34.1 Multiple Spotting Technology; Performing Multiplex Assays on a Single Microarray
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To increase throughput and reduce consumption of precious samples, efforts have been made to transfer molecular assay systems to the microchip format using conventional microarrays, microfluidic systems and chips bearing microwells (Angenendt et al., Analytical Chemistry 2004, 76, 2916–2921). However, all three formats lack the possibility to screen several analytes against several immobilized binders at a time or require complicated liquid handling, surface modifications, and additional equipment (Angenendt P., Drug Discovery Today 2005, 10 (7), 503–511). We have developed a new technology, which enables multiplex assays on a standard microscope slide without the requirement for wells or tubes to separate the samples. The so-called “multiple spotting technique” (MIST) requires only standard microarray surfaces and machinery and comprises the transfer of a substance onto a surface and subsequent spotting of the second compound on the same spot, on top of the first substance (Angenendt et al., Analytical Chemistry 2003, 75, 4368–4372).

While MIST can be used for true multiplexing in the context of protein and antibody microarrays, we have adapted the principle to the high-throughput identification and characterisation of monoclonal scFv’s derived from phage-display selections (Angenendt et al., Analytical Chemistry 2004, 76, 2916–2921). Moreover, we have performed enzymatic activity and inhibition assays and were able to detect as little as 35 enzyme molecules per spot (Angenendt et al., Proteomics 2005, 5, 420–425). To extend the principle to more complicated enzymatic reactions, we are currently performing cell-free protein biosynthesis from unbound DNA templates to allow the facile generation of high-density protein microarrays from DNA microarrays.

34.2 AFM Nanotechnology with Biospecific Irreversible Fishing Is the Way to Reverse Avogadro Number
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The development of methods, which help to reveal disease markers on the concentration level 10–15 M and lower, is the most perspective course in medical proteomics, particularly in early diagnosis. The best approach to address this problem is based on combination of biospecific fishing with AFM chips nanotechnology.

BSF/AFM technology major characteristics:
1) molecule-ligate immobilization on surface of AFM; 2) biospecific fishing of partner out of complex biological mixture with the formation of specific complexes; 3) identification of complexes by AFM.

Reversible biospecific fishing of marker proteins out of complex biological mixture has concentration sensitivity threshold AFM/BSF about 10–15 M. The irreversible biospecific fishing decreases threshold sensitivity up to 10–18 and lower, theoretically up to Avogadro reciprocal number. The most interesting approach to realize this scheme is the usage of photoactive to discover molecular proteins nuclear acids and virus particles in biological sample. The examples of revelation of some proteins and individual viruses and virus particles in hepatitis B and C sera are given.

This work was supported by the program “Medical Diagnostic Systems of the Future,” Support was also provided by the NSch 325.2003.4, by INTAS Grant # 01–470.

34.4 High Density Peptide Chip for Quantitative Measurements of Protein Binding and Enzymatic Activities
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We have developed in situ synthesis of high density microarrays of peptides and peptidomimetics (peptide chips) [1, 2]. We have since established the microfluidic peptide chip as a miniaturizing platform. The challenging issues in making peptide chips a practical tool for understanding biology, drug discovery, and diagnostics are quality of synthesis, specificity in reported activities, and ability for quantitative measurements. We will present the results of the work which involves our intensive effort in:

Development of the method for monitoring and analyzing quality of peptide chip synthesis
Improvement in peptide chip synthesis
Development of the methods for quantitative analysis of (a) the specific binding of antibodies/proteins to peptides on chip and (b) kinase enzymatic activities against substrate peptides on chip.

Our presentation should demonstrate novel applications of peptide chips that can be implemented as routine laboratorial processes.


34.5 AFM and Optical Biosensor Nanotechnology in Revelation of Protein Complexes

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Atomic force microscopy and optical biosensor methods were applied for investigation of protein enzymatic monoxygenase systems: cytochrome P450cam, 2B4 and P450sc-c-systems and antigen/antibody systems as well. Binary and triple complexes, forming in cytochrome P450cam and cytochrome P4502B4 systems, were revealed and visualized owing to atomic force microscopy methods and kinetically characterized by optical biosensor method. It was possible to characterize protein partners interaction both in oxidated conditions and in conditions of hydroxylation due to the optical biosensor method. It has been shown that optical biosensor and mass spectrometry in a combination enable not only selectively fished out membranous protein partners out of complex biological mixtures, but identify membranous proteins complexes as well.

This work was supported by the RFBR Grant # 05-04-48690, 02-04-49057, NSch 325.2003.4, by INTAS Grant # 01–470, and by the Janssen Research Foundation.
Development of Protein Chips for Analysis by Ellipsometry

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Current methods for detecting interactions between probe and target molecules in microarray systems employ fluorescence readers that require tagging of probe molecules with fluorescent label. Tagging probe molecules with fluorescent label can affect the specificity and binding affinity of probes to targets. Ellipsometry is a label-free optical technique that measures the change in the polarisation state of light reflected off a surface. If a film at a surface undergoes a change in thickness then the reflection properties will also change. Measuring the change in reflection properties allows for detection of an actual change in the film’s thickness to a sensitivity of 2 angstroms or better and allows for immediate qualitative detection of target-probe interactions. Microarrays are typically produced on glass or silicon wafers. The optical properties of silicon in the visible spectral region are more desirable for sensitive detection by ellipsometry. Microarrays that are produced on silicon typically employ silanation chemistry for covalent attachment of biological molecules to the surface. Silane layers are not always uniform, which is a disadvantage for using ellipsometry to detect the change in thickness of a film on a surface. We report the production of silicon wafers that have an epoxy-terminated alkyl monolayer covalently attached to the silicon surface giving a uniform and reflective substrate that shows promise as a microarray system that can be analysed using imaging ellipsometry. Covalent immobilisation of protein and subsequent binding of antibody is demonstrated in this system.

Protein Microarray Technology; Technologies and Applications and Market

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Following the completion of the human genome sequencing project well-established DNA microarrays and sophisticated bioinformatics platforms allow scientists to take a global view into biological systems. In today’s proteome era, the time is ready for protein microarrays to screen entire genomes for proteins that interact with particular factors, catalyse particular reactions, act as substrates for protein-modifying enzymes and/or as targets of autoimmune responses.

Due to miniaturisation, microarrays can analyze many parameters in parallel and only require minimal amounts of reagents and sample. Besides planar microarray systems bead based flow cytometry approaches are perfectly suited for the multiplex detection of target molecules, especially when only a small number of parameters have to be determined simultaneously.

Protein microarrays can be grouped according to different formats and different types of applications. Currently, forward-phase protein microarrays are the most frequently used microarray assay formats. They can be used for the simultaneous analysis of a large number different parameters from distinct samples. Examples of forward-phase protein microarrays include antibody arrays and miniaturized sandwich immunoassays that are used to identify and quantitate target proteins and protein affinity assays that are used to study the interactions between proteins and immobilized binding molecules such as proteins, peptides, low molecular weight compounds, oligosaccharides, or DNA. Reverse-phase arrays are used to determine a distinct set of parameters in a large collection of tissue or cell samples, or sample fractions that are immobilized in a microarray format on a solid support. Highly specific antibodies are used to profile a large number of different samples simultaneously for the presence or absence of distinct target proteins.

Appropriate sensitivity, reproducibility, robustness and automation have to be demonstrated before this technology will be suitable for high-throughput applications within the field of proteomic research. In this presentation the current stage of the different types of protein microarrays will be analyzed and future challenges of this cutting-edge technology will be critically discussed.
High-throughput Identification of Arabidopsis MAP Kinase Substrates Using Protein Microarrays

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Protein microarrays have become a powerful tool to screen proteins for expression and molecular interactions in high throughput (LaBaer and N. Ramachandran, Curr. Opin. Chem. Biol. 2005, 8, 14–19; Kersten et al., Plant Mol. Biol. 2003, 52, 999–1010). We applied this technology to analyse the phosphorylation of Arabidopsis proteins by MAP kinases. Therefore we constructed a cDNA expression library from Arabidopsis. Using protein microarrays, putative expression clones from the initial library were selected and rearrayed into a sub-library (~5,000 clones). These clones were sequenced and this information was used to create a database of Arabidopsis proteins (~1,700 uniclones). These proteins of the set were purified and robotically arrayed onto coated glass slides. These protein microarrays were used for phosphorylation studies using active MPK3 and MPK6. 48 potential substrates of MPK3 and 39 of MPK6 were identified. Nearly all of the 48 MPK3 substrates were verified by other in vitro methods. One of the identified MPK6 substrates, ACS-6 was recently shown to be the first plant MAPK substrate in vivo (Liu and Zhang, Plant Cell 2004, 16, 3386–90) thus demonstrating the reliability of our method. Furthermore, transcription factors, transcription regulators, splicing factors, receptors, histones and ribosomal proteins were identified as in vitro substrates, indicating that regulation in response to MAPK signalling is very complex and not restricted to the transcriptional level. The rapid in vitro preselection of kinase substrates using our novel proteomic assay will facilitate and accelerate more elaborate in vivo studies in the future.

The Use of Protein Microarrays for the Validation of Phage Display Antibody Selections within the German NGFN Initiative Antibody Factory

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Within the framework of the German National Genome Research Network (NGFN), the “Antibody Factory” is an initiative to develop technologies for the generation of recombinant antibodies by phage display applicable in proteome research. The ultimate goal is to obtain a set of modularised methods, which allow the assembly of a generation pipeline for antibodies for functional genomic studies on a proteome-wide scale. One of the modules under development is concerned with monitoring the selection process and the specificity of phage-displayed antibodies. The set up is protein microarray based and allows examining the specificity of individual selection rounds (poyclonal entities) for sufficient enrichment, as well as the evaluation of monoclonal entities for cross-reactivity on recombinant proteins. To further increase the throughput of antibody screening and specificity determination, we have applied the Multiple Spotting Technique (MIST; Angenendt et al., Anal. Chem. 2003, 75, 4368–4372). This makes it possible to evaluate a large number of antibody entities on a set of different proteins on a single chip. Theoretically, MIST can be scaled up to yield around 10,000 data points at a time. While the observed sensitivity of the assay is comparable with standard enzyme-linked immunosorbent assays, MIST is in respect of reduced consumption of valuable protein targets and reagents, as well as the speed of analysis by far superior.

Development of Quantitative Protein Detection Arrays

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A reliable method to quantify proteins is mandatory to monitor changes that occur on the protein level in health and disease. Ideally, this method should also provide information regarding the posttranslational modification, e.g., phosphorylation. The method should be sensitive and robust so that alterations can be monitored in a high-throughput format.

In order to establish a protein array-based method for the quantitative detection we immobilized capture antibodies on slides. Detection is performed in a sandwich ELISA format. Secondary antibodies are labeled with infrared-dyes for detection. The strength of specific signals will be determined by ratio imaging and the results will be compared to analysis by quantitative immunoblotting. The quality control is based on statistical analysis.

This technique will be applied for the generation of time resolved quantitative data for systems biology approaches. In the future we plan to use quantum dots with different emission spectra to facilitate simultaneous detection of multiple proteins/posttranslational modifications.

Quantitative MHC Expression Profiling Using Reverse Phase Protein Microarrays

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The MHC (major histocompatibility complex) displays self and foreign antigens on the cell surface for the recognition by T cells. In tumors, cells often accumulate somatic mutations in the MHC gene cluster. This can lead to alterations of the MHC expression. In a consequence, a deficient anti-tumor immune response can make such cells invisible for the immune system. Reverse phase protein microarrays (RPPMs) allow the efficient protein profiling of large numbers of samples in parallel under comparable experimental conditions (“reverse screening”). Arrays are generated from complex samples (e.g., cell or tissue lysates) printed on the chip surface. Each microspot contains the whole proteomic content of the sample. Arrays are probed for the analytes-of-interest in an easy direct immunoblot assay. With only limiting amounts of starting material, hundreds of arrays can be produced for a comprehensive study (Kramer et al., Current Protocols in Protein Science, 2005). Zeptosens was chosen as the technology platform since it combines high sensitivity and reproducibility. In this study, RPPMs were applied for the quantitative analysis of MHC expression in different tumors. Arrays comprised lysates of clinical tumor and healthy tissue samples from 33 patients. In addition, dilution series of recombinant MHC monomers were co-printed onto the arrays as standards for the quantification. Three different human cell culture samples were added as positive controls. FACS- and Western Blot analysis were employed to generate Reference data. The assays provided excellent sensitivity and a wide range of linear assay signals. This study demonstrates that RPPMs are novel tools for proteomic research. The approach is further applied to examine quantitative profiles of low abundant disease and tumor markers.
cloned them for recombinant expression in N. meningitidis. This bacterium is present in 10–15% of the population only 1/100,000 persons get meningitis. One trait of the bacterium is present in 10–15% of the population only 1/100,000 persons get meningitis. One trait of N. meningitidis is the presence of variable-phase genes that are suspected to enhance its ability to cause an invasive disease. To detect the immune responses to phase-variable expressed proteins, we applied protein microarray technology for the screening of meningitis patient sera. We amplified all 102 known phase-variable genes from N. meningitidis serogroup B strain MC58 by PCR and cloned them for recombinant expression in E. coli. With this approach, we were able to express and purify 67 recombinant proteins representing 66% of the annotated genes. These were spotted robotically onto coated glass slides to generate protein microarrays, which were screened using sera of patients suffering from meningitis, as well as healthy controls. From these screening experiments, 47 proteins emerged as immunogenic, exhibiting a variable degree of seroreactivity with some of the patient sera. Nine proteins elicited an immune response in more than three patients, with one of them, the phase-variable opacity protein OpaV, showing responses in 55% of the patient sera. This is the first time that protein microarray technology has been applied for the investigation of genetic phase variation in pathogens. The identification of disease-specific proteins is a significant target in biomedical research, as such proteins may have medical, diagnostic, and commercial potential as disease markers.

Very High Sensitivity Micron Scale Microfabricated Nano-ESI Tips Compatible with Standard Analytical Chromatography Fittings

We have already shown the feasibility of original on-chip nanoelectrospray emitter tips based on the idea of a capillary slot rather than a capillary tube. In this context, we go further here by presenting a silicon-based integrated system which incorporates a high performance nanoelectrospray emitter tip coupled to a closed microfluidic microchannel etched in silicon. The aspect ratio of the cantilever is ca. 400 and both the width and the height of the tip are in the low micron range. This design allows outstanding electrospray ionization performances as the charge concentration in the droplet is inversely proportional to the size of the tip. The design of the input of this microchannel is compatible with the use of standard capillary tube-based hardware (Upchurch®). In addition, we have developed a mechanical holder which is compatible with the standard source housing of mass spectrometers in order to simplify the use of such micromachined objects. Mass spectrometry tests of such devices have revealed very high performances in terms of ionization voltage and molecular identification. There are two important results to be highlighted: firstly, the ionization voltage value could be decreased to 0.5 kV without any marked degradation of both the analysis quality and signal-to-noise ratio and secondly, the aqueous concentration could be increased to 95% without any marked degradation in either the analysis quality or the signal-to-noise ratio. This allows the preservation of molecular interactions and thus enables the study of proteins and complexes in their native conditions. On the other hand, when using classical solvent and voltage these ESI tips are at least one order of magnitude more sensitive. Applications on proteomic human samples will be presented.
Serum Microarrays for Screening of Protein Levels

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Protein microarrays are still in a rather early phase in the development towards robust and established global procedures. They have so far mainly been utilized with immobilized proteins or antibodies for a broad analysis of a single sample. The reverse set-up, where many complex samples are spotted, can be used for simultaneous analysis or screening of one or a few molecules in large amounts of samples.

We aim to develop and establish high-density reverse phase protein microarrays as versatile tools for screening and profiling of serum. The aim is to generate a robust, accurate and sensitive microarray platform which is able to screen thousands of clinical serum samples for their content of the relative amount of specific proteins of clinical relevance. Several reports have been published on reverse phase protein microarrays, but no one on serum microarrays.

An initial pilot study will here be described, where 2000 serum samples were spotted in triplicate onto a microarray and analyzed for their content of IgA. All samples had previously been analyzed with nephelometry for their IgA levels and a comparison of the array data corresponded rather well with the clinically measured data. The sensitivity in this not completely optimized study was found to be roughly 0.1–1 μg/ml, which is promising and the robustness, in terms of median CV, was found to be as low as 2.5%.

A Novel Protein-Chip for Analysis of Protein-Protein Interaction Using DLC Plate

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Since proteins interact with other proteins to perform their particular cellular task, analysis of the protein-protein interaction is important to determine the function of proteins. Protein-protein interaction can be analyzed by several methods such as yeast two-hybrid system, affinity purification techniques and protein chip. Among them, protein chip is considered one of the most promising tools for high-throughput analysis of protein-protein interaction. In conventional technique, proteins expressed by DNA (RNA) or natural proteins have been purified to immobilize on the chemically modified chip plates. However, purification of proteins is laborious and time-consuming, and purification of a number of proteins is often impossible. This is a limiting factor to perform the high-throughput analysis of protein-protein interaction.

We developed a novel protein-chip plate (diamond-like carbon coated (DLC) stainless steel plate) for high-throughput analysis of protein-protein interaction. Proteins separated by gel electrophoresis can be electro-botted onto the DLC plate, of which surface is modified with N-hydroxysuccinimide ester, to produce a high-density of protein chip. Proteins are immobilized covalently on the DLC plate with high blotting efficiency (50–70%). Proteins extracted from the cells are probed with the proteins on the DLC plate, and the interacted proteins can be detected by mass spectrometry such as MALDI-TOF MS using the DLC plate as a MALDI sample target. This technique has a great potential of high-throughput analysis of proteins interacted with thousands of proteins separated by two-dimensional gel electrophoresis. On the other hand, proteins transferred on the DLC plate can be digested with lysylendopeptidase and the digests on the plate can be measured directly by MALDI-TOF MS to identify the proteins. This could be a useful technique for high-throughput identification of the gel-resolved proteins.
Mass Spectrometry Identification of Interaction between Beta-Amyloid Peptide and Its Inhibitor LFFPD

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Here we describe a strategy using mass spectrometry and a gold surface peptide array to identify specific peptide-peptide interaction. This affinity capture surface should avoid protein loss and considerably simplify sample preparation for mass spectrometry analysis as it allows washing steps and mass spectrometry analysis directly on the chip. Moreover, the high sensitivity of mass spectrometry is well suited to on-chip study.

The β-amyloid is a self-aggregating protein found in Alzheimer’s disease and is thought to play major role in the disease process. In this study, a peptide known as an inhibitor of the β-amyloid was synthesised with N-terminal cysteine residue in order to achieve a specific attachment to a gold surface via thiol-gold-chemistry. The methodology of self-assembled monolayers was employed and the peptide monolayer formed was characterised by AFM.

The peptide array was used in two kinds of interaction experiments:
- with a solution of synthesised β-amyloid,
- with a crude brain protein extract.

In both experiments the solutions were incubated on the peptide monolayer. After the incubation step, the gold surfaces were washed and dried. Using a direct mass spectrometry analysis on the gold surfaces we could identify peaks corresponding to the β-amyloid, which demonstrate the specific interaction between this peptide and its inhibitor.

We recently synthesised β-amyloid (1–40) derived with a N-terminal cysteine residue to build a new peptide array to try to characterise interactions between β-amyloid and proteins from crude brain extract.

Peptide-based Proteomics Using Array Technology


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Within the last few years, methods based on DNA microarray technology have been adapted to the analysis of proteins. Today, protein arrays hold the potential to become a key technology for proteome analysis. We have developed an approach for peptide based proteomics using array technology (pepART). Here specific signature peptides for disease-associated marker proteins are synthesised, serving as easily accessible antigens for the generation of peptide-specific antibodies. These antibodies are then utilised as capture molecules in protein microarrays. Complex protein mixtures from cells or tissue samples are degraded into peptide fragments by specific proteases and incubated on the antibody arrays. The respective signature peptides will bind to their corresponding anti-peptide antibodies. This allows a simultaneous peptide based identification and quantification of proteins of interest at high sensitivity. However, a prerequisite for this approach and most types of protein arrays is the availability of stable capture molecules that presently limits the use of protein arrays (Poetz et al., Proteomics 2005, in press). These binding molecules, e.g. antibodies, should bind their targets with high specificity, selectivity and affinity. Binders can be generated to nearly any given target molecule by semi-automated in-vitro methods at high speed using novel recombinant antibody technologies and comprehensive antibody libraries, e.g. HuCAL (Human Combinatorial Antibody Library; see http://www.morphosys.com). Currently the bottleneck in the production process is not the selection, but the characterisation of the obtained binding molecules. We will present our efforts for antibody characterisation, including a peptide based system for epitope mapping. In addition results from proof-of-principle experiments will be presented, demonstrating the feasibility of the pepART approach.
Towards Single Cell Fingerprinting in Microfluidic Device Format; Cell Manipulation, Protein Separation, and Label-free Detection

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Single cell analytics for proteomic analysis is considered as a key method in the framework of nanosystems biology which allows novel proteomics without being subjected to ensemble-averaging, cell-cycle or cell-population effects. We have developed and demonstrate first results of a single cell analytical method for protein fingerprinting which combines a structured microfluidic device with latest optical laser technology for single cell manipulation (trapping and steering), free-solution electrophoretical protein separation and (label-free) protein detection. We focus on three main issues. Firstly, single biological target cells were trapped, injected, steered and deposited by means of optical tweezers in a poly(dimethylsiloxane) (PDMS) microfluidic device, and consecutively lysed at a predefined position. Secondly, separation and detection of fluorescent dyes, amino acids and proteins was achieved with laser induced fluorescence detection in the visible (488 nm) as well as in the deep UV (266 nm) spectral range for label-free, native protein detection. Minute concentrations of 100 fM injected fluorescein could be detected in the visible and a first protein separation and label-free detection could be achieved in the UV spectral range. Whereas the fluorescein detection sensitivity in the visible spectral range corresponds to roughly 50–100 analyte molecules, which is well below the anticipated number of low abundant proteins in a cell, the first protein separation (avidin and lysozyme) with label-free UV-detection has currently a detection sensitivity in the pH8 range and has to be improved in the future. Thirdly, first analytical experiments with single Sf9 insect cells (Spodoptera frugiperda) in this tailored microfluidic device demonstrate distinct electropherograms with a single component peak of the GFP-construct protein proving the validity of the single cell analytical concept. This allows novel and fascinating single cell experiments for nanosystems biology in the future.

Isolation of Highly Specific Antibodies for Use in Sandwich Antibody Microarray Analyses

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Protein microarrays are expected to become an indispensable tool in basic and applied biological research as well as medical diagnostics. More than 10,000 proteins can be spotted onto a single microscope slide which makes massive parallel analysis feasible and the use of proteins and reagents highly economical. Furthermore, antibodies represent ideal protein capture molecules due to their high robustness and stability as well as their relative high specificity against their antigens. In the configuration of a sandwich assay using a pair of antibodies from two different hosts against a target protein, neither proteins nor the protein binding antibodies have to be modified with detection labels.

However, cross-reactivities of antibodies with other proteins in a complex biological sample may severely limit the number of proteins/antibodies that can be evaluated on a single array. To address this problem and to open other sources for highly specific antibodies, we wanted to exploit the comprehensive and diverse antibody pools available from polyclonal sera.

For the development of such an approach, we profited from our experience in epitope mapping: A series of overlapping antigen-derived peptide fragments are synthesized in a macroarray format onto a cellulose membrane (SPOT synthesis). Antibodies from a polyclonal serum bind their specific linear epitope (peptide fragment) and thereby separate into epitope specific antibody pools. Antibodies eluted from the peptide spots are used as detection antibodies in sandwich antibody microarray analyses and tested systematically for their affinities and specificities to their antigens in the context of a complex cellular protein extract. The most suitable antibodies were then selected for purification in larger quantities by peptide affinity chromatography.
A Protein Interaction Array in Quantitative Characterization of SCFV Antibodies Using a Peptide Panel Approach

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Objectives: The aim of the study was to perform a parallel quantitative analysis of scFv-peptide interactions, to understand the effects of affinity maturation and mutations on antibody specificity.

Results: This study was performed on a new protein array from Biacore, which uses SPR technology for label-free detection of protein interactions. The instrument configuration enables in-flow immobilization and quantitative determination of kinetic rate constants. Here, it was used to investigate how terminal truncations and point mutations in 32 peptides derived from the GCN4 transcription factor affected binding to 3 scFvs. Consequently, 96 ScFv-peptide interactions were analyzed and kinetic rate constants derived, enabling the importance of each residue to the interaction between the original peptide and the scFvs to be determined. The rate constants provided novel insights into the mechanisms of the interactions and showed marked differences in how recognition and stability of the complex are differently affected by changes to the peptide sequence.

Conclusion: The protein interaction array provides new possibilities for kinetics-based characterization of relatively large numbers of antibody-peptide interactions. The peptide panel approach offers precision epitope mapping, coupled with the possibility of mechanistic insights provided by the resolution of binding into on- and off-rates. The approach described here should be valuable in a range of proteomics-related applications, including improved selection of antibodies as protein localization and quantification tools, and in the characterization of protein-protein interactions in functional proteomics studies.

Identification of Small Molecule Protease Inhibitors Using a Microarray-based PNA Detection System

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Proteases play a myriad of roles in living systems, from the post translational processing of pro-enzymes or receptors to the degradation of proteins. A fundamental aspect of studying protease function is identifying specific tools to modulate the proteolytic activity. When placed in the context of a cells proteome the complexity of solving this task is magnified. In order to study proteolytic enzymes in complex biological systems, we have developed a microarray based method that can serve as a platform to study the inhibitor preferences of proteases on a miniaturized scale. In our system, split-and-mix chemistry is used to synthesize small molecule protease inhibitors that are encoded with a peptide nucleic acid (PNA) tag. The PNA tag not only encodes the synthetic history of the small molecule, but also enables the spatial deconvolution of the small molecules on a DNA microarray. Here we describe the application of this method to study cysteine proteases.
**34.24**

Antibody Microarrays; Technology and Analysis of Serum Proteomes from Cancer Patients

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Antibody-based microarray is a high-throughput technology with great potential in global proteome analysis and protein expression profiling. This tool will provide new opportunities for diagnostics, biomarker discovery, drug target identification and insights into disease biology. In our laboratory, we have successfully developed a high-performing antibody microarray technology platform based on recombinant antibody fragments. Initially, several basic but critical key technical issues have been addressed, including probe design, surface design, array format, sample handling, and analytical principles to demonstrate the specificity, sensitivity and stability of the set-up. In particular, the single-frame work concept in scFv design (SinFab) has been developed for microarray application, yielding >8 month on-chip-stability, assay sensitivity in the fM range, and direct analysis of labeled complex proteomes. This antibody microarray set-up has been used in a variety of applications, such as serum/cell proteome analysis of low as well as high abundant analytes. Recent projects focusing on cancer proteomics, using SinFab microarrays, have been performed, using sera from different patients suffering from solid tumors, such as e.g. pancreatic carcinomas. The optimized antibody microarray platform, as well as data from the clinical proteome analysis, will be presented.

**34.25**

Fabrication of a Protein Array Using Microcontact Printing and Its Electrochemical Detection

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This paper described a new method for throughput immobilization and electrochemical detection of mini-volume protein samples. Agarose was used to prepare elastomeric micropatterned stamps with 4x6 spots, on which 24 protein samples of 3µL individual volume were added onto each spots for the sample transfer procedure. Microcontact printing was performed by positioning the elastomeric stamps onto the array with 24 gold electrodes modified by dithiobisuccinimidyl propionate (DSP) self-assembly monolayer (SAM). In this case, the proteins were coupled and immobilized on the activated gold surface with N-hydroxysuccinimid (NHS) ester group. The resulting array was further incubated with horseradish peroxidase (HRP) labeled antibodies and then reacted with the substrate (3-Amino-9-Ethylcarbazole, AEC). The electrodes coupling with the HRP-labeled antibody would catalyze the substrate to produce insoluble precipitation. The array electrodes were measured by electrochemical impedance spectroscopy. In our experiment human IgG as a model sample was employed to be transferred onto the array electrode and impedance spectroscopy was carried to assay the electrodes. A good correlation relationship between the values of impedance and the concentration of protein samples was obtained for hIgG in the range of 0.2–20 pg, and the detection limit could reach 0.2 pg.

**34.26**

Protein Profiling and Phosphorylation in Breast Cancer Cells with Antibody Microarrays

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High-throughput monitoring the expression profiles of proteins by antibody micro-arrays can provide crucial information about the physiological status of organisms and can help to identify disease-specific biomarker candidates. The principle of competition between target molecules for binding to nitrocellulose-arrayed antibodies has been used to assess differential expression of proteins in breast cancer cells with one-color (competitive displacement) and two-color fluorescence detection methods. Labeled and unlabeled molecules possess different physico-chemical properties which affect the performance of one-color detection, whereas epitope inaccessibility in protein complexes can prohibit the assessment of competition by both detection methods. In addition, antibody cross-reactivity, target protein truncation and abundance, as well as cellular compartment of origin are major factors that affect protein profiling. Binding assays, performed under different conditions, allowed to conclude that two-color method is better suited to study differential expression of breast cancer proteome. Antibody micro-array data show the functional linkage between the ErbB2 receptor and AP-2 transcription factors in MDAMB-231 and SKBR3 cell lines and highlight unexpected differences in G1 cyclin expression (Yeretssian et al., Molecular and Cellular Proteomics 2005, 4). Micro-arrays have also been supplied to study complex networks involved in several signal transduction pathways. Reverse phase protein arrays have also been prepared to monitor phosphorylation status of target proteins after treatment of breast cancer cells with some natural anticancer agents. We show that a valuable biological information can be obtained by combining the two high-throughput tools taking their respective advantages as a prerequisite to prognostic of breast cancer progression.