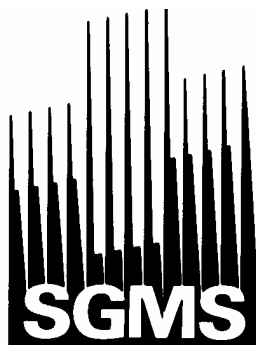


Swiss group for mass spectrometry
Schweizerische Gruppe für Massenspektrometrie



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

Newsletter

SGMS Meeting 2006

and

General Assembly 2006

Mercure Hotel, Beatenberg-Interlaken

November 2 and 3, 2006

11:15

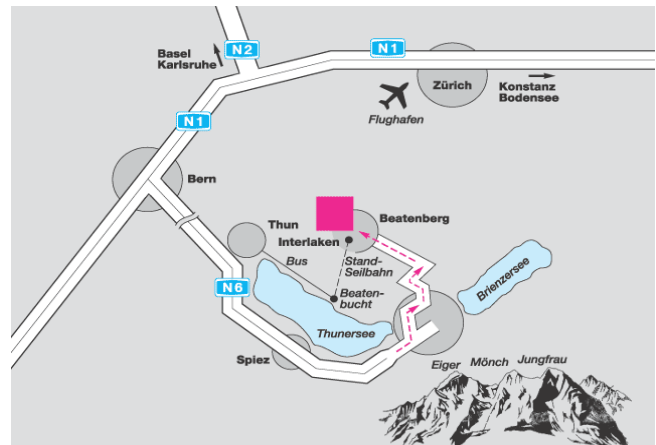
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Travel information

The Beatenbucht-Beatenberg mountain railway goes up every 20 minutes at :05, :25 and :45 till November 19, 2006 (033 841 0 841).

By car: Take highway no. 6 from Berne to Thun, then no. 8 direction Interlaken. Right before reaching Interlaken follow directions to Gunten/Unterseen/Beatenberg.



In Beatenberg: The Mercure Hotel Beatenberg-Interlaken is located at the very far end of Beatenberg - probably the longest village in Europe. Parking at the Hotel is free of charge. Please check-in first at the lobby.

By train (travel date November 2, 2006):

Genève: IC2517 at 07:10, Lausanne 07:45 and Fribourg 08:34 then Bern.

Basel: IC861 at 08:00 arriving in Bern at 08:56 then Bern.

Schaffhausen: IR2561 at 07:07 then Zürich.

Zürich: IC912 at 08:00 then Interlaken. Please stay in the train. It will continue to Interlaken West.

Bern: IC912 at 09:06 arriving in Interlaken West at 09:56.

Interlaken: Bus 107 in front of the main station at 10:10.

Arrival at 10:38 in front of the Mercure Hotel Beatenberg-Interlaken

Dear all

Following an initiative by Renato Zenobi and Marc Suter the SGMS board has submitted for the 19th International Mass Spectrometry Conference to be held in Switzerland in 2011 or 2012. We were assisted in the submission process by FORSCHUNG LIVE, a professional event organizer at ETH Zurich. The deadline for submission was July 15th, 2006. The Centre International de Conférences Genève has been selected as Conference Site.

The submission was backed by the Swiss Chemical Society and we already had commitment for some financial support. Academia, industry and two of our neighbouring countries were represented on the scientific committee.

The IMSF board informed in Prague that the IMSC keeps its triennial cycle.

There were 4 competing bids – Geneva, Kyoto, Torino and Paris. The presentation was well received but the national affiliates voted for Kyoto. After many years in Europe the IMSC 2012 goes to Asia. In the name of the SGMS I would like thank to Renato, Marc and all the others for the great work and effort made to get the IMSC 2012 to Geneva. It is hard to loose but might be that we will get another chance for 2015 the 20th IMSC?

After more than 10 years activities Hansjörg Walter decided to resign from the SGMS committee. Since he has joined the committee he represented the SGMS towards the SCS. The committee could always count on him as a chair person for a session, organizing a social event like a boat trip on the lake of Thun or just for running errands. His inputs and comments were always of great value. It was great having him on board of the SGMS committee. I would like to thank Hansjörg for his commitment to the SGMS and I wish him all the best for the future.

Andreas Stämpfli

November 2, 2006

11:15 – 11:30 Welcome Notes**Starting Session:****Chair: Andreas Stämpfli**

**11:30 - 12:15 The Eye and the Mind in modern plant morphology,
exemplified by morphological "misfits" of tropical waterfalls**

Rolf Rutishauser**12:30 - 14h00 Lunch****Session 1****Chair: Jean-Luc Wolfender**

**14:00–14h45 On the Role of
Free Radical Reactions in Electron Capture Dissociation**

Peter O'Connor

**14:45 - 15:05 2D-LC-MS/MS based functional proteomics approach for the
analysis of stress response in aquatic model organisms.**

Marc J-F Suter

**15:05 – 15:25 Taxonomic characterization of halophilic archaea by mass
spectrometry.**

Laurent Bigler

**15:25 – 15:45 Phosphorylation of the mediator complex: is Cdk8 the sole
operating kinase?**

Manuel Tzouros**15:45 - 16:15 Coffee Break****Session 2****Chair: Marc Suter**

**16:15 – 16:35 Opportunities and limits of the combination of linear ion trap
with Orbitrap analyzer to detect and identify contaminants in
environmental water samples.**

Juliane Hollender

**16:35 – 17:15 Significantly Improving Quantitative LC-MSMS Analysis for
Large Number of Analytes (>500) with Scheduled-MRM**

Yves LeBlanc

17:20	General Assembly
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19:00 Apéro

20:00 Blüemlisalp Dinner Buffet

November 3, 2006

Session 3	Chair: Laurent Bigler
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08:30 - 09:15 Microchip developments for sample preparation and MALDI-TOF MS interfacing

Thomas Laurell

09:15 - 09:35 New MS/MS experiments in the c-trap (curved trap) of an Orbitrap mass spectrometer.

Paul-Gerhard Lassahn

09:35 - 09:55 Extractive electrospray ionization mass spectrometry: Concept, instrumentation and applications.

Huanwen Chen

09:55 - 10:15 The separation of protein ion charge states and their associated fragments using a travelling wave IMS device.

Jennifer Burgess

10:15 - 10:45 Coffee Break

Session 4	Chair: Stephan Brombacher
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10:35 - 11:20 Strategies for the LC/MS analysis of non-polar compounds

Uwe Karst

11:20 - 11:40 Comparison of dissociation constant of calmodulin-melittin complex determined using surface plasmon resonance, circular dichroism and mass spectrometry.

Sonal Mathur

11:40 - 12:00 Therapeutic drug monitoring of antiretroviral drugs using LC-MS.

Katharina M. Rentsch

12:00 - 12:20 Analysis of oligosaccharides by capillary-scale high-performance anion-exchange chromatography with pulsed amperometric detection (CHPAEC-PAD) and on-line electrospray-ionization ion-trap mass spectrometry (CHPAEC-ITMS).

C. Bruggink

12:20 - 12:40 Desorption Electrospray Ionization Mass Spectrometry (DESI-MS): New Developments in Toxicology and Pharmaceutical Analysis

Luc Alexis Leuthold

12:40 - 12:45 Closing Remarks

The Eye and the Mind in modern plant morphology, exemplified by morphological "misfits" of tropical waterfalls

Rolf Rutishauser

***Institut für Systematische Botanik
Universität Zürich***

Biological morphology tries to elucidate body plans ("bauplans") of plants and animals. It is mainly comparative morphology (organography), dealing with concepts in order to compare the organs of different organisms. — There are various groups not fitting the bauplan of typical flowering plants. Such morphological "misfits" deviate considerably from the classical root-stem-leaf (or root-shoot) model known from most ferns and seed plants. The presentation will focus on river-weeds (family Podostemaceae) as morphological misfits among dicotyledonous flowering plants. They mainly occur in tropical waterfalls and river rapids, e.g. in Cameroon, Ghana, India and Mexico. Analyses of the genome (DNA) indicate that the river-weeds (podostemads) are closely related to St. John's wort (*Hypericum*) that also occurs in Europe! The podostemads are enigmatic for people interested in developmental genetics and evolution, for botanists studying their morphology and ecology: Which adaptations allow the river-weeds to survive in their extreme habitats? Most podostemads transformed their "roots" into green ribbons or disk-like crusts that are fixed to rocks inside the torrents. The example of the river-weeds exemplifies how botanists progress in their biodiversity research: (i) analysis of the plant forms; (ii) identification of the genera and species; (iii) listing the molecular and morphological characters into a data matrix; and (iv) phylogenetic reconstruction using computer programs. There are complementary views (perspectives) serving as heuristic devices for the elucidation of evolutionary and developmental aspects of plant forms, e.g. the holographic paradigm anticipated by AGNES ARBER (1879 – 1960), the continuum model and process morphology proposed by ROLF SATTLER. — The talk will finish with artificial life, i.e. virtual plants that only grow and flower inside computers.

On the Role of Free Radical Reactions in Electron Capture Dissociation

Peter O'Connor

***Boston University School of Medicine
Department of Biochemistry***

The mechanism of electron capture dissociation is a matter of substantial debate at the moment. The original, non-ergodic mechanism implied one electron captured equals one backbone cleavage. This was clearly disproved in an experiment that performed ECD on doubly charged cyclic peptides, leading to the proposal that ECD initiates a free radical reaction cascade. Since then, a series of experiments have been performed to test this free radical cascade mechanism.

First, if the free radical cascade is true, then it implies that the radical can move through the molecule prior to cleavage. Since the best site for a radical in a peptide is on the alpha carbon of glycine, peptides were synthesized with deuteroglycine. ECD of these peptides showed that the deuterium atoms moved, which is observable as a mass shift. Clearly the radicals migrate during ECD.

Second, radical migration is presumed to occur on a >1 microsecond timescale, suggesting that ECD proceeds through a long lived radical intermediate. A fairly simple, double resonant experiment can probe lifetimes on the >10-100 microsecond timescale, and this was applied to a series of peptides to see if some of the fragments observed were products of a long lived intermediate that exists for at least that long. New results will be presented to explore this, but in general, it appears that many or most (but not all) of the ECD fragments appear on a >1 msec timescale. Clearly both

fast and slow mechanisms exist, but the fastest reactions that can currently be probed this way are slower than 10 microseconds.

Third, if radical propagation occurs on a slow timescale, then it can be modulated by adding groups to a peptide that contain large, distributed pi-bonded systems which should act as radical traps by resonantly stabilizing the radical. Coumarin can be easily tagged onto the molecule, providing such a system. ECD of a peptide with such a radical trap indicates that backbone fragments are suppressed, but side chain fragments are increased. Furthermore, double resonance shows that most of the side chain fragments are very slow, on the millisecond timescale.

Thus, the free radical cascade mechanism has withstood challenge by a series of experiments designed to test it. While the theory cannot be proven, it has not yet been disproved, and further experiments are planned to test it further.

2D-LC-MS/MS based functional proteomics approach for the analysis of stress response in aquatic model organisms

Marc J-F Suter and Victor J Nesatyy

***Eawag, Swiss Federal Institute of Aquatic Science and Technology
CH-8600 Dübendorf***

A current, powerful and frequently used method for the assessment of integrative responses on the molecular level is the analysis of whole organism gene expression profiles, called transcriptomics. Inside the cells in organisms, the RNA molecules (transcripts) are subsequently translated into proteins, each protein having its specific physiological functions. Protein expression profiles can be assessed as well, in what today is called proteomics. In this approach, the protein expression of exposed organisms is compared to a control. If a certain protein is observed at higher concentrations in the exposed organism, then it is part of the cellular stress response. Proteomics is by now an established and sensitive tool for identifying protein markers for (multiple) stress that can provide insights into the underlying modes of action and can be used to assess exposure. Usually a 2D separation technique is needed to reduce the complexity of a protein extract. This has traditionally been 2D gel electrophoresis, but more recently stacked columns have allowed 2D LC separation culminating in the multidimensional protein identification technology (mudPIT) (Washburn et al, 2001). More importantly, the data produced needs to be matched with in silico digested proteins derived from the genome of the organism in question. Commercial as well as open-source programs are available that match proteins identified from MS data with proteins in the database.

Using this technique we could demonstrate that zebrafish eggs show different induction patterns when comparing eggs exposed to cadmium or 17 β -estradiol. Our most recent results will be presented.

Taxonomic characterization of halophilic archaea by mass spectrometry

Laurent Bigler¹, Urs Stalder¹, Fatemeh Ghorbani¹, Huda Al-Ajmi², and Heiko Patzelt²

¹University of Zurich, Institute of Organic Chemistry, CH-8057 Zurich

²Department of Chemistry, Sultan Qaboos University, Al-Khod 123, Sultanate of Oman

Increasing numbers of extremely halophilic “salt-loving” microorganisms - both archaea and bacteria - are isolated from salt lakes and saline marshes in many parts of the world. These organisms, which grow well at NaCl concentration up to 4M, appear to play a vital ecological role in their habitats and show great promise for applications in environmental biotechnology [1]. The de-replication and ultimately the taxonomic characterization of halophilic cells from natural isolates, however, still remains laborious and expensive. We present here a rapid and facile identification method, based on the fingerprint comparison of whole-cell MALDI mass spectra.

Archaea, isolated from oil-contaminated sites in the saline deserts of the Sultanate of Oman [2], were cultivated in halophilic standard media (15-30% NaCl) and were shown to efficiently degrade aliphatic hydrocarbons. The cells were harvested and – without any extraction steps - applied onto the sample plates of the MALDI mass spectrometer (“cell smear method”) [3]. Spectra were recorded both in the positive and negative detection mode, leading to reproducible sets of data for the polar membrane lipid fractions in the negative and for the short peptide/protein domain [4] in the positive mode. Combined, the two sets of mass data constitute an unambiguous fingerprint for a particular cell type. Control experiments, where membrane lipids were extracted and analyzed by LC-MS methods, confirmed the MALDI results but

were much more time-consuming. The direct MALDI analysis of whole cells is thus the method of choice for the rapid characterization of the archaeal species in complex natural isolates.

- [1] R. Margesin, F. Schinner, *Extremophiles*, 2001, 5, 73-83.
- [2] H. Patzelt, Hydrocarbon Degradation under Hypersaline Conditions, in: N. Gunde-Cimerman, A. Oren, A. Plemenitas (eds.) *Adaptation to life at high salt concentrations in Archaea, Bacteria, and Eukarya*, Springer, Berlin, 2005, pp 105-122.
- [3] P. Krader, D. Emerson, *Extremophiles*, 2004, 8, 259-268.
- [4] C. Fenselau, P. Demirev, *Mass Spectrom. Rev.*, 2001, 20, 157-171.

Phosphorylation of the mediator complex: is Cdk8 the sole operating kinase?

Manuel Tzouros, Albert Heck and Jeroen Krijgsveld

***Biomolecular Mass Spectrometry, Utrecht University
Sorbonnelaan 16, 3584 CA, Utrecht, The Netherlands***

The yeast Mediator complex is an assembly composed of 21 proteins that acts as a transcriptional regulator of RNA polymerase II (pol II) [1]. For instance, Mediator operates as a repressive regulator of gene transcription when an additional module composed of four proteins — the Srb8-11 module — is associated to it. The cyclin-dependent kinase Cdk8 (or Srb10), part of the Srb8-11 module, is responsible for the phosphorylation of several Mediator subunits and is thus a key player in modulating the function of the complex. Some recent investigations have shed light on the presence of extra kinases capable of modifying Mediator.

We have chosen a phospho-proteomics approach to map the modification sites due to Cdk8. We used metabolic labeling (^{15}N) to compare the Mediator subunits expressed by a wild-type and a CDK8-deleted yeast strain. The complex was selectively isolated from the yeast lysates by tandem-affinity purification and submitted to an in-solution trypsin digestion. The resulting digest was fractionated by off-line strong cation exchange (SCX) chromatography and phosphopeptides were enriched using titanium dioxide (TiO_2). Identifications were performed by nanoLC-MS/MS using the LTQ-FT or -Orbitrap operating in the MS2/MS3 or multistage activation mode. The corresponding signals for the “light” (^{14}N) and “heavy” (^{15}N) phosphopeptides originating from both yeast strains were integrated and the ratios obtained were used to evidence the contribution of Cdk8. The data clearly support that Cdk8 is not the sole kinase responsible for the phosphorylation of Mediator subunits and further investigations need to be undertaken to determine their identities.

[1] S. Björklund, C. M. Gustafsson, Trends Biochem. Sci. 2005, 30, 240.

Opportunities and limits of the combination of linear ion trap with Orbitrap analyzer to detect and identify contaminants in environmental water samples

Juliane Hollender, Heinz Singer, Kathrin Fenner

***Department of Environmental Chemistry, Eawag, Überlandstr. 133
8600 Dübendorf***

The challenges in analysing polar organic chemicals and their transformation products in environmental samples by LC-MS are twofold. First, the generally low but nevertheless potentially toxicologically relevant concentrations, which usually lie in the ng/L range, require enrichment, separation of the matrix and sensitive detection. The second challenge is the identification of unknown peaks in LC-MS, where the interpretation of fragmentation pattern without large spectra libraries as those available for GC-MS is more difficult. In recent years the combination of LC-TOF (providing accurate mass measurements to generate elemental compositions of ions) with LC ion trap (providing structural information from fragmentation studies) has a few times been applied in non-target-screening of environmental samples (Thurman et al., 2005; Hernandez et al., 2004; 2005). The limitation of TOF instruments in comparison to quadrupoles is their lower sensitivity, which hampers the detection and identification of analytes at low concentrations (Hernandez et al., 2005).

The scope of our ongoing study is to determine the potential of the new hybrid system linear ion trap and Orbitrap analyzer to detect and identify polar organic contaminants like pesticides and their metabolites in environmental water samples without using reference standards. Surface and groundwater samples were enriched and analyzed by LTQ Orbitrap using

automatic data dependent scanning, enabling the simultaneous acquisition of high resolution MS spectra and several MS/MS scans of the most abundant mass peaks. Screening for stable transformation products was focused by intensive literature surveys as well as the use of chemical fate models and biodegradation pathway prediction tools to produce a list of transformation products that are likely to occur. We managed to identify several pesticides and their transformation products in the lower ng/L concentration range by this method, but it is still an important issue to achieve sufficient detection sensitivity. Upon further refinement, the approach presented opens up an avenue to more realistically assess water quality with regard to emerging contaminants and their transformation products and, at the same time, to refine risk assessment methods to include transformation products.

Hernández, F., Ibáñez, M., Sancho, J.V., Pozo, O.J., 2004. *Anal. Chem.* 76, 4349-4357.

Hernández, F., Pozo, Ó.J., Sancho, J.V., López, F.J., Marín, J.M., Ibáñez M., 2005. *TrAC* 24, 596-612.

Thurman, E.M., Ferrer, I., Zweigenbaum, J.A., García-Reyes, J.F., Woodman, M., Fernández-Alba, A.R., 2005. *J. Chromatogr. A*, 1082, 71-80.

Significantly improving quantitative LC-MS/MS analysis for large number of analytes (>500) with scheduled-MRM

Yves LeBlanc,

S Sciex, Concord, Canada

The selectivity and sensitivity of triple quadrupole systems have made them the instrument of choice for quantitative LC-MS/MS analysis. The main reasons for this acceptance is their high duty-cycle when operated in multiple reaction monitoring (MRM) mode. However, as the number of analytes monitored increases, the accuracy and precision of peak areas decreases significantly due to an effective decrease of the duty-cycle and sensitivity of the instrument. The present work report on the concept of Scheduled-MRM to improve the data collection per unit time. Using retention time (RT) information, only the required MRM transitions are monitored over a given time window. This approach enables the ability to monitor a significantly larger number of MRM's (>500) during an LC analysis while sustaining appropriate quantitation characteristics (CV and accuracy).

Microchip developments for sample preparation and MALDI-TOF MS interfacing

Thomas Laurell

***Department of Electrical Measurements
Lund University, Sweden***

Miniaturization strategies for protein sample preparation prior to mass spectrometry readout has clear benefits and is currently a focus area within the microchip and microfluidics field. The strategies along this line as pursued by the Lund Nanobiotechnology group will be reviewed. Piezodispensing as a means for precise sample deposition and on-spot enrichment as well as chip integrated solid phase extraction linked to MALDI-TOF MS readout will be discussed. A new approach to high capacity on-MALDI-target sample preparation - (ISET Integrated Sample Enrichment Target) - will also be presented.

New MS/MS experiments in the c-trap (curved trap) of an Orbitrap mass spectrometer

Paul-Gerhard Lassahn

Spectronex AG, Hochstrasse 48, CH - 4002 Basel

Injection of ions into the Orbitrap mass analyzer is not an easy task. Ions packets must enter the trap in a very short pulse, they must get highly focused and at the same time the ion beam must not suffer from space charge effects. The c-trap seems to be an ideal interface for fulfilling all these needs.

At the same time the c-trap expands and improves the capabilities of the Orbitrap itself in several ways. The c-trap is an ideal storage device for calibrants selected from known background signal and ensures mass accuracies in the Orbitrap of 1 ppm or better on a routine basis. In addition the c-trap is a collision cell of its own and is able to yield complementary information to MS/MS obtained in the linear ion trap of the Orbitrap mass spectrometer. Due to the dynamics of the collision process the spectra are similar to MS/MS obtained in a triple quad mass spectrometer and in addition they do not suffer from any low mass cut-off.

The talk highlights some important characteristics of the curved trap and discusses recent results obtained in the different MS/MS modes on the Orbitrap in our laboratory.

Extractive electrospray ionization mass spectrometry: concept, instrumentation and applications

Huanwen Chen^{1,2}, Andre Vetor², Graham Cooks², Renato Zenobi¹

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Switzerland***

***²Chemistry Department, Purdue University, West Lafayette, IN 47907
USA***

Conventionally, extraction is performed offline before sampling and ionization in mass spectrometry. In a novel extractive electrospray ionization source (EESI) (schematically shown in Figure 1), on-line droplet-droplet extraction occurs when a sample spray intersects a reagent electrospray; this makes online extraction and ionization happen simultaneously, and allows continuous mass spectrometric direct analysis of trace amounts of compounds in complex matrices for extended periods of time.

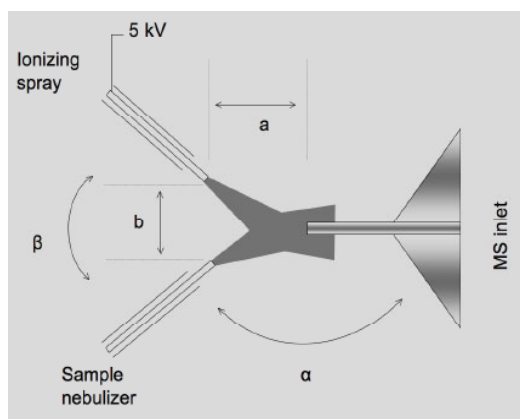


Figure 1 Schematic diagram of extractive electrospray ionization showing ionizing and sample sprays (the distance a , b and the angles α , β are adjustable).

Initially, EESI was demonstrated by using a homemade EESI source coupled to a LTQ mass spectrometer. Analytical performance has been shown with an ion trap mass analyzer. Recently, EESI has been implemented with a

commercial ESI-QTOF-MS instrument without any hardware modification, making EESI available to a more users with ease.

Intrinsically, flexible configuration of the EESI source allows it to tolerate complex matrices and require no sample pretreatment for real time monitoring of various samples. Trace components present in raw urine, milk, serum, drug powder, E. coli strands and breath aerosol samples have been successfully detected. Atmospheric pressure ion/molecule reactions and ion-ion reactions have also been demonstrated in EESI-MS. Potential applications include but not limited to proteomics, biomedical, clinical diagnosis, metabolomics, microbiological applications, pharmaceutical products and process monitoring, food analysis, environmental analysis, explosives detection, homeland security and forensics science.

The separation of protein ion charge states and their associated fragments using a travelling wave IMS device

Jennifer Burgess¹, Steven D Pringle¹, Kevin Giles¹, Iain Campuzano¹, Stormy L Koeniger²; Stephen J Valentine³; Robert H Bateman¹, Sam Merrenbloom², David E Clemmer², Georges Froidevaux⁴, Robert Funck⁴ and Jan Claereboudt⁵

¹Waters MS Technologies Centre, Manchester, UK

²Department of Chemistry, Indiana University, Bloomington, IN

³Predictive Physiology and Medicine, Bloomington, IN

⁴Waters AG Switzerland, Rapperswil, Switzerland

⁵Waters Central Europe, HRMS Division, Zellik, Belgium

Electrospray mass spectrometry is a firmly established tool for the identification of proteins, via the analysis of complex tryptic peptide mixtures. It has also proven to be an extremely powerful technique for determining protein structure by mass analysis at the intact protein level. Often the complexity of the associated mass spectrum limits the information content that can be obtained from the data and additional stages of separation prior to analysis by mass spectrometry are desirable. The potential of using ion mobility spectrometry adds another, orthogonal, dimension of separation to the MS experiment, providing separation of species by their associated mobility, or drift time, a factor which is dependant upon ion size, shape and charge. Consequently, it is possible to separate co-eluting isobaric species which exhibit different drift times. We have combined a travelling wave ion mobility (TWIMS) device within a quadrupole orthogonal acceleration time-of-flight mass spectrometer, enabling ion mobility separations to be combined with electrospray mass spectrometry at high sensitivity.

We have investigated the potential of this configuration for the separation and subsequent mass analysis of multiply charged protein ions and to

separate fragments derived from these multiply charged species, following CID. If mobility separation of fragment ions was efficient, this would reduce mass spectral complexity and facilitate detection and subsequent identification.

In this study, two new ion mobility spectrometry (IMS) techniques have been used to separate the protein ion fragments. These techniques will be compared and contrasted, and the potential of IMS for biological applications will be discussed.

Strategies for the LC/MS analysis of non-polar compounds

Uwe Karst

***Westfälische Wilhelms-Universität Münster
Institut für Anorganische und Analytische Chemie***

In recent years, liquid chromatography/mass spectrometry (LC/MS) has become one of the most powerful analytical techniques for qualitative and quantitative analysis. This is mainly due to the invention of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). With these interfaces, polar compounds are typically detected by protonation in the positive ion mode and by deprotonation in the negative ion mode.

Non-polar analytes, however, which are particularly well separated in reversed-phase HPLC, are either detected with unsatisfactory results or not at all under these conditions. Therefore, strategies to improve the ionization for these compounds have to be developed. Some respective approaches are shown within this presentation.

One promising approach is the electrochemical conversion of the analytes to more polar or even charged products, which are then readily accessible by ESI or APCI. We have developed an on-line LC/electrochemistry/MS method with post-column oxidation of the analytes occurring in a commercial "coulometric" cell, which provides for up to quantitative conversion of the analytes prior to the mass spectrometer. Examples from different applications areas will be presented.

Another approach is based on an electron-capture effect, which is observed for selected nitroaromatic compounds when using the APCI interface as a source of low-energy electrons in the negative ion mode. Depending on the structure of the compound to be analyzed, either a dissociative or a non-

dissociative electron capture effect is observed. The electron capture effect competes with the classical deprotonation in LC/MS and provides for additional structural information on the analytes.

A third approach has been directed to the coordination of inorganic ions to analytes with limited polarity under formation of charged adducts. This has been explored with respect to the addition of Ag^+ ions after separation of rubber vulcanization accelerators.

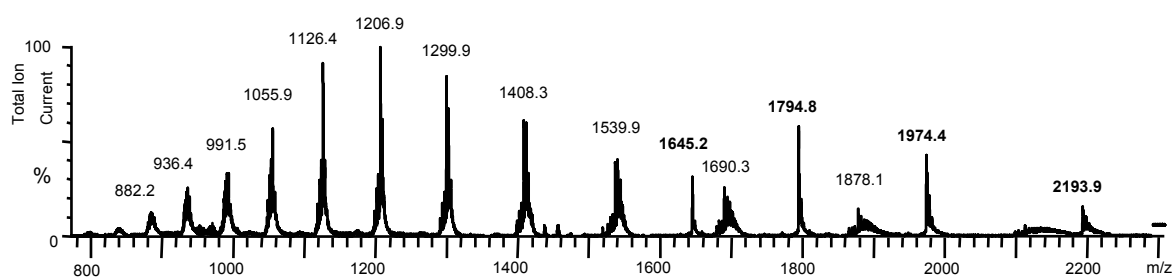
Comparison of dissociation constant of calmodulin-melittin complex determined using surface plasmon resonance, circular dichroism and mass spectrometry

Sonal Mathur¹, Michael Scott², Renato Zenobi¹

¹Department of Organic Chemistry and Applied Biosciences, Wolfgang-Pauli-Strasse-10, ETH Zurich, CH-8093 Zurich, Switzerland

²Functional Genomics Center, UNI ETH Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

Mass spectrometric methods have been used for studying biomolecular interactions since decades [1]. In particular, electrospray ionization (ESI) is a promising tool for the determination of binding affinities of noncovalent complexes. This work is focused on the comparison of standard (surface plasmon resonance and circular dichroism) and mass spectrometric techniques (ESI-MS and MALDI-SUPREX) for the determination of binding strength of these noncovalent interactions. Calmodulin-melittin complex was selected as model system, which shows a high binding affinity of 3 nM with 1:1 stoichiometry in the presence of Ca²⁺ [2].



The results obtained in this study show a good agreement between ESI-MS ($K_d = 5 \pm 4$ nM) and surface plasmon resonance ($K_d = 6 \pm 3$ nM) data. An ESI spectrum showing multiply charged peaks of the free protein and the complex (**bold letters**) is shown above. Preliminary experiments with circular dichroism give high affinity in low nanomolar range. However, the SUPREX

analysis indicates a lower affinity in micromolar range. Instrumental and experimental conditions for each technique were also optimized during the course of this study. As a conclusion, this investigation clearly demonstrates the potential of mass spectrometric methods for the quantitative determination of noncovalent interactions.

- [1] J. M. Daniel, S. D. Friess, S. Rajagopalan, S. Wendt, R. Zenobi, *Int. J. Mass Spectrom.* 2002, 216, 1.
- [2] M. Comte, Y. Maulet, J. A. Cox, *Biochem. J.* 1983, 209, 269

Therapeutic drug monitoring of antiretroviral drugs using LC-MS

Katharina M. Rentsch, Ursula Gutteck-Amsler, Arnold von Eckardstein

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Prospective and retrospective studies have provided some evidence of the clinical and virological benefit of incorporating therapeutic drug monitoring (TDM) into routine patient care. The bases for using TDM with today's knowledge are data demonstrating considerable inter-individual variability in the concentration of antiretroviral drugs among patients and data demonstrating relationships between drug concentrations and responses, either virological or toxicological. Because antiretroviral therapy consists always of a combination of different drugs, analysis can be simplified if different drugs are measured at the same time. Therefore, LC-MS or LC-MS/MS is nowadays the analytical method of choice. Due to the high specificity of the mass spectrometric detection the times of analysis can be shortened and the risk of interferences can be minimized as compared to UV detection.

In 2003 we have published an LC-MS method ⁽¹⁾ for the quantification of amprenavir, efavirenz, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir after solid-phase extraction. In the meantime atazanavir and tipranavir have been introduced into the market. In the process of including them in our analytical procedure we have reduced the sample volume, simplified sample preparation and shortened the chromatographic run time. The result of this optimisation process will be presented.

Sample preparation consisted in the addition of a solution of the internal standard (proteinase inhibitor analogue) in acetonitrile to 100 µl serum which

resulted in protein precipitation. After centrifugation the supernatant was diluted with buffer before injection into the HPLC system. The different drugs were analyzed by reversed-phase chromatography and detected by negative or positive atmospheric pressure chemical ionization (APCI) mass spectrometry.

Depending on the target concentrations in patients, the calibration curves of the new method were linear in the range of 0.01 – 30.0 mg/l. The limit of quantification was accordingly between 0.01 and 0.3 mg/l. The imprecision was < 10% and the accuracy 92 – 108%. The performance data of the new and simplified method are comparable or even better to the published numbers and demonstrate that this method allows the quantification of 10 different proteinase inhibitors or non-nucleoside reverse transcriptase inhibitors in patients with HIV infection.

1. Rentsch, K. M. Sensitive and specific determination of eight antiretroviral agents in plasma by high-performance liquid chromatography-mass spectrometry *J Chromatogr B Analyt Technol Biomed Life Sci* 2003, 788, 339-350.

Analysis of oligosaccharides by capillary-scale high-performance anion-exchange chromatography with pulsed amperometric detection (CHPAEC-PAD) and on-line electrospray-ionization ion-trap mass spectrometry (CHPAEC-ITMS)

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High-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is an established technique for the selective separation and analysis of underivatized carbohydrates. The miniaturization of chromatographic techniques by means of capillary columns, and on-line coupling to mass spectrometry are critical to the further development of glycan analysis methods, which are compatible with the current requirements in clinical settings.

A prototype system has been developed based on a Dionex BioLC equipped with a microbore gradient pump and a PEEK flow splitter, a FAMOS micro autosampler, a modified electrochemical cell for on-line capillary PAD and a capillary column (380 μm i.d.) packed with a new type of anion-exchange resin. This system operates with sensitivity in the low fmol range. In addition, an on-line capillary desalter has been developed to allow direct coupling to a Bruker Esquire 3000 ion-trap mass spectrometer with an electrospray ionisation interface (ESI-IT-MS). Both systems have been evaluated using standard oligosaccharides as well as urine from children with various lysosomal oligosaccharide storage diseases. Our data indicate that the robust and selective anion-exchange system in combination with ESI-IT-MS for structure confirmation and analysis provides a powerful platform which is complementary to existing nano-/capillary LC-MS methods for analytical investigations of oligosaccharides from biological samples.

Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) : New Developments in Toxicological and Pharmaceutical Analysis

Luc Alexis Leuthold, Emmanuel Varesio and Gérard Hopfgartner

Desorption Electrospray Ionization (DESI) is an ambient ionization technique introduced in 2004, allowing the sampling of surfaces under ambient conditions, without the use of any matrix. Jet propelled droplets generated by an electrospray hit the surface to be analyzed, generating analyte ions sampled by the mass spectrometer.

From then on, numerous examples have been published, usually for the detection of relatively high amounts of material (micrograms) on simple or relatively complex matrices, such as drug tablets, biological tissues or plant material; or low amounts of material (down to picograms) on simple matrices as clean surfaces or paper. Detection of relatively low amounts of analytes in complex matrices such as biological fluids or tissues still needs to be investigated and is of great challenge, due to sensitivity and selectivity issues.

Following our report on the analysis of commercial pharmaceutical and Ecstasy tablets [1] and our work on the analysis of compounds of toxicological interest in Dried Blood Spots (DBS) [2], new results will be shown on DESI-MS/MS in toxicological and pharmaceutical analysis, including direct analysis of LSD on paper strips and current work on DBS. The sensitivity issue and reasonable applications will be assessed.

- [1] L. A. Leuthold, J.-F. Mandscheff, M. Fathi, C. Giroud, M. Augsburger, E. Varesio and G. Hopfgartner. *Rapid Commun. Mass Spectrom.*, 2006; 20: 103-110.
- [2] L. A. Leuthold, J.-F. Mandscheff, E. Varesio and G. Hopfgartner. Poster WP49, 54th ASMS Conference on Mass Spectrometry and Allied Topics, 28.5-1.6.2006, Seattle, United States.

2006 General Assembly of the SGMS

Thursday November 02, 2006

~ 17:20 h

Mercure Hotel Beatenberg-Interlaken

Agenda

1. Nomination of the scrutineers.
2. Approval of the minutes of the 2005 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 2007 membership fee.
7. Admission of new members.
8. News from the SCS - HJ. Walther.
9. News from ESMS - L. Bigler.
10. News from the IMSS – A. Staempfli
11. Election of the President and the members of the committee.
12. SGMS homepage - M. Suter.
13. Individual proposals.
14. Miscellaneous.

Individual proposals must be sent to andreas.staempfli@roche.com before October 19, 2006.

The President

Andreas A. Staempfli

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