Topic Introduction

Protein Microarrays: Flexible Tools for Scientific Innovation

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Protein microarrays have emerged as a powerful tool for the scientific community, and their greatest advantage lies in the fact that thousands of reactions can be performed in a parallel and unbiased manner. The first high-density protein microarray, dubbed the “yeast proteome array,” consisted of approximately 5800 full-length yeast proteins and was initially used to identify protein–lipid interactions. Further assays were subsequently developed to allow measurement of protein–DNA, protein–RNA, and protein–protein interactions, as well as four well-known posttranslational modifications: phosphorylation, acetylation, ubiquitylation, and SUMOylation. In this introduction, we describe the advent of high-density protein microarrays, as well as current methods for assessing a wide variety of protein interactions and posttranslational modifications.

PROTEIN MICROARRAYS

In 1983, Tse-Wan Chang spotted a variety of antibodies onto a glass slide in a matrix formation to study “the potential of simultaneous multiple determinations of specific cell surface antigens in one reaction incubation” (Chang 1983). This study represents one of the first examples of what we today refer to as a microarray. Over the next 10 years, this concept was transformed and applied to the generation of DNA microarrays, which allow detection of mRNA expression levels for thousands of genes in parallel. Yet although DNA microarrays provide a useful platform for biological study, they cannot imitate the function of what many consider the pillars of cellular signaling—proteins. As research indicated that mRNA levels do not always associate with protein expression levels, array technology evolved to overcome these limitations through the creation of protein microarrays (Gygi et al. 1999; Zhu et al. 2001; Kopf and Zharhary 2007). In 2001, the first study using high-density protein arrays was presented by the Snyder research group at Yale (Zhu et al. 2001).

The first “proteome” microarray contained approximately 5800 full-length yeast open reading frames (ORFs) individually expressed in yeast. Each ORF was cloned into a yeast high copy expression vector (pEGH-A), which produces proteins that are fused to glutathione S-transferase-polyhistidine (GST-HisX6) at their amino termini, and can be expressed using the inducible *GAL1* promoter. For a detailed protocol describing the protein purification method used to create the array, see the study by Zhu et al. (2001). In brief, yeast cells were grown at 30°C to an OD₆₀₀ of 1.0 and induced for 6 h with

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galactose at a final concentration of 2%. After induction, the yeast were harvested in a 96-deep-well plate and frozen at -80°C . (Cell pellets can be stored in this condition for up to 1 yr.) Lysis buffer containing protease inhibitors and 0.5-mm zirconia beads was added to the pellets, and the yeast cells were sheared by vigorous shaking in a standard hardware store paint shaker. The supernatant of the lysate was collected after centrifugation and transferred to 96-well filter plates (sealed at the bottom with agar), where it was incubated with Sepharose glutathione beads for 1 h to immunoprecipitate the overexpressed protein. After several wash steps, proteins were eluted with buffer containing 40 mM glutathione and collected in a 96-well PCR plate. Eluates were then re-arrayed into a 384-well plate for use with a commercial protein microarrayer. (Because the elution buffer contains 30% glycerol, proteins can be stored in this format for up to 2 yr at -80°C .)

One benefit of using yeast as a means of protein purification for the study was that all proteins were in their native environment. Thus, even the largest and smallest proteins could be purified, including membrane proteins; many of these bound phosphoinositides while printed on the array, signifying that they were properly folded (Zhu et al. 2001). Because the array contained $\sim 80\%$ of the known yeast proteome, almost all of the protein classes were represented, and $>80\%$ of the strains produced detectable amounts of fusion proteins. Lysis buffer containing 0.1% Triton resulted in proteins free of lipids, and the strict washing conditions ensured that other cellular proteins that might noncovalently interact with the purified protein were washed away.

The protein microarrays were fabricated using a commercial robotic system. A standard array indicated that 90% of the spots contained 10–950 fg of protein, an amount detectable using a glutathione S-transferase (GST) antibody. Robotic microarrayers (such as the Bio-Rad VersArray Chip-Writer Pro System) use metal pins containing a small capillary on the tip to collect purified proteins from the 384-well plate and disperse them onto a glass surface in a very systematic and precise manner. The glass surface can either be aldehyde-treated, in which case the fusion proteins attach to the surface through primary amines at their amino termini or other residues of the protein (random orientation), or nickel-coated, which orients the fusion proteins uniformly through their HisX6 tag. The latter can produce better signal-to-noise ratios, but the lack of a covalent modification means that reactions performed on the slide cannot withstand harsh washing conditions (e.g., wash buffers containing sodium dodecyl sulfate [SDS]). Arrays fabricated in this manner can be stored for up to 1 yr at -80°C .

At present, there are no commercially available yeast proteome arrays, but some laboratories fabricate them in-house for their own purposes (e.g., the Heng Zhu laboratory at Johns Hopkins), so obtaining these arrays is generally accomplished through collaborative efforts between laboratories. Currently, the only organism with commercially available “proteome” arrays is human. The array available from Life Technologies contains more than 9000 human proteins and costs $\sim \$1000$ per array.

Detecting interactions on a protein array typically involves one of two methods—fluorescent dyes or radiography. Fluorescent dyes are frequently used to detect binding interactions between the proteins printed on the array and a fluorescently labeled binding protein. Fluorescently labeled antibodies can also be used to detect a wide variety of modifications (e.g., acetylation, SUMOylation, and ubiquitylation). Both fluorescence-based methods require a microarray scanner such as the GenePix 4000B for data acquisition. These machines scan standard microarrays by obtaining fluorescent signal at clearly defined wavelengths. Some can be used for multiplex assays by detecting multiple wavelengths for multiple dyes at the same time. Radiography can be used to detect modifications for which antibody specificity is too poor for use on an array (e.g., phosphorylation). This method simply requires standard film and a high-quality tabletop scanner capable of a resolution of 4000 dpi. Once an array has been scanned, microarray analysis software (such as GenePix 7.0) must be used to score and interpret the data. This is the most time-consuming step of the experiment and requires the most precision. Once the data are scored, simple foreground:background ratios can be obtained and a putative hit list can be generated. Because of the immense amount of data obtained, many laboratories collaborate with bioinformaticians to draw more useful conclusions and/or narrow their findings for further validation studies.

APPLICATIONS

The versatility of protein microarrays, coupled with their ability to allow characterization of thousands of proteins in a parallel and high-throughput manner, has resulted in great strides by the scientific community. By assessing interactions between thousands of proteins, many laboratories have been able to identify binding consensus motifs (Zhu et al. 2001; Lu et al. 2012) and phosphorylation motifs (Newman et al. 2013) and assess antibody specificity (Jeong et al. 2012)—all of which would be incredibly difficult using other methods. Arrays offer advantages over traditional yeast two-hybrid screens, which only take place in the nucleus and are thus severely limited in the breadth of interactions that can be observed. Mass spectrometry approaches have been very powerful for the identification of posttranslational modifications but are also time-consuming and not feasible with low-abundance proteins. Another limitation is the lack of direct evidence for the upstream enzyme(s) responsible for catalyzing the addition of the modification. Protein microarrays overcome these hurdles by providing a wider scope of proteins that can be assessed and identifying interactions even with limited protein amounts.

However, microarrays are not without their disadvantages. Array experiments are performed as *in vitro* assays, so although an interaction or modification is observed, it may not necessarily be present in a cellular context. Also, many methods that require antibodies are limited by antibody specificity, so careful consideration and methodology (e.g., proper controls) must be used when using antibodies for detection. Because of this, further characterization is required for all experiments involving protein microarrays.

Even with these disadvantages, protein microarrays can be used to examine a wide variety of interactions, including protein–protein, protein–DNA, protein–RNA, and protein–lipid interactions. We previously provided a detailed protocol for **Characterization of Protein–DNA Interactions Using Protein Microarrays** [Hu et al. 2011]). Our current protocols include **Protocol: Characterization of Protein–Protein Interactions Using Protein Microarrays** (Paul et al. 2015), **Protocol: Characterization of RNA–Binding Proteins Using Protein Microarrays** (Song and Zhu 2015), and **Protocol: Characterization of Lipid–Protein Interactions Using Nonquenched Fluorescent Liposomal Nanovesicles and Yeast Proteome Microarrays** (Lu et al. 2015). Although these experiments involve non-covalent binding, functional assays can also be performed on the substrate proteins in the presence of an enzyme. In addition to the aforementioned binding assays, we have also provided a method for detection of phosphorylation, acetylation, ubiquitylation, and SUMOylation that can be adapted for any microarray experiment; see **Protocol: Posttranslational Modification Assays on Functional Protein Microarrays** (Neiswinger et al. 2015).

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