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New developments for printing proteins as microarrays

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1. Introduction

DNA chip technology changes the way scientists study gene expression. DNA chips can be used to measure the expression patterns of thousands of genes in parallel. They can also be used to monitor the changes in gene expression in response to drug treatments. They allows rapid and cost-effective screens for mutations and sequence variations in genomic DNA and they provide novel insights into fundamental biological processes of human diseases at the molecular level. In principal microarray technology enables both, probing of the genome and proteome. But arraying of proteins is more difficult than arraying of DNA because they have to maintain their correctly folded conformations. The fabrication of protein arrays is therefore particulary challenging and protein arrays lagged behind in development because of the more complex coupling chemistry, the instability of the immobilized protein, and far weaker detection signals.

2. Genomics

A simple array experiment has five basic steps:

1. The target oligonucleotides are spotted onto a substrate
2. The sample RNA is isolated
3. The cDNA is synthesized, a procedure that also involves fluorescent labeling for later detection
4. The labeled probe is hybridized to target the oligonucleotides on the substrate
5. The results are imaged and analyzed

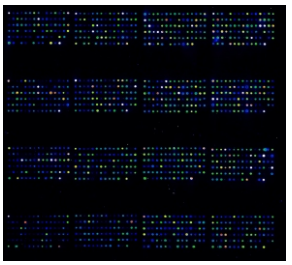

Fig. 2: Expression analysis of 1350 genes

3. Proteomics

Samples are investigated by:

1. Protein arrays
2. 2D-gel electrophoresis
3. Mass spectrometry

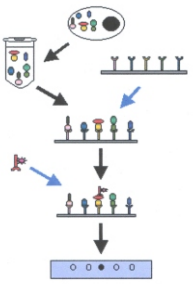

Fig. 3: Antigen-Antibody interaction

4. Projects

- Cell cycle analysis in synchronized yeast cultures
- Development of antibody arrays for proteomics
- Analyzing protein expression profiles
- Monitoring protein-protein interactions
- Identifying protein posttranslational modifications
- Screening the substrates of protein kinases
- Examining the protein targets of small molecules
- Proteomic analysis as a function of bioprocess cultivation

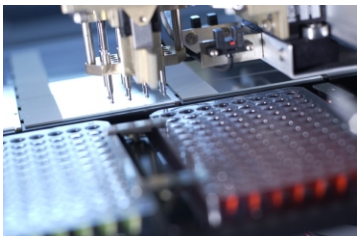

Fig . 1: The Affymetrix 427 arrayer

5. Results and Discussion

The aim of the project is to provide a modern scientific and technology platform based on biochips for proteome analysis. The major significance of the present work is to develop new membrane surfaces (activated nylon, nitrocellulose and others) that remains the most ideal surface for protein arrays and to transfer the established coupling chemistry developed by DNA arrays by the future use of aptamers that combines the advantage of arraying DNA with the specificity of antigene-antibody interaction and shows potential for proteome analysis.

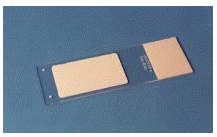

Fig. 4: Spotting on Nitro-cellulose and Nylon

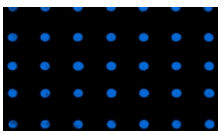

Fig. 5: Spotting on Nylon Filters

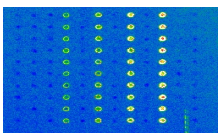

Fig. 7: Hybridisation on Nitrocellulose

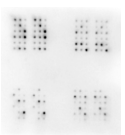

Fig. 8: Antigen Detection with Chemiluminescense