Abschlussbericht
Projekt Nr. 54857

ESF Exploratory Workshop
Protein Microarray Technology

European Scientific Foundation

15 January 2002

NMI
Naturwissenschaftliches
und Medizinisches Institut
an der Universität Tübingen

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1. Overview

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ESF Workshops Spearheading European
Basic Biomedical and Clinical Research - 2001

ESF EMRC Exploratory Workshop
Beyond the human genome sequence

Protein Microarray Technology

07. – 09. October 2001

at the
NMI Natural and Medical Sciences Institute at the University of Tuebingen

Organised by

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2. Summary

The rapid progress in complete genome sequencing allows the definition of the entire potential protein complement of organisms, the proteome. In order to analyse the actual level of protein expression and to to determine the state of posttranslational modifications, new protein identification and quantification techniques have to be developed. The aim of this workshop is to combine the efforts of European research institutes to improve array-based approaches for proteomics and diagnostics.

3. Introduction

The rapid progress in whole genome sequencing (e.g. Hattori et al. *Nature* 405: 311-319, 2000) allows to define the entire potential protein complement of organisms, the proteome. Genome wide studies of gene expression analysis are nowadays possible at the mRNA level using DNA microarray technologies, differential display PCR and serial analysis of gene expression. But the activities in living cells are carried out by proteins. Since mRNA levels and protein expression are not necessarily correlated and posttranscriptional modifications (e.g. phosphorylation, dephoshorylation) are important for the regulation of protein activity, these methods have intrinsic limitations. The best established and most widely applicable approach to study protein expression directly is the densitometric analysis of protein extracts separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Identification of proteins within a certain spot can be achieved using mass spectral data, either based on peptide masses from protein digests or obtained from fragmentation spectra from individual peptides. This is a very time consuming and costly approach. In order to analyse the actual level of protein expression and the state of post-translational modification, novel protein identification and quantification techniques have to be developed which achieve the accuracy and the speed of mRNA expression analysis. One possibility to determine the amount of spe-

cific proteins and to detect changes in co- and posttranslational modifications are array based proteomic approaches (recently discussed by Alabala and Humphery-Smith, *Curr Opin Pharm Proteomics* 1: 680, 1999). Capture molecules e.g. antibodies directed against specific target proteins are immobilised in a microarray format in a fashion analogous to DNA microarrays. Cell extracts labelled quantitatively by fluorescent or radioactive markers are incubated on the array. Bound proteins can be detected with the same instruments as used for DNA microarray technology. Such protein chips will provide a powerful and reliable platform for extending molecular analysis beyond the limitations of DNA chips. In medical research, microarray ELISAs will accelerate immune diagnostics significantly. The highly parallel fashion of analysis will allow the determination of tumour markers with only a minimum amount of biopsy material. Therefore, new possibilities for monitoring treatment and therapy will be developed with this emerging technology.

4. Content of the Workshop

The aim of this workshop was be to combine efforts of research institutes to improve every important step of array based protein microarray assays. The following topics were presented by the participants and their potential for proteomics and diagnostic approaches were discussed.

- **Theoretical concept of miniaturization**
- **Surfaces**
- **Immobilisation strategies**
- **Large scale expression and purification strategies of recombinant proteins.**
- **Large scale peptide synthesis and purification facilities; use of synthetic peptides as capture molecules and substrates for enzyme specific arrays.**
- **Improvement of target labelling procedures.**
- **Binding assay optimisation.**
- **Array based detection methods, limitations and improvement.**
- **Protein/peptide microarrays for diagnostics and pharmaceutical research.**
- **Market expectations for protein/peptide arrays.**

5. Summary

In summary the participants agreed that array based technology beyond DNA chips will accelerate basic research in the area of protein-protein interaction and will allow protein profiling from limited numbers of proteins up to array based proteomic approaches (Emili and Cagney, *Nat. Biotechnol.* 18: 393-397, 2000). Protein and peptide arrays can be used to analyse enzyme substrate specificity and to measure enzyme activity on different kinds of substrates (Arenkov et al., *Anal. Biochem.* 278**:** 123-131, 2000). Analysis of protein function and protein interaction studies using array based approaches will lead to a better understanding of the living cell (Abbott, *Nature* 402: 715-720, 1999). In pharmaceutical industry protein arrays can be integrated in target identification and validation processes. They will be of great value for diagnostic purposes enabling the analysis of multiple disease parameters in parallel with a minimum amount of biopsy material. The workshop was an excellent possibility for scientists from different research areas such as proteomics, biochip technology and oncology to discuss potential interactions and research activities among each others. Although first microarray experiments were performed and discussed by Roger Ekins already in 1989 (Ekins, R.P., *J. Pharm. Biomed. Anal*. 7: 155, 1989), recent advances in biochip technology are coming mainly from research institutes and companies based in the United States. Traditionally, research in proteomics has been strong in Europe and this basis should be used to step into the post genomics area. To strengthen the European position in the developing field of protein array technology the available scientific resources have to be combined. Specific projects and interactions among euro§pean scientists could be supported by funding of the EU and of other European funding organisations. Protein array technology is a rapidly expanding field and Euorpe has the strength to play a major role in the field of protein microarray technology.

6. Workshop programme

Sunday, 07.10.01

16:00	**Welcome of the participants** Thomas Joos, Marianna Minkowski
17:00 - 18:00	**Opening Lecture: Microarray Technology** Roger Ekins
18:00 – 19:00	**Protein Microarray Technology, Assay Development** Thomas Joos
19:30	***Dinner*** *Ratskeller Tuebingen*

Monday, 08.10.01

08:30 - 8:35	Chair: Prof. Dr. Siegfried Neumann,
08:40 - 10:00	**Surfaces** Erik Wischerhoff
10:00 - 10:30	***Coffee Break***
10:30 - 12:00	**Generation of Capture Molecules** Peter Lindner Dr. Ai Ching Lim Markus Templin
12:00	***Lunch***

PROTEIN MICROARRAY TECHNOLOGY

ESF EXPLORATORY WORKSHOP 07.10. – 09.10.01 in Tuebingen
T. Joos, NMI Reutlingen, Germany
I. Humphery-Smith, Pharmaceutical Proteomics, Utrecht, The Netherlands

13:00 - 14:30	**Array based proteomics** Mike Taussig Rosalind Jenkins
14:30 - 16:00	**City tour Tuebingen**
16:00 - 16:30	***Coffee Break***
16:30 - 19:00	**Protein Microarrays** Dolores Cahill Jörg Hoheisel
19:30	***Dinner at NMI***

Tuesday, 09.10.01

08:30 – 10:00	**Applications for Protein microarrays** all participants
10:00 - 10:30	***Coffee Break***
10:30 – 12:00	**General Discussion**
12:00	***Lunch***

End of Workshop

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