EuroBiochips: spot the difference!

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The IBC *EuroBiochips 2001* conference (5–8 June 2001, Munich, Germany) reviewed the current state-of-the-art miniaturization and high-throughput technologies and comprised ~40 presentations, the majority of which was by European speakers. The conference focused on three main technologies: nucleic acid arrays, protein arrays and microfluidic technologies. The term 'biochip', which was defined neither before nor during the conference, could be, and was, applied equally well to each of the technologies presented; and, despite the sometimes ambiguous definitions, all presenters agreed on the following: miniaturization is the key.

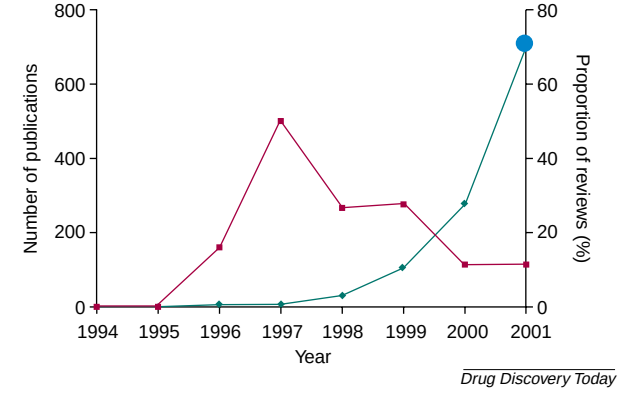

Figure 1. DNA microarray publications (red). Value for 2001 (blue) is an estimate, based on the first two quarters of 2001. Proportion of reviews (green) among total publications on the subject.

Why miniaturize?

There are several obvious reasons: miniaturization allows the packing of more reagents into the same volume. For example: hundreds of different protein-affinity reagents on a chip instead of old-fashioned western blots and Sepharose affinity-columns; hundreds of thousands of DNA hybridization reactions on a single glass-slide instead of old-fashioned Southern and northern blots; and an integrated laboratory inside an ordinary CD-ROM-like disk.

Other, not as obvious but equally important, advantages of miniaturization include the potential to increase reaction kinetics as a result of smaller reaction-volumes, and significantly increased surface:volume ratios[1]. In addition to these advantages, most of the microfluidic applications allow capillary forces to manipulate the reagent solution, and where capillary force is insufficient for pumping and mixing the reagents, spinning of the whole CD-like laboratory will finish the job! In terms of costs, miniaturization is also attractive because less reagents are needed for smaller size reactions. Capillary channels the size of a single DNA molecule can now be produced on a large scale. This could enable single-molecule applications to be carried out routinely. However, there is little data available yet on how DNA, and other large biological molecules, would behave under such conditions.

Nucleic-acid arrays

Only a few years ago DNA arrays seemed to be technology of the future; now researchers are frequently spotting DNA on slides and membranes. Although the patent situation is complex, as was outlined by Dominique Trosch and Bernard Ganahl (Reinhardt Sollner Ganahl, Kirchheim bei Munchen, Germany), the market does appear open and usable. After an initial period, characterized by intensive speculations (illustrated by the abnormally high proportion of published reviews over research papers; Fig. 1), DNA microarrays almost became a commodity, but uptake and use of this technology in drug discovery has not been as fast as some DNA arraying companies would have liked.

There are two major types of arrays – oligonucleotide-based (short nucleic-acid fragments) and cDNA based (longer DNA-fragments). Both are successfully used in industry (e.g. Affymetrix, Santa Clara, CA, USA; and Incyte, Palo Alto, CA, USA) and in more academic do-it-yourself settings (e.g. catalogue and custom arrays from MWG-Biotech, Edersberg, Germany; and Agilent Technologies).

Although the initial costs for a preprinted DNA chip might seem prohibitive, in comparison to the staff, equipment, information-technology support and quality control required to manufacture them in-house, it is cost-effective to first know how many experiments are to be performed. Two cheaper approaches described at this conference are RNA Chip™ and Atlas™ technologies from ClonTech Laboratories (Palo Alto, CA, USA). These relatively inexpensive approaches provide a convenient disease-profiling technology-platform and could be used without large initial investments in expensive equipment.

Now that DNA chips have become more reproducible, efforts are being focused on improving speed and sensitivity, as highlighted by the advent of new

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assay techniques and bioinformatics. Zeptosens (Witterswil, Switzerland) have collaborated with Qiagen (Chatsworth, CA, USA) to apply planar wave-guide technology to DNA chips. EMBL–EBI (European Molecular Biology Institute–European Bioinformatics Institute; Cambridge, UK) and IBM (White Plains, NY, USA) have embarked on data analysis systems to extract meaningful data from massive and complex data sets. With the current increase in use, DNA-array technology is expected to become a routine application (such as DNA electrophoresis) in 2–3 years for HTS of SNPs, mutational analysis and as a first step in the screening of differentially expressed targets.

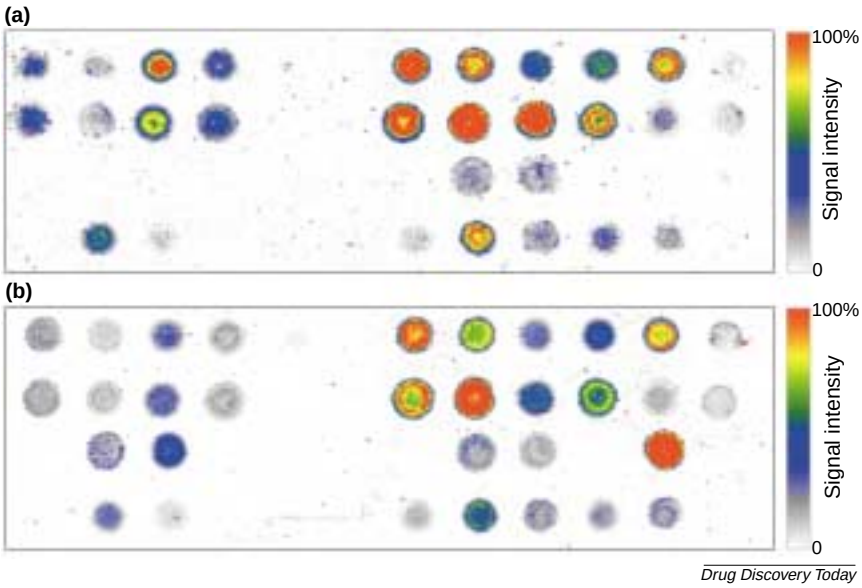

Figure 2. Spot the difference: Typical output of a differential protein expression analysis. Two different cell lysate samples **(a)** and **(b)** were separately labelled and hybridized to identical arrays of 48 different different affinity capture reagents (antibodies). This image is reproduced, with permission, from the presentation by Oxford Glycosciences (UK) Ltd (Oxford, UK).

Protein microarrays – evolution or revolution?

The review by the chair of this session (Thomas Joos, Natural and Medical Sciences Institute (NMI); University of Tuebingen, Reutlingen, Germany) and the first presentation by Hanno Langen (Roche, Basel, Switzerland) introduced this session that discussed recently developed technologies and those of the future, rather than protein-chip derived data.

To make an array of biological molecules, the molecules either have to be available and purified (before spotting) or be synthesizable *in situ*. This task is relatively easy with nucleic acids, for which most of the sequencing information is available, and there are only four different, but chemically similar, bases. Furthermore, nucleic acids are not only information carriers, but also perfect affinity reagents. For these reasons, nucleic acids have attracted a lot of attention and are widely used in a variety of applications, including microarray-based techniques. Millions of DNA fragments can be made cheaply and immobilized to result in high-throughput, massively parallel diagnostic applications.

So, why bother with proteins? Although DNA is an essential information carrier and mRNA is an equally vital messenger molecule, the majority of biological interactions in our cells are performed by proteins. Genomic DNA can be analyzed for single nucleotide polymorphisms (SNPs) and mutations and mRNA expression levels can be analyzed in a quantitative manner by a variety of techniques (including arrays). However, a correlation between the level of mRNA expression and the expression and function of respective proteins is limited. Protein turnover rate in the cell depends on the rate of protein synthesis (which is the only cellular process dependent on mRNA concentration) and protein degradation. The concentration and function of proteins also depends on the immediate cellular environment and on post-translational modifications. Therefore, it is the level of protein expression and post-translational modification that is a better marker of biological processes in a cell. This is why proteomics is a superior tool in disease diagnostics and drug-discovery applications.

To date, the use of two-dimensional (2D) gels coupled to MS has provided most quantitative proteomics data (as presented by Hanno Langen of Roche and Jon Terrett of Oxford Glycosciences, Oxford, UK). This approach has resulted in massive databases of protein sequences and their expression levels, and enables the selection of proteins or protein isoforms, which can be used in disease diagnostics or other related applications. But why have protein arrays not progressed along with DNA arrays? The answer is because there are no complete sets of affinity reagents with the required specificities and affinities [although Ciphergen (Fremont, CA, USA) showed some progress with its non-selective protein-binding matrices using various binding conditions]. This is a result of two factors. First, the absence of information on exactly which protein and which post-translational modification pattern is expressed differentially under particular conditions. (Having access to such data enables the choice of relevant protein targets.) Second, a protein array

for quantitative proteomics is effectively an array of high-affinity reagents, for example, antibodies. The development of each antibody requires significantly more time and resources than, for example, the synthesis of an oligonucleotide or purification of a PCR product. Therefore, it is unlikely that a generic set of affinity reagents against all proteins (even from one species) will be available in the near future, thus, to date, publications on protein-chip technology comprise more reviews than primary research papers.

However, this is not the only obstacle in making useful protein chips. DNA can be dried, rehydrated, frozen and boiled, and even partially degraded without the loss of its information content (its unique sequence identifier) or its high-affinity binding to its complementary strand. Proteins, except in rare cases, will not withstand such harsh treatment. Further, unlike nucleic acids, which can attach to the surface of a solid support in any direction, protein-affinity reagents must be correctly oriented and active sites must be easily accessible. In addition, the protein 'hybridization' steps cannot be carried out under denaturing conditions, unlike DNA hybridization, and this creates additional difficulties with background false-positive staining.

Other important factors include the choice of affinity templates [e.g. antibodies versus protein–RNA fusions (presented by Phylos; Lexington, MA, USA)] and the variety of assay options such as quantitative proteomics (Oxford Glycosciences), protein–protein interactions and functional genomics and proteomics (Biacore, Uppsala, Sweden).

It was concluded, therefore, that the development of protein arrays requires additional efforts as well as the 'know-how' element. There is no doubt that protein-array based proteomics is advantageous over the traditional approaches based on 2D gels and/or chromatography coupled with MS. Protein arrays can enable the scale-up of analysis, both by running multiple affinity-recognition steps in a single chip and by reducing the time of analysis (Fig. 2). Protein-affinity arrays should also improve reproducibility and enable fully quantitative protein-expression analysis. It is not known, however, whether the development of protein arrays will follow the way paved by DNA-array developers, by continued improvements in the surface-immobilization techniques, further increase in the affinity and density of the immobilized antibody and widespread acceptance of these new technologies. Early evidence from this conference suggests much greater variability in the style of protein arrays in all areas including surfaces, arrayers, affinity reagents, assays, analyses and array-readers.

Reference

1 Ekins, R.P. and Chu, F.W. (1991) Multianalyte microspot immunoassay – microanalytical 'compact disk' of the future. *Clin. Chem.* 11, 1955–1967