking for protein-protein interactions, protein function, presence of certain proteins, changes in activation of a protein, substrate proteins or compounds, or total activity.

To array proteins of interest we will need to isolate those proteins and then array them on some surface. Several strategies exist to isolate proteins on a genomic scale.

If all genes have been identified, the proteins can be expressed as fusion proteins. The protein fused to the protein of interest can be used as an affinity tag to aid in protein purification. Currently the process is to use two tags to purify the protein with the removal of at least one tag during the purification process. This is Tandem Affinity Purification (TAP).

Tags can be specific proteins such as glutathione S-transferase, hexyl histidine, calmodulin binding protein, protein A, maltose binding protein, FLAG, etc.

The gene for the fusion protein is expressed in some system and the fusion protein purified.

Heterologous expression is the expression of the protein in a system other than which the protein comes from. Most common systems are E.coli and the insect cell line Sf9.

Homologous expression is best but often difficult.

Problems

- Protein cannot be expressed.
- Protein is insoluble and not active
- Cofactors necessary for protein function are not incorporated
- Lack of appropriate modifications or processing
- Does not necessarily take into account alternative splicing

What do we have to consider when we want to array proteins?
1. Protein stability
2. Attachment methods
3. Accessibility of the protein to substrate or vice versa
4. Activity

**Stability and Activity**

These are related.
Do we want a functional protein on the array and is it necessary?

Currently most proteins are arrayed using a glycerol solution (up to 40%) when placed on the slide. Glycerol provides a stabilizing effect on proteins and prevents complete drying of the sample.

Sometimes it is not necessary to have a functional protein such as the case when probing with antibodies to the protein. Still having a properly folded protein capable of activity often helps in antibody probes.

Activity is required of arrays checking for function of a protein.

**Attachment Methods**

Attachment of the protein to the support should not change the protein structure, and can be accomplished by various chemical methods on the market.

Attachment by most chemical methods is random and can therefore hide the active site. Addition of a specific tag to the proteins to be arrayed can lead to specific attachment to the slide.

Attachment chemistry may have spacers to offset protein from surface or a fusion protein can be used to attach the protein to the slide. Both of these will increase availability of the protein to the substrate. Fusion protein can also be used to bind protein to support through specific interaction.
Protein Arrays

Let’s begin by considering the experiment we want to do and decide on the appropriate array for that experiment.

Example 1  Determining the specificity of an antibody

One paper discusses using the proteome from yeast, ~5000 proteins, and plating this on a micro array. The proteome is then probed with “purified” antibody that has been conjugated to a fluorescent molecule. The array is then read as usual.

Several interesting results came from this experiment.

1. The micro-array was more sensitive to interaction than western blots.
2. Cross reactivity appeared to come from regions of similar sequence.
3. Cross reactivity could not be predicted from the sequence. Many cases similar sequences did not give cross reactivity.
4. In some cases cross reactivity may have been due to protein used for antibody production having another interacting protein bound to it or due to common binding epitope.

Example 2  Using antibodies to probe for proteins

Make an array of antibodies and use it to probe for the presence of a protein. The sample to be probed must be labeled with a fluorescent molecule or secondary labeled antibodies must be used.

Antibodies could probe for

1. total protein of one kind
2. modified protein
3. specific mutants
4. certain compounds

Relative intensity would be determined by

1. amount of protein present
2. cross reactivity
3. binding constant
Example 3      Determining the Substrate for an Enzyme

This can be done in two ways. Either array a series of enzymes on the array and look for modification of the substrate or array a series of substrates and probe with different proteins.

Case A      Determining Specificity of Kinase.

1. The genome is arrayed on a slide and a series of kinases are used to probe the array.
2. The presence of phosphorylated protein can be assayed with several different methods.
   a. One is to use fluorescent dye that binds to phosphate group.
   b. A second is to use MALDI-TOF to determine if phosphorylation has occurred.
3. A variant of this is to array all the proteins and then subject it to a complex of proteins from the cell and see which ones will be phosphorylated.

This same approach can be used to look at protein-protein interactions

1. Use the protein array and treat with a mixture of proteins from the cell.
2. After washing (different wash condition will give different specificity) use MALDI-TOF to determine which peptides bound to the arrayed proteins.
3. A variant of this would be to bind carbohydrate moieties to the slide and then measure which proteins bind to which carbohydrate structure.

Case B      Determining DNA Sequence Binding Specificity

1. Different DNA aptamers are arrayed onto the slide.
2. The DNA binding protein is fluorescently labeled
3. Probe slide and look for reaction
Case C  Determining the class of a protein

1. Bind all proteins to the array or all putative proteins for a function.
2. Create a substrate that will covalently link to the class of enzyme when it is acted upon.
3. Have this compound linked to a reporter molecule like biotin.
4. Use anti-biotin antibodies coupled to a fluorescent probe.

Reverse Phase Protein Arrays

This is a new terminology for one mode of arraying information.

1. Spot an array of extract from different sources in a dilution series on a slide.
2. The different sources could be a series of patients, different treatments of a system, etc.
3. Probe the arrays with a series of labeled antibodies to specific proteins. In one paper the antibodies are to proteins involved in regulation of cell growth.
4. Correlate the pattern of response to some outcome or parameter.

One universal problem with antibody based assays is the specificity of the antibody and the binding constant. Both of these will affect the results obtained from antibody based assays.

References:


