Scientific Forum

Program Organized by the Swiss Chemical Society (SCS) Halle 1, Saal "Luzern"

The Swiss Chemical Society (SCS) as founder and ideological partner of the ILMAC has chosen ***a new concept for the Scientific Forum.*** The organization committee of the SCS under the chairmanship of Dr. Heiner Kern has dropped the idea of running parallel sessions. Short, but efficient papers from Swiss and international experts are now the goal. These will be presented in morning sessions only and no posters are planned. Thus, the entire afternoon is available to visit the exhibition and to allow plenty of time for individual discussions with the experts of the exhibiting companies and make new contacts or improve and deepen existing relations with manufacturers and their Swiss representatives.
To enable ***Swiss Chemical Society members*** to visit the Scientific Program as well as the Fair, ***tickets are offered free of charge by the Swiss Chemical Society.*** SCS members can obtain their tickets online using the Priority Code 292-FT38L66X at *www.ilmac.ch/online-ticket* (the service is available from April onwards). A day ticket includes entrance to all additional events and forums as well as the SCS Scientific Forum (see 'Eintritt*' on page 148)

Each day of the Scientific Forum is devoted to a different topic:

- **Tuesday morning, May 24, 2005:** 'New Tools in Nanosciences for Biodiagnostics and Bioapplications' are discussed on the first day.
- **Wednesday morning, May 25, 2005:** 'The Fate of Drugs in the Body – Mechanisms and Predictions for Uptake, Distribution and Elimination of Pharmaceuticals' is the subject.
- **Thursday morning, May 26, 2005** will concentrate on the Life Sciences, in particular on the exciting field of protein. 'From Proteomics to Systems Biology' is the title of the day.
- **Friday morning, May 27, 2005,** demonstrates the power of Trends in Bioanalysis: 'Nuclear Magnetic Resonance Spectroscopy (NMR) and Mass Spectroscopy (MS)'. NMR and MS complement each other in capturing metabolic data, both in terms of sensitivity and the capability for quantitative pattern determination.

  The *highlight* not only of this session but of the whole scientific part of the ILMAC 2005 will be the lecture by Prof. *Kurt Wüthrich*, Nobel Laureate in Chemistry 2002, entitled 'From Structural Biology to Structural Genomics'.
- **Special Evening Event on Wednesday, May 25, 2005:** The very successful Swiss biotech start-up company Actelion will be presented by its CEO: 'A New Global Player in the Biotech Industry?'

Tuesday May 24, 2005, 10.00–12.50

New Tools in Nanosciences for Biodiagnostics and Bioapplications

Chairperson: *Hans-Joachim Güntherodt*, Institut für Physik, Universität Basel

10.00–10.45 Keynote Lecture
Martin Hegner, Institut für Physik, Universität Basel
'Multifunctional Cantilever Arrays for Chemical and Biological Sensing'
Abstract 1

10.45-11.10 *Andreas Wild*, Universitätsaugenklinik Basel
'Novel Optical mRNA Biosensor'
Abstract 2

11.10–11.35 *Emmanuel Delamarche*, IBM Zürich, Research Laboratory, Rueschlikon
'Soft Lithography for Bioanalytical Applications'.
Abstract 3

11.35–12.00 *Renato Zenobi*, Dept. of Chemistry and Applied Biosciences, ETH Zürich
'Molecular Analysis on the Nanometer Scale'
Abstract 4

12.00–12.25 *Manfred Stanzel*, Siemens AG, Erlangen, Germany
'*quicklab* - Electrical Biochip System'
Abstract 5

12.25–12.50 *Chyi-Cheng Chen*, DSM Nutritional ProductsLTD, Basel
'Vitamin E Nanoparticle for Beverage Application'
Abstract 6

Abstract 1
Multifunctional Cantilever Arrays for Chemical and Biological Sensing

N. Backmann[a], J. Zhang[a], K. Gfeller[a], N. Nugaeva[a], A. Bietsch[a,b], H.P. Lang[a,b], A. Plückthun[c], C. Gerber[a,b], M. Hegner[a]
[a]Institute of Physics, University of Basel, NCCR Nanoscale Science; [b]IBM Research Lab, Zürich; [c]University of Zürich

Biosensing tools are currently undergoing a further stage of development. Increasing efforts have therefore been put into the development of cantilever-based sensors for the detection of physical phenomena and chemical and biological reactions. Microfabricated silicon cantilevers arrays offer a novel parallel label-free approach where ligand-receptor binding interactions occurring on the sensor generate nanomechanical signals like bending or a change in mass that is optically detected *in situ*. The elegance of these sensing methods is that the detection of an analyte requires no labeling, as well as that the various application fields only differ in the functional layers on the cantilever interface. The detection scheme remains common for all the different applications. In principle, any detection method, which is based on molecular recognition, is able to be implemented.

Detection of multiple unlabelled DNA/RNA simultaneously down to picomolar concentrations within minutes is demonstrated. This DNA sensitivity is suitable for detection of specific unlabeled gene fragments within a complete genome (gene fishing). Currently we are able to 'fish' individual gene fragments within a whole genome (rat or human) and have the ability to see whether a gene is turned on or off upon a specific external signal (Interferon) supplied in cell cultures. Such an approach allows non-labeled, non-amplified genetic analysis within a complete genome and will provide a new tool for biomarker screening.

We report on new styles of bio-/chemical cantilever activation for sensing applications. To enhance the sensitivity for protein detection we use scFv fragments and antibody mimicries (*i.e.* ankyrins) which can be tailor made and optimized for enhanced binding strength towards the target of interest.

Selective active growth of micro-organism is detected on micron sized cantilevers within two hours. Potential applications include: Fast antibiotic susceptibility testing and fast fungal spore detection in food industry and bio-destruction of materials.

References:

- H.P. Lang *et al.*, 'Nanomechanics from atomic resolution to molecular recognition based on atomic force microscopy technology', *Nanotechnology* **2002**, *13*, R29–R36.
- R. McKendry *et al.*, 'Multiple label-free biodetection and quantitative DNAbinding assays on a nanomechanical cantilever array', *Proc. Natl. Acad. Sci. USA* **2002**, *99*(15), 9783–9788.
- Y. Arntz *et al.*, 'A label-free protein assay based on a nanomechanical cantilever array', *Nanotechnology* **2003**, *14*, 86–90.
- K. Gfeller *et al.*, 'Rapid biosensor for the detection of selective growth of *Escherichia coli*', *Appl. Env. Microbiol.* **2005**, *71*, in press.

Abstract 2
Novel Optical mRNA Biosensor

Andreas Wild
Universitätsaugenklinik Basel

The search for molecular markers that *predict the prognosis* or the *response to a gene-specific therapy* of individual patients is a major focus in the emerging field of 'molecular medicine' which will have particular impact in cancer treatment. During the last years, much progress has been made in this field. For example, the drug Herceptin® which is a monoclonal antibody that blocks the Her2 receptor mediated tumor growth in metastasic breast cancer has become a paradigm for the feasibility of targeted therapy. Numerous additional molecular markers will be identified in the near future. This knowledge prompts for the development of reliable and sensitive devices for the detection and quantification of therapy target genes with single-molecule sensitivity.

To date, diagnostic methods include detection of DNA copy numbers (*e.g.* by fluorescence *in situ* hybridization, quantitative PCR, Southern blotting), RNA expression (RT-PCR, RNA *in situ* hybridization, Northern blotting) or protein levels (immunohistochemistry (IHC), Western blotting). Such techniques share the disadvantage that they are *time consuming* and *expensive*, and require *extensive pretreatment of samples*. In addition, these assays have only limited capabilities for the parallel investigation of multiple markers.

A simple fiber-optical device has been developed that allows detection of the presence of *single* mRNA copies in native body fluids, such as *e.g.* blood. It is based on existing

technologies combining molecular beacons, optical fibers and ultra-sensitive fluorescence detection. Due to the use of smart probes, no prior fluorescence labeling of samples is necessary. The device is easy to use, robust, fast and highly specific and thus ideally suited for application in a clinical environment and therefore has the potential to outrange today's techniques in molecular diagnostics. Future developments will be directed towards a parallelization of the approach.

Abstract 3

Soft Lithography for Bioanalytical Applications

Emmanuel Delamarche
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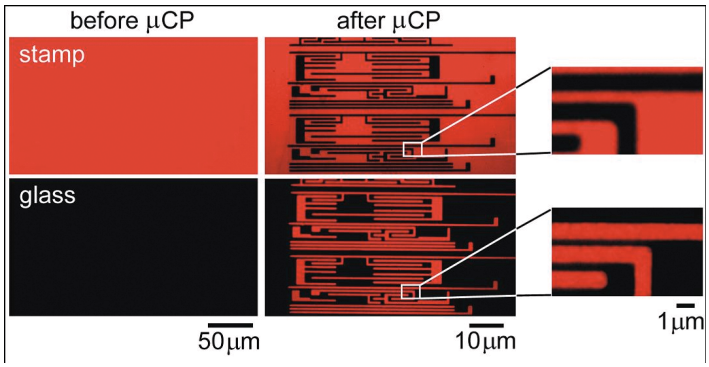
The famous trend known as Moore's law experienced in the microelectronics industry could in principle also occur in biology. Microtechnology has in fact started to impact many areas of biology and medicine by providing new sensing and analytical tools, the means to pattern biomolecules on surfaces, and systems to handle sub-microliter volumes of liquids. Immobilizing ligands on surfaces is a first step in many bioassays, a prerequisite for the design of bioelectronic devices, and valuable in certain combinatorial screening strategies. Biological activity measurements on surfaces depend on the mode of transfer, the immobilization, and the molecular orientation of surface-bound biomolecules. In biosensors, molecules are often immobilized on a solid surface, where they function as specific ligands for biomolecules such as enzymes, antigens, antibodies, and DNA.

Our research on 'soft lithography' for experimental biosciences at the IBM Zurich Research Laboratory entails a set of techniques derived from microcontact printing (μCP). The original concept of μCP is that a patterned elastomer – the stamp – can be inked and placed in contact with a surface to locally deliver a self-assembling monolayer of resist to a surface [1]. In our quest to explore the use of μCP to nanoscale technologies, we derived methods based on μCP and microfluidics that can be used to miniaturize biological assays [2][3]. We expect such miniaturized assays to be faster than conventional methods, conservative in their use of samples and reagents, capable of screening many analytes in parallel, and sensitive. We use μCP, for example, to pattern proteins down to the level of a single protein molecule on surfaces, to form very large arrays of surface-immobilized single vesicles, or to retrieve cell adhesion molecules from complex fluids, and to guide the attachment and growth of neurons on surfaces. Similarly, we use microfluidic networks to pattern proteins on surfaces with great accuracy and to localize all the steps – capture of analytes, rinses, and binding of detection antibodies – needed for surface fluorescence immunoassays. These methods stand as proof-of-concept, and there are now tangible applications in which the level of miniaturization provided by soft lithography techniques will benefit applications in diagnostics, biotechnology, and life sciences in general.

These fluorescence microscope images reveal the presence of TRITC-labeled antibodies on a stamp after inking and the accurate transfer of the antibodies onto a glass surface in the regions of contact between the glass and the stamp. Here, the pattern present on the stamp is not visible until printing is done.

[1] A. Kumar, H.A. Biebuyck, G.M. Whitesides, *Langmuir* **1994**, *10*, 1498–1511.
[2] A. Bernard, E. Delamarche, H. Schmid, B. Michel, H.R. Bosshard, H.A. Biebuyck, *Langmuir* **1998**, *14*, 2225–2229.
[3] D. Juncker, H. Schmid, U. Drechsler, H. Wolf, M. Wolf, B. Michel, N. de Rooij, E. Delamarche, *Anal. Chem.* **2002**, *74*, 6139–6144.



Abstract 4

Molecular Analysis on the Nanometer Scale

Renato Zenobi
Department of Chemistry and Applied Biosciences, ETH Hönggerberg, Zürich (zenobi@org.chem.ethz.ch)

Researching the nanometer scale is currently of great relevance in many branches of modern science and engineering. As nanoscience and nanotechnology develop, powerful nanodiagnostic tools capable of recording chemical/molecular information with good spatial resolution will become increasingly important. While it is possible to obtain topographic and conductivity information with atomic resolution using STM and AFM, these tools typically yield no molecular information at all. Elemental analysis with <100 nm lateral resolution is possible using other methods such as Auger microscopy, but the determination of the *molecular* composition of a sample with a resolution <1 μm is still very challenging.

We are developing scanning near-field optical microscopy (SNOM) for nanoscale molecular analysis. SNOM is the 'optical member' of the family of scanning probe microscopies and is based on a subwavelength light source that is scanned above the object of interest at a distance of a few nm. In the optical near field, the illuminated area is not limited by diffraction, but merely by the size of the illumination source (50–100 nm). SNOM imaging can be done nondestructively and under ambient conditions [1]. Two main lines of research are pursued in our group:

(i) *SNOM* with aperture probes designed for high optical transmission and able to sustain pulsed laser radiation, for performing laser ablation mass spectrometry with subwavelength resolution. The design and capabilities of a second-generation SNOM-MS instrument that combines laser ablation at ambient conditions, transport into a vacuum chamber, an efficient post-ionization/ion storage stage, and mass analysis by time-of-flight mass spectrometry will be presented.

(ii) *Apertureless SNOM,* employing external laser irradiation and a metallic tip 'dipped' into the laser focus, to greatly enhance the local field for 'tip-enhanced' Raman spectroscopy (TERS). The spatial resolution is in this case determined by the curvature of the metallic tip, and is often well below 50 nm. Our aim is the reproducible production of robust and highly enhancing tips for TERS applications. Very recently, enhancement factors above 10^{10} were demonstrated in our laboratory, using the so-called 'gap mode'. This should open the way for single molecule Raman spectroscopy on surfaces with exquisite spatial resolution.

Questions that are being addressed with these methodologies include the determination of the composition of thin solid films, the characterization of novel materials and components of molecular electronics such as carbon nanotubes, the investigation of biological objects in their natural environment, and the study of chemical reactions on the surface of heterogeneous catalysts.

[1] M. De Serio, V. Deckert, R. Zenobi, 'Looking at the Nanoscale: Scanning Near-Field Optical Microscopy', *Tr. Anal. Chem. (TRAC)* **2003**, *22*, 70–77.

Abstract 5

quicklab – Electrical Biochip System

Manfred Stanzel, H. Barlag, P. Paulicka, D. Kühn, K. Friedrich, W. Gumbrecht
Siemens AG, Erlangen, Germany

Molecular diagnostics is becoming increasingly important for the identification of diseases. Siemens intends to implement an entire lab-on-a-chip in a smartcard similar to conventional credit cards. The aim is to manufacture the *quicklab* system, at low costs as a general-purpose, mass-market analytical product on the basis of already existing production technologies. The development is intended to be used in doctors' surgeries and in clinical laboratories, with the smart card being inserted into a laptop-sized terminal which controls the analytic process and provides a readout of the results.

On the *quicklab* card of today, DNA is automatically extracted from a drop of blood or other body fluid. The genomic material is amplified using standard PCR-techniques. The products are analyzed during an 'DNA-ELISA' process resulting in a direct electrical readout.

The *quicklab* card is designed as a microfluidic system composed of channels, chambers and passive valves. Capillary forces draw a small volume of the injected drop of blood into a channel. Chemicals break down the cells in minutes, meanwhile the cellular DNA is bound to magnetic beads. Removal of cell debris and PCR-inhibiting substances takes place during flow of the beads into a small chamber, suitable for PCR. During the thermocycling amplification process, the products are labeled using biotinylated primers. After PCR the DNA product reaches the detection chamber, where the biosensor with individual gold electrodes is located.

The different analytical electrodes of the sensor are coated with DNA-oligonucleotid-catchers specific for wild type or mutation genotypes. On locations where the sample to probe DNA-hybridization takes place, this interaction is electrochemically detected using a two-step (ELISA) process. Streptavidin-alkaline-phophatase binds to the biotinylated DNA, amplified during the PCR, and converts *p*-aminophenylphosphate to *p*-aminophenol which is electrochemically oxidized at the noble metal electrode. Because of the continuous enzymatic conversion of *p*APP to *p*AP the oxidation current increases, resulting in a linear slope which can identify the starting DNA-hybridization process.

Abstract 6

Vitamin E Nanoparticle for Beverage Application

Chyi-Cheng Chen, Gerhard Wagner
DSM Nutrional Products Ltd., Basel

Nanotechnology appears increasingly in various commercial applications. This presentation will give an example of solving a beverage vitamin fortification problem based on nanotechnology. Vitamin E is one of the key ingredients in functional beverages. However, beverage fortification of vitamin E, being a fat-soluble vitamin, presents a real technical challenge.

The most critical issues in vitamin E beverage fortification are in the following areas.

- The dispersed vitamin E droplets rise to the top to form a whitish ring (ringing).
- The dispersed vitamin E droplets increase the beverage turbidity and alter beverage appearance.
- The ingredients used to facilitate vitamin E dispersion impart an objectionable taste.
- The ingredients used to facilitate vitamin E dispersion are subject to regulatory issues.

None of the existing vitamin E products on the market can avoid all four problems. Therefore, a new product is specifically designed to meet the customer's needs. The problems with existing products, design rationales of a new product, product development approach and applications of the new product are to be discussed as outlined below.

I. Product Design of a Nanoparticle Product
  A. Problems with existing products
    1. Poor physical stability
    2. Increasing beverage turbidity
    3. Sensory problem
    4. Regulatory concern
  B. Technical Rationale of the Design of a New Product
    1. Solution for poor physical stability
    2. Solution for minimizing turbidity
    3. Raw Material Selection
    4. The New Product Profile

II. Product Development
  A. Production Process
  B. Formulation

III. Product Application Examples

Wednesday May 25, 2005, 10.00–12.30

The Fate of Drugs in the Body – Mechanisms and Predictions for Uptake, Distribution and Elimination of Pharmaceuticals

Chairperson: *Hans Peter Märki*, F. Hoffmann-La Roche, Basel

10.00–10.45 Keynote Lecture
Jean-Michel Scherrmann, Hôpital Fernand Widal, Paris, France.
'Expression and Functional Role of Multidrug Resistance Transporters at the Blood-Brain Barrier'
Abstract 7

10.45–11.15 *Joerg Huwyler*, F. Hoffmann-La Roche, Basel.
'Relevance of P-Glycoprotein for the Development of CNS Compounds'
Abstract 8

11.15–12.00 *Alex Avdeef*, pION Inc., Woburn MA, USA.
'Prediction of Rodent *in situ* Brain Uptake Using an *in combo* Model, Based on Double-Sink PAMPA (Parallel Artificial Membrane Permeability Assay)'
Abstract 9

12:00–12:30 *Bernard Faller,* Novartis, Basel.
'Combination of *in silico* and Experimental Approaches in Lead Discovery Profiling'
Abstract 10

Abstract 7

Expression and Functional Role of Multidrug Resistance Transporters at the Blood-Brain Barrier

Jean-Michel Scherrmann
INSERM U705; UMR CNRS 7157; Universités Paris 5 et Paris 7, Hôpital Fernand Widal – 200 rue du Faubourg Saint-Denis, 75475 Paris cedex 10, France

The blood-brain barrier (BBB) ensures that many potential therapeutic agents cannot reach the central nervous system (CNS). The BBB is defined by the microvasculature of the brain, which consists of a monolayer of polarized endothelial cells connected by complex tight junctions. The function of the BBB is dynamically regulated by various cells, including astrocytes, neurons and pericytes. The net uptake of a drug by the brain *via* the BBB depends on the overall difference between the uptake and efflux processes. The uptake is controlled by several factors, including the systemic disposition of the drug and the properties of endothelial cells. Therefore, the permeability of endothelial cells and their capacity to metabolize drugs actively control the amounts of drug crossing the BBB in both directions. Permeability is controlled by several properties of the endothelial cells. There is no paracellular movement of drugs because of the tight junctions linking the endothelial cells, but small lipophilic drugs (MW < 600) may enter the brain by penetrating the lipid membrane of the endothelial cells. The passive diffusion of a drug depends on its blood/brain concentration gradient and its lipid solubility, but is inversely related to its degree of ionization and its molecular weight. The recent discovery of active carrier-mediated transporters that are not involved in transporting substrates from the blood to the brain, but from the brain to the blood, has greatly reinforced the barrier properties of the BBB. Most of these transmembrane proteins are in luminal or abluminal membranes of the endothelial cells and control the uptake of numerous drugs. They belong to the superfamily of the ATP-binding Cassette proteins (ABC). Thus many amphipathic cationic drugs are carried by at least one ABC protein, the P-glycoprotein (ABCB1) at the luminal pole of the BBB. Several Multidrug Resistance Associated proteins (MRPs, ABCCs) are also expressed on the brain microvessel membranes and are mainly involved in the efflux of anionic compounds including phase 2 metabolites. Recently, we have demonstrated that ABCG2/BCRP is like P-glycoprotein, present and functional at the BBB. The basal expression is sufficient to limit the brain uptake of mitoxantrone and prazosin so that ABCG2 is a newly identified factor limiting the permeability of the brain to drugs and a potential source of drug–drug interactions. Like the drug metabolism enzymes, the ABC transporters are inductible and affected by genetic polymorphisms reinforcing their role in the variability of CNS drugs. The ABC transporter network contributes to neuroprotection and must be considered as a very critical target in the design of CNS pharmaceuticals.

Abstract 8

Relevance of P-Glycoprotein for the Development of CNS Compounds

Joerg Huwyler
F. Hoffmann-La Roche, Basel

P-glycoprotein is an energy-dependent drug efflux transporter localized at cellular barriers such as the blood-brain barrier. P-glycoprotein is an extensively characterized and prominent member of the superfamily of the ATP binding cassette (ABC)-containing proteins. During the last years, evidence has accumulated demonstrating that such drug transporters may have the potential to interfere significantly with absorption, tissue distribution, and elimination of their respective substrates. In particular, P-glycoprotein has the potential to limit brain uptake of its substrates as shown by experiments using P-glycoprotein deficient knock-out mice. It is therefore important to identify P-glycoprotein substrates during an early phase of the drug discovery process in order to limit the impact of this drug carrier on brain penetration and efficacy of potential CNS drugs.

During the presentation, the following points will be addressed:

- First, why is it important to consider P-glycoprotein during the CNS-drug discovery process?
- Second, how can we measure P-glycoprotein interactions to identify substrates of this transporter?
- Third, how should we interprete the results from our experiments and how can we extrapolate from *in vitro* assays to the *in vivo* situation in humans?
- Fourth, what are possible problems and critical points to consider to allow for better predictions?

Abstract 9
Prediction of Rodent *in situ* Brain Uptake Using an *in combo* Model, Based on Double-Sink PAMPA

Alex Avdeef
pION INC., 5 Constitution Way, Woburn, MA 01801 USA; aavdeef@pion-inc.com.

We have measured the Double-Sink PAMPA (parallel artificial membrane permeability assay) values of about 50 CNS active drugs, including amitriptaline, buspirone, chlorpromazine, colchicine, diazepam, haloperidol, loperamide, maprotiline, methadone, morphine, nalbuphine, nortriptyline, procyclidine, protriptyline, quinidine, sumatriptan, verapamil, vinblastine, and vincristine. The intrinsic permeability values will be compared to mouse *in situ* brain perfusion data already published in the literature, as well as other rodent perfusion data collected under conditions where P-gp was inhibited. The correlation improves when hydrogen bond acidity and basicity are added as molecular descriptors, along with the PAMPA values, in a procedure called '*in combo*'. The BBB prediction model significantly differs from that used to predict intestinal absorption.

Abstract 10
Combination of *in silico* and Experimental Approaches in Lead Discovery Profiling

Bernard Faller
Novartis Institutes for BioMedical Research, CH–4002 Basel

A number of molecular properties of interest for ADME profiling are obtained from *in vitro* assays of various complexity and throughput. At the same time, a number of these properties can be calculated and therefore the question how to combine the two approaches needs to be addressed. We will not discuss the traditional question when to calculate and when to measure but focus on the added value one can generate by combining calculations and measurements. This concept of gaining knowledge through combining measurements with computations will be illustrated with permeability and lipophilicity as examples

Wednesday May 25, 2005, 17.00–17.45

Special Evening Event

Chairperson: *Hans Peter Märki*, F. Hoffmann–La Roche Ltd, Basel

17.00 *Jean-Paul Clozel*, Actelion Pharmaceuticals Ltd. Allschwil
'ACTELION: A New Global Player in the Biotech Industry?'
Abstract 11

Abstract 11
ACTELION: A New Global Player in the Biotech Industry?

Jean-Paul Clozel
Actelion Pharmaceuticals Ltd., Allschwil

Actelion Ltd. is a biopharmaceutical company focused on the discovery, development, and commercialization of synthetic, small-molecule drugs as innovative treatments to serve high unmet medical needs. Founded in 1997, the company's headquarters and research and development operations are based in Switzerland and since 2000 its shares have been listed on the SWX Swiss Exchange. Actelion has two marketed products and is financially independent having started to generate net positive cash flow from ongoing operations during 2003. As of 24 February 2005 it has a market capitalization of CHF 2.6 billion.

The first of Actelion's two marketed products, Tracleer® (bosentan), a dual endothelin receptor antagonist (ERA), is currently the only oral treatment for pulmonary arterial hypertension (PAH), a chronic, life-threatening disorder that severely compromises the function of the lungs and heart. Tracleer® is marketed in the United States, the European Union as well as Australia, Switzerland, Canada, Israel, Hong Kong, Malaysia, Singapore, and Brazil. The second product is Zavesca® (miglustat), a small molecule oral therapy for the treatment of type 1 Gaucher disease, a rare debilitating metabolic disorder. Zavesca® has been approved in the European Union, Israel, the United States and Canada and is now marketed by Actelion in the EU and the US.

Actelion has a proven track record in drug discovery and has significant expertise in two research platforms – G-protein coupled receptors (GPCRs), which are proteins embedded in the cell wall that receive various chemical messages, and aspartyl proteases, a class of natural enzymes. These play important roles in a number of human diseases, many of which are associated with endothelial dysfunction. In particular, Actelion scientists were among the first to work in the field of endothelin receptor antagonists, including its lead product, Tracleer®.

In September 2003, Actelion acquired Axovan AG, a research company in Allschwil, Basel. In December 2003, Actelion and Merck & Co formed an exclusive worldwide alliance to discover, develop, and market new classes of renin inhibitors.

Actelion has been built around a strategy of biotech innovation and pharmaceutical discipline expressed as: maximization of R&D; maintaining global marketing rights on its products; and maintenance of technical innovation. Its R&D model is a promising pipeline of novel compounds and a balanced development portfolio.

The company has also grown its global commercialization infrastructure over the past few years' with over 900 employees worldwide and subsidiaries in key markets in Austria, France, Germany, Greece, Italy, Spain, Switzerland, Netherlands, UK and Ireland, the Nordic countries and the USA, Canada, Brazil, Australia, and Japan.

Thursday May 26, 2005, 10.00–12.30

From Proteomics to Systems Biology

Chairperson: *Beat Wipf*, F. Hoffmann-La Roche Ltd, Basel

10.00–10.40 Keynote lecture
Bruno Domon, ETH Zürich
'Systems Biologyteomics'
Abstract 12

10.40–11.10 *Hans Voshol*, Novartis AG, Basel
'Proteomics for Drug Discovery'
Abstract 13

11.10–11.50 *Hanno Langen*, F. Hoffmann-La Roche AG, Basel
'Proteomics Strategies for Pharmaceutical and Diagnostic Research and for Biomarker Discovery'
Abstract 14

11.50–12.10 *Matthew Kennedy*, Waters Corporation, ALMERE, The Netherlands
'A Multiplexed LC-MS Approach For Simultaneous Qualitative and Quantitative Proteomics'
Abstract 15

12.10–12.30 *Matthias Boese*, Bruker Optics GmbH, Ettlingen, Germany
'Biopharmaceutical Formulations Studied by FTIR' Spectroscopy
Abstract 16

Abstract 12
Systems Biology and Quantitative Proteomics

Bruno Domon
Institute for Molecular Systems Biology, ETH Zürich

The study of entire proteomes is a very challenging task due to the complexity of the samples, characterized by a considerable number of proteins with concentrations spanning over a wide range (ten orders of magnitude in case of serum samples), and by a broad structural diversity, including splice variants, mutations, and post-translational modifications.

Mass spectrometry is central to protein analysis and the progress of the past decade has been crucial to the advances in proteomics. New technologies such as ion cyclotron resonance (or FT-MS) and quadrupole-linear ion trap have expanded the analytical capabilities by decreasing limit of detection while increasing dynamic range, and by enhancing accuracy of measurements and thus quality of the results.

The large volume of data generated requires sophisticated bioinformatics tools to process and analyze the data, and organize the results. A key component of proteomics studies is sample preparation, which includes effective fractionation (at the protein and/or peptide levels) to reduce complexity, and labeling with stable isotopes to perform quantitative analyses. New labeling techniques based on the tandem mass tags allow multiplexed analyses, which improve quantification and increase analysis throughput.

Identification and validation of biomarkers as well as the dissection of biological networks are among the main focus of proteomics. Systems biology, by integrating the proteomics information with orthogonal data sets collected by genomics and metabolomics platforms, will lead to a better understanding of biological networks, diseases, and thus medicine.

Abstract 13
Proteomics for Drug Discovery

Hans Voshol, Jan van Oostrum
Novartis Institutes for BioMedical Research, Basel

In the 10 years since the term proteomics was first coined, the spectacular progress in the field of mass spectrometry has ensured that only rarely the identification of a protein of interest will be a limiting factor in proteomics. Now that a whole range of proteomic technologies is available, focus should be shifted on using them to generate novel insights, *e.g.* in pharmaceutical research in order to elucidate disease processes and discover new drug targets. A prerequisite is the ability to choose from a broad spectrum of proteomic tools and customize the toolbox for each question. This will be illustrated by our approach to bacterial proteomics, which shows that comprehensive proteome analysis is feasible, when the complexity of the system is limited. The other pillar of a successful proteomics philosophy is to evolve from a focused technology platform to an integral part of a functional genomics environment. Only this can ensure that protein expression data are confirmed and validated in appropriate model systems and ultimately translated into new therapeutics and/or diagnostics.

Abstract 14
Proteomics Strategies for Pharmaceutical and Diagnostic Research and for Biomarker Discovery

Hanno Langen
F. Hoffmann-La Roche Diagnostics, Basel

Proteomics is a key technology for the discovery of biomarkers that are required for pharmaceutical research and diagnostics. These markers can be found by massive parallel investigation of biological samples, preferably directly using diseased tissue.

In order to obtain sufficient sensitivity, multidimensional protein fractionation schemes have to be employed, whereas statistical significance is achieved by the comparison of large numbers of samples.

This strategy imposes limitations on the employed technologies. Thus, gel image comparison, as well as manual curation of mass spectrometric identification results are not feasible for large scale biomarker studies. We will show that meaningful data interpretation is possible only with high accuracy in protein identification so that false positive identifications will not obscure the true differences. In our group we have developed alternative solutions to the problem of protein quantification which employ data redundancies built into the experimental design of the biomarker study.

Examples of successful biomarker discovery including the methodology for pre-validation and validation will be shown.

Abstract 15

A Multiplexed LC-MS Approach for Simultaneous Qualitative and Quantitative Proteomics

Matthew Kennedy
Waters Corporation, Almere, The Netherlands

The completion of the Human Genome Project has allowed the rapid identification of large numbers of Human proteins using mass spectrometry in combination with bioinformatics. The separation of complex protein mixtures in large proteomic studies has, most commonly, been achieved by 2D-PAGE.

However, recent advances in both HPLC and mass spectrometry instrumentation have allowed the analysis of protein complexes which have not been separated on a two-dimensional gel. These experiments involve separation of the complex digest mixture by microcapillary liquid chromatography connected to an instrument capable of data directed analysis (DDA™) between the MS and MS/MS modes.

In this case the mass spectrometer will identify the peptide ions as they elute from the HPLC column and select each precursor ion individually for MS/MS. This makes DDA-enabled MS/MS a serial process, independent of which ionization technique or mass analyzer is used. Protein identification is then achieved *via* databank searching of the ESI-MS/MS data, providing qualitative information on the proteins that are present. Hundreds of MS/MS spectra can be acquired in a fully automated fashion, resulting in the identification of significant numbers of proteins, including low copy number proteins, from a single LC/MS/MS experiment.

A common goal of these experiments is the qualitative identification of proteins from simple or complex mixtures of biological origin. However, a significant problem in either the gel-based or non-gel based approach described above is the ability to compare relative expression levels of identical proteins between samples. This requires excellent chromatographic reproducibility, selectivity and specificity.

This talk will introduce a novel multiplexed approach to proteomics that is capable of both qualitative protein identification, and relative quantification across sample sets. The methodology (Waters Protein Expression System) is based upon an exact mass LC/MS experiment on a Q-Tof mass spectrometer. During the course of an LC/MS run the Q-Tof is programmed in the MS mode to cycle between low and elevated collision energy acquisitions. The resolution and mass measurement accuracy (< 5ppm RMS) obtained from the Q-Tof is key in providing additional specificity for the analysis of complex tryptic digests, allowing confident protein identification.

The starting point for the Waters Protein Expression System is reproducible sample preparation. The key elements comprise protein solubilization, using the novel surfactant Rapigest™ SF, followed by reduction, alkylation and subsequently enzymatic digestion using sequencing grade trypsin. Samples of digested protein are subsequently analyzed with highly reproducible online HPLC or UPLCTM separations with an electrospray Q-Tof mass spectrometer.

During the HPLC run, as peptides elute from the column, the Q-Tof instrument is switched alternately at one-second intervals between low and elevated collision energy with argon in the hexapole collision cell. The MS spectra obtained at different collision energies are stored in separate functions. During data acquisition the quadrupole, MS1, is not mass selective but rather operates in the radio frequency (rf) only mode. Thus, the quadrupole operates as an rf ion guide and passes all ions to the hexapole gas cell. The first data function (**MS**) at low energy shows only the normal pseudomolecular ions, whilst the second at elevated energy (**MSE**) shows their associated fragments. At no point during the experiment are precursor ions selected using the quadrupole as in the traditional product ion MS/MS acquisition.

As the mass spectrometer is continually alternating between low and elevated energy on the gas cell, and all ions are passed to the oa-Tof for mass measurement. This results in exact mass measured fragment ions, that can potentially be observed for every peptide precursor ion present in the low energy TOF dataset. Therefore, the two acquired data functions contain the entire set of exact mass measured precursor and product ions that can be formed by fragmentation within the collision cell and represent a '**Global Expression Dataset**'.

Sequence information can thus be obtained on a large number of peptide species at a single point in time. An added benefit of this rapid switching is that the chromatographic integrity of the peptide elution profile is maintained. This results in highly reproducible peptide ion intensities and peak areas from one experiment to the next, as a greater sampling rate means more data points across the chromatographic peak. Typically, each individual sample will be analyzed in triplicate to obtain clear statistical evidence for the presence of low abundance ions. This allows relative quantification to be obtained between two, or more, sample sets.

The acquired Global Expression Datasets are processed using an algorithm that employs a maximum likelihood technique. This determines the exact mass, intensity, retention time and estimates precision for each eluting peptide species from an LC/MS dataset. Peak areas can then be normalized to an endogenous or exogenous internal standard. This provides the first output which can then be used for relative quantification, as exact mass retention time (**EMRT**) signatures are generated for every eluting species detected. Output of these EMRT signatures to programs such as Spotfire allows data visualisation, or statistical treatment/clustering of the datasets to be performed.

Further bioinformatic processing identifies the apex of each peptide ion identified in the low energy data function, and interrogates the MSE data function, acquired with an elevated collision energy, to reveal an associated set of exact mass fragment ions. By combining the exact masses of the peptide molecular ions and the associated fragment ions, a highly specific set of time-resolved masses are generated that define that peptide. These time-resolved sets of exact masses are searched against a databank using a proprietary peptide fragmentation model. Each matching protein in the databank is given a probability and confidence value.

The results are displayed in an interactive browser and list the matched protein sequences ranked by their probability. This display also allows visualization of the associated peptides that match to each protein sequence and display the relevant fragment ion data.

The expression ratio of proteins having a significant probability can then be determined across two or more sample sets, by comparing peak intensities of the relevant monoisotopic peptide masses. The probabilistic technique also provides a measure for the uncertainty of the ratios. Batch processing can be used to compare control with experimental samples.

An example will illustrate the power of the Waters Protein Expression System.

Abstract 16
Biopharmaceutical Formulations Studied by FTIR Spectroscopy

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Methods analyzing conformation and in particular conformational changes of proteins are essentially required for the efficient optimization of protein formulation. Fourier transform infrared spectroscopy (FTIR) seems to be very promising for studying protein stability due to its ability to analyze samples under physical conditions that include aqueous solutions, non-aqueous solutions, and dry powders. As known for many years (for a review see [1]), FTIR sensitively detects conformational changes of proteins which are correlated with protein aggregation and/or protein denaturation, and even permits certain changes to be assigned to the secondary structure. Since the typical acquisition time of modern instruments for protein measurements is only about one minute [2], FTIR facilitates a rapid insight into the quality of different biopharmaceutical formulations.

In this paper the stability of diverse biopharmaceuticals, *e.g.* therapeutic antibodies, was studied by FTIR spectroscopy. The results were compared with these of other biophysical methods (DSC, SEC, CD) to demonstrate advantages and limits of this technology.

[1] A. Barth, C. Zscherp, *Quart. Rev. Biophys.* **2002**, *35*, 369.
[2] A. Wittemann, M. Ballauf, *Anal. Chem.* **2004**, *76*, 2813.

Friday May 27, 2005, 10.00–12.40

Trends in Bioanalysis: Nuclear Magnetic Resonance Spectroscopy and Mass Spectroscopy

Chairperson: *Georg Fráter*, Givaudan Schweiz AG, Dübendorf

10.00–10.30 Keynote lecture
Hans Senn, F. Hoffmann-La Roche, Basel
'Metabonomics: Metabolic Profiles and Biomarkers in Pharma Research'
Abstract 17

10.30–10.50 *Till Kühn*, Bruker Biospin AG, Fällanden
'A New Dimension for Routine NMR in Pharmacological Research'
Abstract 18

10.50–11.30 *Markus Rudin*, Institute for Biomedical Engineering, University of Zürich/ETH Zürich
'Imaging Applications in Drug Discovery and Development'
Abstract 19

11.30–12.00 *Adelbert Roscher*, LMU University of Munich, Germany
'High-Content Screening of the Metabolome by Quantitative MS–MS'
Abstract 20

12.00–12.40 *Kurt Wüthrich*, The Scripps Research Institute, La Jolla, USA and Institute of Molecular Biology and Biophysics, ETH Zürich (Nobel Prize in Chemistry, 2002)
'The NMR View of Proteins – From Structural Biology to Structural Genomics'

Abstract 17
Metabonomics: Metabolic Profiles and Biomarkers in Pharma Research

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Metabolic profiling technologies, often named synonymously as metabonomics or metabolomics, allow the comprehensive mapping and fingerprinting of cellular and systemic biochemical profiles by unbiased spectroscopic detection, mainly NMR and LC-MS. Thereby, 'all' endogenous and drug-related metabolites present in biological samples can in principle be detected at once. The aim of such procedures is to extract latent biochemical information in the form of metabolite profiles and trajectories, *i.e*. observing time-dependent changes in the metabolite pool of the biological samples. Observed differences in endogenous metabolite patterns and/or identified biomarkers thereof reflect 'actual' biological events in the network of metabolic pathways which are ultimately closely related to tissue histology. The metabolic portraits are of high diagnostic and predictive value in pharmacology and toxicology. Metabolomics provides as such a complementary aspect and useful connection between other '-omics' platforms (transcriptomics/proteomics) and the actual metabolic state and tissue histology.

An advantage of the method is that it can equally be applied in pre-clinical and clinical environments, as easily accessible biofluids, *e.g.* urine and serum, can directly be investigated. The non-invasiveness of the sampling procedure of these biofluids also allows the study of time-dependent metabolite patterns and biomarkers. This is of paramount importance because metabolic or other 'omic' consideration at fixed time points can be misleading. Thus, longitudinal measurements in individual animal or patients will improve the predictive value of metabolic patterns/fingerprints or identified biomarkers thereof, both in the preclinical and clinical setting. In summary, the power and robustness of the method relies on three basic properties: 1) There is almost no sample preparation involved; 2) The spectroscopic methods, especially NMR, are extremely reproducible; 3) The nature of the data reflects the actual physiological phenotype.

In this talk it will be shown how metabonomic analyses of body-fluids can be used in early drug development in pre-clinical research for predicting general organ toxicity (such as liver toxicity or kidney toxicity) but also for monitoring more specific effects such as phospholipidosis, mitochondrial effects, peroxisome proliferation, changes of the steroidal biosyntheses, and changes of the gut microflora. Thereby, not only changes of single biomarkers are monitored, but also simultaneous regulations of several metabolites (patterns) within pathways are investigated. Comparing these changes with an extensive database containing metabolic profiles of other studies annotated with toxicology and pharmacology data, allows the new study to be classified

in the context of this multidimensional space. Multivariate procedure are used, *e.g*. to predict toxicology effects on an organ level from metabonomic data. It is also demonstrated how metabonomics can be used to identify new biomarkers, which allow conclusions to be drawn about mechanistic effects. In the clinical environment, the non-invasiveness and ease of sampling biofluids for metabonomics ideally complements the pre-clinical studies and bridging biomarkers may be applied.

Abstract 18

A New Dimension for Routine NMR in Pharmacological Research

Till Kühn[a], Peter Neidig[a], Matthias Niemitz[b], Duncan Farrant[c], John Hollerton[c], Marcus Bauser[d], Ulrich Rosentreter[d], Peter Schmidt[d], Götz Schlotterbeck[e], Alfred Ross[e], Josef Schneider[e], Hans Senn[e]
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It has been shown that the quality of small molecule screening depositories, as used in pharmaceutical companies today often is very poor and efforts have been made to improve their quality. Here we show new approaches to use NMR to address three mayor issues in this respect.

Purity control of the compound: it has to be assured that the compound is pure and not a mixture that might contain side product which could lead to false positives.

Identity control: The identity of the compounds has to be assured in order to define a lead structure from a screening hit.

Concentration determination of the screening solution is important since high throughput screening (HTS) is based on affinity tests, where the accurate knowledge of the concentration is crucial to determine reliable ID50 affinity values.

The purity and identity of the compounds in the depository is important, since these compounds are used for lead finding and might produce bio-activity hits for a given target. Many false positive hits result from wrong assignments and bad quality control in these compound depositories. Therefore hit validation is needed and this typically is a very time consuming and cost intensive step.

Here we present a new NMR method and a laboratory setup, which addresses the ***hit validation directly by NMR***. Only a few micro-litres of compound depository stock solution are needed for the NMR-based structure verification. The NMR experiments typically can be run in less than three minutes in normal (protonated) DMSO, even on a 400 MHz instrument. The whole process such as sample preparation, sample handling, and the NMR experiment itself is automated for high throughput.

Since the industrial HT lead discovery screening is based on affinity tests, it is very important to have good knowledge about the compound concentration. Today's quantification methods are either not always applicable or very inaccurate. For chemo-luminescence nitrogen detection (CLND), nitrogen has to be present in the molecule and some structural patterns may lead to wrong quantification results. Weighing very small amounts can be inaccurate by up to +/–150% especially when salt, polymer beads from solid-phase synthesis, or residual solvent are present. Here we present an ***automated NMR method that gives rise to accurate quantification*** results along with the NMR spectrum, which may be obtained – as mentioned above – in full automation on few micro litres of the screening compound stock solution.

In order to keep cost and time for the hit validation as low as possible it is important to improve the quality of the compounds that enter the compound depository in the first place. Here we also present ***new NMR related hard- and software*** that allow the automated NMR based synthesis control and yield determination of parallel synthesis runs as they appear in typical medical chemistry laboratories.

Abstract 19

Imaging Applications in Drug Discovery and Development

Markus Rudin
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Classical imaging modalities such as X-ray CT or magnetic resonance imaging (MRI) provide structural and function information for diagnostic purposes, or more generally for disease phenotyping. Applications of such approaches to drug discovery were focused on the lead optimization phase by quantitatively evaluating the effects of therapy on structural or functional abnormalities. Typical readouts were the morphometric parameters characterizing a pathology, *e.g.* the infarct volume following cerebral infarction, the increase in the myocardial mass as a consequence of chronic hypertension, or the volume of a neoplastic lesion in experimental cancer [1]. Alternatively physiological parameters (*e.g.* tissue perfusion) have been evaluated as potential indicators of a therapy response.

Structural or functional abnormalities are a consequence of aberrant molecular events occurring at a cellular and sub-cellular level. For instance, genetic mutations may lead to the activation of oncogenes. In general several mutations combined with clonal selection are required to cause malignant tissue transformation and tumor proliferation [2]. From both a diagnostic and therapeutic point-of-view the visualization and quantification of such processes, *e.g.* pathological levels of receptor expression or dysfunctional signal transduction pathways, would be highly desirable.

In the last decade tools have been developed that allow some of these questions to be addressed [3]. Target-specific or *molecular imaging* methods use target specific reporter systems that generally consist of a target-specific moiety, *i.e.* a high affinity binding molecule, and a reporter group, which could be a radioisotope, a paramagnetic or superparamagnetic contrast agent or a fluorescent dye. Three criteria have to be considered when designing such ligand constructs: i) they should have a *high specificity, i.e.* show good affinity to their target and little or no unspecific binding; ii) they should provide *high sensitivity* as their molecular targets are commonly at low concentration (sub-micromolar), which implies the use of a sensitive imaging modality and methods for amplification of the reporter signal; iii) the probes should reach their target site, which might involve penetration of several biological barriers. Molecular imaging is a rapidly evolving interdisciplinary scientific field [3].

Being non-invasive, imaging applications aim also at facilitating the translation from preclinical to clinical phases of drug development; therapeutic concepts applied in disease models should be rapidly validated in a small patient population. Translational imaging applications rely on modalities that can be used both in the animal and in man. A critical aspect for such applications is the identification and validation of biomarkers, *i.e.* chemical, biochemical, physical or structural parameter with predictive quality for clinical outcome.

Summing up, imaging methods have become indispensable tools for the preclinical and clinical drug evaluation. It is anticipated that the development of molecular imaging approaches will significantly enhance the potential of imaging both as a diagnostic modality and for the drug developer.

[1] M. Rudin, N. Beckmann, R. Porszasz, T. Reese, A. Sauter, '*In vivo* magnetic resonance in pharmaceutical research: current status and perspectives', *NMR Biomed.* **1999**, *12*, 69–97.
[2] C.J. Cornelisse, 'Genes and Cancer', *Medica Mundi* **2003**, *47*, 28–33.
[3] M. Rudin, R. Weissleder, 'Molecular imaging in drug discovery and development', *Nature Reviews Drug Discovery* **2003,** *3*, 123–131.

Abstract 20

High-Content Screening of the Metabolome by Quantitative MS–MS

Adelbert A. Roscher[a], Armin Graber[b], Klaus Weinberger[b], Steven Ramsay[b]
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Metabolite profiling technologies comprise a range of advanced analytical and data processing tools, with the objective of utilizing potential markers for disease diagnosis, treatment efficacy or toxicity as a result of comparison of small molecule components of biological systems. Non-targeted qualitative LC/MS and NMR metabolite profiling approaches have widely been used to discover metabolite differences between disease/control and treatment groups. However, the difficulties to gain metabolite quantitation and/or direct identification by these approaches is usually needing cumbersome follow-up investigations.

We have recently devised technology and procedures that allow simultaneous quantitative targeted MS/MS analysis of > 800 metabolites from minute quantities of biological samples such as 3 μl of dried blood, 20 μl of serum/plasma, < 100.000 cultured cells or other sources. Samples are prepared by fully automated solid-phase extraction, derivatized when needed, and analysed by FIA in combination with MRM, precursor and NL scans. Relative quantification is achieved by reference to a wide range of appropriate internal standards. The information content obtainable by this technology in a wide range of key pathways of intermediary metabolism (*e.g.* amino acids, acylcarnitines, sugars, glyco- and phospholipids) was evaluated in comparison with non-targeted qualitative LC/MS.

A placebo-controlled blinded preclinical trial was conducted for metabolic characterization of a disease mouse model. Six groups were studied consisting of healthy and diseased mice either treated, untreated or placebo-treated. The qualitative approach revealed approximately 2000 peaks per spectrum in full ion scans, and enabled discrimination of the groups and selection of putative biomarker candidates by PCA. Although, the loading plots showed the m/z and retention time pairs that contributed the most to the separation of the groups, interpretation was cumbersome and needed significant additional effort to identify corresponding metabolites. Additionally, concerns about reproducibility of chromatography and standardization of spectra alignment and normalization are all known inherent problems of this approach. In contrast, quantitative targeted metabolite profiling concentrates on analytes that are predefined, annotated, and detected by MRM, precursor and neutral loss scans. This approach combined with fully automated sample preparation by a liquid-handling system allows standardization in micro-titer format showing high reproducibility and low CVs. Up to 1000 metabolites were obtained from each compartment and comparison of the groups enabled identification of the animal disease model and facilitated the immediate biochemical characterization of the drug effects. From this experiment, we may conclude that both approaches are complementary. The qualitative LC/MS profiling method performs well as an exploratory screening tool.

The new quantitative method, however, offers added performance and information features: For a very broad array of metabolites relative quantitation can be obtained enabling high resolution of dose-response relationships in functionally relevant pathways to give pharmacodynamic drug signatures. Most metabolites can directly be annotated so as to enable fast and direct biochemical and biological interpretation. Moreover the analytical robustness and speed of the approach should enable routine high-content screening and standardized wide-spread use of metabolomics applications.