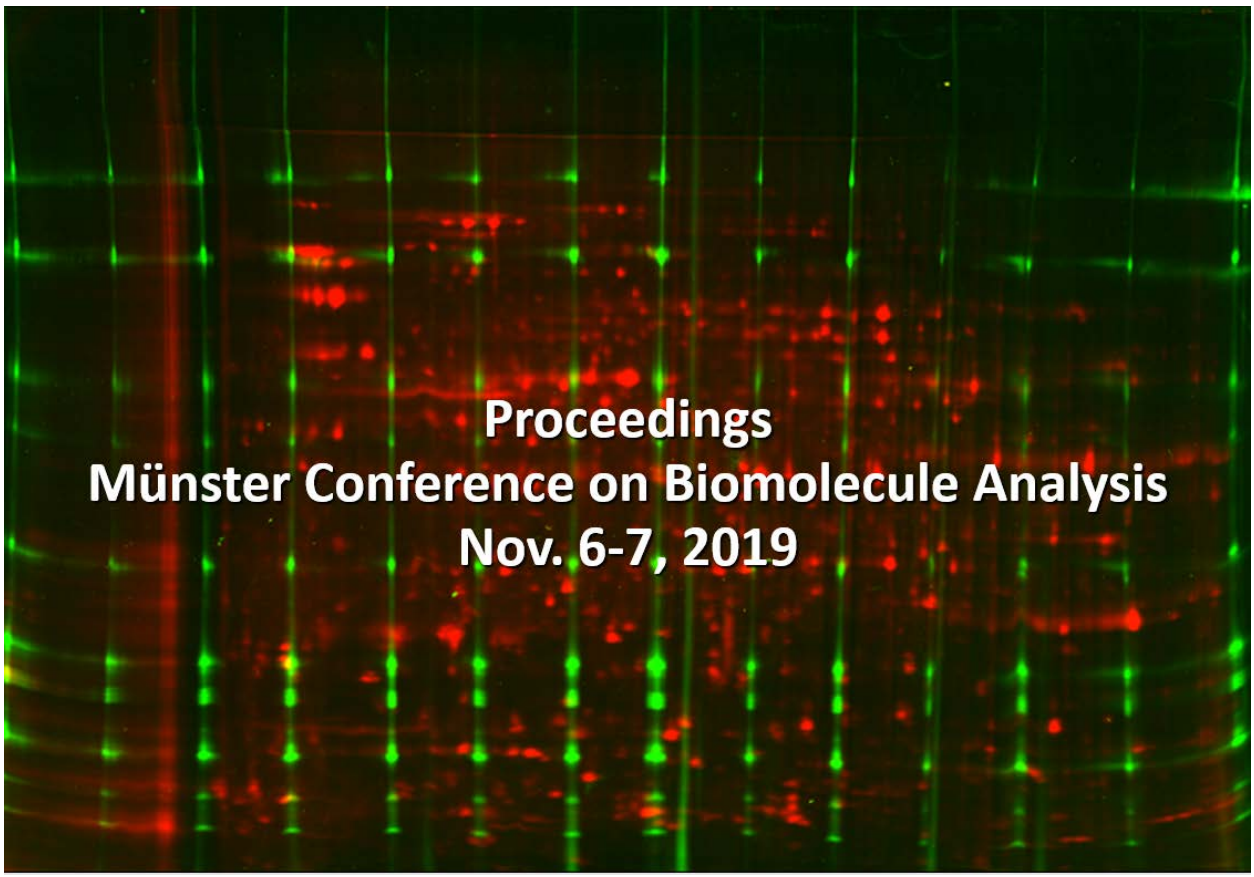

Proceedings
Münster Conference on Biomolecule Analysis
Nov. 6-7, 2019

Organized by the Core Unit Proteomics
of the Interdisciplinary Center for Clinical Research Münster
Prof. Simone König & Dr. Rita Naskar

Preface

The Münster Conference on Biomolecule Analysis 2019 focused on personalized medicine. Members of the Stanford University hPOP (human personal profiling) consortium, Stefanie Hauck (Munich) and Manuel Fuentes Garcia (Salamanca), started the discussion by presenting a number of clinically oriented proteomics projects. Allan Stensballe (Aalborg) spoke about phenotyping of multiple biofluids for liquid biomarkers for diagnostics and personalized medicine. Klaus Berger, the head of the Institute for Epidemiology in Münster, reported on the large population study NaKo, which is currently conducted in Germany. More technically oriented, Klaus Kratochwill (Vienna) contributed insights to the improved Western blot technology his group developed and Felix Engel (Erlangen) spoke about biofabrication towards functional heart tissue.

The conference also provided a platform for companies to showcase their products and interact with customers in workshops. The international event successfully met the increasing interest in proteomics, protein and shot-gun analysis technologies and their interplay with clinical research and provided a valuable information source in particular for Ph.D. students.

Since 2004, the Core Unit Proteomics (CUP) of the Interdisciplinary Center for Clinical Research Münster has organized the event as an annual series of bioanalytical conferences.

Münster, Nov. 11, 2019
Simone König

Poster Abstracts

Direct fluorescence in-situ PCR (FISP) for analysis of forensic and medical relevant markers and spatially visualizing genetic information

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In-situ PCR is a technique, which was developed in 1990s for the detection of genetic and transcriptomic information, spatially resolved. This method can be used on a variety of cells and tissues. Single cells, FFPE tissue, fresh frozen tissue and cell cultures can be used for analysis. In the primal methodology, labelled dUTP nucleotides were incorporated in the PCR product, which were localized using immunochemistry. This sets limitations in number of markers, which can be analysed and performing all steps is time consuming and labour intensive. Here, we provide a method were fluorescently labelled nucleotides or primers were incorporated instead. This gives the opportunity to analyse the expression in whole sections. Additionally subsequent analysis of the resulting PCR product is possible.

Monitoring enzyme activity in Complex Regional Pain Syndrome (CRPS) by thin-layer chromatography of substance P enzymatic fragments

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CRPS is an inflammatory disease, which is developed by 3 – 5 % of patients after limb fracture. Patients show a local rise in temperature, redness of the skin and edema at the affected limb. Additionally, CRPS-patients experience strong pain ultimately resulting in an impairment of everyday life. In order to understand CRPS, neuropeptides such as substance P and bradykinin (inflammatory mediators) are at the center of investigations. An increased concentration of these mediators has been found in CRPS patients as well as reduced enzymatic degradation.[1-3] For monitoring of enzyme activity an assay was developed, which uses substance P (modified with a chromophore) and thin-layer chromatography for separation of degradation products. After incubation of the dabsylated peptide (at Lys3, K3DSP) with serum, fragments K3DSP(3-8) and K3DSP(3-11) were detected and quantified. K3DSP(3-11), in particular, was a good indicator for the enzyme activity. The assay was used to compare CRPS-patients with control groups. A difference in enzymatic activity between CRPS-patients and healthy controls in enzyme activity could be shown. The assay is simple to use and low-cost. It requires only small amounts of serum (1-8 µl). Large cohorts can be tested in a time efficient manner.

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[2] F. Birklein, M. Schmelz, Neurosci. Lett. 2008, 437:199–202.
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Metabolomics-based identification of urinary biomarkers for tomato juice intake

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Maintenance and improvement of human health as well as prevention of diseases are substantially influenced by the diet. The ability to establish a conclusive correlation between intake of different food items and associated health effects is consequently of high relevance. A major challenge faced herein is the objective and quantitative determination of the dietary intake outside of a controlled study environment. The analysis of dietary biomarkers, i.e. food constituents or derived metabolites quantifiable in biofluids after intake of the respective food, has great potential in this regard. However, the number of established dietary biomarkers is very limited so far and a comprehensive validation is still necessary in many cases.
The objective of this project was to identify potential biomarkers for the consumption of tomato products. For that purpose, a dietary intervention study was conducted to compare the urine metabolome of a study cohort between a tomato-free diet and after intake of a single dose tomato juice based on an LC-HRMS metabolomics approach [1]. Herein, novel metabolites of the steroidal glycoalkaloids esculeoside B-1 and B-2 were putatively identified as potential biomarkers. Further metabolites characterized as potential biomarkers were the glucose-derived *β*-carboline alkaloids tangutorid E and tangutorid F as well as their glucuronidated derivatives. The occurrence of both *β*-carboline alkaloids was additionally investigated in several food samples within this project by applying an LC-MS/MS-based stable isotope dilution assay; tomato products were found to contain the highest amounts of tangutorid E and F [2]. Lastly, novel imidazole alkaloids were detected in the study participants' urine after tomato juice intake and showed a huge potential as biomarkers for tomato intake. These alkaloids were successfully synthesized, structurally characterized and quantitatively determined in several tomato products for the first time [3]. Currently, the applicability of all identified metabolites as valid biomarkers for tomato intake is evaluated in the course of a further dietary intervention study.

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diaPASEF: toward the ideal mass analyzer with data-independent acquisition and parallel accumulation – serial fragmentation

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Data-independent acquisition (DIA) promises reproducible and accurate protein quantification across large sample cohorts. The mass spectrometer typically cycles through many isolation windows covering a broader *m/z* range of interest. Current methods utilize only about 1-3% of all available ions. In principle, all ions could be utilized by parallel ion storage and sequential release from the TIMS device into a Q-TOF mass analyzer. Here, we asked if the PASEF principle could be transferred to DIA. Whole-cell proteomes extracted from a human cancer cell line were analyzed via nanoLC coupled to a prototype timsTOF Pro (Bruker). We adapted the instrument firmware to perform data-independent isolation of multiple precursor windows within a single TIMS separation (100ms). We tested multiple schemes for precursor selection window size and placement in the *m/z*-ion mobility plane. Analysis of the four-dimensional data space has been incorporated into OpenSWATH. For ion mobility-aware targeted data extraction, we used a project-specific library from 48 high-pH reverse-phase peptide fractions acquired with PASEF. As ion mobility and mass are correlated, a large proportion of the peptide ion current can be covered by scanning diagonal lines in the *m/z*-ion mobility space. We derived multiple diaPASEF acquisition schemes from the density distribution of about 130,000 precursors present in the library. TIMS provides highly precise measurements of collisional cross sections (CCS) with CVs << 1% in technical replicates. After linear alignment, CCS values extracted from the diaPASEF runs deviated < 2% from the library. In triplicate 120min runs of 200ng HeLa digest each, we quantified over 7000 proteins at a 1% FDR. Fragment ion-based quantification was very reproducible with a median CVs of 10% and a pairwise mean Pearson correlation >0.96. The diaPASEF method captures and utilizes a very large proportion of the available ion current, approaching the ideal mass analyzer.

Simulation of the metabolism of the synthetic cathinone MDPHP by means of electrochemistry/mass spectrometry

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In 2017, the European Monitoring Centre for Drugs and Drug Addiction identified 670 'new psychoactive substances' (NPS) in European countries.[1] NPS are chemically synthesized substances that aim to mimic the effects of common drugs such as cannabis or amphetamines. The chemical structure of the NPS is modified in such a way that the new substance is no longer subject to the respective drug laws of the European member states. The intake of these so-called designer drugs has increased significantly in recent years, affecting the physical and mental conditions of the consumer. Among the NPS, synthetic cathinones make up the vast majority of distributed substances. Since the mid-2000s, ring-substituted cathinone derivatives have appeared on the European market. One of the latest known compounds is 3',4'-methylenedioxy-α-pyrrolidinohexiophenone (MDPHP). Only little research has been conducted on MDPHP regarding pharmacology, toxicity and metabolism.
The rapid variation and distribution of these drugs and the lack of appropriate analysis and detection methods evolve into an ever increasing NPS consumption. In order to establish screening methods, knowledge of the metabolism is necessary, since the substances are mostly excreted via the urine in form of their metabolites. Electrochemistry (EC) coupled with mass spectrometry (MS) is a suitable tool to generate transformation products analogue to the metabolites in a simple and fast manner. Furthermore, the instrumental setup enables the formation and detection of possible intermediates and products without disturbances caused by biological matrices or using animal trials.
In this work, MDPHP was electrochemically transformed in a thin-layer cell equipped with a boron-doped diamond working electrode to simulate its metabolic transformation. The transformation provides oxidation and reduction products detected online by time-of-flight (TOF)-MS. Thereby, hydroxylation of MDPHP was observed. In the reductive mode, the keto-group of MDPHP changed to an alcohol. Via liquid chromatography, the resulting transformation products were separated and isomers were observed. Fragmentation experiments were conducted in order to elucidate the respective structures.
In summary, the results of this investigation demonstrate that EC/MS can serve as an appropriate pre-screening method to generate and detect oxidation and reduction products of synthetic cathinones to counteract the development and spread of the designer drugs.

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pyRNAms enables semi-automated high-throughput nucleoside mass spectrometry data analysis for the common user

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Chemical tRNA modifications are central for fine-tuning translation dynamics and their absence has severe consequences for protein homeostasis and is implicated in human diseases like cancer and neurodegeneration. Consequently, their identification and accurate quantification is required to unravel their biological roles. The current gold standard for the analysis of chemical modifications in RNA is liquid chromatography coupled tandem mass spectrometry (LC-MS/MS). However, the high sensitivity and accuracy of modern MS instruments generates very complex datasets that are challenging and time-consuming to analyze. In particular large experimental setups with hundreds of measurements, e.g. in a clinical context, require a reliable high-throughput method for data analysis and downstream evaluation.

Here we present pyRNAms, a platform-independent Python module tool for the automated analyses of untargeted nucleoside MS data. pyRNAms offers an easy-to-use and versatile toolbox for rapid nucleoside identification and quantification, statistical validation and visualization. Importantly, pyRNAms was designed to analyze any metabolomics MS dataset and can handle every existing labeling technique. This universality is based on pyQms, a software that we previously developed for the accurate quantification of any biomolecule in complex high-resolution mass spectrometry data. pyRNAms can easily be used by any researcher, enabled by an accessible graphical user interface, which allows to analyse and explore the datasets. At the same time, a command line interface allows experts to perform in-depth analyses. Thus, pyRNAms will raise tRNA modifications into the omics era and will pave the way for the reliable detection of novel nucleoside modifications.

Dimethyl fumarate treatment alters T cell metabolism in multiple sclerosis

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Dimethyl fumerate (DMF) is an immune-modulatory drug for treatment of relapsing remitting multiple sclerosis (RRMS). Although DMF treatment leads to beneficial clinical effects, around 17% of patients develop a lymphopenia characterized by sustained decrease of lymphocyte counts within blood. In parallel, DMF-treated patients exhibited decreased frequencies of memory T cells (T_M) while naïve T cells (T_N) were less affected. Interestingly, it is known that murine T_M cells reveal an increased metabolic capacity compared to T_N cells.

In order to investigate the molecular mechanism of DMF in different T cell subsets, we examined the metabolic effects and their potential link to cellular stress-response leading to apoptosis. Therefore, T cells were isolated from MS patients before/during DMF treatment and healthy donors. To determine functional metabolism and metabolite composition of T cells Seahorse technology and untargeted metabolomics were used, respectively. Apoptosis was assessed by flow-cytometric analysis of caspases 3/7 and mitochondrial stress-response was determined by fluorescent redox sensors.

T cells from DMF-treated MS patients displayed a decreased mitochondrial respiration as compared to pretreatment. *In vitro* administration of DMF reduced mitochondrial respiration in T_M and T_N subsets, however, T_M cells were more susceptible than T_N cells due to their 2-fold increased metabolic capacity. Furthermore, mitochondrial stress-response illustrated by elevated ROS levels was enhanced in DMF-treated T_M but not T_N cells corroborating again that T_M cells display an enhanced sensitivity towards DMF. Accordingly, we observed an increased apoptosis rate in T_M cells as compared to T_N cells in DMF-treated patients.

Collectively, these data illustrate that DMF treatment significantly reduces mitochondrial respiration in all T cell subsets. Importantly, T_M cells display an enhanced sensitivity and stress-response following DMF treatment, which might be a potential mechanisms explaining the increased apoptosis and vulnerability of T_M cells in DMF-treated MS patients.

Analysis of aflatoxins using miniaturised capillary liquid chromatography system with novel absorbance-fluorescence detector

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Aflatoxins are group of fungal pathogens produced by molds of *Aspergillus sp.* which commonly contaminate cereals, oilseeds, tree nuts, dry fruits and spices. Aflatoxins are toxic substances with immunosuppressive, mutagenic, and carcinogenic effects, and due to their frequent incidence and toxicity they represent health hazard to humans. Several analytical techniques have been used in the past for their detection and quantification in different matrices, including LC/MS, CE/MS as well as HPLC with fluorescence detection [1, 2]. Commercial HPLC/FLD systems are robust but bulky instruments intended for laboratory use. Herein, we introduce miniaturised and portable instrument with low solvent consumption and waste production. The system consists of simplified capillary LC scheme based on the single piston pump and selector valve connected via capillary which serves as a solvent and sample loop [3]. Selector valve allows automated flow path change, such that solvents and sample are sucked by the pump and consequently pumped on capillary column (0.02 × 150 mm packed with Fortis H2O 3□m particles). The setup is equipped with the newly developed compact optical detector which is capable of simultaneous multi-wavelength absorbance and fluorescence monitoring. Detector uses light emitting diodes (LEDs, 365 nm, 265 nm) as the light source, lab-made L-shaped silica capillary detection cell [4] (50 nL, optical path 1 mm), and CCD spectrometer as the light detector.
We applicated this system for analysis of aflatoxins – standard mixture of B1, B2, G1 and G2, as well as for samples obtained from wheat grain inoculated with *Aspergillus sp.* and *Fusarium sp.* cultures. Presented results demonstrate the capability of miniaturised capillary liquid chromatograph to perform fast (up to 3.5 min) and repeatable baseline separation of aflatoxins in standard mixture, as well as in complex matrices. The LOD was 0.8 µg/L of G2 and B2, or 6 µg/L of G1 and B1 aflatoxins.

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Method development for the determination of δ-aminolevulinic acid-induced protoporphyrin IX in blood

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Glioblastoma multiforme (GBM) is the most lethal type of brain cancer and also the most common one[1]. In order to improve tumor resection, δ-aminolevulinic acid-induced fluorescence-guided resection (ALA-FGR) is used in neurosurgery[2]. It is a powerful tool, because ALA induces protoporphyrin XI (PPIX) in the tumor tissue. PPIX is a direct precursor of heme and has two characteristic emission peaks at 635 and 704 nm due to its conjugated π-system. A method was developed to determine the PPIX levels in blood samples in order to investigate whether ALA-induced PPIX can be a biomarker for *high grade gliomas* (HGGs)[3]. It is based on the purification of blood samples by protein precipitation with acetonitrile (ACN) and subsequent separation and analysis by using high performance liquid chromatography (HPLC) coupled to an ion trap mass spectrometer used in MRM mode. The present method reduced the necessary starting volume for whole blood samples to 1/5 (200 µL) with respect to earlier efforts[4]. In addition, the total measurement time could be reduced from around 7 hours to only 10 minutes by switching from a capillary LC to a normal LC system with a higher flow rate. Consequently, PPIX eluted from the column more quickly. By using a Poroshell column with superficially porous, core-shell particles, moreover, tedious washing steps could be abandoned. Preliminary results indicate higher PPIX levels in whole blood of patients suffering from HGG, who received ALA prior to venipuncture, in comparison to healthy results. The detection limit of PPIX can be reduced by a factor of 100 by using a state-of-the-art triple-quadrupol instrumentation as shown in preliminary experiments. This will have the advantage that even smaller blood volumes will be required for analysis.

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A longitudinal study of human α_{S1}-casein content and its phosphorylation of breast-milk from two mothers during the first 7 months post-partum

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Autoantigen human α_{S1}-casein is the least studied breast-milk casein[1]. Unphosphorylated α_{S1}-casein was reported as TLR4-agonist[2,3]. These effects were abolished by phosphorylation of α_{S1}-casein (P-α_{S1}-casein)[3]. This suggested two roles of breast-milk α_{S1}-casein, an immune-active unphosphorylated and a nutritional phosphorylated one. Using targeted LC-MS with a Q-TOF instrument, we demonstrated that breast-milk of the first week *post-partum* contained between 3 and 540 µg/ml α_{S1}-casein [4] and phosphorylation-occupancy varied between mothers [5]. For further insights on the impact of α_{S1}-casein in immune system development, we evaluated the occurrence of unphosphorylated and phosphorylated α_{S1}-casein in breast-milk of two mothers (M1, M2) during the first 7 months *post-partum* using UPLC-MS^E. Concentration of α_{S1}-casein in breast-milk increased with time of lactation from day one until 7 months from 168 µg/ml to 272 µg/ml (M1) and from 137 µg/ml to 177 µg/ml (M2). In contrast to α_{S1}-casein, the total breast-milk protein content decreased with time of lactation. Furthermore, the phosphorylation of α_{S1}-casein decreased with time of lactation from day one to 7 months of lactation from 81% to 29% (M1) and from 62% to 47% (M2). Phosphorylation of all breast-milk protein was constant. In conclusion, this pilot study showed that concentration of TLR4-agonistic α_{S1}-casein increased with time of lactation, whereas content of phosphorylated α_{S1}-casein decreased. This lactation-time-related change in phosphorylation of breast-milk α_{S1}-casein suggests a difference in function during the infants' immune system development.

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LC-MS/MS based human biomonitoring to assess the individual mycotoxin exposure

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Mycotoxins are toxic secondary metabolites produced by various species of moulds infesting food and feed during production, processing and storage. As the naturally occurring contaminants are responsible for different health effects, the assessment of human mycotoxin exposure is of great importance. Mycotoxin exposure is often estimated on basis of data on average food contamination and average food consumption.
As differences in nutritional habits or the heterogenous distributions of mycotoxins in foodstuffs are difficult to monitor, the analysis of mycotoxins and their metabolites in physiological samples has gained increased acceptance in order to obtain exposure information at the individual level.
The major challenges in these biomarker-based approaches are low analyte concentrations and interfering matrix compounds, so that usually sample cleanup is necessary. Common approaches of sample preparation like solid-phase extraction or immunoaffinity cleanup allow to increase sensitivity and specificity but are very time consuming and therefore unsuitable for a high sample throughput. Furthermore, the broad structural diversity of all analytes cannot be covered.
The detection and quantitation of mycotoxins and their metabolites is enabled with modern techniques of liquid chromatography-tandem mass spectrometry (LC-MS/MS). At the Institute of Food Chemistry of the WWU Muenster, routine methods for the analysis of mycotoxins in physiological samples using simple and fast sample preparation protocols have been established. To that end, for the analysis of 27 mycotoxins and mycotoxin metabolites in human blood samples, a rapid multi-mycotoxin approach based on dried blood spots (DBS) and dried serum spots (DSS), has been developed. Samples are collected by heel, ear, or finger pricking as well as by spotting a defined volume of blood or serum onto filter paper followed by extraction [1].
For human urine samples two techniques of sample preparation are applied: A simple dilute and shoot (DaS)-approach with only centrifugation and dilution of the sample prior to HPLC-MS/MS analysis is used for the analysis of 23 mycotoxins and mycotoxin metabolites [2]. Recently, a new dried-urine-spot (DUS)-based method with additional enzymatic hydrolysis for the determination of 16 analytes of interest has been developed. The sample concentration, hydrolysis of mycotoxin metabolites and separation of matrix components result in an increased sensitivity of the DUS-method compared to the DaS-method, allowing for instance to monitor the average DON exposure in European consumers with higher confidence. The above-mentioned approaches were applied for the analysis of various sample cohorts to assess the individual human mycotoxin exposure.

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Profiling of small polar metabolites in yeast cells by means of capillary ion chromatography mass spectrometry

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Metabolomics is gaining an important role as a technique for the simultaneous identification and quantification of small molecules in biological systems. By monitoring analytes from pathways in the primary metabolism, the efficient functioning of the cells can be observed. Considering the anionic characteristics of these metabolites, it can be deduced that their analysis is a challenging task, since common chromatographic separation techniques such as reversed phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC) hyphenated to mass spectrometry (MS) have shown insufficient retention or selectivity for the small polar or charged analytes.
Especially for ionic metabolites the separation by capillary ion chromatography (Cap-IC) provides an appropriate alternative. Herein, the compounds are separated based on their chemical affinity to charged quaternary amine groups on the stationary phase and eluted by a potassium hydroxide gradient. By neutralizing the saline eluent with an electrochemical suppressor, the Cap-IC can be hyphenated to a mass spectrometer to immediately detect eluting analytes.
Through the application of this system for the analysis of twenty metabolites, which are included in glycolysis, pentose phosphate pathway or the citrate cycle, a separation and quantification was achieved using high-resolution MS applying negative electrospray ionization. The deprotonated molecular ions could be detected and used for the metabolite profiling of *Saccharomyces cerevisiae* extracts, which is also known as bakers yeast and one of the most utilized eukaryotic model organisms.

Neuroprotective effects of the novel specific NMDAR GluN2B subunit antagonist WMS – identifying potential central players during neuroinflammation

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Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system (CNS) and one of the major causes of long-term disability in young adults. Its pathophysiology is characterized by immune-mediated demyelination, chronic neurodegeneration as well as glutamate excitotoxicity. In the mediation of glutamate excitotoxicity, the Ca^{2+}-influx via N-Methyl-D-Aspartate (NMDA) glutamate receptors plays an important role: High levels of glutamate may cause excessive stimulation of these receptors leading to increased intracellular calcium levels and finally the activation of neuronal apoptotic cell death. However, since these receptors are also essentially contributing to learning and memory by enabling long-term potentiation (LTP) and long-term-depression, current treatment approaches targeting NMDA receptor activity show severe side effects.

In our study we are investigating the neuroprotective potential of the exclusive inhibition of the NMDA receptor subunit GluN2B by its specific antagonist WMS 14-10. WMS 14-10 was invented in the Department of Pharmaceutical and Medicinal Chemistry of the University of Muenster as a derivative of ifenprodil. Compared to ifenprodil, WMS shows a higher affinity, selectivity and half-life thereby increasing its therapeutic potential while decreasing possible adverse events. Liquid chromatography-mass spectrometry verified that WMS can cross the blood-brain barrier. In experimental autoimmune encephalomyelitis (EAE), the mouse model for multiple sclerosis, WMS treatment significantly ameliorated especially the later phase of the disease course and its remission. For the evaluation of potential side effects of WMS, we examined its effect on LTP via electrophysiological measurements of murine hippocampal brain slices *ex vivo*. Excitatory postsynaptic potentials were mildly reduced by WMS.

Since inflammation of the CNS leads to the destruction of myelin-forming oligodendrocytes, astrogliosis and neuronal damage, we are further focusing on elucidating the roles and intercellular interactions of the most prominent CNS resident cells and their influence by WMS. While fluorescence-activated cell sorting at disease maximum as well as flow cytometric bead arrays of stimulated microglia *in vitro* showed reduced microglial activation and cytokine secretion upon WMS treatment, peripheral cells were not affected. We also analyzed the Influence of WMS on glutamate excitotoxicity and microglia-mediated neuronal cell death by the use of different apoptosis assays *in vitro*. Here, WMS protected neurons from glutamate-induced cell death.

In conclusion, our results show that the specific inhibition of the NMDAR subunit GluN2B has a promising neuroprotective potential. Microglia are known to play a pivotal role in inflammatory neurological diseases. The significant effect of GluN2B inhibition in these cells may indicate a new pathway during neuroinflammation. Currently we are complementing our data with functional network analyses of oligodendrocytes, microglia, neurons and astrocytes using single-cell proteomics and transcriptomics. Our aim is to dissect the underlying mechanisms and the potential roles of these cells.

Bioimaging of heavy metal hyperaccumulating plants by means of LA-ICP-MS

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In recent years, bioimaging of animal or human tissue has become an important analytical procedure to answer biological and medicinal problems, and well-established protocols have been generated. For plants, however, only few such protocols are available.
Laser ablation inductive coupled plasma mass spectrometry (LA-ICP-MS) is a powerful method to uncover the lateral distribution of elements among a sample. Using a laser, the sample is ablated and turned into a fine aerosol. Via gas flow, the sample is introduced into the ICP-MS system where it is ionized and measured. By screening the sample and enabling time-dependent acquisition for each line, an image is generated. It contains information about the lateral localization and absolute intensity of all elements measured.
In this work, freshly harvested leaves were glued onto a sample plate and analyzed via LA-ICP-MS. Due to the heterogenic thickness and topology of the samples, the instrumental parametrization proved to be much more complex compared to evenly cut thin slices that are usually measured via LA-ICP-MS. Increasing the energy of the laser led to blockage of the transfer lines, while energies set too low did not ablate properly. Additionally, since the leaves were not dried, complete adherence to the sample plate was of utmost importance. Any motion or detachment of the sample would disturb the lateral accuracy and lead to loss of local information.
Using the biopolymer hydroxyethyl cellulose (HEC), the leaves could be glued onto the sample plate as flat as possible. By focusing the laser beam on topologically lower regions of the sample, superjacent regions could still be ablated to a satisfying level. Thus, images generated in this study were able to show the distribution of naturally abundant as well as artificially added elements in the samples up to spot sizes of 50 µm.

The impact of immunoglobulin G1 Fc sialylation on backbone amide H/D exchange

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The usefulness of higher-order structural information provided by hydrogen/deuterium exchange-mass spectrometry (H/DX-MS) for the structural impact analyses of chemical and posttranslational antibody modifications has been demonstrated in various studies. We here present results demonstrating the applicability of the H/DX-MS technique to monitor conformational changes of specific Fc glycosylation variants produced by in vitro glyco-engineering technology. A trend towards less H/DX in Fc Cγ2 domain segments correlating with larger glycan structures could be confirmed. Furthermore, significant deuterium uptake differences and corresponding binding properties to Fc receptors between α-2,3- and α-2,6-sialylated Fc glycosylation variants were verified at sensitive levels.