Protein arrays

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Protein Microarray Platform: Functional Arrays

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[1]. Function-based microarrays study biochemical properties of the proteins printed on the array, examine protein interactions and enzyme activity. These arrays can be used to screen a particular class of enzymes with a potential inhibitor to examine binding selectivity, to identify unintended binding targets of a drug that might indicate possible side-effects and to explore interaction networks that might identify biochemical pathways[1].

The challenges for developing function-based microarrays include protein stability and functionality on slide surface, high-throughput protein production and purification, creation of comprehensive expression library, and the inter- and intra-slide variability of protein concentration[2]. In addition, proteins have a variety of chemistries, affinities and specificities, and may require post-translational modifications or multimerisation to be functional[2].

The results of high-throughput methods require subsequent validation and, much like the way quantitative PCR validates gene expression results obtained from DNA microarrays; western blot analysis is used to validate protein microarray data[1].

Detecting protein-protein interactions

Typically, the detection of protein interactions requires either an analyte specific reagent (like an antibody), which is only available for a limited fraction of the proteome, or a labelled query molecule, which can be expensive and tedious for large studies. One of the disadvantages of these detection methods is that mostly only protein-protein interactions can be detected, and that is only if any protein modifications (such as a fusion tag) do not interfere with its functionality. The ability to detect protein interactions with non-protein biomolecules will depend on the development of label-free methods for measuring interactions[4]. 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[4].

Briefly, monitoring changes in the local index of refraction (LIOR) can be used to detect and characterise interactions between the immobilised protein and the query molecule. Changes in the LIOR alter the plasma wave established on the surface of the sensor and are measured optically[4]. Carbon nanotubes or nanowires are functionalised wires and their conductance changes as the query molecule binds to immobilised protein. MEMS cantilever sensors have strips of silicon material at one end with a capture molecule (immobilised protein) attached. The analyte that binds to the microcantilever is detected by measuring the bending of the cantilever as a result of surface stress, either using optics or vibration detection. Wu and colleagues demonstrated the use of MEMS cantilevers for the detection of prostate specific antigen (PSA) in its free and complexed form at clinically relevant concentrations and conditions[6].

To further address the issue of specificity, Gavin and colleagues have described a new 3-dimensional protein array (methacrylate polymer elements) that directly interfaces with a matrix-assisted laser desorption-ionization mass spectrometer (MALDI-MS) [5]. This study demonstrated the use of this technique in detecting antibody-antigen interactions, enzyme interactions and enzyme-inhibitor interactions in complex biological samples[5].

Real-time 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[4]. The primary challenge of label-free detection is specificity as both adsorption to the sensor surface and non-specific binding to the immobilised protein are sources of non-specific binding[4]. Unlike the DNA microarray platform, increasing the binding stringency is not the ideal solution for protein microarrays as only strong interactions, and not weak transient ones, will be detected.

Functional Array Production

The selection of microarray slide type depends on whether or not the proteins are modified. Amine reactive slide coatings do not require the protein to be modified for effective immobilisation as the proteins are attached by chemical linkage of functionalised groups. The advantage of this is that proteins are immobilised randomly and the disadvantage is that the proteins are bound close to the surface and linkage may affect folding[1]. In order to attach proteins to nickel- or avidin-coated slides, 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[2]. Fusion proteins can also be used to assist with high-throughput affinity purification. The disadvantage of fusion proteins is activities that require free N- or C-terminal ends may be adversely affected[1].

One of the challenges when printing protein arrays involves keeping the proteins functional during array manufacture. This can be achieved if the proteins are kept in cold storage, arrayed in spotting solution containing 30-35% glycerol, and since glycerol is hygroscopic, print at 28-30% relative humidity[2].

Self-assembling protein arrays

As an alternative to producing, purifying, and then spotting proteins on an array to generate a protein microarray, self-assembling protein microarrays have been described[3]. The benefit of these arrays is that they are made just before the hybridisation experiment thus minimising protein denaturation. These arrays are made by printing cDNAs encoding the target proteins at each feature of the microarray. The proteins are transcribed and translated by a mammalian reticulocyte lysate with T7 polymerase and immobilised on the arrays by epitope tags fused to the proteins[3]. Also known as nucleic acid programmable protein arrays (NAPPA), self-assembling protein microarrays 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. As with other high-throughput protein interaction techniques, there are some technical challenges including; the possibility that bridging proteins or inhibitors from the cell-free expression system may have a role in some interactions; the use of peptide tags may block important binding domains; and some post-translational modifications may be absent from NAPPA[3].

References:

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