Protein Array Factsheet

What are antibody arrays?

Antibody arrays are a type of protein microarray (capture arrays). Antibodies can be covalently immobilized to activated array surfaces that can then be used to measure the abundance of thousands of different proteins in samples from cells to biological fluids. Control and experimental proteins are differentially labeled with fluorescent molecules and hybridized to the array.Antibody microarrays can be used to screen protein-protein and receptor-ligand interactions.

What are the challenges in making antibody arrays?

One of the biggest challenges in making antibody arrays is the availability of high-affinity, high-specificity antibodies and the creation of a comprehensive antibody library. High-throughput antibody production and purification is another challenge. It is also important (and difficult) to ensure that arrayed proteins are stable and maintain their 3-dimensional structure.

How are proteins “captured” by antibody microarrays detected?

Proteins can be detected by direct labeling or sandwich assays.

Direct labeling involves adding a fluorescent tag to all analytes. The advantage of this method is that multiple samples can be assayed simultaneously, for example, two states could be compared by labelling each with a different colour label. The disadvantage of this method is that samples are chemically modified by the addition of the fluorescent marker; this could produce cross reactive analytes, that may lead to false readings, and could result in higher background.

Sandwich assays involve the use of two antibodies, each recognizing different epitopes of the same protein. The advantage of this method is that specificity is increased and, since it is not necessary to label the sample, the likelihood of cross reactive analytes being produced decreases. The disadvantage of this method is that two antibodies are required for detection of each analyte and it is more difficult to multiplex.

How are the “hits” from antibody arrays validated?

Western blot analysis is often used to validate antibody microarray data.

What are some practical applications of antibody microarrays?

Antibody arrays can be used to monitor protein abundance in cancer cells following radiation treatment or used to identify potential biomarkers. Mathur et al. used the antibody-array technique to identify DNA repair proteins that were upregulated by ischemic preconditioning in a myocardial infarction model.

What are peptide microarrays?

Peptide microarrays are comprised of biologically active small synthetic peptides in a high-density format. Peptide microarrays are another form of capture microarray, as the peptides replace the antibodies on the array surface and, like antibodies, are capable of binding proteins in a specific manner. Peptide microarrays are another approach used for molecular immune diagnostics.

What are the advantages of peptide microarrays?

In terms of developing a capture-based protein microarray, peptide arrays have many advantages over antibody arrays including cost, greater resistance to denaturation, automated synthesis and purification of peptides, and the fact that peptides can be synthesized with non-natural functionalities (that would allow the addition of linkers to immobilize the peptides in certain orientation on the array surface).

What are function-based protein microarrays?

The protein microarray platform can be divided into two general strategies, one that is used to predict protein abundance and the other to identify protein function. Function-based microarrays study biochemical properties of the proteins printed on the array, examine protein interactions and enzyme activity.

What can functional protein microarrays used for?

Function-based protein arrays can be used to screen a particular class of enzymes with a potential inhibitor to examine binding selectivity; a potential drug could be used to probe many enzymes to identify unintended binding targets that might indicate possible side-effects; interaction networks might identify biochemical pathways.

What are the challenges in making functional protein microarrays?

The challenges for function-based microarrays include the stability and integrity of the proteins on slide surface, time and cost constraints to produce and purify proteins, and the methods used to attach the protein to the surface (immobilization without affecting function). Another consideration is that some proteins may require post-translational modifications or multimerization to be functional.

What type of slide should be used for making protein microarrays?

The selection of microarray slide type depends on whether or not the proteins are modified. Amine reactive slides do not require the proteins to be modified for immobilization as the proteins are attached by chemical linkage of their functional groups. The disadvantage is that the proteins are immobilized in a random orientation and are bound close to the surface (which may affect folding). Nickel- or avidin-coated slides can be used to immobilize proteins, however, fusion proteins with

histidine residues or biotin, respectively, is required. The advantage of using fusion proteins is that attachment to the slide is exclusively at the affinity tag and thus there is a uniform orientation of the functional domains away from the array surface. The disadvantage of fusion proteins is that protein function that requires free N- or C-terminal ends may be adversely affected.

What are self-assembling protein microarrays?

Self-assembling protein microarrays, also known as nucleic acid programmable protein arrays (NAPPA), are made by printing cDNAs encoding the target proteins at each feature of the microarray. The proteins are transcribed and translated by a cell-free lysate (such as mammalian reticulocyte lysate with T7 polymerase) and immobilized on the arrays by epitope tags fused to the proteins. These arrays are able to detect protein-protein interactions as the proteins on the array and the query protein can be transcribed and translated in the same extract.

What are the benefits of self-assembling protein microarrays?

The benefit of these arrays is that they are made just before the hybridization experiment, thus avoiding much sporadic protein denaturation.

What are the challenges in making self-assembling protein microarrays?

Some of the technical challenges involved with making self-assembling protein microarrays include the possibility that bridging proteins or inhibitors from the cell-free expression system may have a role in some interactions and the use of peptide tags may block important binding domains.

What are label-free detection methods and why do we need them?

Emerging tools for real-time label-free detection of protein interactions on functional protein microarrays include using probes of local index of refraction, carbon nanotubes and nanowires, and microelectromechanical systems (MEMS) cantilevers. (Ramachandran, N. et al. Emerging tools for real-time label-free detection of interactions on functional protein microarrays. FEBS, 2005, 272:5412-5425). The ability to detect protein interactions with non-protein biomolecules will require the development of label-free methods of detection.

What are the challenges of label-free detection?

Label-free detection methods need to be compatible with high-throughput methods, be able to detect small molecules, be able to detect interactions with molecules present at very low concentrations, and have a wide dynamic range. The primary challenge of label-free detection is specificity. Adsorption to the sensor itself and non-specific binding to the immobilized protein are both sources of non-specific binding.