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Progress in protein and antibody microarray technology

Philipp Angenendt

The success of genome sequencing projects has led to a shift from the description of single molecules to the characterisation of complex samples. At the same time, there is growing interest not only in studying organisms at the genomic level, but in the characterization of their proteome. Such a task would not be possible without the availability of appropriate technologies. Protein and antibody microarray technologies are, in addition to two-dimensional gel electrophoresis followed by mass spectrometry, two of the most propitious technologies for the screening of complex protein samples. Nevertheless, to succeed, protein and antibody microarrays have to overcome their current limitations. This review aims to introduce these new technologies and highlights their current prospects and limitations.

The basic concept of microarray technology (see Glossary) was initiated by the ambient analyte model of Ekins and colleagues [1–3], which states that 'microspot' assays that rely on the immobilisation of interacting elements on a few square microns should, in principle, be capable of detecting analytes with a higher sensitivity than conventional macroscopic immunoassays. On the basis of such ideas, and boosted by the completion of whole-genome sequencing projects, DNA microarray technology rapidly became the first application of this model [4,5]. However, the realization that the investigation of genetic information could not provide sufficient insight to understand complex cellular networks, as well as the missing close relationship between mRNA and protein abundance, eventually led to the development of a comparable technology for the analysis of proteins [6–8]. To achieve this task, antibodies, being natural binders of proteins, were immobilised in an array on a solid support to create antibody microarrays. In parallel, protein microarray technology evolved for the study of protein interactions and modifications. Although such arrays are envisaged to become a valuable tool for tasks such as the characterisation of enzymes [9,10] or antibody specificity [11,12], as well as for the elucidation of gene function [13,14], many limitations of the technology are still unsolved and prevent protein microarray technology from reaching its full potential. These limitations include the generation of content and the conservation of protein functionality during immobilization, as well as the provision of the required absolute and relative sensitivity.

Originally, protein assays were developed in the format of enzyme-linked immunosorbent assays (ELISAs) [15] in microtiter plates with up to 96 wells. Owing to their robustness and sensitivity, ELISAs soon became the gold standard for protein quantification and were adapted to 384-well plates for increased throughput and decreased consumption. One of the first steps to further increase the complexity of ELISA experiments was performed by Mendoza and colleagues [16], who created arrays of 144 elements in each well of a 96-well microtiter plate. This novel approach allowed for multiplex screening of different samples against each array set. One of the next steps in the development of high-content microarrays comprised the production of

Philipp Angenendt
German Cancer Research Center,
Functional Genome Analysis,
Im Neuenheimer Feld 580,
69120 Heidelberg,
Germany
e-mail: p.angenendt@dkfz-heidelberg.de

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TABLE 1

	Surface chemistry	Surface coating	Refs
2D slides	Amine slides	Amine groups	[29–31]
	Aldehyde slides	Aldehyde groups	[29,31]
	Epoxy slides	Epoxy groups	[30,31]
	Mercapto slides	Mercaptopropyltrimethoxysilane groups	[31]
	MaxiSorb slides	Polystyrene-based modified surface	[29]
3D slides	Hydrogel slides	Modified polyacrylamide gel	[26,103–105]
	Agarose slides	Agarose gel	[27]
	Polyacrylamide gel	Polyacrylamide gel	[25,29,100]
	FAST slides	Nitrocelluose-based matrix	[10,28,30,100,106]
	SuperProtein slides	Hydrophobic polymer	
Other	PEG-epoxy slides	Polyethylene glycol layer with reactive epoxy groups	[10,30,100,106]
	Dendrimer slides	Dendrimer layer with reactive epoxy groups or carbonyldiimidazole	[30,107,108]
	BSA-NHS slides	Bovine serum albumin activated with *N*-hydroxysuccinimide	[19]
	Ni-NTA slides	Nickel-nitrilotriacetic acid complex	[95]
	DMA-NAS-MAPS slides	*N,N*-dimethylacrylamide, *N,N*-acryloyloxysuccinimide, [3-(methacryloyl-oxy)propyl]trimethoxysilyl copolymer	[109]
	Streptavidin slides	Streptavidin	[32,33]
	Avidin slides	Avidin	[110]

arrays by high-density spotting of bacteria onto nitrocellulose filters [17]. The bacteria expressed and secreted antibody fragments that were subjected to a filter-based ELISA for the identification of antibody fragments that were specific for the tested antigens. The last step with regard to miniaturisation came along with the spotting of purified proteins and antibodies on coated glass slides. The rigid structure of glass slides allowed an increase in feature density and permitted quantitative assays with diminished amounts of sample solution [18,19].

GLOSSARY

Aptamer: DNA or RNA molecule that has been selected to allow specific detection of other molecules.
Chemiluminescence: Luminescence resulting from a chemical reaction; for example, the oxidation of luciferin in fireflies.
Ellipsometry: A method of measuring the thickness of thin films or large molecules based on the detection of phase shift during reflection of a plane of polarized light.
Epitope: Also known as the antigenic determinant. A localized region on the surface of an antigen that antibody molecules can identify and bind.
Kelvin nanoprobe detection: A detection method that uses the principles of Kelvin physics and atomic force microscopy (AFM). The nanoprobe measures the current generated when two materials, one subjected to vibration, are connected. When contact occurs, the equilibration of the Fermi levels of the two substrates leads to a current.
Microarray: Microarrays consist of large numbers of molecules distributed in rows in a very small space with spot sizes <250 μm. Microarrays permit scientists to simultaneously characterize complex analyte solutions with regard to many features.
Macroarray: Array with spot sizes >300 μm.
scFv (single-chain Fv fragment): Antigen binding part of an antibody consisting of a heavy and a light chain that were genetically modified to be connected by a flexible linker.
Surface plasmon resonance: The observation of polarization of light reflected from a surface that is coated with one of the binding partners. The change in polarization provides, in real-time, a measure of binding of the second partner.

As an alternative format to microarrays, the xMAP technology of Luminex Corporation (http://www.luminexcorp.com) is rapidly evolving. In contrast to conventional microarray technology, xMAP does not rely on the spatial separation of capture molecules on glass slides, but instead uses beads for immobilization that are colour-coded by different ratios of two fluorescent dyes. During readout, two different fluorescent signals are recorded, with one signal arising from the fluorescent reporter molecule that monitors the binding event and the other originating from the colour code of the bead for identification of the capture molecule. This combination allows multiplex analysis of up to 100 different species in a liquid environment without any washing steps [20,21].

Surface coatings

As the objective of protein and antibody microarray technology is the study of interaction partners, the provision of optimal binding conditions is a crucial feature of the microarray support. In previous years PVDF (polyvinylidene fluoride) membranes were the support of choice for high-density protein macroarrays [22,23] and microarrays [12]. The demand for even higher densities as well as the need for decreased sample consumption and quantification led to the application of glass slides as solid supports for microarrays. However, the key requirement of a high binding capacity with low unspecific background and low variability remained unchanged. In addition, the shift from DNA to protein and antibody microarrays also necessitated an additional and even more challenging task: the provision of a surface that accommodates proteins of varying composition and structure in such a way that their three-dimensional structure, functionality and binding

sites are retained. Such a task is not only important for immobilisation of the relatively stable antibodies, but becomes crucial for the detection of protein–protein interactions on protein arrays.

To meet the requirements of protein and antibody microarrays, several surfaces have been proposed, which can be broadly divided into three major groups (Table 1; Figure 1). The first comprises two-dimensional (2D) plain glass slides (Figure 1a), which are activated with a variety of coupling chemistries such as aldehyde, epoxy or carboxylic esters [24]. Slides with these surfaces bind proteins and antibodies either by electrostatic interactions or through the formation of covalent bonds. Although they offer several advantages, such as a strong attachment combined with low variation, they suffer from rapid evaporation of the liquid environment as well as the close protein surface contact, which may affect the three-dimensional structure. An alternative is three-dimensional (3D) gel or membrane-coated surfaces (Figure 1b), such as polyacrylamide [25,26], agarose [27] and nitrocellulose [28]. These surfaces bind protein mainly by physical adsorption and have the advantage that they are expected to be most favourable with regard to preservation of the native protein conformation. However, large variations in signal intensity are a disadvantage of these surfaces [29]. The third group includes surface coatings (Figures 1c–e), such as dendrimer or avidin slides, which mix both concepts mentioned above. They do not have a visible 3D structure, and cannot be considered as two-dimensional because they display a supramolecular structure on their surface.

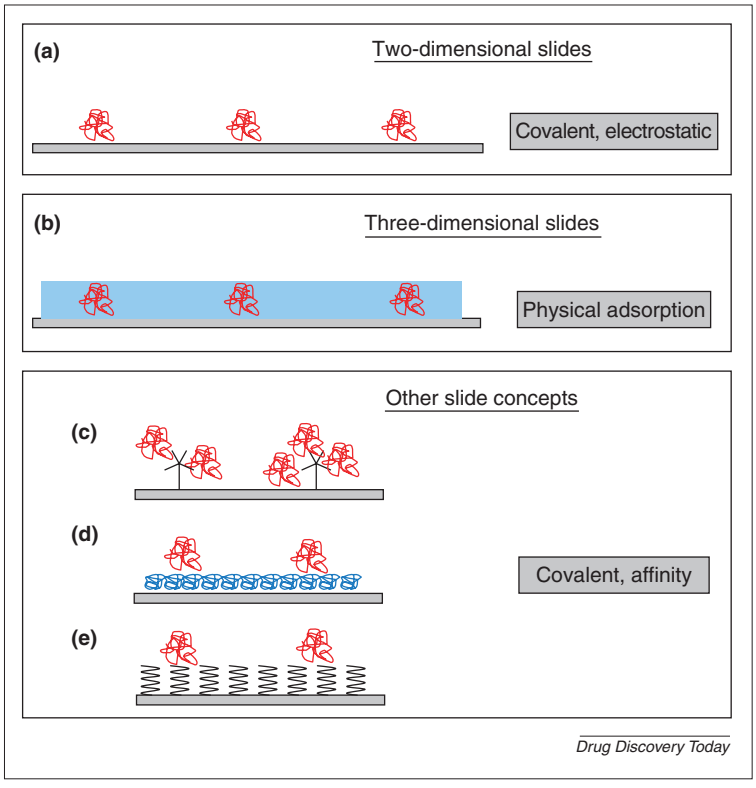

FIGURE 1

Concepts of slide surface coatings for protein and antibody microarrays. The figure displays three classes of surface coatings and lists their major binding modes. **(a)** Two-dimensional slide surfaces bind proteins directly to the surface, whereas **(b)** three-dimensional slide surfaces retain proteins by physical adsorption within the gel structure. Other slide surface concepts comprise surface coatings such as **(c)** dendrimer slides, **(d)** avidin or dendrimer slides and **(e)** polyethylene glycol-epoxy slides. As these surfaces do not display a three-dimensional gel, but provide a supramolecular structure, they cannot be classified into the other groups. Moreover, the binding mode of such surfaces might be different, as they bind proteins in an oriented mode via affinity interactions.

A comparison of the different surfaces for the generation of protein and antibody microarrays was recently performed, highlighting distinct differences in the performance of surfaces [29–31].

Different immobilisation strategies were evaluated by Wacker, Schröder and Niemeyer [32], who compared the immobilisation of antibodies by DNA-directed immobilisation (DDI), direct spotting, and streptavidin-biotin attachment. The study revealed that DDI and direct spotting led to the highest signal intensities, with DDI displaying the best spot homogeneity and reproducibility as well as the lowest consumption of antibody. Nevertheless, DDI is disadvantaged by the additional efforts arising from the separate preparation of DNA–protein conjugates for each antibody. The effects of the orientation of antibodies and Fab were also investigated by Peluso and colleagues [33]. Within their study they detected an upto 10-fold increase in analyte-binding capacity of slide surfaces that promoted oriented immobilisation.

Assay conditions and detection

The optimisation of assay conditions is another major challenge for microarray technology. Experience from DNA microarrays has shown that the elucidation of assay conditions that allow optimal binding of all molecules present in the analyte is still quite a challenging task, even for such a uniform molecule as DNA [34]. The transfer of microarray technology to the protein amplifies the problem and becomes crucial with increasing content on the array. Another challenge is the absolute and relative sensitivity that can be obtained on microarrays. Although the theoretical detection limit of a microspot assay was predicted to be a few femtograms or less [35], it has been difficult to generate detectable signals in the low picogram range even with artificial one-antibody one-antigen test systems [36]. In complex solutions, the relative sensitivity can be expected to be lower.

Besides the provision of an optimal surface coating and the optimisation of assay conditions, the sensitive detection of bound samples is another key parameter of every microarray experiment. Detection can be achieved in two ways: directly, by using labelled binding molecules, or indirectly without any modification of the binder (Table 2). Direct labelling is mainly performed either radioactively, using isotopes such as ^{125}I or ^{3}H, or fluorescently using Cyanine, Alexa or Oyster dyes. Even though radioactive labelling is traditionally one of the most sensitive labelling

TABLE 2

	Commonly used agents	Method	Advantages	Disadvantages
Direct labelling	Fluorophore-NHS ester	Direct attachment of dye to the analyte	No additional incubation steps necessary	Reproducible labelling of complex protein solutions is difficult Labelling can alter the structure of the molecule Removal of unbound dye is necessary
Indirect labelling	Fluorophore–secondary antibody conjugates Fluorophore–protein A or L conjugates Specific-tag specific antibody conjugates Fluorophore–streptavidin conjugates TSA: horseradish peroxidase–secondary antibody conjugate, fluorophore–tyramide conjugate	Incubation with labelled generic binders that are often species- or tag-specific TSA: enzymatic formation of tyramide radicals that attach to the phenol moiety of tyrosine residues	Labelled generic binders can be obtained commercially No labelling step necessary Enzyme–antibody fusions are available commercially	Requires additional incubation step All analytes require a common tag or motif to which the generic binder can bind Stringent washing steps are required
Enzymatic labelling	RCA: antibody–primer conjugate, DNA circle, polymerase, fluorescent oligonucleotide probes	RCA: extension of primer and hybridization of labelled oligonucleotide probes	Enzyme can be attached directly or indirectly to the analyte	Higher variations can arise owing to different incubation times

Abbreviations: NHS, *N*-hydroxysuccinimide; RCA, rolling circle amplification; TSA, tyramide signal amplification.

procedures, it has become a trend to sequentially replace radioactive labelling by other detection methods. Reasons for this are mainly the risks of radioactive contamination, problems associated with proper waste disposal and, most of all in the area of microarrays, incompatibility with high-throughput screening technologies. By contrast, fluorescent dyes are becoming more and more popular and are nowadays the method of choice for labelling and detection of molecules in microarray applications. In addition, the development of bright and pH stable dyes with narrow emission and excitation spectra promotes their use for multicolour applications on microarrays. Such multicolour detection schemes are especially beneficial, as microarray measurements are not quantified absolutely but relatively, so that simultaneous detection permits direct comparison of different samples without interchip variations. A promising addition to established labelling techniques are semiconductor quantum dots, which are brighter and more stable than organic dyes. Wu and colleagues [37] successfully applied such quantum dots for the labelling of proteins on cells and within the cytoplasm and nucleus.

Indirect labelling generally involves generic binders, such as species-specific labelled secondary antibodies for the detection of primary antibodies or labelled protein L for the detection of recombinant antibody scFvs (see Glossary). This approach is advantageous, as it represents the most effortless labelling method in serum or antibody specificity screenings on protein microarrays. Although the risk of unspecific binding of the generic binders is a disadvantage, the indirect detection does not entail the risk of changing the properties of the analyte, as direct labelling methods do. Indirect labelling methods are also beneficial in sandwich assays, in which the antibody–antigen complex is detected by another antigen-specific antibody. Sandwich assays have the advantage that they increase the specificity of antibody arrays. Nevertheless, they are more expensive and mainly limited by the cross-reactivity of the antibodies, which restricts the amount of different antibodies that can be applied simultaneously. Cross-reactivity is also a major limitation of complexity in antibody microarray technology. As all antibodies bear the risk of displaying cross-reactivity to both proteins with and without sequence homology [38], the chances of measuring interactions arising from cross-reactivity increases with analyte and array complexity.

A valuable addition to labelling procedures is enzymatic signal amplification. Schweitzer and colleagues applied this concept to the development of a new methodology named rolling circle amplification (RCA). RCA relies on the enzymatic extension of a primer–antibody conjugate followed by hybridisation of labelled probes to the generated DNA strand [39–41]. Enzymatic amplification of signals can also be done using the tyramide signal amplification (TSA) system, which catalyses the formation of short-lived tyramide radicals that attach to the phenol moiety of tyrosine residues. [42]. As an alternative to fluorescent detection, Huang and colleagues applied a chemiluminescence (see Glossary) approach for the sensitive detection of multiple cytokines [43,44].

Apart from these established labelling methodologies, other technologies are being adapted for use in protein

microarray technology. These include real-time detection by surface plasmon resonance (SPR; see Glossary), intrinsic time-resolved UV fluorescence [45], mass spectrometry [28], as well as ellipsometry [46] and Kelvin nanoprobe detection (see Glossary) [47]. Although advances with regard to throughput have been made for SPR and mass spectrometry [48,49], neither of these technologies is currently suitable for high-throughput applications.

Another totally different detection scheme exploits the characteristics of different molecule classes, such as DNA and proteins. One of the most promising developments in this context are aptamers (see Glossary), which are small nucleic acid molecules that specifically bind proteins [50]. As aptamers are non-proteinaceous, a generic protein stain can be used to label the spots that have bound proteins [51]. Alternatively, detection based on the structural changes that occur in aptamers upon binding can be envisaged [52,53]. In this method, aptamers are labelled with two different fluorophores at both ends, and the detection relies on the binding event that leads to a structural change inducing a quenching or fluorescence resonance energy transfer event.

Generation of content

One of the key challenges for the success of protein and antibody microarray technology is the provision and generation of content. To date, this step represents a major hurdle, as there is no simple way of generating large and diverse sets of proteins or antibodies.

The complexity of this task is influenced by the application and its requirements with regard to nativity of the immobilised substances. Protein chips are used for a large variety of applications, such as screens of the immune response [54]. One example where nativity of the proteins involved is not an issue, is protein arrays for the purpose of immunoprofiling sera obtained from patients suffering from autoimmune diseases [55]. Furthermore, domains that readily bind unstructured sequences present in peptides or unfolded proteins, such as PDZ, EF-hand, SH3 and WW domains, can also be applied for screening of protein or peptide arrays. However, most applications that are based on protein–protein interactions require a more cooperative contact from both binding partners. In this case the immobilised proteins must be presented in a native state.

The production of proteins using cDNA libaries in *Escherichia coli* with subsequent purification remains the gold standard. To meet the requirements of protein microarrays and supply a large number of different proteins, the protocols were adapted to high-throughput expression in a fully automated system [56]. The purification is mainly based on short affinity tags to either the N or C terminus of recombinant proteins and involves immobilised metal affinity chromatography (IMAC) to Ni(II)-nitrilotriacetic acid (Ni-NTA) or Co(II)-carboxymethylated aspartic acid affinity media [57]. The addition of tags, such as the His-tag, does not severely change the properties of recombinant proteins and increases the molecular weight by less than 1 kDa. Moreover, the affinity media is readily available, selective and stable enough for protein purification under strong denaturing conditions [58,59]. This is important, as many eukaryotic proteins form inclusion bodies upon expression in *E. coli*, and require solubilization by denaturing chaotropes like guanidinium or urea for purification. Additionally, inexpensive materials, the purity after a single step, and high scalability have made the use of IMAC purification the standard procedure for high-throughput protein purification in the context of expression libraries [60].

As an alternative to *E. coli,* other hosts such as *Pichia pastoris* [61] and *Saccharomyces cerevisiae* [62] have been tested for high-throughput protein expression. Such cultures increase the expression yield of soluble proteins, improve folding and provide all post-translational modifications. Nevertheless, they suffer from the more intricate handling and purification requirements in comparison with *E. coli*.

Cell-free expression has become an alternative to cell-based systems for high-throughput applications. In cell-free systems, proteins are expressed from cDNA templates [63], which can be easily generated by PCR and stored. Moreover, such systems can be easily down-scaled [64], which is an advantage as they are rather expensive, or they can be used for the direct synthesis of proteins on microarrays [65].

An interesting alternative to the purification and subsequent spotting of proteins was introduced by Madoz-Gurpide and colleagues [66], who prepared cell lysate from adenocarcinoma cell lines and fractionised the protein extract first by anion exchange and then by reverse-phase liquid chromatography. The obtained fractions were then characterised by mass spectrometry and immobilised on a microarray. The special advantages of such protein samples is that they have the correct post-translational modifications, which allows further properties to be assayed in comparison to proteins from recombinant sources.

Antibody chips, which harbour a large collection of specific binder molecules, such as antibodies, antibody fragments, affibodies, engineered binding proteins, phage particles or even aptamers, are mainly used to specifically capture and quantify components of complex samples. As the binders can be considered the active binding partner, these must retain their specific binding properties. Therefore, it is necessary to select antibodies that maintain their functionality and to optimise protocols for the immobilisation, storage and assays [18,67]. Another important issue is the source of antibodies. Although a large variety of monoclonal antibodies are commercially available, the costs associated with hybridoma technology and production are too high to use them for routine antibody array production. Display technologies, like phage display, are a suitable alternative. Antibody fragments can be selected and produced using inexpensive media and purification

methods [68,69] and can be spotted directly as phage particles [70]. To further increase the quality of antibodies, new *in vitro* strategies have been developed using ribosomal display [71,72] or mRNA–protein fusions [73]. At the same time, binding scaffolds other than immunoglobulin domains, such as affibodies [74], lipocalin [75], fibronectin [76] or repeat domains [77], are being explored to meet the requirements associated with antibody array technology [78]. Finally, oligonucleotides can be selected to bind certain antigens [79]. These so-called aptamers are especially interesting because of low production costs and easy handling.

Recent applications

Although protein and antibody microarray technology are at an early stage of development, several applications in areas such as autoantibody profiling, cancer research or signal pathway characterisation highlight their potential.

In the area of autoimmune profiling, Robinson and colleagues [55] fabricated arrays containing 196 distinct biomolecules, comprising proteins, peptides, enzyme complexes, ribonucleoprotein complexes, DNA and post-translationally modified antigens. With such arrays, they characterised sera from eight human autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis. Quintana and coworkers [80] used protein microarrays consisting of 266 different antigens to confirm that the future response of mice to induced diabetes could be predicted by immunoglobulin G autoantibody repertoires.

Characterisation of cytokine release was investigated by Lin *et al.* [81], who quantified human cytokines, chemokines, growth factors, angiogenic factors and proteases in estrogen receptor positive and negative cells. Turtinen and coworkers [82] studied the effects of different amphotericin B formulations on cytokine release from THP-1 leukemic monocytes and showed that tumor necrosis factor-α and interleukin-8 levels correlated well between the antibody microarray and quantitative ELISA measurements.

The investigation of antiviral antibody responses to vaccine trials with a simian-human immunodeficiency virus (SHIV), a model for human immunodeficiency virus (HIV), was done by Neuman de Vegvar and collegues [83]. They produced antigen microarrays with 430 different proteins and overlapping peptides spanning the whole SHIV proteome and identified eight immunodominant epitopes (see Glossary). Xu *et al.* [84] studied HIV-1-mediated apoptosis of neural cells and used protein microarrays for the identification of upregulated host-cell factors in infected macrophages.

Cancer research is currently one of the largest areas of application for protein and antibody arrays. Serum screening was performed in several studies to characterise the serum and plasma of patients suffering from diverse cancers, such as colon, lung or nasopharyngeal cancer [54,85–87]. All studies demonstrated the applicability of arrays to this field and led to the identification of known or new potential biomarkers. In another application, mutations and polymorphisms of p53 were functionally characterised with regard to their DNA-binding capacity on protein microarrays [88]. Paweletz *et al.* [89] created organ- and disease-specific microarrays using reverse-phase protein arrays, which were created by immobilisation of the whole repertoire of patient proteins. Such arrays were then applied to quantify the phosphorylated status of signal proteins and to monitor cancer progression from histologically normal prostate epithelium to prostate intraepithelial neoplasia and invasive prostate cancer. A similar approach was used in the group of Petricoin [90,91], who used reverse-phase arrays for the profiling of signal pathways in prostate and ovarian cancer.

First methodologies for the analysis of membrane proteins were developed by Fang *et al.* [92], which generated membrane protein arrays for analysis of the ligand-binding properties of receptors. The microarrays consisted of an array of G-protein-coupled receptors and adrenergic receptors, which were employed for the subtype-specific detection of a cognate antagonist analogue specific for β-adrenergic receptors. Because important members of signalling pathways, such as receptors and their associated signalling molecules, are membrane located, systematic analysis of membrane proteins provides a valuable task for proteomics.

Additionally, efforts were made to monitor enzymatic reactions on a chip. As a first step, kinase activity was measured quantitatively on a peptide chip. Houseman *et al.* [93] applied SPR, fluorescence, and phosphorimaging for the detection of phosphorylation and evaluated three inhibitors quantitatively. Zhu *et al.* [94] used protein chips bearing microwells to analyse nearly all of the protein kinases from *S. cerevisiae*. Many novel activities were found as well as 27 protein kinases with an unexpected tyrosine kinase activity. The same group investigated protein–protein interactions on a large scale through the production of the first proteome chip [95]. For the generation of the chip, 5800 open reading frames of yeast were cloned and the corresponding proteins were overexpressed in *E. coli*, purified and spotted. The resulting protein microarray was used to screen for interactions with calmodulin and phospholipids and allowed the identification of binding motifs. Phosphorylation was also studied in plants by Kramer *et al.* [10], who identified 21 potential substrates of barley CK2α kinase. Protein and antibody microarray technology is not limited to the areas mentioned above, but has been applied in other areas such as the investigation of neurodegenerative disorders [96], the correlation of cell phenotype with surface markers [97], identification of chromatin-related proteins [98] as well as the mapping of WW domains [99] and generation of *Arabidopsis* protein chips [100].

A new approach, named multiple spotting technology (MIST), was developed by Angenendt and coworkers [101].

MIST allows the multiplex analysis of different analytes on a single chip. In a first spotting, biomolecules such as antibodies, enzymatic substrates or antigens are transferred to a slide and the slide is blocked to prevent non-specific binding. The analyte, which is expected to contain an interaction partner to the reagent that was applied to the chip in the first spotting, is then transferred to the chip by a second spotting. Because the buffer used for the second spotting also contains a hygroscopic additive, total evaporation of the liquid is prevented and liquid reaction entities are maintained. After spotting, the slides are washed and scanned in a conventional microarray scanner. This procedure is different from standard procedures, as it does not apply the analyte by total incubations, but allows the transfer of a multitude of different analytes to different spots. In a first study, the enzymatic activity of enzymes such as alkaline phosphatase, β-galactosidase or cathepsin D was measured [9] by the transfer of a fluorogenic substrate in a first spotting and the spotting of different concentrations of enzyme in a second spotting on the very same spot. The same technology was also applied to facilitate and speed up the screening of large populations of recombinant antibody fragments [102].

Conclusions

The wide variety of different applications in which protein and antibody microarrays are employed reflects the versatility of the technology and underlines the urgent need for technologies that are capable of high-throughput analysis of proteins and antibodies. Although several limitations currently hinder widespread use, it can be expected that advances such as protein production on the chip and the generation of large full-length expression libraries will facilitate the generation of protein microarrays. This will promote the application of protein arrays for qualitative measurements, such as interaction or modification screenings. By contrast, the widespread application of antibody microarrays for the quantitative analysis of complex protein solutions still requires modifications and careful optimisation to overcome the limitations with regard to sensitivity and cross-reactivity. Although several studies have demonstrated the applicability for small-scale projects, it is currently not foreseeable when the first large-scale translational profiling projects can be conducted. Until then, the benefits of microarray technology, such as low analyte consumption, will help to facilitate the analysis of complex samples in small-scale applications.

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