

Swiss group for mass spectrometry  
Schweizerische Gruppe für Massenspektrometrie



Groupe suisse de spectrométrie de masse  
Gruppo svizzero di spettrometria di massa

## Newsletter

# SGMS Meeting 2008

and

## General Assembly 2008

**Dorint Resort Blüemlisalp  
Beatenberg**

**November 6<sup>th</sup> and 7<sup>th</sup>**

**11:15**

---

In this Newsletter:

Travel information	2
Letter of the President	3
Program of the SGMS Meeting 2008	4
Abstracts of the meeting	7
Changes in the committee	33
Preliminary Agenda of the General Assembly 2008	34
SGMS Committee	35
Our Sponsors	last page

---

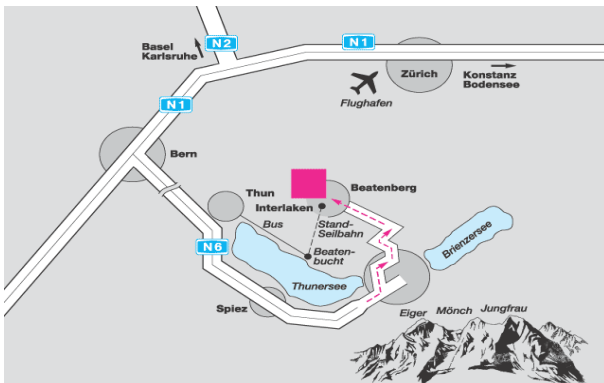
## Travel information

### by car

**Basel** (A3/E60)→Zürich→A2/Bern→A6/Interlaken ↓ Beatenberg

**Zürich** (A1)→Basel/Bern→A1/Bern→A6/Interlaken ↓ Beatenberg

**Genève** (A9/A12)→Lausanne→A1/Bern→A6/Interlaken ↓ Beatenberg



**by train** train leaves (as of May 13, 2008)

**Geneva:** IC 717 at 07:45, track 4  
**Lausanne** at 08:20  
**Fribourg** at 09:04  
**Bern** at 09:26, track 7  
 then see *Bern* below

**Basel:** Cisalpino at 08:30 arrives in **Bern** at 09:27, track 3  
 stay in the train until Thun

**Zürich:** IC712 at 08:32  
**Bern** at 09:29, track 4

**Bern:** Cisalpino at 09:35, track 3  
 arriving in **Thun** at 09:52

**Beatenbucht:** take Bus 21050 at 10:01  
 the bus will arrive in **Beatenbucht** at 10:32  
 take cable car to **Beatenberg** at 10:44

Of course there are trains via Interlaken to Beatenberg. More info can be found at [www.sbb.ch](http://www.sbb.ch) !

Dear all

Do you remember the great talks we had at the 25<sup>th</sup> anniversary meeting of the SGMS? Staffan Nielson with his levitator making chemistry in a floating bubble? Graham Cooks' shoebox sized, 10 kg handheld miniature mass spectrometer? Richard Caprioli showing 3-D protein images of substructures of mouse brain? Phil Marriott talking about speed limits in GCxGC MS?

I am pretty sure that some of you do! But surely all participants do remember the really unforgettable social event at the Grand Hotel Giessbach, the boat trip on Lake Brienz with the astonishing An Lär, the marvellous Belle Epoque Hall, and the splendid dinner? Just Perfect! ... It's time to say good bye! ...

It's time for a change, to make place for a new president leading the SGMS.

And there will be other changes too. Due to his new professional commitment Thomas Läubli has decided to resign from his position as secretary of the SGMS by the next general assembly.

In addition there is still the vacancy since Hansjörg Walther resigned two years ago. Since then the committee consists of 6 members only, leaving an open position for a member at large.

At the upcoming general assembly the committee will propose to go for another bid for hosting the 2015 IMSC in Switzerland. Marc Suter and Renato Zenobi again plan to co-chair the meeting. More detailed information will be given at the general assembly.

Last but not least I would like to thank all the committee members for all their contributions and help during the last 6 years. It was a pleasure to work with you. Of course I got also lots of support from the SGMS community, encouraging hand shakes and sometimes a free drink at the bar.

Andreas Stämpfli

## ***Program***

Thursday 2008-11-06

---

<b><i>Session 1</i></b>	Chair: Andreas Stämpfli, F. Hoffmann-La-Roche, Basel
11:15 - 11:30	<b><i>Welcome</i></b>
11:30 - 12:15	Eduard Arzt <b><i>Biomimetic adhesion surfaces</i></b>
12:30 - 14:00	<b><i>Lunch</i></b>
<b><i>Session 2</i></b>	Chair: Marc Suter, EAWAG, Dübendorf
14:00 - 14:45	Gary Siuzdak <b><i>Mass-based metabolomics from solution and surfaces</i></b>
14:45 – 15:05	Yury O. Tsybin <b><i>Top down mass spectrometry advances in protein quantitation based on stable isotope labeling and protein-metallodrug binding characterization</i></b>
15:05 – 15:25	Berthold Lausecker <b><i>Quantification of a peptide down to the low pg/mL concentration level using three different LC-MS/(MS) approaches</i></b>
15:25 – 15:45	Michael Przybylski <b><i>CREDEX-MS: Molecular elucidation of carbohydrate recognition epitopes in lectins and glycosylated proteins by proteolytic excision- mass spectrometry</i></b>
15:45 – 16:15	<b><i>Coffee break</i></b>

---

<b>Session 3</b>	Chair: Lauren Bigler, University of Zürich
16:15 – 16:35	Anton Kaufmann <b><i>Are LC/MS-MS fragmentation patterns unequivocal confirmation criteria?</i></b>
16:35 – 16:55	Ratna Karuna <b><i>LC-APPI-MS FOR THE ANALYSIS OF 27-HYDROXYCHOLESTEROL AS CANDIDATE BIOMARKER FOR ATHEROSCLEROSIS</i></b>
16:55 – 17:15	Matthias C. Jecklin <b><i>Rapid affinity classification of kinase inhibitors by mass spectrometry</i></b>
	<b><i>Just a very, very short break (!do not leave!)</i></b>
17:20	<b><i>General Assembly</i></b>
19:00	<b><i>Apéro</i></b>
20:00	<b><i>Dinner Buffet</i></b>
22:30	<b><i>Muh-Bar</i></b>



**Eiger, Mönch and Jungfrau**

Friday 2008-11-07

---

<b>Session 3</b>	Chair: Stephan Brombacher, Novartis Pharma, Basel
08:30 - 09:15	Mario Thevis <b><i>Liquid chromatography – mass spectrometry in sports drug testing</i></b>
09:15 – 09:35	Therese McKenna <b><i>An additional dimension of separation - MALDI MS Imaging with high-efficiency ion mobility</i></b>
09:35 – 09:55	Bich Claudia <b><i>DNA-nuclear receptor interaction studied by mass spectrometry</i></b>
09:55 – 10:15	Alexis Nazabal <b><i>High-Mass MALDI ToF Mass Spectrometry and Chemical Cross-linking for interaction analysis</i></b>
10:15 - 10:45	Coffee Break
<b>Session 4</b>	Chair: Yury Tsibin, EPFL, Lausanne
10:45 - 11:30	Roman Zubarev <b><i>Pathway Analysis in Expression Proteomics: Toward Pathway Search Engine</i></b>
11:30 - 11:50	Martin Vollmer <b><i>Analysis and quantitation of pharmaceutical drugs using different HPLC-chip approaches</i></b>
11:50 - 12:10	Heike Schäfer <b><i>Exactive – Combining Qualitative and Quantitative Analysis in One Run</i></b>
12:10 - 12:30	Stephen Guilfoyle <b><i>In-Mouth and In-Nose Analysis of Volatile Flavour Compounds in Chewing Gum, using SIFT-MS</i></b>
12:30 - 12:50	Arnd Ingendoh <b><i>A Novel Ultrahigh-Resolution Mass Spectrometer for both LC and GC Coupling</i></b>
12:50 – 13:00	<b><i>Closing Remarks</i></b>

---

## ***Biomimetic adhesion surfaces***

***Eduard Arzt***

***Scientific Director and Chairman (CEO)  
INM - Leibniz Institute for New Materials  
Saarland University  
Germany***

Adhesive joining with molecular (van der Waals) interactions without chemical glue is presently receiving much attention because of many potential applications. Research on how insects, spiders and geckos stick to surfaces has inspired a new paradigm: fibrillar surfaces with appropriate design can show much higher adhesion performance than flat surfaces. The insight gained in studying biological systems can be transferred to the development of optimized artificial attachment devices. By systematic variations of fiber diameter, aspect ratio and contact shape, we have produced, on a laboratory scale, artificial structures with adhesion strengths similar to the gecko. Further advances with switchable adhesion ("smart adhesives") have been demonstrated and may lead to interesting applications in medical products, sports good, construction materials and microfabrication.

## ***Mass-based metabolomics from solution and surfaces***

***Gary Siuzdak***

***The Scripps Research Institute  
Center for Mass Spectrometry  
10550 North Torrey Pines Road  
La Jolla, CA 92037, USA***

Quantitative global analysis of endogenous metabolites from cells, tissues, fluids or whole organisms - metabolomics, is becoming an integral part of functional genomics efforts as well as a tool for finding diagnostic biomarkers. Where the genome and proteome is largely enabled by the predictable fragmentation pattern of peptides, metabolomics is complicated by the tremendous chemical diversity of metabolites. The experimental aim in our global metabolomics studies is to obtain a comprehensive quantitative with an unbiased view of the metabolome. We have explored multiple novel mass spectrometry platforms for metabolomics including both solution-based approaches and surface-based mass spectrometry, such as nanostructure-initiator mass spectrometry (NIMS). These platforms will also be presented in the context of specific biochemical applications such as neonate screening, gut microbial influence, and tissue imaging.

## ***Top down mass spectrometry advances in protein quantitation based on stable isotope labeling and protein-metallodrug binding characterization***

***Yury O. Tsybin<sup>1</sup>, Thibaut Douche<sup>1</sup>, Adrien Schmid<sup>1</sup>, Michael Affolter<sup>2</sup>  
Martin Kussmann<sup>2</sup>, Christian G. Hartinger<sup>1</sup>, Alexander Egger<sup>1</sup>  
Paul J. Dyson<sup>1</sup>***

***<sup>1</sup> Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne,  
Switzerland***

***<sup>2</sup> Nestle Research Centre, Vers-chez-les-Blanc, Switzerland***

High performance mass spectrometry platform based on Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) with 11 T superconducting magnet has recently been installed at EPFL's Department of Chemistry. Herewith we will present recent results in the domain of top down protein analysis carried out in collaboration with Nestle Research Center and Medicinal Chemistry group of EPFL.

Top-down high-resolution mass spectrometry of ANIBAL stable-isotope labeled proteins<sup>1</sup> has revealed a high sample complexity and has indicated specific advantages over the bottom-up approaches and the necessity for the high resolving power of FT-ICR MS. Preliminary analysis of derivatized ubiquitin has revealed a population of 10- to 12-fold aniline labeled protein molecules, which corresponds to 83% to 100% yield of theoretically possible derivatization. Complementary to bottom-up MS, the top-down MS approach allows localization of ANIBAL labels on a protein sequence in heterogeneous protein-label mixture to indicate specific protein structural preferences for chemical derivatization. We are applying this analytical approach, improved by coupling to LC, to the quantification of proteins from biological tissues.

---

<sup>1</sup> Panchaud A. et al., Mol Cell Prot 2008 (7) 800-812

We have demonstrated for the first time that for metal-based drugs, e.g. cisplatin, transplatin and oxaliplatin, the primary binding sites on ubiquitin and model oligonucleotides could be directly determined by top-down FT-ICR-MS and the uncertainties arising from post-digestion reactions from the bottom-up approach have been eliminated. The top-down approach is likely to become the standard method for determining metal binding sites to proteins and DNA, especially in the domain of metal-based drugs, and it should ultimately provide the data that would facilitate drug design and discovery.

## ***Quantification of a peptide down to the low pg/mL concentration level using three different LC-MS/(MS) approaches***

***B. Lausecker, K. Heinig***

***F.Hoffmann-La Roche Ltd, Non-Clinical Safety, Basel Switzerland***

Pharmaceuticals industry is facing demanding challenges with respect to keep their productivity at a level which guarantees revenues in the double digit range. One option that started more than 10 years ago is to complement the small molecule portfolio with large therapeutic bio-molecules. While the tools for the quantification of low molecular drugs have been improved significantly over the last 10 to 15 years by the implementation of LC-MS, ELISA as the first choice for the quantification of e.g., mABs has not much improved and no quick and powerful alternative has shown up. The aim of the presentation is to discuss which strategies using LC-MS technologies could be used to quantify bio-molecules in order to support the drug R&D compared to the common used ELISA technologies. A peptide which shares to certain extent small molecule and large biomolecule properties will be used to demonstrate advantages and the limitations of the LC-MS approaches. Data will demonstrate that for the peptide low pg/mL quantification limits could be achieved using different sample preparation and LC-MS setups. The LC-MS methods will be compared with respect to sample volume needed, sample preparation time and instrument run time. Other mass spectrometric features as the use of high resolution MS and high-field asymmetric waveform ion mobility spectrometry (FAIMS) are evaluated with respect to the assays sensitivity and specificity.

## ***CREDEX-MS: Molecular elucidation of carbohydrate recognition epitopes in lectins and glycosylated proteins by proteolytic excision- mass spectrometry***

***Michael Przybylski<sup>1</sup>, Adrian Moise<sup>1</sup>, Hans-Christian Siebert<sup>2</sup>  
Hans-Joachim Gabius<sup>2</sup>***

***<sup>1</sup>Laboratory of Analytical Chemistry and Biopolymer Structure Analysis,  
Department of Chemistry, University of Konstanz, Germany***

***<sup>2</sup>Department of Physiological Chemistry,  
Ludwig-Maximilians-University of Munich, Germany***

The emerging relevance of glycan-encoded information in a plethora of biological events directs increasing attention to interaction structures between bioactive glycan determinants and their endogenous receptors, such as lectins<sup>2</sup>. Structures of carbohydrate complexes with lectins and antibodies have been determined in a few cases by X-ray crystallography and NMR, however both methods are limited by large amounts and high purity of material required. We report here a new direct method for molecular mapping of peptide motifs in lectin carbohydrate domains (CRD) by the combination of proteolytic excision of protein-carbohydrate complexes and mass spectrometry (CREDEX: Carbohydrate-REcognition-Domain-EXcision). The CREDEX-MS method was applied to the identification of CRDs of human galectin-3 and galectin-1, for which X-ray crystal structures of their lactose complexes have been determined. Lactose was covalently coupled to epoxy-activated Sepharose, galectins added to the affinity matrix and the lectin complexes digested using trypsin. After removal of unbound fragments, competitive elution of remaining affinity-bound gal-peptides with lactose followed by MALDI-MS provided the identification of specific peptides, gal-

---

<sup>2</sup> Gabius HJ, Crit. Rev. Immunol. 2006, 26: 43-80.

3(152-162) and -(177-183), and gal-1(37-48) and (64-73), in complete agreement with CRDs from the crystal structures. In the same manner, two specific, discontinuous gal-3 peptide epitopes were identified for blood group tri-A<sup>3</sup>. Their specificity was confirmed by affinity-MS<sup>4</sup> of the synthetic CRD peptides, and by inhibition studies with gal-3 in human lymphoma cells. Most recent applications to CRD identifications of hitherto unknown lectin-carbohydrate complexes ascertain the CREDEX-MS approach as a powerful tool for the direct determination of CRDs in solution, suggesting a wide range of applications to define contact sites for lectin ligands of human sugar receptors in biological material.

---

<sup>3</sup> Moise A, Siebert HC, Gabius HJ, Przybylski M., Proc. Natl. Acad. Sci. USA 2008, submitted; Przybylski M. et al., 2008, Patent applications.

<sup>4</sup> Macht M, Marquardt A, Deininger SO, Damoc E Kohlmann M, Przybylski M Anal. Bioanal. Chem. 2004, 378: 1102-1111.

## ***Are LC/MS-MS fragmentation patterns unequivocal confirmation criteria?***

***Anton Kaufmann; Kantonales Labor Zürich***

Relative abundances of product ions as obtained by LC/MS-MS experiments, are often used to confirm the presence of trace analytes in complex matrices. Such ratios are mostly obtained by measuring multi reaction monitoring (MRM) traces followed by the integration of the resulting chromatographic peaks. Maximum tolerable deviations of such MRM ratios (between a suspected peak in a sample and in a standard) have been written into the European legislation (2002/657/EC).

Samples were analyzed in the author's laboratory where a chinolone (dicloxacillin) was present. However, the relative peak intensity between the observed ratio in the sample strongly deviated from the ratio observed in the standard. This would have led to a false negative finding. A detailed investigation showed that deviating ratios were not caused by endogenous compounds which happened to produce one of the monitored transitions. Matrix effects were observed, which suppress certain MRM traces. Although matrix induced signal suppression is well known in LC/MS-MS, no such matrix induced deviations of MRM ratios were reported in the literature. Furthermore, a shift of MRM ratios could also be observed when analyzing matrix free standards under different source temperatures. A closer evaluation of a dicloxacillin ESI spectra  $[M+H]^+$   $m/z = 400$  showed the presence of the double charged analyte ( $m/z = 200.5$ ). This interpretation could be confirmed by a number of experiments.

A double charged ion indicates that there are two different sites of protonation. This lead to the theory that the analyte molecule can also be

single protonated at these two different locations, resulting in two different, isobaric ions. These two ions can not be separated in the first quadrupole. However, the presence of a charge at different sites will affect the stability of the resulting ions. Hence the fragmentation in the collision chamber will be different. It is likely that factors like the presence of the matrix or the change of the desolvation temperature in the interface will affect the ratio of these two differently protonated ions.

Experiments were performed, where none and very intense in source collision induced fragmentation was provoked. A very intense fragmentation regime leaves only a small precursor ion abundance. If there is a stability difference among the two postulated isobaric ions, then only the more stable one is expected to survive. Selecting the  $[M+H]^+$  precursor in the first MS stage and maintaining identical fragmentation conditions, produced - as postulated - very different product ion spectra under the two source collision regimes. Two different fragmentation paths could be elucidated, supporting the theory of two isobaric precursor ions.

The reported observation is of significance for analytical work, where the confirmation of a suspected compound relies on MRM ratios. The use of tolerances as defined by the mandatory Commission Decision 2002/657/EC can lead for certain compounds to false negative findings.

## ***LC-APPI-MS FOR THE ANALYSIS OF 27-HYDROXYCHOLESTEROL AS CANDIDATE BIOMARKER FOR ATHEROSCLEROSIS***

***Ratna Karuna, Arnold von Eckardstein, Katharina M. Rentsch***

***Institute of Clinical Chemistry, University Hospital Zurich  
Raemistrasse 100, CH-8091, Zurich, Switzerland***

Due to its role in maintaining whole-body cholesterol homeostasis, 27-Hydroxycholesterol is a potential biomarker for atherosclerosis and reverse cholesterol transport. Evaluation of this candidate biomarker in plasma samples of humans and animal models requires a sensitive and robust analytical method.

APPI (Atmospheric Pressure Photoionization) is the state-of-the-art of ionization techniques interfacing liquid chromatography and mass spectrometry (LC-MS) system. With the help of toluene as dopant and MeOH as LC eluent, APPI has improved the sensitivity of the analysis, in comparison to the published LC-APCI-MS method, allowing the quantification of 27-hydroxycholesterol from as little as 15  $\mu$ L plasma with a limit of quantification (LOQ) of 40 ng/ml plasma. The method was validated also for the quantification of 27-hydroxycholesterol from 50  $\mu$ L plasma, with LOQ of 10 ng/ml. A further advantage is that no prior derivatization is needed, unlike the previously established LC-ESI-MS or GC-MS methods.

Preliminary results from analyses of plasmas from different knock-out mice show the potential of 27-hydroxycholesterol as a novel biomarker of atherosclerosis.

## ***Rapid affinity classification of kinase inhibitors by mass spectrometry.***

***Matthias C. Jecklin,<sup>1</sup> David Touboul,<sup>1</sup> Rishi Jain,<sup>2</sup> Estee Naggar Toole,<sup>2</sup> John Tallarico,<sup>2</sup> Paul Ramage,<sup>3</sup> and Renato Zenobi<sup>1</sup>***

***<sup>1</sup> Department of Chemistry and Applied Biosciences, ETH Zürich, CH-8093 Zürich, Switzerland***

***<sup>2</sup> Novartis Institutes for Biomedical Research, Global Discovery Chemistry, Lead Synthesis & Chemogenetics, Cambridge MA, USA***

***<sup>3</sup> Novartis Institutes for Biomedical Research, Protein Structure Unit, CH-4002 Basel, Switzerland.***

Protein kinases have emerged as a major drug target in the last years. Since more than 500 kinases are encoded in the human genome, cross-reactivity of a majority of kinase inhibitors causes problems for the design of specific drugs. Tools are required for a rapid classification of inhibitors according to their affinity for a certain target in order to refine the search for new, more specific lead compounds. We are comparing different nanoelectrospray mass spectrometry (nanoESI-MS) based methods to quantify binding affinities and qualitatively determine, by competition experiments, the affinity of several clinical inhibitors. Our results are then compared with standard IC<sub>50</sub> measurements as well as with literature.

All samples (2 proteins: Lck and p38, and 17 clinical inhibitors including BIRB796, Iressa, Tarceva, VX-745, SB202190, PP1, PP2) were provided by Novartis. All samples were diluted in ammonium bicarbonate (10 mM, pH 7.9). A chip-based nanoESI robot (NanoMate, Advion Biosystems) was fitted to a Q-ToF Ultima (Waters). Different MS-based methods were compared in order to qualitatively classify the binding affinities of the compounds to the protein targets.

The first method has been introduced by Tjernberg et al. [Anal. Chem. 2004, 76 (15), 4325], and the second method was developed in our lab [Wortmann et al., JMS, 2007, in press].

Method 1 is based on monitoring the noncovalent complex signals of two different inhibitors competing for binding to protein. The signal of the protein complexed with the tighter binder will appear at higher intensity compared to the protein complexed with the weaker binder. By increasing the concentration of the weaker binder the tighter binder can be displaced, indicated by a change in the ratio of the noncovalent complex signals. In this manner, the binding affinity order obtained for p38 was: BIRB796 > VX-745 > SB202190 > BIRBanalogue5 > PD-173074, and for Lck: CGP076030 > BIRB796 > PP1  $\approx$  PP2 > CGP062464. Since this method allows a determination of the binding strength (KD), the results can be compared to IC50 measurements.

Method 2 is based on ligand depletion. With the mass spectrometer tuned for small molecule detection, the signal of two inhibitors is monitored for different concentrations of protein. As the inhibitors are competing for the binding pocket, their relative intensity changes with increasing protein concentration. In this manner the binding affinity order obtained for p38 was: VX-745 > SB202190 > BIRBanalogue5  $\approx$  Bay43-9006 > BIRBanalogue4 > PD-173074, and for Lck: Bay43-9006 > CGP062464  $\approx$  CGP076030 > PP2 > PP1 > Tarceva.

With few exceptions, the results of both methods agree well. The advantages and disadvantages of the used methods will be discussed. The qualitative binding orders obtained are compared to standard IC50 measurement using Caliper protein kinase profiling system (Caliper Life Sciences, Hopkinton, MA, USA).

***Liquid chromatography – mass spectrometry  
in sports drug testing***

***Mario Thevis***

***German Sport University Cologne  
Institute of Biochemistry  
Carl-Diem Weg 6  
50933 Cologne  
Germany***

LC-MS(/MS) has gained considerable importance in sports drug testing due to its capability to measure thermolabile compounds with great sensitivity and specificity in complex biological matrices such as blood or urine. Numerous assays formerly established using GC-MS approaches were transferred to LC-MS-based methods, and complementing procedures particularly for peptide hormones such as insulins or corticotrophins were developed. Consequently, the instrumental equipment of doping control laboratories has changed over the last decade, and with the growing number of prohibited compounds and methods of doping, modified or additional screening methods are currently utilized to cope with the 'creativity' of cheating athletes and the virtually infinite pool of new therapeutic agents with potential for misuse in sports. A selection of analytical methods developed for doping control purposes will be presented and outline the particular challenge that either the compounds or their legal availability represent to sports drug testing authorities.

## ***An additional dimension of separation - MALDI MS Imaging with high-efficiency ion mobility***

***Marten F Snel, Keith Compson, Emmanuelle Claude, Therese McKenna, James Langridge***

***Waters Corporation, Manchester, UK***

Introduction: Imaging Mass spectrometry is an emerging tool in proteomics, lipidomics and metabolomics. Biomolecules (i.e. proteins, lipids and drugs) are analysed directly from a tissue section, providing spatial information.

It can provide complementary information to traditional costly and time consuming techniques, such as autoradiography. The two main instrumental challenges for the mass spectrometric analysis of tissue samples are sensitivity and specificity, i.e. how well the compound of interest can be distinguished from background ions. A means of increasing the separating power of a MALDI imaging experiment is the use of high efficiency ion mobility spectrometry, coupled with time-of-flight mass spectrometry which offers a new dimension of separation. Using this technique it is possible to separate different compound classes.

Methods: The samples studied were thin sections of animal tissue. Sections of 12 $\mu$ m thickness were produced using a cryotome and deposited onto thick aluminium foil. Several coats of  $\alpha$ -cyano-4-hydroxycinnamic acid matrix were evenly deposited onto the samples using an airbrush, and the samples were subsequently mounted onto MALDI target plates. The tissue areas were selected and imaged by MALDI IMS-MS. All data were acquired on a MALDI hybrid orthogonal acceleration time-of-flight mass spectrometer. After acquisition IMS-MS data were evaluated in software to export regions of drift

time vs  $m/z$ . Data were converted into Analyze file format and subsequently analysed using BioMap (Novartis, CH).

Results: It is desirable to increase the specificity of the imaging experiment, typically achieved by adding additional dimensions of separation, but, unlike complex samples in the liquid phase, where a number of additional separation and clean-up techniques such as liquid chromatography, affinity based depletion etc. are well developed, for tissue samples clean-up protocols are limited. Here we show how ion mobility separation can be used to provide a additional dimension of separation, post ionisation and hence can be utilised in a MALDI imaging experiment. The feasibility of this approach has been shown previously<sup>5</sup>, here we further develop this method through the use of a high efficiency ion mobility separation device.

We will show data demonstrating that different compound classes, such as peptides and lipids can be separated, as well as examples where the intensity contribution of MALDI matrix ions could be eliminated from an ion intensity image. Furthermore we will show examples of ion mobility separation of isobaric peptides generated by on tissue digestion of formalin fixed paraffin embedded samples.

---

<sup>5</sup> 1McLean JA, Ridenour WB, Caprioli RM. Profiling and imaging of tissues by imaging ion mobility-mass spectrometry. *Journal of Mass Spectrometry*. 2007, 42 (8): 1099-1105.

## ***DNA-nuclear receptor interaction studied by mass spectrometry***

***BICH Claudia<sup>1</sup>, BOVET Cédric<sup>1</sup>, ROCHEL Natacha<sup>2</sup>, PELUSO-ILTIS Carole<sup>2</sup>, NAZABAL Alexis<sup>1,3</sup>, WENZEL Ryan<sup>1,3</sup>, MORAS Dino<sup>2</sup> and ZENOBI Renato<sup>1</sup>.***

***<sup>1</sup> Department of Chemistry and Applied Biosciences, Zurich, Switzerland***

***<sup>2</sup> Institut de Génétique et de Biologie Moléculaire et Cellulaire CNRS, Illkirch, France***

***<sup>3</sup> CovalX AG, Technoparkstrasse 1, Zurich, Switzerland***

Nuclear receptors, such as retinoic acid receptor (RAR), interact not only with their ligands but also with other types of receptors. Previous biological analyses (gel-shift) have shown that two coactivators and a specific DNA sequence bind to the receptor but also induce a partial homodimerization of RAR. Mass spectrometry (MS) has been shown to be a powerful technique to analyze changes such as these. Here, two different methods were used to study the interactions: nondenaturing nanoelectrospray (nanoESI- MS), under soft conditions, and high-mass matrix-assisted laser desorption ionization MS (MALDI-MS) combined with chemical cross-linking.

The RAR protein was incubated with either coactivators peptides (PF108 and PF124) or with various DNA sequences (DR5, A9 and C9) then analyzed with high-mass MALDI MS and nanoESI-MS. Prior to high-mass MALDI analysis, a cross-linking protocol (K200, CovalX, Switzerland) was used to stabilize the noncovalent complexes. MALDI mass spectra were acquired on a TOF mass spectrometer (Reflex IV, Bruker Daltonics, Germany) equipped with a high-mass detection system (HM1, CovalX, Switzerland). ESI mass spectra were acquired on a Q-TOF Ultima mass spectrometer (Waters, UK) equipped with a chip-based nanoESI system (Nanomate, Advion Biosciences, USA) after an overnight incubation of RAR with DNA or peptides.

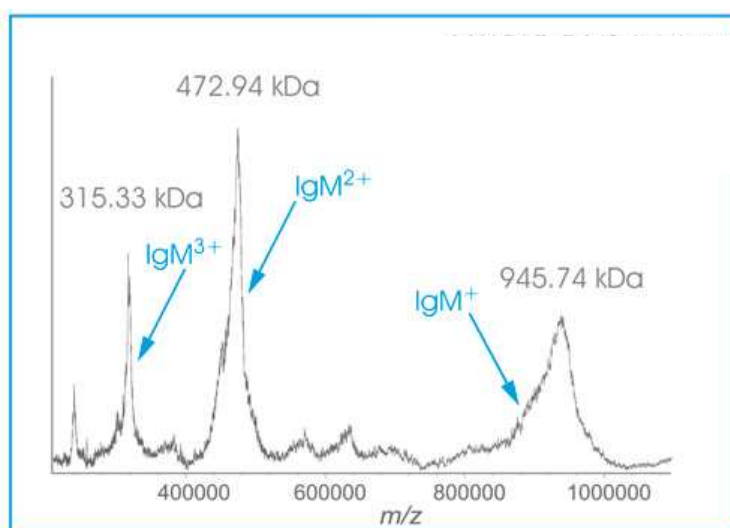
When measured alone, the RAR showed no significant homodimer judging from results obtained with both MS methods. The single strand (A9 and C9) and double strand (DR5) DNA fragments were detected by both of these methods. NanoESI revealed that PF108 and PF124 bind to RAR under native conditions. After chemical cross-linking, the high-mass MALDI MS spectra show a binding for PF124 but not for PF108. In both cases, the RAR dimer was still not observed. A complex between protein RAR and the double strand DR5 was detected with nanoESI. After cross-linking the high-mass MALDI showed that RAR binds the single strand DR5. Moreover, DNA induced the dimerization of RAR and this dimer could bind not only the single strand but also the double strand. Using nanoESI, we were able to detect the RAR-DR5 complex in the presence of the peptides PF108 and PF124. This complex was not detected using high-mass MALDI. The specificity of the binding between RAR and DR5 was tested by incubating RAR with other single strand DNA (A9 or C9). These DNA fragments did not bind to RAR and did not induce a dimerization.

## ***High-Mass MALDI ToF Mass Spectrometry and Chemical Cross-linking for interaction analysis***

***Alexis Nazabal, Benoit Plet, Ryan Wenzel***

***CovalX AG, Technoparkstrasse, 1, CH-8005, Zürich, Switzerland***

The analysis of intact protein complexes by mass spectrometry is still challenging. Here we present an approach based on high-mass MALDI ToF mass spectrometry and chemical cross-linking. To circumvent the problem of dissociation when using MALDI ionization, a specific cross-linking protocol has been developed to stabilize covalently the samples. To solve the problem of detection, we are using a specially developed high-mass detection system, allowing sub- $\mu\text{M}$  detection up to 1000 kDa. The use of this methodology presents a number of advantages: Sensitivity (sub- $\mu\text{M}$ ), tolerance for samples impurity, speed. We will present with details the high-mass technology used and show comparison spectra with MCP detection, the technology used in most of standard MALDI ToF instruments. We will also present examples of applications of this methodology in the field of protein complex analysis (intact protein complexes ranging from 40 to 1000 kDa), antibody characterization (Interaction analysis, Sandwich assays, Epitope mapping), Therapeutic protein aggregates analysis and drug discovery.



High-Mass MALDI ToF MS analysis of IgM (945.74 kDa; 300 nM) using CovalX Hm1 high-mass detection system

***Pathway Analysis in Expression Proteomics:  
Toward Pathway Search Engine***

***Roman Zubarev***

***Biological Mass Spectrometry  
Uppsala Biomedical Centrum  
Husargatan 3  
SE-75 123 Uppsala  
Sweden***

Analytical Pathway Biology strives to develop a tool that uses proteomics datasets as an input and yields activated signaling pathways as an output. Such a “pathway search engine” (PSE) will find application in many fields, from fundamental studies to search for novel disease biomarkers. The task of PSE development is far from trivial as straightforward mapping of up- or down-regulated proteins onto signaling pathways usually produces incorrect results. We have developed a novel approach to pathway identification (Zubarev et al., *J. Proteomics*, 2008, 1, 89-96), and have extended this approach to keynodes. Examples of application of this approach to different biological systems will be demonstrated.

## ***Analysis and quantitation of pharmaceutical drugs using different HPLC-chip approaches***

***Martin Vollmer and Lukas Trojer***

***Agilent Technologies Waldbronn***

Quantitation of drugs and drug metabolites in the early drug discovery phase is usually challenged by small sample volume and complex biological matrices. Studies using small animals and microdosing would reduce significantly costs and enhance throughput.

HPLC-chip/MS provides an easy to use tool to achieve superior sensitivity without the limitations usually encountered by conventional Nano-HPLC/MS due to integration of connections, valving, and separation on a single polymeric chip. Robustness, leak-tightness, repeatability and chromatographic performance are therefore significantly increased.

In addition pre-concentration and sample cleanup which is normally conducted by a separate SPE step can directly be performed on the HPLC-chip enrichment column, prior to transfer of the sample to the on-chip separation column.

Novel chip designs developed for the analysis of a wide range of pharmaceutical molecules (from hydrophilic to hydrophobic) were tested. In order to cover for a wide polarity range chips with C18 packing material were compared against chips containing different HILIC and ionexchange materials. Combinations of orthogonal techniques were investigated and advantages and disadvantages will be discussed regarding simplification of DMPK workflows.

Since nanoflow-setups bear the drawback that runtimes are usually significantly longer than with standard flow setups, concepts will be presented which outline processing of samples on chip in order to obtain acceptable runtimes and throughput.

## ***Exactive – Combining Qualitative and Quantitative Analysis in One Run***

***Heike Schäfer, Paul-Gerhard Lassahn***

***Thermo Fisher Scientific, Reinach, Switzerland***

Compound screening in biological or environmental samples is becoming increasingly important in analytical sciences. Often, a sample can contain hundreds or even thousands of potentially important compounds like pesticides, metabolites or toxins in complex matrices. The goal is the identification and quantification of the screening-candidates in one single analytical run. The charm of the analytical method is further increased, if it is able to yield information of unknown compounds in the same experiment. This combination of quantitative and qualitative analysis in a single run is one of the new challenges in LC-MS development.

So far, iontrap, triple stage quadrupole, TOF and FT instruments are used to do this type of experiment, each with its own advantages and limitations; such as dynamic range, scan speed sensitivity or selectivity. Instrument price might be a barrier, and price often excludes high performance techniques like FT-MS.

In an ideal world high resolution HPLC in combination with a fast-scanning high resolution Mass Spectrometer yielding superior sensitivity and a wide dynamic range could be the answer to these needs. The ability to yield accurate mass information would greatly facilitate the identification of unknowns and would certainly increase the value of the analytical procedure. The talk discusses a new member of the Thermo Orbitrap product series (Exactive) that fulfills many of the described features. It is based on Orbitrap

technology, and it features all the characteristics of this revolutionary MS technique.

In the present study we have used the new instrumental setup to develop a workflow for screening of complex biological samples. It could be shown, that the detection and quantification of hundreds of components is possible in a single run. Comparison of medium and high resolution MS data clearly showed the benefit of resolution for screening applications. A new screening software is used permitting fast component detection, identification and quantification.

## ***In-Mouth and In-Nose Analysis of Volatile Flavour Compounds in Chewing Gum, using SIFT-MS***

***Stephen Guilfoyle (1), Greg Francis (1), Vaughan Langford (2),  
Matthias Herzog (3)***

***(1) Syft Technologies UK Ltd, Daresbury Innovation Centre, WA4 4FS, UK***

***(2) Syft Technologies Ltd, Middleton, Christchurch, New Zealand***

***(3) Brechbuehler AG, CH-8952, Schlieren, Switzerland***

Release of volatile flavour compounds from food as it is chewed and swallowed is a very complicated process, because it depends on the properties of the food itself and the physiological characteristics of the person who is eating. In order to better understand how flavour is released and perceived, the last decade has seen substantial work published on real-time analysis of flavour compounds in vivo as they are eaten.

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is a newly commercialised technology that analyses gas samples for volatile organic compounds (VOCs) and certain inorganic compounds. It can accurately detect and quantify these compounds in real time at very low concentrations (often to parts-per-trillion levels), even at breath humidity. These characteristics make it ideally suited to in vivo flavour release measurements as food is consumed.

In this work we describe the first application of Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) to the in vivo analysis of several chewing gum flavours (peppermint, spearmint and fruity). We find that SIFT-MS readily detects and quantifies key flavour compounds both in static headspace experiments and in real time as gum is chewed. In flavour release measurements, individual chewing events are observed on the breath-by-

breath concentration profiles, demonstrating the high speed and sensitivity of the technique.

Flavour release measurements also enable SIFT-MS to differentiate release rates of flavour compounds from different formulas of gum.

## ***A Novel Ultrahigh-Resolution Mass Spectrometer for both LC and GC Coupling***

***Arnd Ingendoh, Sebastian Goetz, Thomas-Arthen-Engeland, Oliver Räther  
Dirk Wunderlich, Pierre-Olivier Schmidt, Carsten Baessmann***

***Bruker Daltonik GmbH, Fahrenheitstr. 4, 28359 Bremen, Germany***

API-QTOFs have been used for LC-MS/MS applications for a long time, mainly because of the combination of MS/MS at high mass resolution and mass accuracy. In the last years new detector and digitizer technology enabled a wide intra-spectral dynamic range of up to five orders of magnitude. Accurate mass and preservation of the isotopic pattern made confident automated formula generation in MS and MS/MS possible. However, identifying and quantifying trace compounds based on accurate mass LC/MS in complex matrices requires much higher resolution. At the same time, the trend to decrease the chromatographic separation times by means of ultra fast LC systems requires MS solutions without a compromise between speed and mass resolution.

With the novel mass spec technology presented here, ultrahigh mass resolution of  $\geq 40.000$  and mass accuracy of  $\leq 1\text{ppm}$  without compromising other performance factors like sensitivity, dynamic range or scan speed is presented in real samples from different metabolomics and screening applications

E.g., samples with complex matrices containing known analytes were separated by means of an ultra fast system. Using a 5 cm column (particle size  $1.8\ \mu$ ) and a 3 minute gradient very narrow LC peaks can be observed ( $< 4$  seconds FWHM). Making use of the combination of accurate mass high resolution and mass position stability, very narrow extracted ion

chromatograms can be generated resulting in a strong decrease of commonly seen noise caused by the matrix.

In most applications, the API methods are utilised for coupling liquid chromatography (LC) systems to the mass spectrometers. Only recently there were some examples reported to couple also gas chromatography (GC) systems to API mass spectrometers. Since Atmospheric Pressure Laser Ionisation (APLI) has been added to the suite of API methods, less polar compounds of higher molecular weight are within reach for GC/MS.

In a simple application matrix we compare the analytical results obtained from the different types of ionisation techniques. Shown will be examples of APLI application like polycyclic aromatic hydrocarbons (PAH), alkylated or hetero-PAH, halogenated oligomeric aromates or complex samples like crude oil.

## ***Changes in the committee***

After 8 years of activities Thomas Läubli has decided to resign from the SGMS committee. Since he has joined the committee he held the position of secretary of the SGMS. The committee could always count on him. Soon after the meetings the minutes were send out. It was great having him on board of the SGMS committee. I would like to thank Thomas for his commitment to the SGMS and I wish him all the best for the future.

In addition the committee would like to extend again to 7 members as it is stated in the statutes.

The committee announces therefore the following nomination to the general assembly:

For President:       Dr. Marc J.-F. Suter, EAWAG, Dübendorf

Secretary:           Mr. Matthias Herzog, Brechbühler AG, Schlieren

Member at large:   Prof. Yuri Tsybin, EPFL, Lausanne

If there should be other nominations/proposals: Please address them directly to: Dr. Andreas A. Stämpfli, c/o F. Hoffmann-La Roche AG, CH-4070 Basel

All nominees will present a short CV summarizing their past, present and future work during the general assembly.

For the committee

Andreas Stämpfli

## ***2008 General Assembly of the SGMS***

**Thursday November 6<sup>th</sup>, 2008**

**~ 17:30 h**

**Mercure Hotel Beatenberg-Interlaken**

### **Preliminary Agenda**

1. Nomination of the scrutineers.
2. Approval of the minutes of the 2007 general assembly.
3. President's report and its approval.
4. Treasurer's report.
5. Auditor's report and approval of treasurer's and auditor's report.
6. Decision on the 2008 membership fee.
7. Admission of new members.
8. Election of the President and the members of the committee.
9. News from the SCS.
10. The SGMS homepage.
11. IMSC Bid 2015.
12. Individual proposals.
13. Miscellaneous.

Individual proposals must be sent to [andreas.staempfli@roche.com](mailto:andreas.staempfli@roche.com) before October 23, 2008.

The President

Andreas A Staempfli

- President* **Andreas A Stämpfli**  
F. Hoffmann-La Roche AG  
PROBMA, Bau 65/112A  
CH-4070 Basel  
andreas.staempfli@roche.com  
Phone +41-61-688 3131
- Vice President* **Jean-Luc Wolfender**  
Laboratoire de Pharmacognosie et Phytochimie  
Ecole de Pharmacie Genève-Lausanne  
Université de Genève  
Quai Ansermet 30  
CH-1211 Genève 4  
Phone +41-22-379 3385
- Secretary* **Thomas Läubli**  
Brechtbühler AG  
Steinwiesenstrasse 3  
CH-8952 Schlieren  
thomaslaeubli@brechtbuehler.ch  
Phone +41-44-732 3126
- Treasurer* **Stephan Brombacher**  
Novartis Pharma AG  
WSJ-145.8.15  
CH-4056 Basel  
stephan.brombacher@novartis.com  
Phone +41-61-324 6808
- Internet* **Marc J-F Suter**  
EAWAG  
Ueberlandstr. 133  
CH-8600 Dübendorf  
marc.suter@eawag.ch  
Phone +41-44-823 5479
- EMS representative* **Laurent Bigler**  
OCI, Universität Zürich  
Winterthurerstr. 190  
CH-8057 Zürich  
lbigler@oci.unizh.ch  
Phone +41-44-635 4286
- SCS representative* **Marc J-F Suter**
- Newsletter* **Andreas A Stämpfli**

Thanks to our sponsors



**Agilent Technologies**



Passion. Power. Productivity.



**VARIAN**

