'. . .the 'Holy Grail' of microarray technology is the in vitro *diagnostics market'*

editorial

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The long and difficult road to the diagnostic market: protein microarrays

The basic principles of microarray technology were described in the early 1980s by Ekins' Ambient Analyte Theory, which states that a tiny amount of binding material (e.g. antibody or other receptor) does not significantly change the sample concentration and can give better sensitivity than conventional assay formats with 100 or 1000 times the amount of binding material [1]. The driving force behind this theory was the quest for increased sensitivity in the determination of low concentrations of diagnostically important substances such as hormones. A second, and more important, factor was the need to process large amounts of information within the genomics field, which could only be accomplished by testing for all possible analytes simultaneously – 'massive parallel testing'. Both requirements resulted in the development of microarrays: high sensitivity following Ekins' Ambient Analyte Theory; and parallel testing offering the possibility of determining thousands of parameters in one single experiment. DNA microarrays, built from different capture oligonucleotides on individual 'features' at a density of tens of thousands per cm^2, are well-established systems that are able to analyze the whole transcriptome in a single experiment [2]. Combined with sophisticated bioinformatics tools, DNA microarrays allow scientists to take a global view of biological systems. In the field of proteomics, microarray technology is applied to analyze gene expression and to study the function of proteins in a multiplexed fashion [3].

With regards to business, the 'Holy Grail' of microarray technology is the *in vitro* diagnostics (IVD) market. IVD could benefit in many ways from the implementation of microarray technology: cost per test, required sample volume, resulting infectious waste, and turn-around-time. Microarrays, being binding assays, can potentially be used in the fields of immunoassays and molecular diagnostics, with immunoassays being the significantly larger market. To be of substantial commercial value, regulatory approval of such miniaturized parallelized assays is necessary. Otherwise, the label 'for research purpose only' would prohibit any major commercial microarray success in the IVD market.

Assay formats of protein microarrays

Protein-microarray-based assays can be grouped according to formats and types of applications. Currently, forward-phase protein microarray assays are the most frequently used format. Using an array of well-defined capture molecules allows the simultaneous analysis of large numbers of different parameters from one sample. Examples of forward-phase microarray assays include antibody

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microarrays that are used to identify and quantitate proteins of interest, and affinity arrays that are used to study the interactions between proteins and immobilized binding molecules such as proteins, peptides, low molecular weight compounds, oligosaccharides or DNA [3].

On a reverse-phase array, which is another assay format, a multitude of different samples such as tissue or cell lysates are immobilized in a microarray format. Each microspot contains the whole proteome repertoire of the tissue or cell. Single soluble probes, such as highly specific antibodies, are used to screen these spots simultaneously for the presence or absence of distinct target proteins. Using replicates of microarrays, sets of parameters in large collections of tissue or cell samples, or sample fractions can be determined [4].

Antibody arrays for protein profiling

One of the ambitious goals within the field of protein microarray technology is array-based proteomics, the complementary proteome array to whole genome DNA microarray using high density antibody arrays to analyze the expression of thousands of proteins in a single experiment. In principle, the binding of capture molecules to target nucleic acids and proteins is similar: both reactions exhibit binding constants in the range of 107–1011 I/M and assays need to be able to handle cross reactivity – the binding of non-target molecules. Design and generation of highly specific protein profiling arrays depend on the availability of specific capture agents. This is not a problem for microarrays involving nucleic acid molecules. Based on the primary sequence of the target DNA, specific DNA capture sequences can be determined according to the well-defined principle of complementary base pairing. In addition, high-throughput oligonucleotide synthesis enables the fast and inexpensive generation of such DNA capture agents. Because DNA molecules are a homogenous class of molecules, assay conditions such as temperature or appropriate buffer systems can be optimized to achieve similar binding conditions for all target molecules of interest. In addition, nonspecific binding events can be detected by including appropriate control spots (for each and every target molecule) in the microarray. Furthermore, sensitivity can be increased by applying analyte amplification processes (like PCR) as a first step of the experiment [2,5].

In contrast to DNA chips, protein-based microarray assays are much more difficult to establish. First of all, it is impossible to predict high-affinity capture molecules for proteins from their primary amino acid sequence, owing to the diverse tertiary structures of proteins and the manifold possibilities of protein–protein interaction. Interaction depends on strong electrostatic forces, hydrogen bonds and hydrophobic or weak van der Waals interactions, but most often all of these interactions in combination. Moreover, post-translational modifications such as glycosylation or phosphorylation impact protein interactions. In addition, proteins often appear in complexes and simultaneously interact with different binding partners. All these factors combined necessitate that protein capture molecules must be generated individually and screened, not only for affinity but also for specificity and cross-reactivity. One of the trickiest problems is to retain the functionality of the immobilized capture molecules by preserving their tertiary structure and their binding sites. This is, of course, far more difficult than attaching oligonucleotides or DNA fragments to a solid support. Finally, no amplification method for proteins is available, which would be equivalent to PCR for nucleic acids. Nucleic acids and proteins also differ in the way signals are detected. Nucleic acid samples can be labelled with low molecular weight markers before the hybridization reaction. Only the amount of label required for a specific assay is introduced and the binding capabilities of the analyte to the immobilized capture molecules remain unchanged. This is not possible for proteins.

Recently, several antibody microarrays made up of hundreds of antibodies have become commercially available (http://www.biochipnet.de). However, antibody arrays are still far from becoming a routine high-throughput proteomic discovery tool, owing to the currently limited content – the number of different capture molecules within an array – and limited sensitivity.

To achieve appropriate sensitivity, the user usually relies on sandwich immunoassay technology, where a second, not necessarily highly specific, antibody linked to a label is added for detection. The higher the concentration of this antibody the higher the nonspecific interaction, and this results in an increase in background. In practice, this limits the number of different features in a protein array – unless the number of different detection compounds can be reduced. As an example, in an array of antibodies directed against specific allergens or autoantibodies in humans, the detection antibody can be only one species, namely an anti-human-antibody. Therefore, protein microarray assays cannot compete with the newer nucleic acid arrays regarding the number of different analytes. However, protein microarrays consisting of focused sets of miniaturized and parallelized sandwich immunoassays are meanwhile well-established for use in basic and applied research (http://www.biochipnet.de).

Protein microarrays and diagnostics

After many years of development, microarrays are still not a significant diagnostic business. Two factors are mainly responsible for that.

- First, most of the development efforts in the past have been directed towards nucleic acid systems. Nucleic acid analysis – despite all of the rosy pictures painted by various consultancy groups –still plays a fairly minor role in the diagnostic market. Looking at market studies, one can only admire the 'hockey stick' tendencies that are shown year after year – only to be shifted along year after year. Nucleic acid analysis does not have the direct medical and/or biological relevance of analyzing molecules such as hormones, blood sugar, cholesterol or tumour markers. Furthermore, it is often difficult to act on information gained by nucleic acid analysis. For example, an individual carrying a mutation in a gene that might predispose for breast cancer might never develop this disease. Or, vice versa, it is possible to develop breast cancer despite the absence of such a mutation. Such types of diagnostic tests lack therapeutic relevance that protein microarrays could deliver.
- Second, the regulatory issue. Manufacturers of a diagnostics test need to ascertain that the correct compound (antibody or protein) is functionally immobilized on the microarray (see the review by Master *et al.* [6] in this issue of *Drug Discovery Today*). These compounds need to undergo all kinds of quality control

procedures. Required regulatory procedures command a substantial share of the development budget of a new test.

What if one of many tests on a microarray needs to be changed – a different antibody for example – or a new test needs to be added? Does this require new regulatory approval of all the other tests? If a customer does not want the result of one specific test on the chip, does this mean he can pay less? If the manufacturer simply blanks out the result the customer does not want and this test indicates a severe medical condition in the patient, is the manufacturer liable? These hypothetical examples show that there are many questions that still need to be addressed before microarrays can penetrate the diagnostics market. In the meantime, existing planar microarrays and bead-based assay systems are already available to provide robust platforms for multiplexed analysis in research applications (http://www.biochipnet.de) [7–9]. Acceptance of protein microarrays based on the sandwich immunoassay format is constantly growing and such types of protein microarray are nowadays broadly used in biomarker screening programmes, to generate panels of disease-specific biomarkers [10]. It will depend on the number of validated disease-specific biomarkers as well as on their therapeutic relevance, whether such assays will be performed using a protein microarray format. Today, in immune or allergy diagnostics, where it is necessary to screen patient sera for the presence or absence of a relatively small number of different types of autoantibodies or antibodies directed against parasitic and viral antigens or allergens, new tests in the microarray format are on their way into the diagnostic market. However, high-content multiplexed analyses, containing hundreds of different capture agents, are still far away.

Medical need, combined with an overall cost reduction, must become the driving force, in order for protein arrays to gain a substantial share of the IVD market. Only then will protein arrays be able to deliver on the high expectations they have raised.

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