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## Newsletter

# SGMS Meeting 2010

and

## General Assembly 2010

**Dorint Resort Blüemlisalp  
Beatenberg**

**November 4<sup>th</sup> and 5<sup>th</sup>**

**! 11:15 !**

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In this Newsletter:

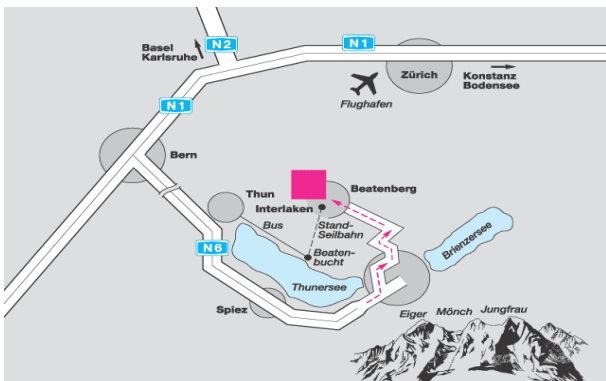
Travel information	2
Program of the SGMS Meeting 2010	4
Abstracts of the meeting	6
Preliminary Agenda of the General Assembly 2010	38
SGMS Committee	39
Our Sponsors	last page

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## 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 September 7, 2010)

#### Geneva:

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

#### Basel:

IC 1063 at 08:28 track 9 arrives in **Bern** at 09:27  
 stay in the train until **Thun**, track 1

#### Zürich:

IC 712 at 08:32  
 arrives in **Bern** at 09:29, track 4 ; then see *Bern* below

#### Bern:

IC 1063 at 09:35, track 3  
 arrives in **Thun** at 09:52

#### Thun:

take Bus NFB 21058 at 10:02  
 arrives in **Beatenbucht** at 10:33  
 take cable car to **Beatenberg** at 10:44  
 arrives in **Beatenberg** at 10:54

it's only a short walk to Dorint Resort Blümlisalp

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

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## ***2010 General Assembly of the SGMS***

**Thursday November 4, 2010**

17:45

**Dorint Resort Blüemlisalp, Beatenberg**

### **Agenda**

1. Nomination of the scrutineers
2. Approval of the minutes of the 2009 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 2010 membership fee
7. Admission of new members
8. Election of the president and committee members
9. IMSC2014
10. News from the IMSF and EMS
11. The SGMS homepage
12. News from the SCS
13. Individual proposals
14. Miscellaneous

Individual proposals must be sent to [marc.suter@eawag.ch](mailto:marc.suter@eawag.ch) before **October 21, 2010**.

The President, Marc Suter

## Program

Thursday 2010-11-04

<b>Session 1</b>	Chair: Marc J-F Suter, Eawag, Dübendorf	
11:15 - 11:30	<b>Welcome</b>	
11:30 - 12:15	Jyotsna Sharma, Texas Tech University, Lubbock, TX, US <b><i>Orchids: models of biological complexity</i></b>	
12:30 - 14:00	<b>Lunch</b>	<b>Restaurant</b>
<b>Session 2</b>	Chair: Yury Tsybin, EPFL, Lausanne	
14:00 - 14:45	Catherine E Costello, Boston University School of Medicine, Boston, MA, US <b><i>Mass spectrometry-based methodologies for investigations of N- and O-linked glycans and their effects on assembly and interactions of cells and organisms</i></b>	
14:45 - 15:05	Arnd Ingendoh, Bruker Daltonik GmbH, Bremen, DE <b><i>In-depth characterization of glycoproteins by ZIC-HILIC enrichment and mass spectrometry</i></b>	
15:05 - 15:25	Maria A van Agthoven, University of Lille, Villeneuve d'Ascq, FR <b><i>Two-dimensional Fourier transform ion cyclotron resonance mass spectrometry</i></b>	
15:25 - 15:55	<b>Coffee break</b>	<b>Lobby</b>



Thursday 2010-11-04

<b>Session 3</b>	Chair: Lauren Bigler, University of Zürich	
15:55 – 16:40	Andrej Shevchenko, MPI, Dresden, DE <b><i>Shotgun lipidomics for cell biology and molecular medicine</i></b>	
16:40 – 17:00	Aurélien Thomas, Geneva University Hospitals, Geneva, CH <b><i>Phospholipidic fingerprinting by on-line desorption of dried spots and quadrupole-linear ion trap mass spectrometry: evaluation of atherosclerosis biomarkers in mouse plasma</i></b>	
17:00 – 17:20	Richard Lock, Waters Corporation, Manchester, UK <b><i>Sites of metabolic substitution: Definitive metabolite structures deduced using ion mobility and molecular modeling</i></b>	
17:20 – 17:40	Michaël Méret, MPI, Potsdam, DE <b><i>Stable isotope-labeled metabolomes - a tool for systems level analysis</i></b>  <b><i>Just a very, very short break (!do not leave!)</i></b>	
17:45	<b><i>General Assembly</i></b>	
19:00	<b><i>Apéro</i></b>	<b><i>Lobby</i></b>
20:00	<b><i>Dinner Buffet</i></b>	<b><i>Conference Room</i></b>
22:30	<b><i>Muh-Bar</i></b>	<b><i>In the Caves</i></b>



spectrometry in order to characterize individual proteins as putative unique and novel markers. This may further contribute to the identification of so far not database registered species

## ***MALDI-TOF mass spectrometry: Applications in food microbiological identification and food safety research***

René Brunisholz<sup>1</sup>, Britta Stoop<sup>2</sup>, Simone Wüthrich<sup>1</sup>, Frank Hesford<sup>2</sup>,  
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MALDI-TOF mass spectrometry for the characterization and classification of fungi and bacteria is based on the detection of mainly ribosomal proteins which are constantly expressed as high-abundant proteins. Resulting protein fingerprints are unique, highly reproducible and mostly independent of culture medium, incubation temperature and growth state. The objective of this study is to efficiently identify undesired and human pathogenic microorganisms in food products of plant origin by this rapidly emerging technology.

Beside using standard workflows, as organic/acid extraction of the isolates, we are investigating in rapid and efficient sample clean-up for reasons of increasing scoring. The mass spectra are acquired using Bruker Autoflex II and Ultraflex II mass spectrometers operating in positive ion linear mode. Data are evaluated using Bruker Biotyper software.

Accordingly, it is shown that MALDI biotyping is a reliable, robust and specific tool for species- and strain-specific identification of microorganisms related to food quality and safety. This has been demonstrated for different fungal and bacterial species. Finest differences between distinct strains could easily be distinguished. Results are compared to biochemical and molecular biological data. The application of a ZipTip clean-up method improved quality of spectra and increased score values.

Currently, we are investigating in protein separation by capillary HPLC and on-target proteolytic digestion approaches followed by MALDI-TOF/TOF mass

Friday 2010-11-06

<b>Session 4</b>	Chair: Jean-Luc Wolfender, University of Geneva	
09:00 - 09:45	Jean-Luc Veuthey, University of Geneva and Lausanne, Geneva, CH <b><i>Capillary electrophoresis and ultra high pressure liquid chromatography hyphenated with MS in pharmaceutical analysis</i></b>	
09:45 – 10:05	Jean-Pierre Chervet, Antec, Zoeterwoude, NL <b><i>Electrochemistry/MS a powerful analytical technique to mimic drug metabolism and other redox reactions in life sciences</i></b>	
10:05 – 10:25	Christian Berchtold, ETHZ, Zurich, CH <b><i>Online breath analysis by mass spectrometry, a possible tool for anesthesia monitoring?</i></b>	
10:25 – 10:45	Katja Heinig, Hoffmann-La-Roche, Basel, CH <b><i>Dried blood spots – a revolution in drug development?</i></b>	
10:45 – 11:15	Coffee Break	<b><i>Lobby</i></b>
<b>Session 5</b>	Chair: Andreas Stämpfli, Hoffmann-La-Roche, Basel	
11:15 – 11:35	Franz Herzog, IMSB, ETHZ, Zurich, CH <b><i>Probing the architecture of protein complexes by chemical cross-linking and mass spectrometry</i></b>	
11:35 – 11:55	Manfred Heller, University of Bern, CH <b><i>Towards label free protein quantification of the plasma membranome</i></b>	
11:55 – 12:15	Luca Fornelli, EPFL, Lausanne, CH <b><i>Tissue transglutaminase-induced deamidation and transamidation of neuropeptides revealed by electron capture dissociation mass spectrometry</i></b>	
12:15 – 12:35	René Brunisholz, FGCZ, University of Zurich, ETHZ, Zurich, CH <b><i>MALDI-TOF mass spectrometry: Applications in food microbiological identification and food safety research</i></b>	
12:35	<b><i>Closing Remarks</i></b>	

## *Orchids: models of biological complexity*



### **Jyotsna Sharma**

Department of Plant and Soil Science  
MS 42122, Texas Tech University  
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Orchidaceae is one of the largest and most highly evolved plant families. Although approximately 70% of the orchid species are of tropical origins ranging in size from a few centimeters to several meters, the rest are native to the temperate and even arctic regions of the planet inhabiting most all natural ecosystems except for the driest deserts. A majority of the tropical and subtropical orchids grow as epiphytes, i.e., growing on top of other plants, whereas those in the temperate regions tend to grow terrestrially and include several non-photosynthetic species. There is at least one known subterranean orchid species. This wide diversity and evolutionary success of orchids is a result of an array of very complex, and often very specific, biological and ecological strategies employed by these organisms. Their highly specialized floral and vegetative structures, pollination mechanisms involving sophisticated chemistry, cryptic growth habits, root morphology, and unique reliance on mycorrhizal fungi are just some of the characteristics that make them outstanding, although challenging, models for studying complex biological interactions and even motivated Darwin to exclusively study their biology. Orchids continue to intrigue commercial and biological explorers alike. One of the highly distinctive features of orchids is their specialized interaction with mycorrhizal fungi. These interactions can range from complete dependence on fungi throughout the life of an orchid to heavy reliance only during certain life-stages or very little reliance on fungi beyond the fully heterotrophic, seed germination stage. Orchid fungus interactions also can either be highly specific or relatively general. Given that up to 30,000 species are

reaction conditions the most abundant reaction product was always the singly-deamidated Glu5-SP. Therefore, we carried out an in vitro assay to compare its agonist potency toward the natural NK1 receptor with the one of unmodified SP.

Based on the results obtained with tTGase-induced modification of SP, the ECD-based methodology is being applied to the study of deamidation and transamidation of amyloid beta peptides, e.g., the full-length amyloid beta 1-40 and its truncated forms, to unambiguously clarify their deamidation and cross-linking sites.

## ***Tissue transglutaminase-induced deamidation and transamidation of neuropeptides revealed by electron capture dissociation mass spectrometry***

Luca Fornelli<sup>1</sup>, Luigino Grasso<sup>2</sup>, Horst Vogel<sup>2</sup>, Adrien W Schmid<sup>3</sup>, Yury O Tsybin<sup>1</sup>

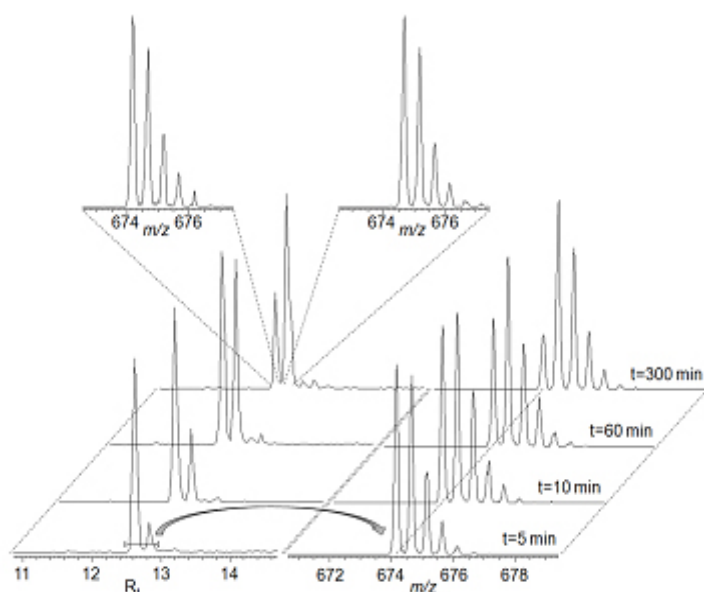
<sup>1</sup> LSMB, Ecole Polytechnique Fédérale de Lausanne, CH

<sup>2</sup> LCPPM, Ecole Polytechnique Fédérale de Lausanne, CH

<sup>3</sup> PCF, Ecole Polytechnique Fédérale de Lausanne, CH

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Upon interaction with peptides and proteins, the ubiquitously expressed enzyme tissue transglutaminase (tTGase) catalyzes glutamine (Gln) deamidation and, in presence of a peptidyl-lysine (Lys), transamidation, which results in the formation of a protease-resistant isopeptide bond. Particularly, recent in vitro studies indicate that tTGase might be involved in the deamidation and covalent cross-linking of neuropeptides and proteins, e.g., amyloid beta and alpha-synuclein, which seem to be related with the onset of neurodegenerative disorders. In the present study we first used the neuropeptide substance P (SP, RPKPQOFFGLM-NH<sub>2</sub>) as a model to determine the catalytic activity of tTGase. We applied reversed-phase liquid



chromatography Fourier transform ion cyclotron resonance mass spectrometry for the reaction products analysis, see Figure.

Electron capture dissociation (ECD) was used to elucidate the sequential deamidation of Gln5 and Gln6 residues in SP and get an insight into the structure of

transamidated SP dimers. Three different dimer populations were detected, distinguished by the number of deamidated Gln residues. Upon all the tested

estimated to belong to the Orchidaceae, a number of ecological strategies appear to exist among orchid mycorrhizae.

However, all orchid fungi identified this far fall into select fungal taxonomic groups only. This presentation will highlight some examples of the intricate orchid-fungal interactions and their consequences for biodiversity. We seek to understand whether fungal distribution determines orchid distribution in natural habitats, and whether the associations of orchids and their fungi are specific or general. Overall, orchid mycorrhizae are relatively underexplored, and there is especially a need to understand their distribution, inter-dependence, and communication mechanisms.

## ***Mass Spectrometry-based Methodologies for Investigations of N- and O-linked Glycans and Their Effects on Assembly and Interactions of Cells and Organisms***



### **Catherine E Costello**

Boston University School of Medicine  
Mass Spectrometry Resource  
670 Albany Street, Rm 511  
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The glycosylation status of cell surface proteins and lipids influences interactions of individual cells and even whole organisms, with one another and with the environment. For example, epithelial cellular adhesion via adherens junctions is mediated by multi-protein complexes. Similarly, cell surface carbohydrates provide critical signals that govern expansion of tumors and activation of growth factors. Assembly of multimers of P0 protein, a major component in myelin is dependent on its glycosylation. Furthermore, changes in cell surface glycosylation, either species-specific or due to genetic mutations, cause changes in each system's susceptibility to microbial infection. We are developing new and improved methods, centered on MS, for detailed structural determinations of glycans and glycoconjugates present as components of these complex mixtures. We are investigating new methods for glycan structural determinations and are utilizing glycomics and proteomics-based approaches to define glycan-dependent interactions and to correlate changes in the phenotypes of individual cells and whole organisms with degrees of glycosylation and differences in glycans.

Acknowledgements: NIH National Center for Research Resources and National Heart Lung and Blood Institute.

short proteinase K digestion covered 67%, including many hydrophobic stretches. Many expensive kits with elaborate affinity methods for the enrichment of plasma membrane proteins are propagated by commercial companies. We have compared the efficiency of some with a very simple two-step centrifugation protocol after microsomal aggregation induced by addition of  $\text{Ca}^{2+}$  ions [Lin et al. (1987) *Biochemistry*, 26, 731]. With this latter, low tech protocol we were able to find many more potential plasma membrane proteins than with any other protocol. The combined trypsin and proteinase K digests increased the sequence coverage of membrane associated proteins.

## ***Towards label free protein quantification of the plasma membranome***

Niurka Meneses Moreno<sup>3</sup>, Natasha Tetkovic<sup>1</sup>, Dimitrios J Fotiadis<sup>2</sup>, Manfred Heller<sup>1</sup>

<sup>1</sup> Department of Clinical Research, University of Bern, CH

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Background: A major proportion (~30%) of the human genes code for membrane proteins. This class of protein fulfills a variety of important cellular functions, such as metabolite or macro-molecule trafficking through a lipid bilayer, homeostasis of membrane potential, energy production, cellular signaling, and last but not least, cell to environment communication at the plasma membrane. Despite their importance, membrane proteins in general, and plasma membrane proteins in particular, are hugely underrepresented in proteome study results. Reasons for this are manifold, e.g. solubility, low abundance, or inaccessibility for proteolysis. Our research focuses on the molecular processes at the surface of endothelial cells exposed to different shear stress qualities. In order to make quantitative prediction of plasma membrane protein changes, there is a clear need to improve plasma membrane enrichment and protein digestion efficiencies.

Methods: Tests for increasing the digestion efficiency of membrane proteins were carried out with a purified urea transporter protein, using trypsin and proteinase K and acid labile detergent. Primary human umbilical endothelial cells and an immortal lymphocyte cell line were used to test plasma membrane enrichment strategies based on affinity capture, e.g. ConA binding, cell surface protein biotinylation, or centrifugation techniques. Proteins were separated by SDS-PAGE and identified by in-gel digestion and LC-MS/MS on an orbitrap system. Gene ontology analyses were used for cellular location prediction of the identified proteins.

Results: The urea transporter (MW ~36 kDa) has nine predicted transmembrane helices. While trypsin digestion resulted in a sequence coverage of only 29%, a

*Notes:*

## ***In-depth characterization of glycoproteins by ZIC-HILIC enrichment and mass spectrometry***

Arnd Ingendoh<sup>1</sup>, Arndt Asperger<sup>1</sup>, Anja Resemann<sup>1</sup>, Andrea Schneider<sup>1</sup>,  
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N- and O-glycosylation of proteins are involved in many cellular processes and may play diverse roles at different sites within one protein. Therefore, the site-specific analysis of protein glycosylation has become an important issue in pharmaceutical and biotechnology applications for fundamental research as well as for quality control. A common technique for glycosylation analysis on the peptide level is LC/MS/MS. However, the analysis is difficult due to low abundance of glycopeptides in complex protein digests, micro heterogeneity at the same glycosylation site, ion suppression effects and competition for ionization by co-eluting (glyco-) peptides. The challenging requirement on mass spectrometric is the elucidation of both the peptide as well as the glycan moiety for a full structural assignment. Described here is a procedure for comprehensive glycosite analysis by specific and quantitative batch enrichment of N- and O-glycopeptides. Complementary 1D or 2D HILIC- and RP-LC setups are coupled to MS for the detection of low abundant glycoforms.

Fetuin, alpha1-acidglycoprotein and Asialo-Fetuin were used to equally represent sialylated and non-sialylated glycosylic structures. In addition, monoclonal antibodies were analyzed as a dedicated example for pharmaceutical QC. Samples were digested with trypsin. The glycopeptides were enriched by a dedicated ZIC glycocapture resin in combination with an optimized buffer system. The intact glycoproteins and the digests were analyzed by various MS technologies (ion trap, MALDI-TOF/TOF or UHR-QTOF) for glycoprofiling and in depth characterization. Mascot searches of the glycopeptide MS/MS spectra provided for the peptide sequence and the localization of the glycosylation site. Searches in glycan

of multi-protein complexes like proteasome or RNA polymerase I at high purity and concentration yielded about 100 – 150 non-redundant distance constraints.

As affinity-purification of protein complexes from human cell lysates results in substantially lower protein concentration and purity we modified our cross-linking protocol allowing the identification of about 100 intra- and inter-protein cross-links on the protein phosphatase 2A complex.

Novel Aspect: Cross-linking of native human protein complexes may facilitate the analysis of protein-protein interactions and complex topologies on a routine basis.

## ***Probing the architecture of protein complexes by chemical cross-linking and mass spectrometry***

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Introduction: Structural biology techniques like X-ray crystallography and NMR spectroscopy have solved structures of individual proteins and subcomplexes, however, failed to reveal a comprehensive picture of the architecture of macromolecular protein assemblies. Our strategy aims at integrating spatial constraints identified by mass spectrometry and structural information obtained by conventional methods. We apply chemical agents to cross-link different amino acid side chains within a single polypeptide chain or two polypeptides in a complex and identify the covalently linked peptides by mass spectrometry. The length of the cross-linker provides a measure for the maximum distance spanned between the two connected residues.

Method: Protein samples are cross-linked with an equimolar mixture of isotopically light and heavy labeled cross-linkers facilitating the identification of all modified peptides by a characteristic isotope pattern. Following enzymatic digestion, cross-linked peptides are enriched over the vast majority of the non-cross-linked species by size exclusion chromatography and analyzed by LC-MS/MS on a LTQ-Orbitrap hybrid instrument. Pairs of isotope-coded peptides are identified by the precursor ion mass shift and the corresponding tandem mass spectra are submitted to the dedicated search engine, xQuest. The experimental product ion spectrum is matched to all possible theoretical candidate spectra derived from a database. The general feasibility of the concept is indicated by the identification of inter- and intra-protein cross-links within purified recombinant protein complexes.

Preliminary Data: Based on the analysis of a mixture of eight model proteins we estimate a false discovery rate of our cross-linking strategy of ~4%. Cross-linking

databases on the same MS/MS spectra completed the characterization of N-linked glycopeptides.

In the high resolution QTOF, discrete changes in the glycosylation patterns even for molecules as large as intact antibodies can be observed. After reduction and alkylation, the molecular weights of the resulting light and heavy chains can be determined with 0.2 ppm mass accuracy. In MALDI-TOF/TOF, the analysis of multi-sialylated glycopeptides was performed. Linear positive ion mode spectra of sialylated glycopeptides provided the respective precursor ion information. In reflector mode, these precursors were selected for MS/MS even though they were not detectable. Rich MS/MS spectra of the sialo-glycopeptides were obtained providing both glycan and peptide fragments. A specific fragmentation pattern allows identifying the MW of the peptide moiety and its proper sequence assignment including the glycosylation site. Additionally, the glycan heterogeneity can be elucidated. In Fetuin, tri-antennary glycan structures with up to four sialic acids were elucidated using MALDI-TOF/TOF and ion trap data. In comparison to direct MS analysis of glycoprotein digests, the enriched samples resulted in MS/MS spectra of higher quality and the detection of more glycopeptides.

## ***Two-dimensional Fourier transform ion cyclotron resonance mass spectrometry***

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In analytical chemistry, the most interesting samples (biological, food, environmental or petroleum samples) are often complex, containing hundreds or thousands different compounds. The high resolving power and mass accuracy of FT-ICR/MS enables the deduction of chemical formulae over a wide mass range and dynamic range, and MS/MS yields more advanced structural information. However, for complex samples this is time consuming, since it requires the fragmentation of all the peaks in the MS spectrum and inaccurate since isolation windows are rarely narrower than 0.1 Da. In 1988, Gäumann et al. [1] developed a pulse sequence for 2D FT-ICR/MS that circumvents ion isolation and yields data in one easily readable 2D mass spectrum. Until now, this method has scarcely been used due to limitations both in acquisition and in data analysis.

We implemented a new version of 2D FT-ICR/MS on a commercial instrument over an analytically useful mass range. The data was acquired on a Bruker Daltonics 9.4 T nanoESI-hQh-FT-ICR/MS with a modified experimental script over a mass range of  $m/z$  86-2000 in both directions, and processed with the NMR data processing shareware NMR Processing Kernel [2] that we modified for 2D FT-ICR/MS.

By using gas-free fragmentation modes, we achieved high resolution in the first dimension, and by acquiring more spectra, we significantly improved the resolution in the second dimension and we resolved isotope peaks along the self-correlation line. Off-diagonal fragmentation peaks were clearly visible and corresponded to fragments obtained in MS/MS spectra. The main source of signal distortion in 2D mass spectra is scintillation noise, caused by fluctuations in the number of ions trapped in the ICR cell [3]. It appears as vertical stripes along the highest

based on our experimental findings, literature data and discussions with internal and external experts.

[1] Barfield M, Spooner N, Lad R, Parry S, Fowles S. Application of Dried Blood Spots combined with HPLC-MS/MS for the quantification of acetaminophen in toxicokinetic studies.

*J Chromatogr B* 870, 32-37 (2008)

## ***Dried blood spots – a revolution in drug development?***

Katja Heinig, Almudena Gajate-Perez, Berthold Lausecker

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Dried blood spots (DBS) is a technique where blood is sampled on filter paper-based cards, dried, discs punched out, extracted and the compounds of interest contained in the blood are analyzed. It is widely applied in neonatal screening of inborn errors of metabolism and in therapeutic drug monitoring for many years. Recently, it has been expanded into the DMPK field within drug development [1], as an alternative to measure plasma concentrations to obtain pharmacokinetic data. This became feasible since analytical techniques have advanced, and LC-MS/MS is now state of the art in drug analysis, with mass spectrometers getting more and more sensitive. Only small sample volumes are needed which provides benefits for toxicology (i.e. reduction of animal numbers), pharmacokinetics (i.e. serial sampling from small animals) and clinics (i.e. enabling of pediatric studies). Transport and storage costs for study samples are significantly reduced. Furthermore, improved stability of otherwise instable compounds could be demonstrated in some cases.

Here we provide an overview of DBS technique, including sampling, handling and analysis aspects and current applications in pharmaceutical drug development. We share the experience with DBS gained at F. Hoffmann-La Roche in bioanalysis, in particular with unstable drugs, pro-drugs or metabolites. Our examples include small molecule drugs and a synthetic peptide. The attempts at reducing the quantification limits and the possibility to provide automation by on-line DBS extraction are presented. The DBS assays were validated, and their quality is compared with standard plasma LC-MS/MS methods. In the conclusion, we evaluate the potentials and limitations of DBS in pharmaceutical development

abundance peaks. Unlike additive noise, it cannot be reduced by accumulating spectra. We used the Cadzow algorithm [4] to minimize the effect of scintillation noise on spectra in order to make them easily interpretable. We hope that with these improvements the revisited Gäumann's 2D FT-ICR/MS will become in a viable analytical technique.

- [1] P Pfändler, G Bodenhausen, J Rapin, M-E Walser, T Gäumann, *J Am Chem Soc* 110 (1988) 5625-5628
- [2] D Tramesel, V Catherinot, M-A Delsuc, *J Magn Res* 188 (2007) 56-67
- [3] G. Van der Rest, A.G. Marshall, *Int J Mass Spectrom* 210-211 (2001) 101-111
- [4] C Brissac, TE Malliavin, M-A Delsuc *J Biomolecular NMR* 6 (1995) 361-365

## ***Shotgun lipidomics for cell biology and molecular medicine***



Andrej Shevchenko

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Lipidomics, an emerging branch of the omics sciences, aims at cataloguing and quantifying the total lipid complement synthesized by a cell, tissue or organism. Shotgun analysis of the lipidome implies that total lipid extracts containing hundreds of molecules from different lipid classes, are directly infused into a tandem mass spectrometer and thousands of MS and MS/MS spectra are acquired in a single run. Individual molecular species are recognized and quantified using their accurately determined masses and characteristic structural fragments.

Shotgun lipidomics approach is appealing: it is rapid, comprehensive and easy to set up at any tandem mass spectrometer. Quantification of lipid species does not involve time-integration because the same analyte is infused into a mass spectrometer. There is ample time to achieve good ion statistics even for minor peaks and the ionization conditions can be tuned to enhance the sensitivity towards barely detectable lipid classes. There is no carry-over between samples and the entire process can be completely automated.

Shotgun lipidomics set up at the same instrumentation platform supports both high-throughput clinical screens and targeted characterization of molecular lipid species from a variety of model organisms from bacteria to humans. However, two major bottlenecks of the shotgun approach are in the limited dynamic range and possible ionization suppression of certain species and in the consistent interpretation of exceedingly complex spectra datasets.

We argue that high resolution tandem mass spectrometers together with the dedicated data interpretation software could overcome these hurdles and support a

Our investigations on different mass analyzers, such as quadrupole time of flight, 3D and linear ion traps indicate that most commercial systems are at the same level of sensitivity and any reasonable enhancement is not easily realized. As a next step in this development we used EESI and APCI to detect compounds like fentanyl, morphine, caffeine and nicotine and determine the limit of detection in the gas phase. Our first evaluation showed that reasonable detection limits in breath (low ppb(V) levels, online detection) is possible for the more volatile narcotics like fentanyl or nicotine. For less volatile compounds, such as morphine or caffeine, detection by online methods seems to be more challenging. Offline experiments with morphine show that the estimated online concentration is at the level of low ppt(V), which cannot be reached at the moment. Further optimization and enhancement of the ionization methods is needed. Online breath analysis for anesthesia in breath is a challenging topic and is directly related to the development of ionization systems. We are still investigating feasible tools for the sensitive and fast ionization of breath.

## ***Online breath analysis by mass spectrometry, a possible tool for anesthesia monitoring?***

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A big challenge in anesthesiology is the dynamic control of drug dosage used to anesthetize a person during surgery. Today, sophisticated patient monitoring is used to control the health status, as well as the level of consciousness of a patient. During surgery, a patient is usually connected to a breathing machine that allows monitoring of his breath. Currently, a patient's breath is monitored for CO<sub>2</sub> saturation as well as isoflurane levels by spectroscopic methods. Unfortunately, no commercial tool is available for the monitoring of metabolites or other drugs in breath such as morphine or fentanyl. Recent developments in ambient ionization technologies suggest using mass spectrometry as a system for the detection of metabolites in breath, such as the narcotics used in anesthesia. Technologies like atmospheric pressure chemical ionization mass spectrometry (APCI-MS), selected ion flow tube mass spectrometry (SIFT-MS), proton transfer reaction mass spectrometry (PTR-MS) or extractive electrospray ionization mass spectrometry (EESI) show very sensitive detection limits and enable direct analysis of compounds in exhaled breath. Recent investigations using offline methods suggest a sensitivity of low ppt(V) is needed to detect narcotics in breath if they are present. At the moment, no technology reaches this sensitivity for online detection of narcotics. In our ambitious research project we try to overcome this problem.

The sensitivity of mass spectrometric methods is an issue of sample preparation, ionization method and mass spectrometer used. If a method is supposed to be considered as online, sample preparation has to be very limited. This means the sensitivity has to be reached by both the ionization method as well as the mass analyzer used.

broad scope of research efforts in cell biology, molecular medicine and nutrition science.

## ***Phospholipidic fingerprinting by on-line desorption of dried spots and quadrupole-linear ion trap mass spectrometry: evaluation of atherosclerosis biomarkers in mouse plasma***

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Phospholipids play important roles in the biochemistry of all living cells, either as the building blocks of membranes or regulators of processes such as homeostasis, metabolism, and signal transduction. This primordial physiological role is highlighted by the numerous diseases in which phospholipids are implicated, such as atherosclerosis, cancer, and Alzheimer's disease. Atherosclerosis is one of the major diseases in humans and is the most common cause of death in western countries. It is induced by a chronic inflammatory response in the subendothelial space resulting from the interaction of oxidized low density lipoprotein (ox-LDL), monocyte-derived macrophages, T cells, and normal cellular elements. In patients with hypercholesterolemia, excess LDL infiltrates the artery at sites of hemodynamic strain. Oxidative and enzymatic modifications of LDL then lead to the release of phospholipids which are able to promote atherogenesis. This work presents a strategy for the evaluation of differences in plasma phospholipid content between atherosclerotic (ApoE<sup>-/-</sup>) and healthy mice from micro volumes (2  $\mu$ L) spotted on filter paper. Dried plasma spots (DPS) were directly desorbed into a triple quadrupole linear ion trap mass spectrometer using a homemade prototype, ensuring high-throughput analysis of dried spots without any sample pretreatment. Multiple positive and negative neutral loss and precursor ion scans were simultaneously acquired in a single loop, allowing oriented fingerprinting until 2700 potential species including phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol

HPLC system, which allows for fully automated screening of numerous samples on phase I and phase II metabolism.

## ***Electrochemistry/MS a powerful analytical technique to mimic drug metabolism and other redox reactions in life sciences***

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Traditional methods to investigate oxidative drug metabolism are based on in-vitro (e.g. microsomes) or in-vivo (urine, plasma, etc.) methods, which are both cumbersome and time consuming. Therefore, mimicking oxidative metabolism using Electrochemistry (EC) is of great interest as a fast and inexpensive alternative.

In this presentation we will show the application of on-line EC/ESI-MS as a powerful tool to simulate various oxidative processes in life sciences. A specially designed  $\mu$ -preparative electrochemical flow cell will be presented. The cell generates sufficient amounts of metabolites, to allow their use as reference material in MS or for structural elucidation by NMR. Moreover the EC/MS approach can be used to measure metabolic profiles of drug compounds or xenobiotics in a very short period of time. Oxidative metabolism (phase I) of different drugs such as amiodarone, acetaminophen, etc., mimicked in the electrochemical cell will be shown, including the formation of adducts, e.g., conjugation with GSH (phase II metabolism).

Recently, the use of EC/(LC)/MS has been extended towards new applications such as: rapid risk assessments of drug-protein binding, signal enhancement in MS, oxidative damage of DNA, electrochemical cleavage of proteins, etc.

Some of these new applications will be discussed in more detail, highlighting the tremendous potential of using Electrochemistry to mimic nature's redox reactions in seconds vs. months or years in nature.

Furthermore, we will demonstrate a novel EC/LC/MS approach, based on the integration of an amperometric thin layer EC cell into the fluidic pathway of an

(PI), phosphatidylserine (PS), and sphingomyelin (SM) classes. The phospholipidic variations between 15 healthy and 15 atherosclerotic mice were evaluated using T-tests, matrix reduction and merging, and principal component analysis (PCA) as a chemometric statistical approach. The discriminating ions in PCA analysis were qualitatively identified in an information dependent acquisition (IDA) manner using enhanced resolution and enhanced product ion scans.

PCA demonstrates a clear clustering between healthy and diseased mice. Regarding the most relevant variables identified, this procedure has confirmed the role of SM and PS classes in atherosclerosis and has established potential biomarkers shown to be significantly up- or down-regulated with the disease.

## ***Sites of metabolic substitution: Definitive metabolite structures deduced using ion mobility and molecular modeling***

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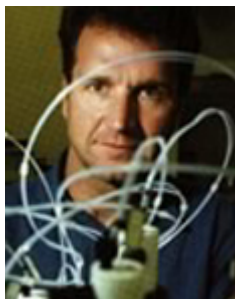
As an analytical technique, mass spectrometry (MS) cannot separate isomeric species or provide conformational information. Structural information can be gained through tandem MS (MS/MS) techniques. There are classes of substituted molecules, such as hydroxylated metabolites which produce identical MS/MS spectra. This is problematic if the exact position of the hydroxylation is required. Ion mobility (IM) has the ability to separate isomeric species, such as hydroxylated metabolites, rapidly (msec) based on differences in their collision cross-section ( $O$ ) in the gas-phase, thus providing specific information on ionic configuration and therefore, the position of the hydroxyl moiety.

Experiments were performed using a hybrid quadrupole/ion mobility/orthogonal acceleration time-of-flight Synapt G2 HDMS instrument. Ion mobility separation was performed at a pressure of 3.2 mbar nitrogen with a wave velocity of 1100 m/sec and a travelling wave amplitude of 40 V was used. Theoretical  $O$  values were calculated using MOBCAL and compared to the travelling wave derived  $O$  values. Ion mobility calibration was undertaken using five singly charged polyalanine ions. Three-dimensional metabolite conformations were explored with the "Systematic Search" protocol in MOE. Ten thousand combinations of possible values for variables were explored. All conformations were minimised with the MMFF94 forcefield. Conformations with energies below 2 kcal/mol were selected for each metabolite.

All three hydroxylated metabolites showed different UPLC chromatographic elution times. However, the MS and MS/MS spectra are all identical, thus hampering identification. The  $O$  value of the parent compound ondansetron and the three hydroxylated metabolites GR60661, GR63418 and GR90315 were measured by

temperature, fused-core particles and sub-2 $\mu$ m particles working under very high pressure (UHPLC). Among the proposed approaches, it has been demonstrated that UHPLC and fused-core particles presented several advantages for the analysis of small molecules as well as large bio-molecules. Therefore, UHPLC-MS with different analyzers can be used to analyze very complex matrices with compounds present at low concentration. The possibilities offered by UHPLC at high temperature (i.e. HT-UHPLC) to further enhance chromatographic performance will be also discussed. Finally, UHPLC-MS/MS and UHPLC-TOF-MS can be attractive in ADME studies at an early stage of the drug discovery process.

## ***Capillary electrophoresis and Ultra high pressure liquid chromatography hyphenated with MS in pharmaceutical analysis***



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In the last ten years, a strong development has emerged in Capillary Electrophoresis (CE) and in Liquid Chromatography (LC) to achieve fast, ultra-fast and highly efficient separations in the pharmaceutical field. In the same period of time, Mass Spectrometry (MS) with different analyzers became the best complementary tool to separation techniques, to further gain selectivity and/or sensitivity, when dealing with complex matrices (e.g. biological fluids and plant extracts).

The use of large bio-molecules is increasing in pharmacy. Therefore, there is a need for efficient analytical techniques for determining these compounds (e.g. proteins). The on-line combination of capillary electrophoresis (CE) with mass spectrometry (MS) is an attractive option for intact protein analysis (i.e., no digestion, no derivatization). On the one hand, CE presents features such as high speed, great efficiency, and low solvent and sample consumptions. Moreover, CE allows working under aqueous conditions and without stationary phase. On the other hand, MS provides selectivity and ability to identification. TOF (time-of-flight) analyzer is particularly well suited to protein analysis, due to high mass range and mass accuracy. For small charged molecules, CE-MS with a simple quadrupole is also a powerful orthogonal analytical tool to LC-MS. Different examples will be given to illustrate the potential of CE-MS in the pharmaceutical domain.

In LC, various analytical strategies have been reported for enhancing the chromatographic performance, such as the use of monolithic supports, high

UPLC-IMS-MS. Theoretically derived  $O$  values for hydroxylated metabolites GR60661, GR63418 and GR90315 are 110.9 Å<sup>2</sup>, 110.4 Å<sup>2</sup> and 109.5 Å<sup>2</sup> respectively. Travelling wave ion mobility derived  $\omega$  values are in very close agreements with the theoretically derived values, thus allowing accurate identification of the hydroxylated metabolites, even those with a  $\omega$  difference of less than 1 Å<sup>2</sup>. No additional structural characterization techniques, such as NMR or X-ray crystallography were required for metabolite structural determination. This is a very time efficient and effective means of identifying drug metabolites directly from biological matrices. Additionally this method can be utilised in a situation where chemically synthesised standards may not be available.

#### Novel Aspect

Ab-initio metabolite conformation calculation and ion mobility derived collision cross-sections, aiding drug and metabolite identification and characterization.

## ***Stable isotope-labeled metabolomes – a tool for systems level analysis***

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Metabolomics aims to study the chemical content of a wide range of organisms, fingerprint of dynamic processes and consequences of specific development conditions. At first introduced for diseases diagnostics until nowadays for system biology understanding.

We have developed a unique strategy for targeted/untargeted qualitative and quantitative metabolomics.  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{34}\text{S}$  labeled plant material, analyzed by three different analytical platforms: (1) Primary metabolites targeted approach using GC-MS, (2) secondary metabolites profiling by on line coupled Ultra Performance Liquid Chromatography with Heated Electrospray Ionization Mass Spectrometry (UPLC-HESI-MS) and (3) lipids content profiling by UPLC-HESI-MS. Information gained have contributed to significantly improve elemental composition annotation (as a first step toward compound annotation).

This robust method is routinely operated in a high throughput fashion. After data generation, tools including statistical analysis and data visualization can provide powerful insights for understanding biological/chemical systems. Applications to biology and chemistry will be described.

*Notes:*