Final Report
ESF Workshop

Protein Arrays – Bridging the Gap Between Physics and Biomedicine

Jena, Germany,
1 – 3 April 2004

Executive Summary

Monitoring cellular activities on an extended scale requires tools with a highly parallel working principle. On the level of gene expression, DNA microarrays have become an indispensible tool in order to gain data about processing and quantification of genetic information within a cell. Cellular activities, however, are executed by proteins. Therefore, transferring the microarray principle onto the protein level is a highly useful strategy to get more insight into generation of protein complexes, protein-ligand-, and protein-protein-interactions on the cellular level.

The field of protein microarrays has recently experienced considerable progress with regard to new technologies in substrate coating, protein immobilization chemistry, and protein labeling methods. In order to exploit the possibilities of protein microarrays optimally, appropriate detection techniques need to be applied. Physical detection of protein-protein interaction on protein microarrays still is in its infancy, in particular, as sophisticated physical detection techniques are often narrowed to specific sample preparation or to limited sample complexity. In reality, typical biological samples still represent a challenge for a specific and sensitive detection of protein-protein-interactions due to frequently undefined and complex composition of protein mixtures and cell extracts, and because of heterogenous physical properties of the respective components.

In general, detection techniques may be separated in two classes: label dependent, and label-free. Advantages of label-dependent techniques include high sensitivity using signal amplification cascades, and also high specificity, but they usually require substantial and expensive preparations like antibody generation or protein engineering. Current label-free methods, in contrast, are much more generally applicable, but often lack sensitivity or sufficient specificity.

This is a motivation for investigating further possibilities for susceptible and specific detection of protein-protein-interaction, as well as for adapting existing methods better to screening and interpretation needs on protein microarrays.

Combining methods from biomedicine and physics also means translation of language and problem solving strategies in either field to the respective other one. Therefore, presentation of results of the own field in a way which allows comprehension by representants of the other will help bridging the gap between the two meanwhile highly specialized fields. This will be certainly of use for further application of protein microarrays in medical diagnosis and for screening purposes.

Scientific content of the event

The contributions at the ESF workshop "Protein Microarrays – Bridging the Gap Between Physics and Biomedicine" offered a broad sprectrum of in-depth investigations on the central topics of protein microarrays: protein immobilization chemistry, theoretical aspects of probe orientation, spotting techniques, in situ protein production techniques, and physical detection methods, i.e. fluorescence detection ellipsometry, surface plasmon resonance, SELDI, or 2D analysis. This was followed by several talks concerning applications in different fields of proteome analysis, ligand screening or cancer analysis.
In particular, browsing along the line of generation, analysis, and application of protein microarrays helped to gain a good overview on current development in protein microarray techniques and analysis.

The workshop started with a introduction on innovative methods in protein immobilization chemistry. C. Niemeyer, Dortmund, Germany, presented dendrimer type molecules, which, after covalent attachment to the substrate, served as spacers for protein immobilization. Proteins immobilized in defined distances to the substrate surface yield high signals, give homogeneous spots, and are suitable for long term storage of microarrays. Further possibilities include DNA directed immobilization of proteins, either covalently, or non-covalently as supermolecular complexes.
HM Striebel, Jena, Germany, talked about protein immobilization for subsequent analysis by fluorescence lifetime spectroscopy. As measurements require UV excitation of the fluorescent amino acids tryptophan and tyrosine, an immobilization chemistry without aromatic functions or conjugated systems is required. Substrate coatings like hydrogels carrying spacers, and NHS-ester mediated crosslinking chemistry help to provide a protein friendly microenvironment for fluorescence lifetime analysis.
An optimal orientation of proteins on microarrays was emphasized by A. Cass, Cambridge, UK. C- or N-terminal peptide fusion was described as a useful way for functionalised protein immobilization. Additionally, protein engineering could be used to enhance binding site affinity to ligands, good longterm stability and correspondingly good signal generation. The principle was demonstrated with phosphate binding proteins.

I. Humphery-Smith, Utrecht, The Netherlands, emphasised importance of control of the parameters leading to multiple protein-protein interactions. From antibody – antigen binding studies he deducted an enormous number of possible interactions under intra-cellular

conditions, probably also causing adverse drug effects. Solutions could be found by carefully modelling probe and target interactions.

HAO Hill, Oxford, UK, described ways for protein immobilization on electrodes for sensor development. Focus was on redox-active proteins at Au(111) surfaces. Aim was to retain immobilized proteins still electroactive. An analytical tool for assaying of surfaces was scanning probe microscopy, which was employed to investigate individual molecules of glucose oxidase under conditions of occurring electroenzymatic reaction.

An attractive way for protein transfer onto protein microarrays was presented by B. Liedberg, Linköping, Sweden. He employed microcontact printing for biochip production. Using elastomeric PDMS stamps he investigated conditions to generate molecular patterns on carboxymethylated dextran hydrogels. Ligand immobilization and characterization were assayed using different analysis techniques i.e. fluorescence and surface plasmon microscopy.

Another way of protein microarray generation was presented by M. He, Cambridge, UK. He described a cell free protein *in situ* arraying method using *in vitro* expression and *in situ* protein immobilization. The produced proteins were immobilized by a tag capturing surface. Further developments of this technique allowed "library against library" screening of potential interacting partners.

An integrated protein production approach was presented by GA Michaud, Branford, CT, USA. He and his co-workers used integrated cloning, expression, purification, and arraying techniques to rapidly represent proteins of particular organisms onto microarrays. A commercially available microarray with more than 4000 proteins was presented.

A central part of the workshop provided insight into sophisticated physical analysis methods for the read out of protein microarrays. Fluorescence based techniques occupied a major share of the talks, but also ellipsometry, or surface plasmon resonance.

T Soukka, Turku, Finland, introduced a technique featuring time-resolved luminescence measurement using lanthanide chelate labelled proteins in a bioaffinity assay. By this mean, temporal resolution can be used additionally to spectral and spatial resolution. In a multilabel assay, each signal can be defined by spectral as well as temporal parameters.

Another approach was presented by E. Schick, Witterswil, Switzerland. He mapped protein-protein interactions by way of a planar waveguide based fluorescence imaging system, which could be used both for DNA and protein microarrays. This was part of a complete protein profiling system, including a microarray analysis software.

P Schellenberg, Jena, Germany, introduced marker-free detection of protein-protein interactions by using an UV-laser. The decay intrinsic fluorescence of the proteins is measured in dependency of the binding or non-binding of ligands. This approach was demonstrated with multiple protein – protein pairs.

A marker-free technique based on imaging ellipsometry was presented by M. Vaupel, Göttingen, Germany. The method was used for investigation of biochips featuring proteins bound to a gold surface on a glass substrate. This approach is highly suitable for high throughput screening purposes. Tunable multilayer substrates were also a promising development.

A range of different detection methods useful for the analysis of protein microarrays as well as for analysis of coated beads was the topic of S. Pabst, Tübingen, Germany. Both kinds of assays were based on capture antibodies. Bead based assays were provided for the analysis of the epidermal growth factor receptor family, array based assays for matrix metalloproteases.

Applications for protein microarrays are an rapidly increasing field. Focus of the talks about microarray applications was on cancer diagnosis, lipid metabolism and general screening purposes.

G. Siest, Nancy, France described protein microarrays for clinical chemistry applications. Lipid profiles in family studies were analysed until yet by classical profiling approaches. These studies would profit from application of apolipoprotein, tumor, and cytokine arrays.

Applications of microarray-related methods, i. E. 2D electrophoresis and mass spectrometry (SELDI) were presented by F. von Eggeling, Jena, Germany, for the identification of tumor markers, particularly those of head and neck cancer tissue.

Cancer related applications were also presented by J. Hoheisel, Heidelberg, Germany. His talk focussed on the selection of particular genes on basis of transcriptional profiling experiments using cancer microarrays, thereby combining methods from genomics and proteomics for cancer analysis.

Results from a data mining project on gene expression in a broad spectrum of cancers were the topic of K.O. Greulich, Jena, Germany. It could be shown that a small number of genes are overexpressed in many types of cancer. The presented data provide the basis for a list of proteins, which could be usefully included into tumor microarrays.

An overview about generation and application of protein microarrays was given by H. Eickhoff, Berlin, Germany. He discussed some of the basic problems which arise during

efforts in automation of protein microarray analyses. Particular attention was drawn to conditions that prevent proteins from denaturing during processing and storage.

Further applications of protein microarrays were presented by W. Ansorge, Heidelberg, Germany. His talk gave a differentiated overview about the possibilities of the use of protein and nucleic acid microarrays. Particularly comparative hybridisations with nucleic acid from human cell lines demonstrate the potential of arrays in biomedical research.

Finally, a theoretical study was presented by F. E. von Eyben, Odense, Denmark, who pointed out ways to investigate obesity by combined application of gene expression and 2D electrophoresis data.

Assessment of the results

Substrates, protein immobilization chemistry

Since the advent of protein microarray technology, quite a spectrum of supports and chemical immobilization methods have been developed. Substrates and immobilization strategies presented at the workshop gave a good overview about currently used methods. A tendency to move from simple protein immobilization schemes towards more sophisticated binding methods like self – assembled structures, dendrimers and fusion tag based proteins is clearly visible. The workshop also provided a good platform for exchange of different approaches in the respective laboratories.

Protein production, spotting techniques

The common way to provide proteins for protein microarrays is their separate cloning and purification before they can be applied on an array. Some of the talks presented on the workshop focussed rather on an integrative way, where protein production, purification, and immobilization is performed *in situ* on a chip. This may become a fast and cost effective way to provide sufficient probe material. Also, the presented way of protein stamping may become an important alternative to current protein spotting procedures.

Physical methods for the read out of protein microarrays

New methods for the read out of protein microarrays occupied a considerable part of the talks presented on the workshop. An important share was provided by fluorescence based detection methods, which may be classified in marker-free and marker-dependent methods. New

approaches of marker dependent fluorescence detection included protein engineering techniques, protein fusion, and coupling of rare-earth chelate functions to proteins. These techniques are not used routinely yet, but have a high potential to provide suitable probes for screening and diagnosis.

Marker free detection methods are either based on the detection of protein – intrinsic fluorescence or on surface plasmon resonance and ellipsometry. The latter have matured into systems, which are employed for routine screening purposes. First applications were presented by representatives of companies participating in the workshop.

A range of further protein detection techniques were presented. These included electrochemical methods, where electroactive proteins were immobilized on electrodes, as well as mass spectrometry and 2D electrophoresis. Mass spectrometry (SELDI) in particular was presented as a way to investigate whole tissues for tumor biomarkers.

Protein microarray applications

Protein microarray may be the method of choice for solving many questions in proteomics and diagnosis. In particular, protein microarrays are suited for clarification and mapping of cellular networks, i. E. signal transduction cascades, phosphorylation networks, and of proteins involved in tumor genesis. This was reflected by a range of talks with the focus on cancer chips, as well as on lipid metabolism. It turned out, that a small number of proteins are overexpressed in most types of cancer, a fact that may facilitate the development of suitable cancer microarrays. There is a tendency visible leading towards higher integrated arrays examining a large subset of the proteome. Approaches probing the proteome and the genome in a combined approach also turned out advantageous for diagnosis.

Outlook

"Protein Microarrays – Bridging the Gap Between Physics and Biomedicine" was a workshop, which was intended to bring together physicists working in fields relevant to protein microarray analysis, as well as scientists in biomedicine working in fields from protein microarray generation to protein microarray application.

The talks were presented throughout clearly and easy to follow by listeners from the "other side" of the field. This gave rise to enlightening discussions between participants, leading to the exchange of theoretical and methodical ways of protein microarray generation and

analysis. In this regard, the aim of encouraging interactions between participants of different scientific background has been reached successfully.

Future aspects of the field will certainly go towards higher integration of protein microarrays, to better map and analyze protein networks in cells or tissues. The number of proteins available for the use on microarrays is increasing continuously. In combination with the impressive spectrum of analysis methods presented at the workshop, this will clearly promote the use of protein microarrays as one of the most promising new tools in biomedicine.

List of Participants

Co-Convenors:

1. **Peter SCHELLENBERG**
   Institut für Molekulare Biotechnologie e.V.
   Beutenbergstr. 11
   07745 Jena
   Germany
   Tel: +49 3641 65 6401
   Fax: +49 3641 65 6410
   Email: schelli@imb-jena.de

2. **Hans-Martin STRIEBEL**
   Institut für Molekulare Biotechnologie (IMB)
   Beutenbergstraße 11
   07745 Jena
   Germany
   Tel: +49 3641 65 6401
   Fax: +49 03641 65 6410
   Email: hms@imb-jena.de

Local Organiser:

3. **Karl-Otto GREULICH**
   Institut für Molekulare Biotechnologie (IMB)
   Universität Jena
   Beutenbergstraße 11
   07745 Jena
   Germany
   Email: kog@imb-jena.de

ESF Representative:

4. **Hui Wang**
   Scientific Secretary - Medical Sciences
   European Science Foundation
   1, quai Lezay-Marnésia
   BP 90015
   67080 Strasbourg Cedex
   France
   Tel: +33 (0)388 76 71 00
   Fax: +33 (0)388 37 05 32
   Email: hwang@esf.org

Participants:

5. **Wilhelm ANSORGE**
   EMBL Heidelberg
   Meyerhofstrasse 1
   Postfach 1022.09
   69012 Heidelberg
   Germany
   Tel: +49 6221 387 355/474
   Fax: +49 6221 387 306
   Email: ansorge@embl-heidelberg.de

6. **Anthony CASS**
   034 Biochemistry
   Imperial College London
   South Kensington Campus
   London SW7 2AZ
   United Kingdom
   Tel: +44 20 7594 5195
   Email: t.cass@imperial.ac.uk

7. **Holger EICKHOFF**
   Scienion AG
   Volmerstraße 7b
   12489 Berlin
   Germany
   Email: Eickhoff@scienion.de

8. **Mingyue HE**
   Discerna Ltd.
   The Babraham Institute
   Babraham Hall
   Cambridge CB2 4AT
   United Kingdom
   Tel: +44 1223 496217
   Fax: +44 1223 496038
   Email: m.he@discerna.co.uk

9. **H. Allen O. HILL**
   Inorganic Chemistry Laboratory
   Department of Chemistry
   University of Oxford
   South Parks Road
   Oxford OX1 3 QR
   United Kingdom
   Tel: +44 1865 275900
   Email: allen.hill@chem.ox.ac.uk

10. **Jörg HOHEISEL**
    Deutsches Krebsforschungszentrum (DKFZ)
    Functional Genome Analysis
    Im Neuenheimer Feld 506
    69120 Heidelberg
    Germany
    Tel: +49 6221 42 4680
    Fax: +49 6221 42 4682
    Email: j.hoheisel@dkfz-heidelberg.de

11. **Ian HUMPHERY-SMITH**
    Department of Pharmaceutical Proteomics
    Utrecht University
    PO Box 80082
    (Sorbonnelaan 16)
    3508 TB Utrecht
    Netherlands
    Tel: +31 30 253 6817
    Fax: +31 30 251 8219
    Email: i.humphery-smith@pharm.uu.nl

12. **Bo LIEDBERG**
Molecular Films and Surface Analysis Group,
Lab. of Applied Physics
Dept. of Physics and Measurement Technology
Linköping University
581 83 Linköping
Sweden
Tel: +46 13 281877
Fax: +46 13 288969
Email: bolie@ifm.liu.se

13. **Gregory MICHAUD**
Protometrix, Inc
688 East Main Street
Branford CT 06405
United States
Tel: +1 203 848-1100
Fax: +1 203 848-1155
Email: Gregory.Michaud@protometrix.com

14. **Derek MURPHY**
Royal College of Surgeons in Ireland
Centre for Human Proteomics
123 St. Stephen's Green
Dublin 2
Ireland
Tel: +353 1 402 8550
Fax: +353 1 402 8551
Email: dmurphy2@rcsi.ie

15. **Christof M. NIEMEYER**
Biologisch-Chemische Mikrostrukturtechnik
FB Chemie
Universität Dortmund
Otto-Hahn Str 6
44227 Dortmund
Germany
Tel: +49 231 755 7080
Fax: +49 231 755 7082
Email: cmn@chemie.uni-dortmund.de

16. **Stefan PABST**
Dept. of Biochemistry
NMI Natural and Medical Sciences Institute
University of Tuebingen
Markwiesenstrasse 55
72770 Reutlingen
Germany
Tel: +49 7121 51530 815
Fax: +49 7121 51530 16
Email: pabst@nmi.de

17. **Eginhard SCHICK**
Zeptosens AG
Benkenstr. 254
4108 Witterswil
Switzerland
Tel: +41 61 726 81 81
Fax: 41 61 726 81 71
Email: Eginhard.Schick@zeptosens.com

18. **Gérard SIEST**
INSERM
U525
Biologie Prospective
Faculté de Pharmacie
Université Henry Poincaré
30 rue Lionnois
54000 Nancy
France
Tel: +33 3 83 352 616
Fax: +33 3 83 321 322
Email: gerard.siest@pharma.uhp-nancy.fr

19. **Tero SOUKKA**
University of Turku
Department of Biotechnology
Tykistökatu 6A 6th
FIN-20520 Turku,
Finland
Tel: +358-2-333-8685
Fax: +358-2-333-8050
Email: tero.soukka@utu.fi

20. **Matthias VAUPEL**
Nanofilm Technologie GmbH
Anna-Vandenhoeck-Ring 5
37081 Göttingen
Germany
Tel: +49 551 99960-25
Fax: +49 551 99960-10
Email: mv@nanofilm.de

21. **Ferdinand VON EGGELING**
Klinikum der FSU Jena
Institut für Humangenetik und Anthropologie
Kollegiengasse 10
07743 Jena
Germany
Tel: +49 36 41 93 55 26 or 28
Fax: +49 36 41 93 55 82
Email: fegg@mti-n.uni-jena.de

22. **Finn Edler VON EYBEN**
Odense University Hospital
Gaedesmuttevej 30
DK-5210 Odense NV
Denmark
Tel: +45 66 145862
Email: finnvon@mail.tele.dk

PROGRAMME

All talks will be 20 min + 10 min discussion

Thursday 1 April 2004

from 14:00	*Registration*
19:30	Get together reception
	Dinner

Friday 2 April 2004

09:00 – 09:15	**Hui Wang**, Strasbourg, France Representative of the Standing Committee for the European Medical Research Councils Presentation of the European Science Foundation (ESF)
	Session 1 - Bioconjugates, Biomodifications
09:15 – 09:45	**Christof Niemeyer**, Dortmund, Germany Self-Assembled Bioconjugates for Biochip Technologies
09:45 – 10:15	**Anthony Cass**, London, UK The role of protein engineering in designing content
30 min coffee break	
	Session 2 - Detection techniques for protein arrays: optical spectroscopy, microscopy
10:45 – 11:15	**H. A. O. Hill**, Oxford, UK Proteins at electrode surfaces: examination by Scanning Probe Microscopy
11:15 – 11:45	**Tero Soukko**, Turku, Finland Time-resolved fluorescent labels in protein recognition
11:45 – 12:05	**Peter Schellenberg,** Jena, Germany Analysing protein microarrays by utilizing intrinsic time resolved UV fluorescence
12:10	Lunch

Session 3 – Detection techniques for protein arrays: ellipsometry, mass spectroscopy

14:00 – 14:30 **Bo Liedberg**, Sweden
Microarray production on polymeric hydrogels using microcontact printing

14:30 – 15:00 **Matthias Vaupel**, Germany
Quality control and kinetics recording on micro arrays with imaging ellipsometry

15:00 – 15:30 **Ferdinand von Eggeling**, Jena, Germany
Biomarker discovery with ProteinChip technology

30 min coffee break

Session 4 – Substrates, coatings, chemistry

16:00 – 16:30 **Holger Eickhoff**, Berlin, Germany
Protein Chip data bridging data sets from 2D Gels and DNA – microarrays: Applications in neurology and immunology research

16:30 – 17:00 **Gérard Siest,** Nancy, France
Will the proteinchips in clinical chemistry be useful in preventive medicine

17:00 – 17:30 **Hans Martin Striebel** , Jena, Germany
Supports and probe immobilization for protein microarray analysis by time resolved UV fluorescence

Dinner/Buffet Restaurant SCALA, Intershop-Tower
City tour from top of the building

Saturday 3 April 2004

Session 5 - Protein array assembly: supports, immobilization techniques

09:00 – 09:30 **Eginhard Schick**, Switzerland
Cell Lysate Arrays- Protein Profiling in Cellular Systems

09:30 – 10:00 **Derek Murphy**, Ireland
Generation and some applications of protein arrays

10:00 – 10:30 **Stefan Pabst**, Tübingen, Germany
Protein Microarray Technology

30 min coffee break

Session 6 - Protein array applications: screening, medical diagnosis, antibody arrays, translation profiling

11:00 – 11:30	**Wilhelm Ansorge**, Heidelberg, Germany Microarray techniques in biomedical research
11:30 – 12:00	**Jörg Hoheisel**, Heidelberg, Germany Combining proteomics and genomics for cancer analysis
12:00 – 12:30	**Gregory Michaud**, New Haven, CT, USA Applications of functional proteome scale microarrays
12:30 – 13:00	**Ian Humphery-Smith**, The Netherlands Proteins arrays and a new paradigm in lead optimization
13:00	Lunch

Session 7 – Micro probe arrays, special applications

14:30 – 15:00	**Mingyue He**, Cambridge, UK Cell-free protein arrays from DNA
15:00 – 15:30	**Karl Otto Greulich**, Jena, Germany Ubiquitous cancer genes – candidates for protein chips?
15:30	**Discussion:** Future prospects
16:30	**End of workshop**
	Dinner

Sunday 4 April 2004

Breakfast

Departure

Statistics

Type of home institution:

University	10
Research Institute	6
Company	5
EFS Representative	1

Level of Profession:

Junior / Postdoc	5
Senior / Professor	11
Industry	5

Country:

Denmark	1
Finland	1
France	2
Germany	10 (incl. convenors and local organizer)
Ireland	1
Sweden	1
Switzerland	1
The Netherlands	1
United Kingdom	3
USA	1