Protein Microarray Technologies for Detection and Identification of Bacterial and Protein Analytes

Christer Wingren and Carl AK Borrebaeck

Abstract

Protein-based microarrays is a novel, rapidly evolving proteomic technology with great potential for analysis of complex biological samples. The technology will provide miniaturized set-ups enabling us to perform multiplexed profiling of minute amounts of biological samples in a highly specific, selective, and sensitive manner. In this review, we describe the potential and specific use of protein microarray technology, including both functional protein microarrays and affinity protein microarrays, for the detection and identification of bacteria, bacterial proteins as well as bacterial diseases. To date, the first generations of a variety of set-ups, ranging from small-scale focused biosensors to large-scale semi-dense array layouts for multiplex profiling have been designed. This work has clearly outlined the potential of the technology for a broad range of applications, such as serotyping of bacteria, detection of bacteria and/or toxins, and detection of tentative diagnostic biomarkers. The use of the protein microarray technology for detection and identification of bacterial and protein analytes is likely to increase significantly in the coming years.

1. Introduction

Entering the post-genomic era, proteomics—the large-scale analysis of proteins—has become a key discipline (Phizicky et al. 2003; Zhu et al. 2003). To this end, the need for (novel) technologies, allowing us to perform rapid and multiplexed analysis of biological samples in a selective, specific, and sensitive manner in various applications, ranging from focused assays to proteome-scale analysis, is tremendous (Yanagida 2002; Hanash 2003; Phizicky et al. 2003; Zhu et al. 2003; Wingren and Borrebaeck 2004). The protein-based microarray is a promising and rapidly evolving technology that may provide us with the unique means to perform high-throughput proteomics (Haab 2001; Zhu and Snyder 2003; Wingren and Borrebaeck 2004; Kingsmore 2006). In this review, we will describe the potential and specific use of protein microarray technology for the detection and identification of bacteria, bacterial proteins, and bacterial diseases. The number of such applications is still low, but is likely to increase significantly as the microarray technology develops.
1.1. Definition and Classification of Protein Microarrays

The concept of protein microarrays is based on the arraying of a small amount (pL to nL scale) of protein in discrete positions in an ordered pattern, a microarray, onto a solid support where they will act as probes, or catcher molecules (Fig. 26.1) (Haab 2001; MacBeath 2002; Wingren and Borrebaeck 2004; Angenendt 2005; Kingsmore 2006). A minute quantity (µL scale) of the biological sample, e.g., serum, is then incubated on the array and any specifically bound analytes can be detected using mainly fluorescence as a read-out system. Adopting a high-performing setup, assay sensitivities in the pM to fM range can be observed, allowing low-abundance analytes to be readily targeted even in complex samples (Pawlak et al. 2002; Wingren et al. 2005; Wingren et al. 2006). Depending on the assay setup at hand, the observed microarray binding pattern can then be converted into, for example, a protein-ligand interaction profile, a (differential) protein expression profile, or even a proteomic map revealing the detailed composition of the proteome at the molecular level (MacBeath 2002; Wingren and Borrebaeck 2004; Kingsmore 2006).

Protein microarrays are frequently divided into two conceptual classes of array approaches, functional protein microarrays (functional proteomics) and affinity protein microarrays (quantitative proteomics) (MacBeath 2002; Poetz et al. 2005). While functional protein microarrays examine the biochemical activity, such as ligand binding properties and reactivity, of a set of immobilized target proteins (MacBeath and Schreiber 2000; Zhu et al. 2000, 2001; Phizicky et al. 2003); affinity microarrays utilize affinity reagents as probes to detect and measure the abundance of multiple proteins in a (semi-) quantitative way (Haab 2003; Wingren and Borrebaeck 2004, 2006a). As will be described in this review, both classes of protein microarrays have been used for detection and identification of bacteria and bacterial protein analytes, and the different protein array technology platforms have already been shown to display great promise within biomedical and biotechnological applications (Table 26.1). In this context, it might be of interest to note that protein-based microarray applications have recently emerged in a similar manner also for the detection of viruses, viral proteins, and viral diseases (Perrin et al. 2003; Yuk et al. 2004; Livingston et al. 2005; Lu et al. 2005; Zhu et al. 2006).

1.2. Functional Protein Microarrays

In general terms, functional protein microarrays have been designed and developed to investigate the biochemical properties, e.g., immunoreactivity (Robinson et al. 2003; Hueber...
| Application                          | Type of Microarray          | Probe Source           | Support             | Mode of Detection                  | Sample                                      | References                     |
|-------------------------------------|-----------------------------|------------------------|---------------------|-----------------------------------|---------------------------------------------|---------------------------------------------|
| 1) Serotyping of bacteria           | Antibody microarray         | Pabs (antisera)        | SuperEpoxy          | Fluorescence                       | Various *Salmonella* strains                 | Cai et al. 2005                   |
|                                     |                             | Epoxy-modified         | TMB / red light     |                                    | Several *E. coli* strains                   | Anjum et al. 2006                   |
| 2) Detection of bacteria            | Antibody microarray         | Mabs                   | *C*<sub>18</sub> derivatized SiO<sub>2</sub> | Impedance spectroscopy             | *Listeria monocytogenes* cells             | Huang et al. 2003                  |
|                                     |                             | Abs                    | Streptavidin modified | Fluorescence                       | *E. coli*                                   | Gehring et al. 2006                |
|                                     | Reversed protein array      | Genetically engineered | gold electrodes     | fluorescence                       | Streptavidin                                | Oh et al. 2006                    |
|                                     | (cell-based array)          | *E. coli* cells        | cellulosic membrane | Fluorescence                       | *E. coli*                                   | Stokes et al. 2001                  |
| 3) Detection of toxins              | Antibody microarray         | Pabs and Mabs          | Avidin-coated borosilicate | Fluorescence (waveguide)          | Several toxins / toxoids                    | Ligler et al. 2003                  |
|                                     |                             | Mabs                   | Epoxy-slides        | Fluorescence                        | Several native toxins                       | Rucker et al. 2005                  |
|                                     |                             | Abs                    | Hydrogel-based      | Fluorescence, MS chemiluminescence | Several plant and bacterial toxins          | Rubina et al. 2005                  |
|                                     |                             | Abs                    | Glass-slides        | Fluorescence                        | Toxic agents                                | Wadkins et al. 1998                |
| 4) Simultaneous detection of bacteria and toxins | Antibody microarray         | Pabs                   | Neutravidin derivatized | Fluorescence (waveguide) | Bacterial, viral, and protein analytes      | Rowe et al. 1999                   |
|                                     |                             | Mabs                   | Neutravidin derivatized | Fluorescence (waveguide) | Bacterial analytes and toxins               | Rowe-Taitt et al. 2000; Taitt et al. 2002 |
|                                     |                             | Abs                    | Neutravidin coated   | Fluorescence                        | Bacteria and bacterial proteins             | Delehanty and Ligler 2002          |
|                                     |                             |                        | SERS-activated metal | SERS microscopy                    | Bacteria and bacterial analytes             | Grow et al. 2003                   |
### Table 26.1. (continued)

| Application                                      | Type of Microarray | Probe Source            | Support                  | Mode of Detection | Sample                          | References          |
|--------------------------------------------------|--------------------|-------------------------|--------------------------|------------------|---------------------------------|---------------------|
| 5) Identification of diagnostic markers           | Protein microarray | *Y. pestis* proteins    | Silylated glass          | Fluorescence     | Serum from immunized rabbits    | Li et al. 2005      |
|                                                  |                    | *N. meningitides* proteins | FAST-slides              | Fluorescence     | Human sera                      | Steller et al. 2005 |
| 6) Disease state differentiation                  | Protein microarray | *M. tuberculosis* proteins | FAST-slides              | Fluorescence     | Human sera                      | Sartain et al. 2006 |
|                                                  |                    | *M. tuberculosis* antigens | Epoxy slides             | Fluorescence     | Human sera                      | Tong et al. 2005    |
| 7) Identification of toxin modulator / regulator | Protein microarray | Yeast/human proteins    | Nitrocellulose           | Fluorescence     | Cytotoxic enterotoxin           | Galindo et al. 2006 |
| 8) Detection of protein signatures associated with bacterial infection | Antibody microarray | Recombinant scFv Ab library | Black polymer Maxisorb | Fluorescence     | Human stomach tissue            | Ellmark et al. 2006b |
| 9) Carbohydrate fingerprinting of bacteria        | Protein microarray | Lectins                 | Nexterion H slides       | Fluorescence     | *E. coli strains*               | Hsu et al. 2006     |
|                                                  |                    |                         | Exopoxysilane modified glass slide | Fluorescence (wave guide) | Glycoproteins                   | Uchiyama et al. 2006 |
|                                                  | Reversed protein array | Carbohydrates           | CodeLink slides          | Fluorescence     | Various model bacteria systems | Disney and Seeberger 2004 |

Abs = Antibodies, not specified whether polyclonal and/or monoclonals; Mabs = Monoclonal antibodies; MS = mass spectrometry; Pabs = Polyclonal antibodies; SERS = Surface-Enhanced Raman Scattering; TMB = 3,3′, 5,5′-tetramethylbenzidine (TMB) peroxidase substrate
et al. 2005; Lueking et al. 2005), and the functional properties, e.g., protein-protein interactions, of the arrayed proteins (MacBeath and Schreiber 2000; MacBeath 2002; LaBaer and Ramachandran 2005; Kingsmore 2006). In particular, the use of cDNA expression libraries as the probe source has been very rewarding (Zhu et al. 2000, 2001; Horn et al. 2006). For example, microarrays composed of 119 of 122 yeast protein kinases (Zhu et al. 2000), 5,800 of 6,200 yeast proteins (Zhu et al. 2001), or 37,200 redundant recombinant fetal brain proteins (Horn et al. 2006) have been designed, fabricated, and successfully applied to perform global protein-protein interaction studies. To date, mainly water-soluble protein analytes have been targeted, but the first microarray design also targeting membrane proteins in the format of intact mammalian cells has recently been published (Deviren et al. 2007).

Functional protein arrays have frequently been applied within the academic research community, but several commercial ventures have also developed such microarrays that are now available on the market. For example, focused microarrays targeting cell signaling proteins and tentative cancer-associated proteins have been launched by Sigma (http://www.sigma.com). In addition, comprehensive microarrays based on over 8,000 human recombinant proteins have recently been released by both Protagen (http://protagen.de) and Invitrogen (http://invitrogen.com).

In the case of bacteria and bacterial protein analytes, various designs of functional protein microarrays have been successfully applied (Table 26.1). In these first examples, microarrays based on a set of human or yeast proteins (Galindo et al. 2006), recombinant bacterial proteins (Li et al. 2005; Steller et al. 2005; Tong et al. 2005), biochemically isolated and fractionated bacterial proteins (Tong et al. 2005; Sartain et al. 2006), neoglycoproteins (oligosaccharides bound to bovine serum albumin) (Tong et al. 2005), and lipopolysaccharides and saccharides (Tong et al. 2005) have been developed and fabricated. In these studies, the first generation(s) of applications for the identification of toxin modulators/regulators (Galindo et al. 2006), disease state differentiation (Tong et al. 2005; Galindo et al. 2006; Sartain et al. 2006), and identification of diagnostic markers (Li et al. 2005; Steller et al. 2005) have been outlined.

1.3. Affinity Protein Microarrays

Affinity protein microarrays have been developed for multiplex protein expression profiling, specifically detecting whether the targeted analytes are expressed and at what (relative) levels (MacBeath 2002; Zhu and Snyder 2003; Angenendt 2005; Wingren and Borrebaeck 2006a). To date, antibodies are the most commonly used probe source for affinity protein microarrays (Wingren and Borrebaeck 2004; Haab 2006; Kingsmore 2006; Wingren and Borrebaeck 2006a). In more detail, microarrays based on polyclonal and monoclonal antibodies (Sreekumar et al. 2001; Miller et al. 2003; Gao et al. 2005; Sanchez-Carbayo et al. 2006), as well as recombinant antibody fragments (Wingren et al. 2003; Pavlickova et al. 2004; Wingren et al. 2005; Ellmark et al. 2006b), have been successfully designed and applied for (disease) proteomics. For example, tentative protein expression profiles associated with disease and clinical parameters have been identified (Gao et al. 2005; Borrebaeck 2006; Ellmark et al. 2006b; Sanchez-Carbayo et al. 2006).

Intense efforts are currently under way to develop the affinity protein microarray technology even further; for a review see Wingren and Borrebaeck (2004); Kingsmore (2006); Wingren and Borrebaeck (2006b, 2006a). In parallel with all the academic efforts, several commercial products have been launched, mainly for focused assays (protein expression profiling) and point-of-care applications (Wingren and Borrebaeck 2004); although larger arrays have also started to emerge on the market (e.g., http://www.clontech.com, http://www.raybiotech.com, http://www.protneteomix.com, http://www.whatman.com, http://www.sigmaaldrich.com). As for functional protein arrays, mainly water-soluble analytes have
been targeted (Wingren and Borrebaeck 2004), but array designs targeting membrane proteins in the format of intact mammalian cells have recently been published as well (Belov et al. 2001, 2003; Ko et al. 2005b, 2005a; Campbell et al. 2006; Ellmark et al. 2006a) (Dexlin, Borrebaeck, and Wingren unpublished).

In Table 26.1, different applications in which various antibody-based microarray designs have been used to target bacteria and protein analytes thereof are listed. So far, the main applications are the detection of bacteria (Stokes et al. 2001; Huang et al. 2003; Gehring et al. 2006; Oh et al. 2006), toxins (Wadkins et al. 1998; Ligler et al. 2003; Rubina et al. 2005; Rucker et al. 2005), or both (Rowe et al. 1999; Rowe-Taitt et al. 2000; Delehanty and Ligler 2002; Taitt et al. 2002; Grow et al. 2003); the serotyping of bacteria (Cai et al. 2005; Anjum et al. 2006); and the detection of protein expression signatures associated with bacterial infections (Ellmark et al. 2006b). To date, the degree of multiplexity differs, with array designs based on a few single probes ranging up to a few hundred, depending on the actual assay at hand.

In more detail, a low degree of multiplexity (≤ 50 probes) may be sufficient to screen and detect toxins, etc., while a high degree of multiplexity (> 100 probes) may, at least initially, be required to detect, for example, disease-associated protein expression signatures.

1.4. Alternative Microarray Setups

Entering the post-genomic era, not only the proteome but also the glycome has gained significant biomedical interest (Shriver et al. 2004; Miyamoto 2006). In recent work this has been explored to single out pathogenic bacteria (Pohl 2006), as well as to detect pathogens (Disney and Seeberger 2004). In these efforts, work has been made to design both lectin microarrays (Hsu et al. 2006; Uchiyama et al. 2006) and carbohydrate microarrays (Disney and Seeberger 2004) (Table 26.1). Cell surface carbohydrates are in fact critical for many seminal interactions that define bacteria as pathogens and symbiotes. By adopting multiplex technologies for carbohydrate profiling, series of bacterial strains could be fingerprinted, based on their carbohydrate patterns, which might be used for identification, etc.

Lectins are sugar-binding proteins of nonimmune origin that contain at least two sugar-binding sites and are commonly used in agglutination tests to screen the bacterial glycome. However, these assays often suffer from inadequate sensitivity and subjective visual read-out. In comparison, MS, NMR, and HPLC-based analysis are alternative but time-consuming assays commonly applied for bacterial glycan analysis. In a recent paper, Hsu et al. presented a lectin-based microarray approach for analyzing the dynamic bacterial glycome (Hsu et al. 2006). The platform was based on 21 lectins on Nexterion H slides, and the arrays were imaged by fluorescence, since the bacteria were directly labeled with SYT 85. The results showed that (1) closely related *E. coli* strains could be distinguished based on their glycosylation pattern, i.e., enabling fingerprinting; and (2) dynamic alterations in the bacteria glycome could be observed. The range of specificities displayed by lectins is currently a bottleneck, which is why other carbohydrate binding probes, e.g., antibodies, may provide an alternative route.

Recently, proof-of-concept was reported for an alternative lectin microarray platform by Uchiyama and coworkers (Uchiyama et al. 2006). They have developed a novel setup that allows observation of lectin-glycoprotein interactions under equilibrium conditions, based on an evanescent-field fluorescence-assisted detection principle. This enables the assay to be performed without washing procedures, a clear advantage considering the relatively weak lectin-glycan interactions.

To examine carbohydrate-cell interactions and to detect pathogens, a carbohydrate microarray has been developed (Disney and Seeberger 2004). In this context, it may be of interest to note that cell-surface carbohydrates are exploited by many pathogens for tissue adherence and entry into host cells. The carbohydrates (e.g., mannose and fucose) were dispensed and
immobilized onto CodeLink slides, and bound bacteria (directly labeled) were detected by fluorescence. Proof-of-principle was shown for this carbohydrate microarray as a means to detect bacteria, as illustrated by different *E. coli* strains. In addition, these nondestructive arrays allow the bacteria to be harvested and tested for, e.g., antibacterial susceptibility.

### 2. Detection of Bacteria and Bacterial Protein Analytes

#### 2.1. Serotyping of Bacteria

Bacteria can be serotyped by determining their somatic (O) and flagellar (H) cell surface antigens. Serotyping is of clinical importance as, for example, many O bacterial serotypes are linked with a number of diseases (syndromes), in that subsets of serotypes, or pathotypes, can cause meningitis, systematic disease, diarrhea, etc. Despite being a primary diagnostic tool, the current methods available, e.g., agglutination tests, suffer from limited throughput, no or low multiplexity, requirement of large sample volumes, and are costly. In two recent publications, the possibility of using antibody-based microarrays for serotyping of *E. coli* (Anjum et al. 2006) and *S. enterica* (Cai et al. 2005) strains have been explored and exploited (Table 26.1).

Anjum and coworkers adopted the ArrayTube platform to develop miniaturized antibody arrays on an epoxy-modified glass surface (Anjum et al. 2006). The authors employed 17 rabbit antisera raised against the most common *E. coli* pathogens (e.g., O157 and O26) associated with disease syndromes in both humans and animals. After adding the *E. coli* cell samples, bound cells were detected by secondary antibodies and signal amplification reagents, and the arrays were imaged by monitoring specific changes in red light transmission. This feasibility study showed that 88–100% of the tested *E. coli* isolates could be correctly classified. In fact, the observed discrepancy was related to poor sample quality rather than to an inadequate identification. Hence, the results implied that the antibody array setup performed well for O serotyping, providing multiplexed, cost-effective, efficient, and accurate typing.

In comparison, Cai et al. developed an antibody array platform based on 35 polyclonal antibodies (antisera) against 20 common *Salmonella* serovars using SuperEpoxy slides (Cai et al. 2005). Numerous fluorescently labeled *Salmonella enterica* strains were analysed and the arrays were imaged using fluorescence as a read-out system. The results showed that the array setup enabled complete serovar identification of 86 of 117 target strains, and partial identification of 30 of 117. Further, all of the 73 analysed nontarget strains (negative controls) were successfully excluded. Hence, an array platform providing a rapid and cost-effective alternative to the traditional agglutination method for *Salmonella* serotyping has been developed.

#### 2.2. Detection of Pathogenic Organisms

Foodborne pathogenic bacteria, such as *E. coli* O157:H7, are responsible for about 80 million illnesses in the United states each year, with thousands resulting in death. So far, the analytical approaches applied for the detection of bacteria have included plate culture, ELISA, and PCR. Hence there is a tremendous need for multiplexed technologies enabling combinations of pathogens to be screened and detected in a simple manner, and efforts to address this issue are underway (Table 26.1).

In recent years, two independent antibody microarray or biochip setups for the detection of *E. coli*, using O157:H as a test system, have been developed (Stokes et al. 2001; Gehring et al. 2006). In the first example, a microfluidics-based antibody biochip-based system was developed for the detection of *E. coli* (Stokes et al. 2001). This reversed affinity protein microarray is based on the exposure of a cellulosic membrane to a sample potentially containing *E. coli*. The bacteria is then immobilized (bound) to the membrane and detected by fluorescently labeled secondary
antibodies. The setup was found to display rapid and selective detection of bacteria with at least three orders of magnitude linear dynamic range. Of note, the assay sensitivity was found to be as low as 20 organisms (in this case *E. coli* O157:H7). In comparison, Gerhing and coworkers have developed a sandwich fluorescent immunoassay in the microarray format (Gehring et al. 2006). Biotinylated capture antibodies were immobilized onto streptavidin-modified Superfrost Gold slides, and the setup was evaluated targeting *E. coli* O157:H7 samples. The applicability of this, so far, low-density setup was outlined and a limit of detection in the $3 \times 10^6$ cells/ml range was observed.

Similar to the setup by Gerhing et al. (2006), Oh and colleagues have developed a reversed affinity protein array setup for the detection of bacteria (Oh et al. 2006). Using a microfluidic device, the cells were electrokinetically immobilized onto gold electrodes and imaged by fluorescence after adding appropriate secondary reagents (antibodies). It should, however, be noted that this setup was not primarily developed for the detection of bacteria, but rather as a new tool for taking advantage of the bacteria to display a capture protein, e.g., a membrane protein, in its natural environment, and thereby increasing its on-chip functionality. In this context, it should be noted that designing and fabricating membrane protein microarrays is in general a major challenge that remains to be fully resolved (Fang et al. 2002a, 2002b; Wingren and Borrebaeck 2004). Hence, this setup might open new avenues not only for the detection of bacteria, but also for designing membrane protein-based microarrays.

In recent work, an antibody microarray setup based on biotinylated monoclonal antibodies was developed and optimized with respect to nonspecific adsorption of bacteria and proteins thereof. The antibodies were immobilized via streptavidin, which in turn was bound to biotinylated bovine serum albumin (BSA) adsorbed onto a C$_{18}$-derivatized SiO$_2$ surface (Huang et al. 2003). The results showed that the dual action of BSA, acting both as a surface blocker and as a probe immobilized, was successful. Directed immobilization and low nonspecific binding were reported.

### 2.3. Detection of Multiple Toxins

Rapid, sensitive, and multiplex detection of biological toxins in clinical samples, food, drinking water, and environmental samples is of great importance in revealing possible infections and contaminations, as well as potential bioterrorist threats (Table 26.1). In the long-term, small, simple, and portable devices (biosensors) would be an attractive format for the instrumentation behind such key applications. In this context, the sample format will also be critical, and assay designs allowing the sample to be directly applied without any significant pretreatment, e.g., fluorescent labeling, would clearly be advantageous.

Four studies have been published, in which the efforts at developing antibody-based microarray biosensors for the detection mainly of toxins have been successfully described (Wadkins et al. 1998; Ligler et al. 2003; Rubina et al. 2005; Rucker et al. 2005). In an early study by Wadkins et al. a planar array immunosensor for the detection of multiple toxic agents was fabricated (Wadkins et al. 1998). Polyclonal antibodies were covalently coupled to derivatized glass slides, and bound toxins, e.g., ricin and staphylococcal enterotoxin B, were monitored using fluorescently labeled secondary antibodies. Assay sensitivities in the 5–25 ng/ml range were observed.

In comparison, an array biosensor, based on monoclonal and polyclonal antibodies, capable of detecting multiple targets on the borosilicate glass surface of a single waveguide, was more recently designed (Ligler et al. 2003). Both competitive and sandwich fluor-immuno setups were developed to enable small, as well as large, molecular weight toxins (e.g., ricin, botulinum toxoids, and trinitrotoluene) to be detected in complex samples, such as food or clinical specimens. Notably, the setup was capable of addressing up to 12 samples at the same
time. The results showed that specific and sensitive (≥0.5 ng/ml) detection of target analytes was accomplished. With additional development of the sensor instrument, this may in the end provide a rapid, fieldable, and low-tech assay for the detection of toxins.

Similarly, Rucker and colleagues have developed competitive and noncompetitive antibody microarray setups for native toxin detection (e.g., diphtheria toxin and anthrax lethal factor) in serum samples (Rucker et al. 2005). In this case, monoclonal antibodies were immobilized on epoxy-slides, and the arrays were imaged using fluorescence. While the competitive assay setup was favored for not having to label the sample, the direct assay benefited from superior sensitivity (low ng/ml vs. high ng/ml). In the end, the choice of setup may be dependent on whether the assay is run in field trials, where a simple assay is desired, or in the laboratory, where more complex assay principles providing higher sensitivity can be applied.

Further, a hydrogel-based monoclonal antibody microchip was recently designed and fabricated by Rubina et al. (2005). The platform was developed with the aim of performing a quantitative immunoassay of a series of plant toxins (e.g., ricin and viscosum) and bacterial toxins (e.g., diphtheria toxin and tetanus toxin). Direct, competitive, and sandwich assay setups were successfully developed and found to be compatible with the platform. In contrast to the previous studies, this system was interfaced with either a fluorescent-, chemiluminescent-, or MS-based read-out system, providing high flexibility. In all cases, the assay sensitivities were found to be in the low ng/ml range, i.e., within the range of sensitivity required in order to be able to perform clinical applications.

2.4. Simultaneous Detection and Identification of Bacterial Proteins and Bacteria

Similar to the work described in Section 26.2.3, antibody-based microarray biosensors have also been used for the simultaneous detection of bacteria and bacterial proteins (e.g., toxins) (Table 26.1), where again technologies for rapid and multiplexed detection will play a key role.

An antibody-based array biosensor composed of three parts, the antibody array (recognition element), an image capture and processing part, and an automated fluidics unit, has been developed by Ligler et al. (Rowe et al. 1999; Rowe-Taitt et al. 2000; Taitt et al. 2002). The capture polyclonal and/or monoclonal antibodies were biotinylated and immobilized on neutravidin-derivatized waveguides. Bound analytes (proteins, glycoproteins, Gram-negative, and Gram-positive bacteria) were detected using labeled secondary (tracer) antibodies. The results showed that assay sensitivities in the mid ng/ml range were readily observed targeting, for example, cholera toxin and B. globigii. Moreover, the assay was demonstrated to be rapid (<15 min) and easy to execute. Taken together, these studies have demonstrated proof-of-concept for an inexpensive and multiplex device for simple detection of bacteria and bacterial analytes. In addition, the setup is in a format amenable to automation and portability. In these first studies, the capture antibody spots were in the 2.5 mm² size range and generated by physically isolated patterning using polymer flow cells. In recent work, the spot size of the biotinylated capture antibodies has been considerably reduced (0.04 mm²) by adopting a noncontact piezo-based dispenser to fabricate the arrays (Delehanty and Ligler 2002). Using confocal microscopy for detection, an assay sensitivity in the low ng/ml range was still obtained. Hence the latter study outlined a way of fabricating high-density arrays for bacterial detection, while maintaining assay sensitivity.

In a recent review by Grow et al. a new biochip technology for label-free detection of pathogens and their toxins was presented and discussed (Grow et al. 2003). The biochip is composed of spots of capture probes (e.g., antibodies) immobilized on a surface-enhanced Raman scattering (SERS) active metal surface. Once the chip has been subjected to sampling and target analytes have been bound, a Raman microscope is applied to collect SERS fingerprints from the spots (pixels) on the chip. This interesting technology has been named
μSERS, as it couples SERS with microscopy. The identification is based on SERS fingerprints, and the authors demonstrated that both Gram-positive and Gram-negative bacteria often could be detected at the strain/subspecies level based on their SERS fingerprints. Further, the SERS fingerprints could also be used to differentiate viable vs. nonviable, e.g., heat- or UV-killed, microorganisms; different physiological states of the bacteria cells, e.g., when cultured under conditions known to affect virulence; and to detect toxins in a specific and sensitive manner. Work is currently under way to develop the Raman microscope instrumentation even further, to enable the simultaneous collection of hundreds or thousands of spectra from discrete positions on the chip with a spatial resolution of 250 nm to 1.5 μm. Future experiments will unravel the potential of this read-out system for protein microarray-based applications.

3. Detection of Diagnostic Markers, Toxin Regulators and Associated Protein Expression Profiles

3.1. Identification of Potential Diagnostic Markers and/or Vaccine Candidates

In two recent publications (Li et al. 2005; Steller et al. 2005), the possibility of using protein microarrays to identify novel potential diagnostic markers and/or vaccine candidates was explored and outlined (Table 26.1). Again, the array format was critical in order to enable sufficient multiplexity and throughput to gain success.

In the first study, a recombinant bacterial protein microarray was fabricated on FAST-slides and used for identification of new potential diagnostic markers for Neisseria meningitides (Steller et al. 2005). The authors succeeded in expressing 67 of 102 known phase-variable genes from N. meningitides serogroup B strain MC58 as recombinant proteins in E. coli. Subsequently, these proteins were used as probes in the array format and applied to screen sera from healthy controls vs. patients suffering from meningitis. The results showed that 47 of these proteins were immunogenic, i.e., that an antibody response had been mounted. Nine proteins were found to be immunogenic in at least 3 of 20 meningitis sera tested, while 1 protein showed a response in 11 of 20 sera. The potential of these N. meningitides proteins for diagnostic purposes remains to be elucidated, but this study clearly outlines the potential of the approach.

Yersina pestis causes plague, which is one of the most feared diseases. Work is ongoing to identify novel vaccine candidates to improve the current plague vaccines. In these efforts, Li et al. have developed a 149-recombinant Yersina pestis protein microarray to profile the antibody response in immunized rabbits, providing a new tool in the search for vaccine candidates and/or diagnostic antigens (Li et al. 2005). The authors found that an antibody response had been elicited against about 50 of the arrayed Y. pestis proteins. Among these 50, 11 proteins to which the predominant antibody response was directed were identified. Taken together, these 11 new proteins show promise for further evaluation as candidates for vaccines and/or diagnostic antigens.

3.2. Disease State Differentiation and Identification of Diagnostic Markers

The evaluation of serological reactivity from healthy vs. nonhealthy patients, in order to allow disease state differentiation and the identification of tentative diagnostic markers, has gained significant attention within the field of disease proteomics (Hanash 2003; Wingren and Borrebaeck 2004; Borrebaeck 2006). Focusing on bacterial related diseases, two independent protein microarray setups, focusing on tuberculosis, have been developed (Tong et al. 2005; Sartain et al. 2006) and applied to perform serological tuberculosis assays (Table 26.1).
Tuberculosis can be diagnosed by microscopy and culture of mycobacteria of the *Mycobacterium tuberculosis* complex from clinical samples. Still, these approaches are associated with limitations and technical hurdles. To be proven valuable, a serodiagnostic approach should (1) display a specificity > 90% (i.e., comparable to microscopy and bacterial cultures), and (2) be able to differentiate/detect multiple disease states.

Interestingly, Tong and coworkers have developed a protein microarray setup based on 54 *M. tuberculosis* antigens on epoxy-slides, and the arrays were imaged by fluorescence (Tong et al. 2005). The probe antigens were obtained from five sources, including biochemical fractionation of *M. tuberculosis* cells/culture fluids, oligosaccharides bound to BSA, purified lipopolysaccharides, purified polysaccharides, and recombinant antigens. The clinical serum samples from healthy controls (non-TB) and tuberculosis (TB) patients were screened for IgG antibodies specific for any of these antigens, e.g., for a serum-specific IgG profile. Based on the analysis of 20 TB sera and 80 non-TB sera, combinations of TB antigens were ranked with respect to specificity and sensitivity of TB detection. The results showed that the highest-ranking TB antigen combination displayed a receiver operator curve (ROC) with an area under the curve (AUC) of 0.95. Of note, a single antigen, Ara\(_6\)-BSA, was found to give an AUC value of 0.90. The authors concluded that the TB antigen microarray provided a rapid and efficient means of finding TB antigens that could be used to discriminate between TB and non-TB patients.

In comparison, Sartain et al. fabricated a TB antigen microarray based on 960 unique fractions, obtained from *M. tuberculosis* cytosol and culture filtrates, by multidimensional protein fractionation (Sartain et al. 2006). TB antigen arrays were fabricated on FAST slides and interfaced with a fluorescent read-out system. Next, serum samples from 12 healthy individuals, 9 noncavitary TB patients, 11 cavitary TB patients, 10 HIV-positive TB patients, and 6 HIV-positive TB-negative patients were analysed. The authors demonstrated that the TB antigen microarray setup provided them with a novel means of assessing antigen recognition profiles (e.g., specific IgG profiles) discriminating between different disease states. In more detail, the different sera were found to display partly overlapping reactivity patterns, e.g., containing antibodies specific for material in the arrayed subfractions, but also distinctly unique patterns. Hence, the results indicated that the setup could be useful for differentiating the different disease states, thus demonstrating the potential of array-based serodiagnostics for tuberculosis.

### 3.3. Identification of Potential Toxin Modulators/Regulators

The use of high-density protein microarrays to examine the protein-protein interaction patterns for bacterial toxins in a multiplex high-throughput manner, is very appealing (Table 26.1). In the end, this may allow for identification of novel toxin modulators and/or regulators.

In a recent paper by Galindo and colleagues, the potential of cytotoxic enterotoxin (Act) of *Aeromonas hydrophila* to bind to human and yeast proteins was investigated by adopting a protein microarray approach (Galindo et al. 2006). To this end, the human and yeast ProtoArrays composed of 1869 human proteins and 4319 yeast proteins, respectively, on nitrocellulose coated slides were used. The study showed that Act was capable of binding nine human proteins and 4 yeast proteins. For three of the interactions, a confirming Western blot analysis was performed. Next, a set of experiments, including small interfering RNA, was performed in order to explore the relevance of the observed interactions. These efforts indicated a potential involvement of galectin-3 and SNAP23 in *A. hydrophila* cytotoxic enterotoxin-induced host cell apoptosis. Hence, by adopting a high-density protein microarray screening approach, the authors were able to present the first report of tentative protein binding partners for Act, as well as potential mediators/regulators for Act-induced apoptosis.
3.4. Screening of Protein Expression Signatures Associated with Bacterial Infection

To date, a major focus has been placed upon using protein microarrays, and in particular antibody-based microarrays for oncoproteomics, with the aim of finding disease-specific (serum or tissue) protein signatures for diagnostics and biomarker discovery, etc. (Wingren and Borrebaeck 2004; Borrebaeck 2006; Kingsmore 2006). In a similar fashion, protein microarrays could be used to find protein expression signatures associated with bacterial infections and conditions (Table 26.1).

In a recent study by Ellmark et al. the authors examined stomach tissue samples from gastric adenoma carcinoma patients using a 127-human recombinant scFv antibody microarray on black polymer Maxisorb slides, interfaced with a fluorescent read-out system (Ellmark et al. 2006b). Of note, these cancer patients are often associated with *Helicobacter pylori* infections. The proteins were extracted from the tissue samples, biotinylated, and analysed on the recombinant antibody microarrays. The platform was found to display an assay sensitivity in the low pg/ml range. Further, the results showed that a 14-protein expression signature associated with *H. pylori* infection could be identified, where 10 analytes were distinctly different from the corresponding protein signature found to be associated with adenoma carcinoma. Taken together, these studies clearly demonstrate the use and potential of antibody (protein) microarray technology for rapid, sensitive, and multiplexed expression profiling of complex samples in order to identify disease-associated protein signatures.

4. Conclusions and Future Perspectives

Taken together, the first generations of protein-based microarray technology platforms for detection and identification of bacteria, bacterial proteins and bacterial diseases, have in recent years been developed. These miniaturized assay platforms include functional protein microarrays as well as affinity protein microarrays, and the designs range from small-scale focused biosensors targeting a few analytes to large-scale semi-dense microarray set-ups for multiplex screening. A broad range of applications, such as serotyping of bacteria, detection of bacteria, identification of toxins, disease state differentiation, and discovery of disease-associated biomarkers, have so far been demonstrated, clearly outlining the potential of the technology. Still, the number of applications is low, but is likely to increase significantly as the microarray technology progress and develops into a robust proteomic technology. In future, protein microarray based applications are likely to play an important role for detection and identification of bacterial and protein analytes.

Acknowledgements

This study was supported by grants from the Swedish National Science Council (VR-NT), the SSF Strategic Center for Translational Cancer Research (CREATE Health), the Alfred Österlund Foundation, and the Great and Johan Kock Foundation.

References

Angenendt P (2005) Progress in protein and antibody microarray technology. Drug Discovery Today 10:503–511
Anjum MF, Tucker JD, Sprigings KA, Woodward MJ, Ehrricht R (2006) Use of miniaturized protein arrays for *Escherichia coli* O serotyping. Clin Vaccine Immunol 13:561–7
Belov L, de la Vega O, dos Remedios CG, Mulligan SP, Christopherson RI (2001) Immunophenotyping of leukemias using a cluster of differentiation antibody microarray. Cancer Res 61: 4483–9
Belov L, Huang P, Barber N, Mulligan SP, Christopherson RI (2003) Identification of repertoires of surface antigens on leukemias using an antibody microarray. Proteomics 3:2147–54
Borrebaeck CA (2006) Antibody microarray-based oncoproteomics. Expert Opin Biol Ther 6:833–8
Cai HY, Lu L, Muckle CA, Prescott JF, Chen S (2005) Development of a novel protein microarray method for serotyping *Salmonella enterica* strains. J Clin Microbiol 43:3427–30
Campbell CJ, O’Looney N, Chong Kwan M, Robb JS, Ross AJ, Beattie JS, Petrik J, Ghazal P (2006) Cell interaction microarray for blood phenotyping. Anal Chem 78:1930–8
Delehanty JB, Ligler FS (2002) A microarray immunoassay for simultaneous detection of proteins and bacteria. Anal Chem 74:5681–7
Deviren G, Gupta K, Paulaitis ME, Schnepp JP (2007) Detection of antigen-specific T cells on p/MHC microarrays. J Mol Recognit 20:32–8
Disney MD, Seeberger PH (2004) The use of carbohydrate microarrays to study carbohydrate-cell interactions and to detect pathogens. Chem Biol 11:1701–7
Ellmark P, Belov L, Huang P, Lee CS, Solomon MJ, Morgan DK, Christopherson RI (2006a) Multiplex detection of surface molecules on colorectal cancers. Proteomics 6:1791–802
Ellmark P, Ingvarsson J, Carlsson A, Lundin SB, Wingren C, Borrebaeck CA (2006b) Identification of protein expression signatures associated with *H. pylori* infection and gastric adenocarcinoma using recombinant antibody microarrays. Mol Cell Proteomics 5:1638–46
Fang Y, Frutos AG, Lahiri J (2002a) Membrane protein microarrays. J Am Chem Soc 124:2394–5
Fang Y, Frutos AG, Webb B, Hong Y, Ferrie A, Lai F, Lahiri J (2002b) Membrane biochips. Biotechniques Dec Suppl:62–5
Galindo CL, Gutierrez C Jr, Chopra AK (2006) Potential involvement of galectin-3 and SNAP23 in *Aeromonas hydrophila* cytotoxic enterotoxin-induced host cell apoptosis. Microb Pathog 40:56–68
Gao WM, Kuick R, Orzechowski RP, Misek DE, Qiu J, Greenberg AK, Rom WN, Brenner DE, Omenn GS, Haab BB, Hanash SM (2005) Distinctive serum protein profiles involving abundant proteins in lung cancer patients based upon antibody microarray analysis. BMC Cancer 5:110
Gehringer AG, Albin DM, Bhunia AK, Reed SA, Tu SI, Uknalis J (2006) Antibody microarray detection of *Escherichia coli* O157:H7: quantification, assay limitations, and capture efficiency. Anal Chem 78:6601–7
Grow AE, Wood LL, Claycomb JL, Thompson PA (2003) New biochip technology for label-free detection of pathogens and their toxins. J Microb Methods 53:221–33
Haab BB (2001) Advances in protein microarray technology for protein expression and interaction profiling. Curr Opin Drug Discov Devel 4:116–23
Haab BB (2003) Methods and applications of antibody microarrays in cancer research. Proteomics 3:2116–22
Haab BB (2006) Applications of antibody array platforms. Curr Opin Biotechnol 17:415–21
Hanash S (2003) Disease proteomics. Nature 422:226–32
Horn S, Lueking A, Murphy D, Staadt A, Gutjahr C, Schulte K, Konig A, Landsberger M, Lehrch H, Felix SeB, Cahill DeJ (2006) Profiling humoral autoimmune repertoire of dilated cardiomyopathy (DCM) patients and development of a disease-associated protein chip. Proteomics 6:605–613
Hsu KL, Pilobello KT, Mahal JK (2006) Analyzing the dynamic bacterial glycome with a lectin microarray approach. Nat Chem Biol 2:153–7
Huang TT, Sturgis J, Gomez R, Geng T, Bashir R, Bhunia AK, Robinson JP, Ladisch MR (2003) Composite surface for blocking bacterial adsorption on protein biochips. Biotechnol Bioeng 81:618–24
Hueber W, Kidd BA, Tomooka BH, Lee BJ, Bruce B, Fries JF, Sanderstrup G, Monach P, Drijfhout JW, van Veenooij WJ, Utz PJ, Genovese MC, Robinson WH (2005) Antigen microarray profiling of autoantibodies in rheumatoid arthritis. Arthritis Rheum 52:2645–55
Kingsmore SF (2006) Multiplexed protein measurement: technologies and applications of protein and antibody arrays. Nat Rev Drug Discov 5:310–20
Ko IK, Kato K, Iwata H (2005a) Antibody microarray for correlating cell phenotype with surface marker. Biomaterials 26:687–96
Ko IK, Kato K, Iwata H (2005b) Parallel analysis of multiple surface markers expressed on rat neural stem cells using antibody microarrays. Biomaterials 26:4882–91
LaBaer J, Ramachandran N (2005) Protein microarrays as tools for functional proteomics. Curr Opin Chem Biol 9:14–9
Li B, Jiang L, Song Q, Yang J, Chen Z, Guo Z, Zhou D, Du Z, Song Y, Wang J, Wang H, Yu S, Wang J, Yang R (2005) Protein microarray for profiling antibody responses to *Yersinia pestis* live vaccine. Infect Immun 73:3734–9
Ligler FS, Taitt CR, Shriver-Lake LC, Sapsford KE, Shubin Y, Golden, J.P. (2003) Array biosensor for detection of toxins. Anal Bioanal Chem 377:469–77
Livingston AD, Campbell CJ, Wagner EK, Ghazal P (2005) Biochip sensors for the rapid and sensitive detection of viral disease. Genome Biol 6:112
Lu DD, Chen SH, Zhang SM, Zhang ML, Zhang W, Bo XC, Wang SQ (2005) Screening of specific antigens for SARS clinical diagnosis using a protein microarray. Analyst 130:474–82

Lueking A, Cahill DJ, Müllner S (2005) Protein biochips: A new and versatile platform technology for molecular medicine. Drug Discovery Today 10:789–94

MacBeath G (2002) Protein microarrays and proteomics. Nat Genet 32(Suppl):526–32

MacBeath G, Schreiber SL (2000) Printing proteins as microarrays for high-throughput function determination. Science 289:1760–3

Miller JC, Zhou H, Kwekel J, Cavallo R, Burke J, Butler EB, Teh BS, Haab BB (2003) Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. Proteomics 3:56–63

Miyamoto S (2006) Clinical applications of glycomic approaches for the detection of cancer and other diseases. Curr Opin Mol Ther 8:507–13

Oh SH, Lee SH, Kenrick SA, Daugherty PS, Soh HT (2006) Microfluidic protein detection through genetically engineered bacterial cells. J Proteome Res 5:3433–7

Pawlak M, Schick E, Bopp MA, Schneider MJ, Oroszlan P, Ehrat M (2002) Zeptosens protein microarrays: a novel high performance microarray platform for low abundance protein analysis. Proteomics 2:383–93

Pavlickova P, Schneider EM, Hug H (2004) Advances in recombinant antibody microarrays. Clin Chim Acta 343:17–35

Perrin A, Duracher D, Perret M, Cleuziat P, Mandrand B (2003) A combined oligonucleotide and protein microarray for the codetection of nucleic acids and antibodies associated with human immunodeficiency virus, hepatitis B virus, and hepatitis C virus infections. Anal Biochem 322:148–55

Phizicky E, Bastiaens PI, Zhu H, Snyder M, Fields S (2003) Protein analysis on a proteomic scale. Nature 422:208–15

Poetz O, Schwenk JM, Kramer S, Stoll D, Templin MF, Joos TO (2005) Protein microarrays: catching the proteome. Mech Ageing Dev 126:161–70

Pohl NL (2006) Array methodology singles out pathogenic bacteria. Nat Chem Biol 2:125–6

Robinson WH, Steinman L, Utz PJ (2003) Protein arrays for autoantibody profiling and fine-specificity mapping. Proteomics 3:2077–84

Rowe CA, Tender LM, Feldstein MJ, Golden JP, Scruggs SB, Macraith BD, Cras JJ, Ligler FS (1999) Array biosensor for simultaneous identification of bacterial, viral, and protein analytes. Anal Chem 71:3846–52

Rowe-Taitt CA, Golden JP, Feldstein MJ, Cras JJ, Hoffman KE, Ligler FS (2000) Array biosensor for detection of biohazards. Biosens Bioelectron 14:785–94

Rubina AY, Dyukova VI, Dementieva EI, Stomakhin AA, Nesmeyanov VA, Grishin EV, Zasedatelev AS (2005) Quantitative immunoassay of biotoxins on hydrogel-based protein microchips. Anal Biochem 340:317–29

Rucker VC, Havenstrite KL, Herr AE (2005) Antibody microarrays for native toxin detection. Anal Biochem 339:262–70

Sanchez-Carbayo M, Socci ND, Lozano JJ, Haab BB, Cordon-Cardo C (2006) Profiling bladder cancer using targeted antibody arrays. Am J Pathol 168:93–103

Sartain MJ, Slayden RA, Singh KK, Laal S, Belisle JT (2006) Disease state differentiation and identification of tuberculosis biomarkers via native antigen array profiling. Mol Cell Proteomics 5:2102–13

Shriver Z, Raguram S, Saishekharan R (2004) Glycomics: a pathway to a class of new and improved therapeutics. Nat Rev Drug Discov 3:863–73

Sreekumar A, Nyati MK, Varambally S, Barrette TR, Ghosh D, Lawrence TS, Chinnaiyan AM (2001) Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins. Cancer Res 61:7585–93

Steller S, Angenendt P, Cahill DJ, Heuberger S, Lehrhach H, Kreutzberger J (2005) Bacterial protein microarrays for identification of new potential diagnostic markers for Neisseria meningitidis infections. Proteomics 5:2048–55

Stokes DL, Griffin GD, Vo-Dinh T (2001) Detection of E. coli using a microfluidics-based antibody biochip detection system. Fresenius J Anal Chem 369:295–301

Taitt CR, Anderson GP, Lingerfelt BM, Feldstein MJ, Ligler FS (2002) Nine-analyte detection using an array-based biosensor. Anal Chem 74:6118:93–103

Tong M, Jacobi CE, van de Rijke FM, Kuijper S, van de Werken S, Lowary TL, Hokke CH, Appelmelk BJ, Nagelkerke NJ, Tanke HJ, van Gijswijk RP, Veusken J, Kolk AH, Raap AK (2005) A multiplexed and miniaturized serological tuberculosis assay identifies antigens that discriminate maximally between TB and non-TB sera. J Immunol 301:154–63

Uchiyama N, Kuno A, Koseki-Kuno S, Ebe Y, Horio K, Yamada M, Hirabayashi J (2006) Development of a lectin microarray based on an evanescent-field fluorescence principle. Methods Enzymol 415:341–51

Wadkins RM, Golden JP, Pristilos LM, Ligler FS (1998) Detection of multiple toxic agents using a planar array biosensor. Biosens Bioelectron 13:407–15

Wingren C, Borrebaeck C (2006a) Antibody microarrays—current status and key technological advances. OMICS 10:411–427

Wingren C, Borrebaeck C (2006b) Recombinant antibody microarrays. Screening. Trends in Drug Discovery 2:13–15

Wingren C, Borrebaeck CA (2004) High-throughput proteomics using antibody microarrays. Expert Rev Proteomics 1:355–64
Wingren C, Ingvarsson J, Dexlin L, Szul D, Borrebaeck CA (2006) Design of recombinant antibody microarrays for complex proteome analysis: choice of sample labeling-tag and solid support. (submitted)

Wingren C, Ingvarsson J, Lindstedt M, Borrebaeck CA (2003) Recombinant antibody microarrays—a viable option? Nat Biotechnol 21:223

Wingren C, Steinhauer C, Ingvarsson J, Persson E, Larsson K, Borrebaeck CA (2005) Microarrays based on affinity-tagged single-chain Fv antibodies: sensitive detection of analyte in complex proteomes. Proteomics 5:1281–91

Yanagida M (2002) Functional proteomics: current achievements. J Chromatogr B Analyt Technol Biomed Life Sci 771:89–106

Yuk CS, Lee HK, Kim HT, Choi YK, Lee BC, Chun BH, Chung N (2004) Development and evaluation of a protein microarray chip for diagnosis of hepatitis C virus. Biotechnol Lett 26:1563–8

Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, Lan N, Jansen R, Bidlingmaier S, Houfek T, Mitchell T, Miller P, Dean RA, Gerstein M, Snyder M (2001) Global analysis of protein activities using proteome chips. Science 293:2101–5

Zhu H, Bilgin M, Snyder M (2003) Proteomics. Annu Rev Biochem 72:783–812

Zhu H, Hu S, Jona G, Zhu X, Kreiswirth N, Willey BM, Mazzulli T, Liu G, Song Q, Chen P, Cameron M, Tyler A, Wang J, Wen J, Chen W, Compton S, Snyder M (2006) Severe acute respiratory syndrome diagnostics using a coronavirus protein microarray. Proc Natl Acad Sci U S A 103:4011–6

Zhu H, Klemic JF, Chang S (2000) Analysis of yeast protein kinases using protein chips. Nature Genetics 26:283–290

Zhu H, Snyder M (2003) Protein chip technology. Curr Opin Chem Biol 7:55–63