DRUG DISCOVERY TODAY TARGETS

Protein biochips: a new and versatile platform technology for molecular medicine

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The human genome has been sequenced and the challenges of understanding the function of the newly discovered genes have been addressed. High-throughput technologies such as DNA microarrays have been developed for the profiling of gene expression patterns in whole organisms or tissues. Protein arrays are emerging to follow DNA chips as possible screening tools. Here, we review the generation and application of microarray technology to obtain more information on the regulation of proteins, their biochemical functions and their potential interaction partners. Already, a large variety of assays based on antibody–antigen interactions exists. In addition, the medical relevance of protein arrays will be discussed.

High-density DNA microarray technology has played a key role in the analysis of whole genomes and their gene expression patterns. The use of oligonucleotide or cDNA arrays to study many thousands of individual genes is now widespread, with applications ranging from the profiling of gene expression patterns in whole organisms or tissues to the comparison of healthy and pathological samples. However, despite the success of DNA microarrays, it is obvious that biological functions are executed by biomolecules such as proteins rather than by DNA itself. Therefore, protein biochips are emerging to follow DNA microarrays as a possible screening tool to identify any protein–ligand interactions. Traditional methods for the analysis of proteomes include two-dimensional gel electrophoresis or chromatography which, when combined with mass spectrometry, enable large-scale separation and identification of proteins, including many of their modifications. These proteomic methods have been applied to the comparative study of expression patterns. For example, comparative studies have been carried out of differentially developed states and of diseased versus normal tissues [1,2] and even of related pathogenic versus non-pathogenic organisms [3]. Such experiments can be referred to as 'unbiased' or 'discovery-oriented' proteomics and lead to large data collections. They contrast with 'system-oriented' proteomics experiments, where only a defined subset of proteins are analysed, such as a family of proteins related by function or sequence or proteins belonging to a common pathway. System-oriented proteomics aims at observing changes in the concentration, localisation or modification of particular proteins of interest. Measuring such quantitative changes requires the development of analytical microarrays. Such analytical microarrays can consist of immobilised antibodies that may capture defined amounts of the corresponding antigen [4]. A second task of system-oriented proteomics is the definition of the biological role of a specific protein, which may involve the identification of interacting proteins or, in the case of enzymes, of specific substrates.

Predominantly, protein biochips will play a role in the discovery-oriented proteomics, but it is also foreseen that they will be implicated in system-oriented proteomics.

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Chip content

For the generation of protein biochips, especially for the discovery-oriented approach, large numbers of biomolecules have to be produced. This is a major technical challenge, calling for highly parallel, preferably automated recombinant expression systems.

High-throughput sub-cloning of open reading frames (ORFs) has been described for the genome of humans, *Saccaromyces cerevisiae*, *Arabidopsis thaliana* and *Caenorhabditis elegans* ([5–9]). Such recombination-based cloning approaches, which lead to collections of individual cDNA expression constructs, are strongly dependent on the progress of genome sequencing projects and the annotation of those sequences [10,11]. This means that uncharacterised proteins will not be represented, limiting this approach as a discovery tool. Additionally, clear determination of the expressed sequence remains difficult because of differential mRNA splicing. For these reasons, this approach has proven most valuable for the production of chips containing proteins from well-characterised organisms, such as *S. cerevisiae* and *C. elegans* [6,8].

The effort of creating individual cDNA expression constructs can be reduced by the generation of arrayed cDNA expression libraries that generate thousands of cDNA expression products in parallel [12–14]. By adding an affinity tag (e.g. His tag or GST tag) to the 5′ end of the cDNA insert, expression clones can be rapidly detected and purified via the detection of their His- or GST-tagged fusion protein. The automated recombinant expression and purification is most frequently performed in *E. coli*, which is a robust and convenient host organism [15–17]. However, many eukaryotic proteins end up in bacterial cytoplasmic inclusion bodies and can only be recovered in denatured form. When immobilising such denatured proteins onto the microarray, the proteins display linear sequence epitopes. Such a retained display of linear epitopes is sufficient for antigen–antibody screening purposes [18–21], but not for investigating the functionality or biological role of proteins. Therefore, eukaryotic expression systems, such as *S. cerevisiae* [7,8,22,23] or *Pichia pastoris*, have been adapted to high-throughput expression and purification [14,24,25]. In addition, these systems are able to perform post-translational modifications of recombinant proteins. Albala and co-workers have applied a baculovirus expression system to the 96-well format and expressed 72 different human cDNA clones in high-throughput format, of which 42% produced a soluble product [26].

Similar to protein biochips, the generation of antibody arrays requires a large number of high affinity, high specificity protein binding ligands (e.g. antibodies), ideally one for each protein of the proteome of interest. For the human proteome, this means the generation of >100 000 protein binders if all the different post-translational modifications are taken into account. Additionally, those binders should be of high quality with respect to binding behaviour, affinity and specificity. Polyclonal antibodies might not be the best choice as their polyclonal state means they might recognize different epitopes in proteins, which could result in more cross-reactivity. Moreover, obtaining thousands of poly- or monoclonal antibodies by mouse immunisation is highly expensive and raises ethical and patent-related questions. Therefore, the use of recombinant antibodies is strongly recommended for high density antibody arrays [27]. Antigen-binding fragments such as Fab or ScFv provide simple antibody formats that can be affinity selected *in vitro* by display technologies, such as phage or ribosome display [28,29]. Alternatively, aptameres, which are single-stranded oligonucleotides, have been systematically evolved by an exponential enrichment (SELEX) process to bind proteins [30]. They appear to be promising new array probes as they can be photo-crosslinked to the recognized proteins with very low background from other proteins in the sample. Because no other proteins are immobilised onto those arrays, non-specific protein stains can be used to detect the ligands [31,32].

Protein microarray applications

Protein arrays comprised of immobilised proteins are an emerging biochip format. Protein biochips have been used for protein–antibody and auto-antibody profiling, for the study of protein–ligand interactions, where the ligand can be either proteins, peptides, DNA or RNA, and for the determination of enzymatic activity and substrate specificity of classes of enzymes.

Protein–antibody interaction analysis

The specificity and cross-reactivity of antibodies has been successfully determined using high-content protein biochips [20,33,34]. As antibodies are used extensively as diagnostic and clinical tools, the characterisation of their binding specificity is of prime importance. Additionally, well-characterised antibodies are essential for the generation of highly specific antibody arrays.

In functional immunomics, antibody–antigen interactions are exploited in medically relevant contexts such as autoimmune diseases. Screening protein arrays with sera or plasma from auto-immune patients would not only allow the identification of potentially new auto-antigens, but also enable the diagnosis and sub-typing of the autoimmune disease on the basis of the presence of specific autoantibodies [20,35]. By combining the cDNA expression library approach with protein microarrays, the humoral auto-immune repertoire of dilated cardiomyopathy (DCM) patients has been profiled and several protein antigens have been determined which are associated with heart failure [18]. In a different approach, a protein array consisting of 196 structurally diverse biomolecules representing major autoantigens was probed with serum from patients with different autoimmune diseases including systemic lupus erythematosus (SLE), Sjögren syndrome and rheumatoid arthritis (RA). There were distinct autoantibody patterns

for the different autoimmune diseases, indicating their suitability for diagnosis [35]. Using a mouse model of type 1 diabetes, a panel of 27 different antigens was determined that allows discrimination between mice resistant or susceptible to cyclophosphamide-accelerated diabetes [21].

It is suggested that autoantigen patterns as determined by functional immunomics may allow predictive medicine. For example, NOD mice spontaneously produce IgG antibodies to the acetylcholine receptor, which is an antigen of pathogenic antibodies in experimental autoimmune myasthenia gravis (EAMG). At that time, there were no reports that NOD mice were susceptible to EAMG induction. To test if the presence of autoantibodies recognizing the acetylcholine receptor has predictive potential, NOD mice were challenged with a standard protocol used to induce EAMG, and the mice developed EAMG [36]. It has to be considered that in many cases patterns of autoantigens/autoantibodies have not yet been assigned to a function in the disease process, as functional studies remain difficult. However, the specificity of the autoimmune response may be an important tool for diagnosis, classification and prognosis.

The diversity of the autoimmune response is a great challenge for the development of antigen-specific tolerising therapies. For example, an increased diversity of autoantibodies in acute and chronic experimental autoimmune encephalomyelitis (EAE), a mouse model for multiple sclerosis (MS), is predictive of a more severe clinical course. It has been shown that the use of DNA vaccines encoding autoantigens such as epitopes of myelin prevent induction of EAE [37–39]. To identify new autoantigens that may act as tolerizing vaccines, an antigen microarray consisting of 232 proteins specific for the myelin proteome was generated and additional proteins were identified as potential targets of autoimmune response in chronic EAE [40]. Such analysis of immune response can also be applied to other diseases. For example, to analyse the humoral immune response to cancer, solubilized proteins from the LoVo colon adenocarcinoma cell line were separated into 1760 fractions, which were arrayed in microarray format and incubated with sera from newly diagnosed patients with colon cancer. One fraction, which exhibited a strong reactivity to colon cancer sera, was subjected to mass spectrometry, leading to the identification of a putative antigen [41]. Another immediate application is the use of 'allergen arrays' to screen for the presence of particular IgE molecules in a patient sample. The traditional approach involves the use of simple extracts from potential allergens. These extracts are commonly used in skin prick tests to determine the possible source of an allergic reaction in the patient. By combining array technology with recombinant allergens (e.g. pollen and fungus proteins), large arrays have been produced that enable fast screening of many allergens simultaneously [42–45]. These arrays can also readily include non-protein allergens (e.g. latex).

Protein–peptide interaction analysis

Protein microarrays are suitable to study protein–ligand interactions in which the ligand can be a protein, peptide, DNA, RNA, oligosaccharide or chemical compound. In a recent approach, the interaction of a restriction enzyme to double-stranded DNA was monitored on a micron-sized monolayer surface using atomic force microscopy [46].

In a discovery-oriented approach, a yeast proteome chip containing 5800 recombinant yeast proteins was generated and used for the identification of known as well as new calmodulin binders [8]. In addition, lipid binding specificity was profiled using phosphoinoside-doped liposomes [46].

In a system-oriented approach, the conserved cytoplasmic motif KVGFFKR from the platelet membrane protein integrin, which has previously been shown to play a critical role in the regulation of activation of the platelet integrin $\alpha_{IIb}\beta_3$, was used for interaction studies [47,48]. The tagged peptide (biotin–KVGFFKR) was screened against a high-density array of ~37 000 *E. coli* clones expressing recombinant human proteins [12,33] and thirteen different proteins were identified as binding the labelled peptide (Figure 1). By peptide pulldown assays and co-precipitation experiments, the interaction between a putative chloride channel (ICln) and integrin $\alpha_{IIb}\beta_3$ has been confirmed [47]. This experiment demonstrates the enormous potential of a protein array approach, not only for the identification of novel protein interactions, but also for the teasing apart of biological pathways in general.

Protein–chemical compound interaction analysis

For drug development, the analysis of the interactions of chemical compounds or important pharmacological targets with proteins is of major interest. For example, the isoxazole derivative Leflunomide (N-(4-trifluoromethylphenyl)-5-methylisoxazol-4-carboxamide, HWA 486) is a immunoregulatory and anti-inflammatory drug with proven *in vivo* efficacy. Strong experimental evidence points to the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH) as the major target of Leflunomide's mode of action [49]. Nevertheless, not all effects of the drug can be explained by the interaction with DHODH. Therefore, by using affinity chromatography, ten so far unknown intracellular potential binding partners of the Leflunomide were identified. Three of these proteins play a key role in the second part of the glycolytic pathway. A further validation by BIACORE® analysis revealed that Leflunomide specifically bound pyruvate kinase, GAPDH, malic dehydrogenase and lactic dehydragenase [50]. It is suggested that protein arrays with high content may have a strong impact on such interaction screening assays as the mode of action of therapeutically interesting drugs may be more deeply explained using these methods. However, additional validation methods such as BIACORE® or pull-down assays are essential.

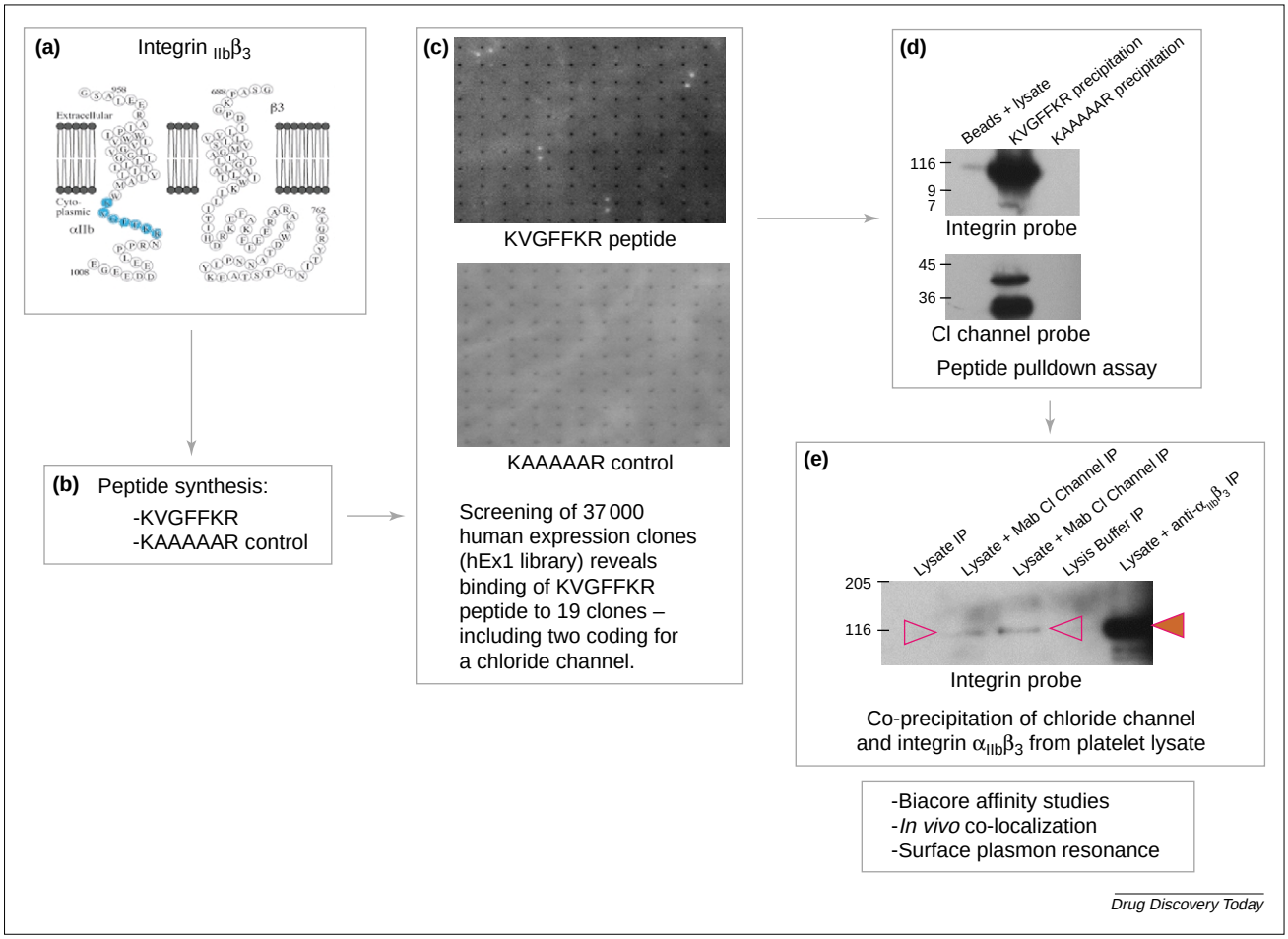

FIGURE 1

Outline of the approach for identification of proteins that interact with the cytoplasmic tail of a membrane protein, in this case a platelet integrin. (a) The structure of integrin $\alpha_{IIb}\beta_3$ is shown [65]. **(b)** A tagged peptide (biotin-KVGFFKR) was synthesized. **(c)** This peptide was screened against a high-density array of 37000 *E. coli* clones expressing recombinant human proteins. 19 clones, coding for 13 different proteins, were identified as binding the labelled peptide. **(d)** Peptide pulldown assays show a strong binding between this labelled peptide and purified proteins isolated from three clones. Of these clones, one codes for a protein which could not be shown to be present in platelets, and two code for a putative chloride channel, ICln. **(e)** Co-precipitation of ICln and platelet integrin has been successfully shown [64].

Enzyme activity analysis

Phosphorylation of proteins by protein kinases plays a central role in regulating cellular processes and it is suggested that this process contributes to many diseases, including diabetes, inflammation and cancer. Therefore, kinases are an important class of drug targets, resulting in a strong interest in identifying new kinases and their substrates. Different enzyme activities including phosphatases, peroxidases, galactosidases, restriction enzymes and protein kinases have been analysed on protein, peptide and nanowell microarrays [7,51–59]. Zhu *et al.* have created protein arrays of *S. cerevisiae* kinases [7]. In this study, a total of 119 known or predicted protein kinases were expressed, purified as GST fusion proteins, arrayed and cross-linked on a protein chip and assayed with 17 different substrates for auto-phosphorylation by treatment with radiolabelled ATP, and new activities have been found [7]. For example, 27 kinases show a phosphorylation activity of poly-Glu-Tyr, indicating that many kinases are capable of phosphorylating tyrosine even if they are members of the serine/threonine family on the basis of sequence comparison. Also, when comparing kinase activities across the different substrates, many kinases were found to phosphorylate one or two substrates. This result was confirmed in another experiment, where the 768 purified proteins acting as putative substrates for barley protein kinase CK2α were immobilised onto the surface. Out of these 768 proteins, 21 potential substrates of CK2α were identified. Most of these proteins represent high mobility group proteins or calreticulin [59].

Conclusions

Applications of protein array technology, such as target identification and characterisation, target validation, diagnostic marker identification and validation, pre-clinical study monitoring and patient typing seem to be feasible. Proteins as drug targets dominate pharmaceutical R&D, with ligand–receptor interactions comprising ~45% and enzymes 28% of the targets [60]. Additionally, many therapeutic proteins, especially humanized antibodies, are in

clinical development, such as Avastin®, Remicade® and Enbrel®. The ultimate application for high-throughput screening would be to test new leads or new targets in a highly parallel manner. Some examples for applications in this direction already exist. Recently, an immunosensor array has been developed that enables the simultaneous detection of clinical analytes [61]. Here, capture antibodies and analytes were arrayed on microscope slides using flow chambers in a cross-wise fashion. This current format is low-density (6 × 6 pattern) but has high-throughput potential, as it involves automated image analysis and microfluidics; it is already being used as a format for enzyme activity testing and other assays [62]. In another study, small sets of active enzymes were immobilized in a hydrophilic gel matrix. Enzymatic cleavage of the substrate could be detected and inhibitors blocked the reaction [53]. More recently, an enzyme array that is suitable for assays of enzyme inhibition has been reported [63]. Initial publications in the area of receptor–ligand interaction studies using a microarray format have shown that the interaction of immobilized compounds and proteins in solutions can be determined [8,50,56]. This technology allows high-throughput screening of ligand–receptor interactions with small sample volumes.

The multi-parallel possibilities of protein array applications have the potential not only to allow the optimization of pre-clinical, toxicological and clinical studies through better selection and stratification of individuals, but also to affect how diagnostics are used in drug development.

References

1 Ostergaard, M. *et al.* (1997) Proteome profiling of bladder squamous cell carcinomas: identification of markers that define their degree of differentiation. *Cancer Res.* 57, 4111–4117
2 Marcus, K. *et al.* (2003) Differential analysis of phosphorylated proteins in resting and thrombin-stimulated human platelets. *Anal. Bioanal. Chem.* 376, 973–993
3 Mahairas, G.G. *et al.* (1996) Molecular analysis of genetic differences between *Mycobacterium bovis* BCG and virulent *M. bovis*. *J. Bacteriol.* 178, 1274–1282
4 Kusnezow, W. *et al.* (2003) Solid supports for microarray immunoassays. *J. Mol. Recognit.* 16, 165–176
5 Wiemann, S. *et al.* (2003) CDNAs for functional genomics and proteomics: the German Consortium. *C. R. Biol.* 326, 1003–1009
6 Reboul, J. *et al.* (2003) *C. elegans* ORFeome version 1.1: experimental verification of the genome annotation and resource for proteome-scale protein expression. *Nat. Genet.* 34, 35–41
7 Zhu, H. *et al.* (2000) Analysis of yeast protein kinases using protein chips. *Nat. Genet.* 26, 283–289
8 Zhu, H. *et al.* (2001) Global analysis of protein activities using proteome chips. *Science* 293, 2101–2105
9 Kersten, B. *et al.* (2003) Generation of *Arabidopsis* protein chip for antibody and serum screening. *Plant Mol. Biol.* 52, 999–1010
10 Heyman, J.A. *et al.* (1999) Genome-scale cloning and expression of individual open reading frames using topoisomerase I-mediated ligation. *Genome Res.* 9, 383–392
11 Walhout, A.J. *et al.* (2000) GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol.* 328, 575–592
12 Büssow, K. *et al.* (1998) A method for global protein expression and antibody screening on high-density filters of an arrayed cDNA library. *Nucleic Acids Res.* 26, 5007–5008
13 Holz, C. *et al.* (2001) A human cDNA expression library in yeast enriched for open reading frames. *Genome Res.* 11, 1730–1735
14 Lueking, A. *et al.* (2000) A system for dual protein expression in *Pichia pastoris* and *Escherichia coli*. *Protein Expr. Purif.* 20, 372–378
15 Büssow, K. *et al.* (2000) A human cDNA library for high-throughput protein expression screening. *Genomics* 65, 1–8
16 Braun, P. *et al.* (2002) Proteome-scale purification of human proteins from bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 99, 2654–2659
17 Brizuela, L. *et al.* (2002) The FLEXGene repository: exploiting the fruits of the genome projects by creating a needed resource to face the challenges of the post-genomic era. *Arch. Med. Res.* 33, 318–324
18 Horn, S. *et al.* Profiling humoral auto-immune repertoire of dilated cardiomyopathy (DCM) patients and development of a disease-associated protein chip. *Proteomics* (in press)
19 Gutjahr, C. *et al.* Mouse protein arrays from a TH1 cell cDNA library for antibody screening and serum profiling. *Genomics* (in press)
20 Lueking, A. *et al.* (2003) A nonredundant human protein chip for antibody screening and serum profiling. *Mol. Cell. Proteomics* 2, 1342–1349
21 Quintana, F.J. *et al.* (2004) Functional immunomics: microarray analysis of IgG autoantibody repertoires predicts the future response of mice to induced diabetes. *Proc. Natl. Acad. Sci. U. S. A.* 101 (Suppl. 2), 14615–14621
22 Holz, C. *et al.* (2002) A micro-scale process for high-throughput expression of cDNAs in the yeast *Saccharomyces cerevisiae*. *Protein Expr. Purif.* 25, 372–378
23 Holz, C. *et al.* (2003) Establishing the yeast *Saccharomyces cerevisiae* as a system for expression of human proteins on a proteome-scale. *J. Struct. Funct. Genomics* 4, 97–108
24 Lueking, A. *et al.* (2003) A dual-expression vector allowing expression in *E. coli* and *P. pastoris*, including new modifications. *Methods Mol. Biol.* 205, 31–42
25 Boettner, M. *et al.* (2002) High-throughput screening for expression of heterologous proteins in the yeast Pichia pastoris. *J. Biotechnol.* 99, 51–62
26 Albala, J.S. *et al.* (2000) From genes to proteins: high-throughput expression and purification of the. *J. Cell. Biochem.* 80, 187–191
27 Holt, L.J. *et al.* (2000) The use of recombinant antibodies in proteomics. *Curr. Opin. Biotechnol.* 11, 445–449
28 Walter, G. *et al.* (2001) High-throughput screening of surface displayed gene products. *Comb. Chem. High Throughput Screen.* 4, 193–205
29 Schaffitzel, C. *et al.* (1999) Ribosome display: an *in vitro* method for selection and evolution of antibodies from libraries. *J. Immunol. Methods* 231, 119–135
30 Jayasena, S.D. (1999) Aptamers: an emerging class of molecules that rival antibodies in diagnostics. *Clin. Chem.* 45, 1628–1650
31 Brody, E.N. and Gold, L. (2000) Aptamers as therapeutic and diagnostic agents. *J. Biotechnol.* 74, 5–13
32 Brody, E.N. *et al.* (1999) The use of aptamers in large arrays for molecular diagnostics. *Mol. Diagn.* 4, 381–388
33 Lueking, A. *et al.* (1999) Protein microarrays for gene expression and antibody screening. *Anal. Biochem.* 270, 103–111
34 Michaud, G.A. *et al.* (2003) Analyzing antibody specificity with whole proteome microarrays. *Nat. Biotechnol.* 21, 1509–1512
35 Robinson, W.H. *et al.* (2002) Autoantigen microarrays for multiplex characterization of autoantibody responses. *Nat. Med.* 8, 295–301
36 Quintana, F.J. *et al.* (2003) Experimental autoimmune myasthenia gravis in naive non-obese diabetic (NOD/LtJ) mice: susceptibility associated with natural IgG antibodies to the acetylcholine receptor. *Int. Immunol.* 15, 11–16
37 Urbanek-Ruiz, I. *et al.* (2001) Immunization with DNA encoding an immunodominant peptide of insulin prevents diabetes in NOD mice. *Clin. Immunol.* 100, 164–171
38 Ruiz, P.J. *et al.* (1999) Suppressive immunization with DNA encoding a self-peptide prevents autoimmune disease: modulation of T cell costimulation. *J. Immunol.* 162, 3336–3341
39 Garren, H. *et al.* (2001) Combination of gene delivery and DNA vaccination to protect from and reverse Th1 autoimmune disease via deviation to the Th2 pathway. *Immunity* 15, 15–22
40 Robinson, W.H. *et al.* (2003) Protein microarrays guide tolerizing DNA vaccine treatment of autoimmune encephalomyelitis. *Nat. Biotechnol.* 21, 1033–1039
41 Nam, M.J. *et al.* (2003) Molecular profiling of the immune response in colon cancer using protein microarrays: occurrence of autoantibodies to ubiquitin C-terminal hydrolase L3. *Proteomics* 3, 2108–2115
42 Hiller, R. *et al.* (2002) Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J.* 16, 414–416
43 Deinhofer, K. *et al.* (2004) Microarrayed allergens for IgE profiling. *Methods* 32, 249–254
44 Jahn-Schmid, B. *et al.* (2003) Allergen

microarray: comparison of microarray using recombinant allergens with conventional diagnostic methods to detect allergen-specific serum immunoglobulin E. *Clin. Exp. Allergy* 33, 1443–1449
45 Wiltshire, S. *et al.* (2000) Detection of multiple allergen-specific IgEs on microarrays by immunoassay with rolling circle amplification. *Clin. Chem.* 46, 1990–1993
46 O'Brien, J. *et al.* (2000) Preparation and Characterisation of self-assembled double-stranded DNA (dsDNA) microarrays for protein:dsDNA screening using atomic force microscopy. *Langmuir* 16, 9559–9567
47 Larkin, D. *et al.* (2004) ICln, a novel integrin αIIbβ3-associated protein, functionally regulates platelet activation. *J. Biol. Chem.* 279, 27286–27293
48 Stephens, G. *et al.* (1998) A sequence within the cytoplasmic tail of GpIIb independently activates platelet aggregation and thromboxane synthesis. *J. Biol. Chem.* 273, 20317–20322
49 Williamson, R.A. *et al.* (1995) Dihydroorotate dehydrogenase is a high affinity binding protein for A77 1726 and mediator of a range of biological effects of the immunomodulatory compound. *J. Biol. Chem.* 270, 22467–22472
50 Mangold, U. *et al.* (1999) Identification and characterization of potential new therapeutic targets in inflammatory and autoimmune diseases. *Eur. J. Biochem.* 266, 1184–1191
51 Reineke, U. *et al.* (2001) Applications of peptide arrays prepared by the SPOT-technology. *Curr. Opin. Biotechnol.* 12, 59–64
52 Burns-Hamuro, L.L. *et al.* (2003) Designing isoform-specific peptide disruptors of protein kinase A localization. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4072–4077
53 Arenkov, P. *et al.* (2000) Protein microchips: use for immunoassay and enzymatic reactions. *Anal. Biochem.* 278, 123–131
54 Bulyk, M.L. *et al.* (1999) Quantifying DNA-protein interactions by double-stranded DNA arrays. *Nat. Biotechnol.* 17, 573–577
55 Houseman, B.T. *et al.* (2002) Peptide chips for the quantitative evaluation of protein kinase activity. *Nat. Biotechnol.* 20, 270–274
56 MacBeath, G. and Schreiber, S.L. (2000) Printing proteins as microarrays for high-throughput function determination. *Science* 289, 1760–1763
57 Uttamchandani, M. *et al.* (2004) Site-specific peptide immobilization strategies for the rapid detection of kinase activity on microarrays. *Methods Mol. Biol.* 264, 191–204
5 8 Angenendt, P. *et al.* (2004) Cell-free protein expression and functional assay in nanowell chip format. *Anal. Chem.* 76, 1844–1849
59 Kramer, A. *et al.* (2004) Identification of barley CK2α targets by using the protein microarray technology. *Phytochemistry* 65, 1777–1784
60 Drews, J. (2000) Drug discovery: a historical perspective. *Science* 287, 1960–1964
61 Rowe, C.A. *et al.* (1999) An array immunosensor for simultaneous detection of clinical analytes. *Anal. Chem.* 71, 433–439
62 Cohen, C.B. *et al.* (1999) A microchip-based enzyme assay for protein kinase A. *Anal. Biochem.* 273, 89–97
63 Park, C.B. and Clark, D.S. (2002) Sol-gel encapsulated enzyme arrays for high-throughput screening of biocatalytic activity. *Biotechnol. Bioeng.* 78, 229–235
64 Aki, T. *et al.* (1997) Identification and characterization of positive regulatory elements in the human glyceraldehyde 3-phosphate dehydrogenase gene promoter. *J. Biochem. (Tokyo)* 122, 271–278
65 Li, Z. *et al.* (2001) A mitogen-activated protein kinase-dependent signaling pathway in the activation of platelet integrin αIIbβ3. *J. Biol. Chem.* 276, 42226-42232