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The Design of Selected Reaction Monitoring Method based on Empirical Spectra Library of Synthetic Peptides for Higher Sensitive Measurements

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Selected reaction monitoring (SRM) known as multiple reaction monitoring (MRM) is a highly sensitive and selective mass spectrometry technique for peptide quantification in proteomics. In MRM-based proteomics quantification, four steps are
required to design any MRM assay; the selection of candidate proteins, the identification of representative peptides, the best selection of MRM transitions pairs (precursor/fragment ion), and the optimization of the instrument parameters, in particular collision energy (CE). In general, there are three ways to select transitions in MRM assays: (i) from shotgun proteomics experiments, (ii) from proteomics MRM databases or (iii) by generating spectral libraries from synthetic peptides. While with shotgun experiments and MRM database the building of MRM assays are straightforward, the optimization of MRM methods with synthetic standards peptides can be time intensive. In the present work, the selection of the optimal MRM transitions (selectivity and sensitivity) and the tuning of instrument parameters were performed using an automated workflow based on synthetic peptides, considering the acquisition parameters (i.e. charge state, CE) and resulting in the selection of most favorable transitions set. One hundred ninety three isotopically labeled (`heavy`) peptides were investigated on a triple quadrupole linear ion trap (QTRAP 5500, AB SCIEX) individually by flow injection analysis (FIA). MS2 spectra were acquired by ramping the CE voltage from 10 to 70 V (steps of 2 V) for precursor ions charge states of 2+/3+ in most cases, and 4+/5+ for longer peptides, taking into account the number of basic residues in the peptide sequence using a batch builder script on analyst 1.6.2 (AB SCIEX). The predominant charge state of each peptide, fragment ion assignments, and CE profiles generation were obtained using in-house developed java-based software (SRMoptimizer). In SRMOptimizer software, transitions and their optimized CE values were generated from MS/MS spectra library for 193 synthetic peptides, in which transitions were selected taking three most intense b and y ions. In order to compare the transitions selection in this approach with other tool, we generate transitions list for all peptides from SRMAtlas (HumanPublic_2012_02). Results show that 60% of similarity between the two sets of transitions was observed, taking into account the difference in the CE values due to the way of calculating or optimizing these values using SRMAtlas or SRMOptimizer software, respectively. To assess the relative effect of optimizing MRM transition by this approach in quantitative manner, the relative intensities of the two sets of transitions were compared. This approach shows about 2- 10 fold higher in the relative intensity, in particular for these peptides with low signal response. Moreover, the optimization of all fragment ions in the MS/MS library will help users to replace non-selective transitions in their MRM assay without having to reacquiring data.

HRMS dereplication, spectral networks and small molecule epigenetic modifiers: tools to decipher cryptic metabolic pathways in fungal microorganisms

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Introduction: The interest of microorganisms as a valuable source of bioactive compounds needs no more justifications. Since the discovery of Flemming's penicillin G from Penicillium notatum at the beginnings of the XXth century to the isolation of the proteasome inhibitor salinosporamide A from Salinospora tropica in 2003, numerous valuable biologically active metabolites have been isolated from microorganisms. Recent insights from the progress in genetics have shed a new light on the biosynthesis of microbial natural products. It is now well known that under classical laboratory culture conditions, microorganisms only express a small proportion of their biosynthetic potential.[1] This phenomenon, known as gene cluster silencing, is very common and has been reported to occur in a vast range of living organisms.[2] Recently, new approaches aiming to address silenced biosynthetic pathways in eukaryotes have appeared. [3,4] One of our research interest is the de novo induction of secondary metabolites stimulated by the co-culture of diverse fungal strains [5,6]. In the present study, induction mechanisms are studied under a different perpective and it is hoped to gain new insights and deeper understanding of communication between fungi at the metabolite level.
Methods: In order to explore the hidden biosynthetic potential of filamentous fungi we used small molecule epigenetic modificators (EM) of various classes (HDACi, DNAMTi) on phylogenetically diverse fungal strains. A metabolomic approach implying UHPLC-HRMS analysis, semi-automated dereplication procedures, MS/MS spectral networks generation and multivariate data analysis was set up to detect the induction of novel metabolites and select promising responding fungal strains for further culture scale-up and in depth metabolite characterisation.
Results: This workflow allowed us to highlight the production of various secondary metabolites not detected in control conditions. The application of HR-MS/MS networking provided valuable information regarding structures of the induced features induced by epigenetic modifications. In particular, the MS/MS networks allowed to reveal that induced features were structurally closely related in one of the treated strain, thus indicating the probable unlocking of a common biosynthetic cluster.

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Hyphenation of SPRi and MALDI MS for Interaction Analysis

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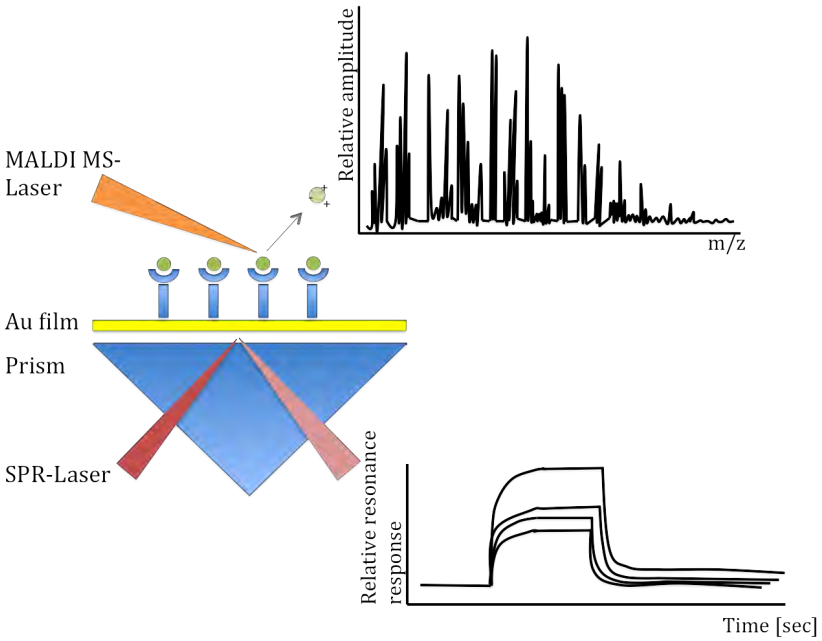
The investigation of new, high-throughput analytical techniques is important in clinical research and diagnostics, for more accurate and targeted determination of biomarkers. To analyze biomolecules in a label-free fashion surface plasmon resonance (SPR) is very suitable and it provides information on binding kinetics (k_{on}/k_{off}) and binding affinity in real time. Working in an array format provides rapid and high-throughput analysis of different interactions in parallel. The coupling of surface plasmon resonance imaging (SPRi) with matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) enables a multiplexed detection and quantification of binding by SPRi on one hand, and on the other hand, characterization of interacting ligands on the molecular level with MALDI MS. [1]



We are investigating the binding specificity of various DARPins for maltose binding protein (MBP). An important advantage of SPRi-MALDI MS is the possibility to measure direct on the plate with no need of elution of the bound analyte. With MS it is even feasible to differentiate between protein isoforms.

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MS-based isolation strategy for rapid targeted purification of antifungal compounds at the preparative scale

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Introduction: The targeted purification of bioactive molecules from complex extracts is of prime importance in the field of drug discovery from natural sources. In this respect, Mass Spectrometry (MS) detection is a key tool enabling the monitoring of specific features for precise fractionation.
Methods: To improve the isolation process efficiency of active natural products, an LC-MS-based purification strategy was developed. First, the chromatographic separation of crude extract was optimized by application of linear gradients at the analytical scale in HPLC-UV-MS. At-line microfractionation, followed by agar overlay bioautography using a *Candida albicans* hypersusceptible strain, was performed to identify the bioactive fractions [1]. The gradient was then geometrically transferred from the analytical to the preparative scale based on chromatography rules and calibration of both chromatographic systems [2]. Finally, an MS-triggered isolation of the localized antifungal compounds was realized with a Flash chromatographic system coupled, via splitter, to a single quadrupole mass spectrometer.
Results: This isolation strategy was applied for the MS-direct purification of the antifungal compound Diplophyllolide A from the Chinese liverwort Chiloscyphus Polyanthos (L.) Cord. The bioactive molecule was directly isolated in large amount, without issued related to MS saturation, thanks to the optimization of the MS splitting geometry. The use of a single quadrupole mass spectrometer coupled to a Flash/Prep LC system (PuriFlash® – MS) appears to be a promising tool for the precise MS-direct isolation of natural products from crude complex extracts.
Conclusions: This rational LC-MS-based methodology has high potential not only for the rapid purification and identification of bioactive NPs that lack of UV chromophore, but also for the isolation of biomarkers identified by UHPLC-MS metabolomics.

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Evaluation of Hadamard Transform Atmospheric Pressure Ion Mobility-ESI-MS for the rapid profiling of isomeric natural products

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Introduction: The efficient analytical profiling of molecules present in plant or fungal extracts represents a key element in natural product research. Commonly these complex extracts are characterized by highly chemodiverse secondary metabolites and by the presence of many multiple isomers. HPLC and UHPLC coupled with mass spectrometry are the most frequent methods used to analyze these types of samples but these techniques sometimes fail to resolve multiple isomers especially when used in a high throughput mode. In such cases, Ion Mobility Spectrometry (IMS) is an attractive alternative for the efficient separation of mixtures containing isomers, without increasing cycle time and with minimal added experimental complexity. In this study, the potential of High Resolution IMS-MS has been evaluated and compared to UHPLC-MS, for the analysis of closely related isomeric flavonoids. Moreover the applicability of IMS-MS has been demonstrated for the fingerprinting of complex mixtures, such as fungal co-culture and mono-culture extracts.

Results: UHPLC-MS and IMS-MS were investigated for the rapid analysis of closely related isomeric flavonoids and their glycosides. On a time scale of a few minutes, the flavonoid aglycones were all separated by ion mobility, but not by UHPLC. The glycosides were better resolved by IMS-MS, but not completely separated by both methods. The ion mobility resolving power was routinely 150, indicating that the system provides sufficient resolution for separation of isomeric natural products even in complex samples. Furthermore, HPLC-IMS-MS and UHPLC-MS were evaluated for the analysis of complex fungal co-culture and mono-culture extracts.

Conclusions: This study demonstrates that isomeric flavonoids can be better resolved by IMS-MS compared to UPLC-MS. Moreover, a significant number of compounds were detected by direct analysis of the fungal extracts using IMS-MS and were not detected by UHPLC-MS or MS only. These results suggest that high resolution IMS is well suited for the separation of isomeric natural compounds (even in high-throughput metabolomics studies) and appears as an attractive alternative to established UHPLC-MS methods.

Add-on Secondary Electrospray Ionizer for, delivering high ionization efficiency of vapors for the Analytical sector and for pre-existing API-M.

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Introduction: Despite the interest in the detection of volatiles by mass spectrometry, currently only two dedicated instruments for the task (SIFT PTR) exist. Notably, no commercial alternative exists for owners of a pre-existing MS with an atmospheric pressure ionization source to turn their instruments into a vapor analyzer.
Secondary ElectroSpray Ionization (SESI) in tandem with Atmospheric Pressure ionization Mass Spectrometry (API MS) has already shown sensitivities below the ppt level for polar vapors. It has been successfully used in different applications requiring the analysis of ambient vapors, with very promising results. In the security field of explosives detection, in which an extraordinary level of sensitivity and selectivity is required, an improved version of this technique (named Low Flow SESI) enabled, in tandem with a Differential Mobility Analyzer, the detection of explosives in cargo containers at the sub-ppq level.
In a collaborative project, we have joined efforts to further develop an Add-on Low Flow SESI platform to bring these improvements to the general MS user. Here, we present the first results of this development.

Methods: The new architecture utilizes the optimized electrostatic and fluid dynamic configuration of the LF-SESI, which enables high efficiency ionization, and is further improved to enable a fast coupling or disassembly to the API-MS, to operate safely at high temperature, and to be compliant with safety and work place regulations. The new ionizer will be coupled to AB Sciex, Thermo, and Waters API-MS instruments. It is composed of three main parts: (i) an interface, which is specific for each type of MS, (ii) a core, which incorporates the ionization chamber, and (iii) an external module, which incorporates the flow and temperature controllers. In the first stage of this development we have evaluated the expected ionization efficiency, and the transmission of ions to the MS.

Results: The mechanical limitations imposed by the MS are compatible with high transmission as long as the LF-SESI electrodes are sharp enough to avoid stagnation regions. The expected result of this development is a vapor ionizer which, due to its versatility, will allow the user to take full advantage of the high sensitivity of LF-SESI, and the fast evolving performances of the MS developped by the main vendors. The new ionizer, if compared with other vapor analyzers, which incorporate a built-in MS, will drastically reduce the acquisition costs for users who already own an API-MS (including the most commonly used LC-MS platforms).

Conclusions: According to our compatibility and transmission studies, the Add-on LF-SESI configuration for general MS is viable.

Novel Aspect: The new Add-on architecture enables high efficiency ionization and high ion transmission, and brings LF-SESI to the general MS user.

Exploring demultiplexing strategies for peptide identification in SWATH spectra: assessment of elution profile similarity

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Data-independent acquisition (DIA) offers several advantages over data-dependent acquisition (DDA) schemes for complex protein digests analyzed by LC-MS/MS: no bias toward high abundance peptides and reproducibility for identification as well as better sensitivity and accuracy for quantification. In SWATH acquisition, a resolving Q1 isolation window (e.g. 20-30u) is stepped repeatedly across an *m/z* range (typically 500-1500). High-resolution LC-MS maps of multiplexed fragment-ion chromatographic profiles are generated from all detectable peptides. At present, SWATH data is mainly processed based on MS information obtained from previous DDA/SRM analyses for peptide identification and quantification. The ultimate aim of this work is to develop an effective processing strategy to exploit SWATH data without using any prior MS peptide knowledge. In order to define an efficient combination which maximises the discrimination of multiplexed fragments from different precursors, we assessed the elution profile similarity of fragments from co-eluting peptides and compared a collection of similarity measures and smoothing algorithms.

We investigated two sample types: a reference mixture of 12 standard proteins and a real biological sample containing a complex protein mixture derived from cell extracts of primary human dendritic cells (hDCs) generated from peripheral blood monocytes. The biological sample was produced in the context of a project aiming to understand the role of hDCs in the coordination of innate and acquired immune responses during HIV-1 infection. This project is based on mass spectrometry and bioinformatics workflows applying a systems biology approach to unravel the host-pathogen response mechanisms by which DCs determine disease outcome. Protein extracts were digested with trypsin and analyzed with a 2D+ NanoLC-Ultra system (Eksigent) hyphenated to a TripleTOF 5600 mass spectrometer (AB Sciex). Data have been acquired in positive mode using both SWATH and DDA methods.

While for a simple peptide mixture spectra demultiplexing was possible, elution profile similarity was not sufficient for demultiplexing SWATH data obtained from the analysis of a relevant biological sample. We are working to find complementary strategies and combine them in a more sophisticated demultiplexing algorithm for peptide identification in SWATH data.

Metabolite screening in plasma based on SWATH data acquisition in UHPLC-MS/MS analysis combined with a high resolution metabolomics library

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Metabolomics studies commonly report changes in the occurrence of hundreds of observed metabolites. To understand the biological meaning of these changes, correct assignment of LC/MS peaks to the known metabolites is essential, however the identification of small molecules from LC/MS data remains a challenge. It ultimately relies on the comparison with reference standards that are often not easily obtainable for metabolites. The currently available MS/MS libraries are of varying quality and dependent on the instrumentation used for acquisition. Furthermore no information is available for relative MS response factors. We generated a MS metabolomic library for 600 metabolites reported in the human metabolome database (HMDB) taking into account MS response adducts, fragment annotation and retention times and used it for identification of metabolites in plasma. High resolution TOF MS and MS/MS spectra were acquired on a Triple TOF 5600 mass spectrometer in ESI positive and negative mode by flow injection analysis of each reference compound. The retention factors of the typically polar metabolites were determined by UHPLC with two RP C18 columns of medium and high polarity and a HILIC column with gradients based on different solvents at various pH values. The results were correlated with predictive retention times using a software package from ACD labs. Human plasma samples were analysed by UHPLC-MS using data independent acquisition (DIA) with SWATH (sequential window acquisition of all theoretical fragment ion spectra) acquisition mode. Metabolite assignments were performed by searching the annotated library and the LC retention time. Our MS metabolomic database includes the MS response for the various adducts such as $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, $[M+K]^+$, $[M-H]^-$, $[M+Cl]^-$ $[M+Na-2H]^-$, $[M+FA-H]^-$, $[M+AA-H]^-$, dimers and losses of H_2O, CO_2 and NH_3 based on TOF MS data. It also includes the observed MS/MS fragments and their structure assigned using a prototype software tool for fragment structure annotation as well as the retention factors determined by RP and HILIC chromatography. Retention times were predicted based on a linear regression between the retention times for a set of model compounds and their log D values. Accuracy was improved when the model compounds were selected based on structural similarities with the compound of interest. This library provides the basis for identifying and quantitating these metabolites in the LC-MS-SWATH data. An annotated high resolution library of human metabolites could be generated considering relative MS response factor and LC retention time. This library could be successfully used to perform metabolite identification in plasma for LC-SWATH MS data.

Analyzing Durable Anti-fungal Resistance Processes in Cerealsby Metabolomics Using UHPLC-HR-MS

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Fungal pathogens cause substantial losses of crop yield every year. Introduction of durable resistance genes in crops is an important strategy to prevent yield loss and to maintain food security. The resistance gene *Lr34* of wheat (*Triticum aestivum*), which is durably conferring resistance to four major fungal pathogens [1, 2], was cloned into barley (*Hordeum vulgare*). The molecular resistance mechanism of *Lr34*, which encodes for an ATP-binding cassette transporter [3], is not known yet.

The objective of our research is to identify the molecular nature of the transported molecule(s) and to understand the mechanisms of *Lr34*-mediated durable resistance.

An untargeted metabolomics approach [4] based on ultra-high performance liquid chromatography high-resolution mass spectrometry has been developed. Control plants were compared with transgenic lines for non-quantitative pattern recognition by statistical evaluation of LC-MS data.

Different classes of metabolites that accumulate to a different level in control and transgenic lines were detected in barley extracts. Characterization of these differential metabolites using accurate mass and MS/MS fragmentation in combination with *in silico* fragmentation and spectral databases will be presented.

A workflow for plant metabolomics based on UHPLC-HR-MS was established for crop tissue extracts and is applied to barley, rice, wheat and sorghum samples. The newly developed metabolomics method enables us to gain valuable insight into the resistance mechanism of this highly relevant wheat resistance gene.

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Proton homodecoupling with enhanced resolution and sensitivity

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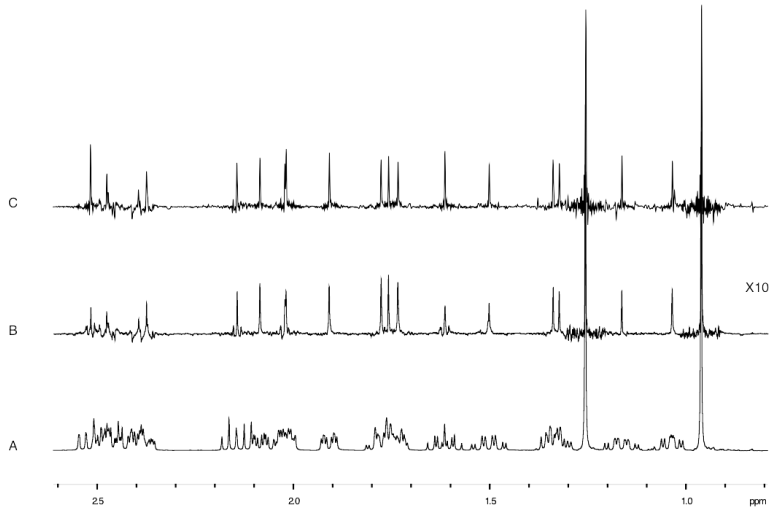
In proton NMR spectra, the coupling structures due to homonuclear scalar coupling often cause signal overlap making it difficult to exploit one- and two-dimensional spectra. The obtention of simplified spectra where multiplets are collapsed into singlets is therefore a quite interesting decades-old challenge [1-4]. In this study, we improved our approach based on spatial encoding and spectral aliasing to quickly obtain high-resolution 2D spectra leading to homodecoupled 1D spectra. The introduction of the Zangger-Sterk element [5] with spectral aliasing allows an enhancement of the spectral resolution and a reduction of the experimental time. The resulting spectra display resolved singlets even for protons 1 Hertz apart. We achieved a sensitivity enhancement of about one order of magnitude using frequency-modulated pulses.



(A) Classical 1D spectrum, (B) Homodecoupled 1D proton spectrum recorded using a non-modulated pulse and (C) Homodecoupled 1D proton spectrum recorded using a 10-frequency-modulated pulse.

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Confined Thin Layer Cyclic Voltammetry for Halide Detection

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Thin layer samples (less than 50 µm) allow one to establish exhaustive electrochemical processes, such as ion depletion, when either controlled current or potential techniques are used. This concept coupled with a perm-selective membrane and coulometric readout has recently explored in our group aiming to achieve calibration-free ion sensing platforms [1]. Both ions (K^+, Ca^{2+}) and polyions (protamine and heparin) were measured using this approach. Only a few microliters of sample are required to accomplish the ion determination, making this a promising analytical tool for clinical samples.

Similarly to the mentioned perm-selective membranes, cation-exchange permselective membranes (i.e, Nafion) are commonly used as efficient separators in several electrochemical sensors. In fact, the use of Nafion membranes for online coulometric desalination of seawater was recently reported by our group [2]. The electrochemical cell was based on a silver wire introduced inside of a tubular Nafion membrane. Using a similar strategy to prepare the electrochemical cell, we aim here to demonstrate the use of thin layer samples measured by cyclic voltammetry in order to analyze samples that contain chloride, bromide and iodine.

We describe the fabrication of a microfluidic electrochemical cell based on an Ag/AgI wire (working electrode) inserted in a tubular Nafion membrane, which limits the sample solution layer to about 20 µm. An Ag/AgI counter-reference electrode was also used. The cell is schematically illustrated in Fig. 1a. By increasing the applied potential, halide ions (X^-) present in the thin layer sample are electrodeposited at the working electrode as AgX while the cationic counterions are transported across the perm-selective membrane to the outer solution.

When thin layer cyclic voltammetry was applied to NaCl, NaBr or NaI using this device, each halide showed a reversible peak due to its electrodeposition at the working electrode surface. Calibration graphs for each halide showed a linear range over nearly four orders of magnitude in concentration. While these halides exhibit the same peak potential when they are analyzed separately, cyclic voltammograms showed a unique peak for each halide when they are analyzed together in mixed samples (see Fig. 1b). While this work aims at replacing argentometric titrations with a more direct analytical approach, it also forms a platform for future new approaches in ion detection.

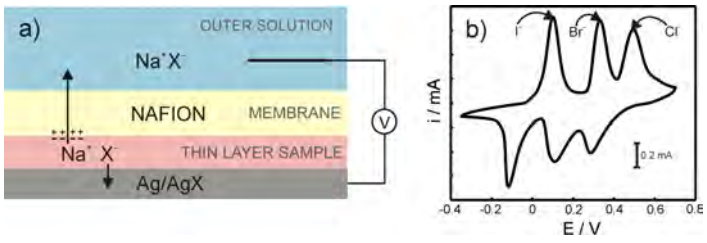

Fig. 1a) Scheme of the cell. **b)** Preliminary cyclic voltammogram of a mixture of 0.033 M NaCl, NaBr and NaI (scan rate = 10 mV/sec).

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Quantitation of Dystrophin in Quadriceps of Treated mdx Mice by LC-SRM/MS

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The *mdx* mouse is a model for Duchenne Muscular Dystrophy (DMD), a severe neuromuscular disorder caused by the absence of functional Dystrophin, a 427 kDa protein linking the cytoskeleton to the extracellular matrix. A promising novel therapeutic approach uses antisense-tricyclo-oligonucleotides (tc-DNA) to induce skipping of the mutated exon 23 in *mdx* mice, leading to restoration of the reading frame and expression of a shortened yet partially functional protein. Unlike other antisense-induced exon-skipping approaches, this treatment also shows improved dystrophin levels in cardiac muscle and in the brain [1]. The development of analogue therapeutics for human patients could lead to a significantly milder disease progression similar to Becker Muscular Dystrophy (BMD), prolonging and easing patients' lives.

LC-SRM/MS provides a sensitive approach to the identification and quantitation of tryptic peptides of a target protein in a complex mixture. Quantitation is achieved by means of stable isotope labelled standard peptides, which exhibit the same chromatographic behavior as their unlabeled counterparts but can be distinguished by mass spectrometry due to their larger mass. Once retention times and MS/MS fragmentation patterns of several tryptic peptides of the target protein have been determined, quantitation can be undertaken with minimal sample preparation prior to the analysis.

In this study, a method has been developed to isolate dystrophin from murine quadriceps samples of wild-type and treated *mdx* mice and to obtain quantitative information about the normal and restored dystrophin levels, respectively. In order to identify proteotypic peptides and determine their retention times on the chromatographic system, isolated dystrophin was digested with trypsin and analyzed by UPLC coupled to an ESI - LTQ Orbitrap mass spectrometer, providing high resolution spectra enabling accurate mass assignment and determination of suitable precursor-product ion transitions. Quantitation was performed on an ABSciex QTrap 5500 LC-MS/MS system using the established transition list to monitor the selected peptides and their beforehand spiked-in isotope-labeled analogues in SRM mode.

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Rapid and sensitive analysis of proteins with CE-SDS-LIF: mass spectrometric characterization of fluorescent labeled proteins

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Capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) with laser induced fluorescence detection (LIF) after derivatization with a fluorescent tag achieves silver stain sensitivity in protein analysis.[1,2] At last year's SCS Fall Meeting we demonstrated CE-SDS-LIF detection limits of 10 ng/ml for BSA through labeling with the fluorogenic reagent 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ) in the presence of the nucleophile CN^- under optimized reaction conditions.[3]

FQ reacts with the primary amine groups in proteins, but not all lysines in a protein are labeled.[2] In order to estimate protein quantities in unknown protein mixtures, comparable to with SDS-PAGE silver staining procedures, the labeling reaction must be better understood. To test different quantification models *e.g.* based on protein concentration or number of lysines, the labeling of over 20 different model proteins with MS is studied. These proteins range in molecular weight from 6 kDa to 150 kDa and have a wide range of lysine/molecular weight ratios. ESI-QTOF-MS is used to determine the number of labels on each protein in both reduced and unreduced forms. The MS data are compared to the signal obtained in CE-SDS-LIF and used to test different models for protein quantification.

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Quantification of La in $CaMnO_3$ by ICPMS for Analysis of PLD Films

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The perovskite-structured compound $La_{1-x}Ca_xMnO_3$ exhibits interesting magnetoresistant properties, depending on what exact stoichiometry it has when formed into thin films via pulsed laser deposition (PLD) [1]. Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) is one of the most efficient and suited techniques for the quantification of the raw material and any thin film derivatives thereof. However, when applying the commonly applied single point calibration method using NIST SRMs, discrepancies in the calculated stoichiometry of up to 12% were observed when comparing the ablation methods line scanning and single hole drilling. Furthermore, the calculated stoichiometries were not accurate, i.e. the sum of lanthanum and calcium was greater than 1, deviating therefrom up to 20%.

Therefore, raw material was digested and analyzed by solution nebulization to gain detailed information about the stoichiometry of the original material used for thin film production. In addition, various quantification procedures based on solution calibration for laser aerosol quantification was applied to determine the La concentration. The results of these analyses of $La_{1-x}Ca_xMnO_3$ analyzed by different technique and under different conditions, will be compared and discussed.

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Tandem mass spectrometric elucidation of the higher-order structure of sugar-modified nucleic acid duplexes

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Great potential lies in antisense oligonucleotides, especially in sugar-modified DNAs, for the treatment of gene-related diseases. Tricyclo-DNA (tcDNA) for instance, a nucleic acid analogue harbouring a three-membered ring system instead of the ribose, was shown to reinstate partial functionality of muscles in mice affected by muscular dystrophy. The structural modification of the sugar-moiety is designed to promote high biostability of the analogue and to increase the selectivity for the mRNA target.

Though a crucial factor for a drug's stability *in vivo*, resistance to enzymatic degradation poses a tremendous challenge to biochemical sequencing of antisense oligonucleotides. Mass spectrometry meets the resulting demand for alternative sequencing methods ideally, because the technique is largely unaffected by structural modifications of the analyte. Sequence coverage of a fully modified tcDNA 15mer could be obtained in a single tandem mass spectrometric experiment.

In addition to resolving the primary structure, mass spectrometric characterization provides insights into the higher-order nucleic acid structure. The presented data on tcDNA:DNA and tcDNA:RNA hybrid duplexes confirm the selectivity of tcDNA for complementary RNA over DNA that had previously been put forward by differences in the melting temperatures of similar duplexes. The gas-phase dissociation pathways of hybrid duplexes include strand separation, backbone cleavage of the single strands as well as ejection of nucleobases and backbone fragments from the precursor, which leads to the detection of truncated duplex ions.

Method development for analysis of (oxygenised) volatile organic compounds in ambient air

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Volatile organic compounds (VOCs) and oxygenised VOCs are important tropospheric trace gases. They are released to the atmosphere by anthropogenic, as well as biogenic sources and comprise a large variety of compounds (from C_2-C_{16}), including alkanes, alkenes, alkynes, aromatic compounds, terpenoids, alcohols, and aldehydes. The mole fractions of these compounds vary between a few ppt (parts per trillion) and tens of ppb (parts per billion) in background and urban environments, respectively. The VOCs and OVOCs have multiple roles in atmospheric chemistry. Of main interest is their contribution to photochemical processes, such as the generation of photo-oxidants, their impact on oxidizing capacity of the atmosphere, and their contribution to secondary organic aerosol production. For this reason, reliable measurements of VOCs and OVOCs are essential and are included in long-term monitoring programmes, such as Global Atmosphere Watch and European Monitoring and Evaluation Programme. In general, OVOCs are more difficult to analyse than VOCs due to their polarity and associated sorption to surfaces, and to their higher affinity to water. Therefore, these compounds are scarcely reported and efforts are done to measure them more frequently and precisely. A promising new analytical concept to measure both VOCs and OVOCs with a gas chromatograph (GC) coupled to two flame ionisation detectors (FID) was developed by Hopkins et al. [1, 2]. We further improved and validated this online GC-FID/FID method. Prior to analysis, ambient air samples are drawn through a condensation trap to remove moisture from the samples, then pre-concentrated on an adsorbent trap, and thermally desorbed to the GC-FID/FID. The main improvements to existing methods include: the optimisation of the condensation trap temperature, the addition of a further chromatography column, the use of a coated capillary column as restrictor, the use of a Deans switch between the two columns (no spilt injection onto two columns), and the optimisation of the GC-temperature program. With this current method, 34 VOCs and 24 OVOCs can be routinely analysed in ambient air samples in one single GC-run. The detection limits for all compounds are in the lower ppt range (10 – 20 ppt). The system is now employed routinely at the station Zürich-Kaserne of the Swiss National Air Pollution Monitoring Network (NABEL).

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Unraveling the requirements for immortality – Description of the alternative lengthening of telomeres type I cell phenotype using microarrays for mass spectrometry.

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Single-cell level mass spectrometry plays an important role in identifying cell-to-cell heterogeneity.[1,2] Cell-to-cell variability is a natural occurring characteristic that manifests itself in all organisms and leads to the development of different cellular phenotypes even in an isogenic cell population.[1,2] Such variation can have medical relevance; as it is the case of cells that undergo a mechanism known as alternative lengthening of telomeres (ALT).[3,4] ALT cell phenotypes are able to achieve unlimited cellular proliferation by overcoming the shortening of telomeres associated with DNA replication, and account for 10-15% of cancer phenotypes.[3]

The current hypothesis for the ALT mechanism is based on stochastic epigenetic switches,[4] thus a metabolomic analysis at the single-cell level can provide new insight not available with any other available analytical mass spectrometry method.[1] A novel method for matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS), called microarrays for mass spectrometry (MAMS), has allowed to achieve sensitivity for single-cell metabolite detection.[2]

Here, using MAMS technology, the metabolic profile of *saccharomyces cerevisiae* wild-type (wt) and telomerase defective cells (ΔTLC1 Type I ALT survivors) was monitored. Our preliminary results demonstrate that MAMS technology offers the unique potential to establish mechanistic links between cellular central metabolism and the requirements for telomere maintenance based on an ALT mechanism.

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Investigation of Primaquine Metabolism and its Effects on the Metabolomic Distribution of Hepatocytes Using a Dedicated LC/MS Platform Including Automated Bligh Dyer Extraction

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Metabolomics is an increasing research field dealing with the investigation of all cellular metabolites, thus, endogenous as well as exogenous low molecular weight compounds, and their varying concentrations upon system stimuli. Targeted and non-targeted liquid chromatography (LC) mass spectrometry (MS)-based techniques are commonly applied to plasma, serum or urine samples for identification and quantification of up- or down-regulated compounds. However, the potential biomarkers searched for and revealed by such approaches may differ from those in the target organ (e.g. liver) if remaining instead of being excreted. Hence, a dedicated LC/MS platform was developed implementing dual-column separation and automated Bligh Dyer extraction to characterize the metabolome of hepatocyte cells. Its changes upon exposure to primaquine were monitored and a potential link to hepatic drug toxicity was investigated.

Hepatocyte samples were first analyzed without adding stress-inducing factors, later treated with different concentrations of the anti-malarial drug primaquine. Cell samples were flash-frozen and mechanically lyzed by cryogenic grinding with glass beads. For extraction of metabolites the Bligh Dyer approach was performed manually or automatically with a PAL RTC system (CTC Analytics). LC/MS analysis of the recovered extracts was achieved via dual-column separation injecting aqueous fractions on a C18 and organic fractions on a C8 column, both coupled to a TripleTOF 5600 (AB Sciex) operated in positive or negative ESI with periodical TOF autocalibration. For MS/MS data, SWATH mode (“sequential window acquisition of all theoretical fragment-ion spectra”) was applied allowing for the integration of qualitative and quantitative analysis (QUAL/QUAN).

Hepatocytes represent a suitable model for studying the hepatic metabolome at an original state as they still contain the various cell organelles comprising exogenous metabolites and endogenous compounds. Different cell concentrations were tested to enable the analysis of a majority of cellular metabolites simultaneously. The hepatocytes were then incubated with primaquine using selected drug concentrations (low µM to
upper pM range) and incubation time points. It was aimed at maintaining the detection of endogenous metabolites while preventing signal superimposition by primaquine and/or related exogenous metabolites. The resulting high resolution (HR) MS2 spectra from acquisition in SWATH mode were evaluated using multivariate statistical tools in combination with database search. Next to commonly known platforms like HMDB and MassBank, an in-house created library was additionally exploited for a more reliable validation of potential hits. Hence, the effects of primaquine on the hepatocellular metabolome distribution can readily be assessed.

Metabolomic changes were considered at cell stage in hepatocytes using a dedicated workflow based on Bligh Dyer extraction and LC/HR-MS in SWATH mode.

Pattern-Based Sensing of Aminoglycosides

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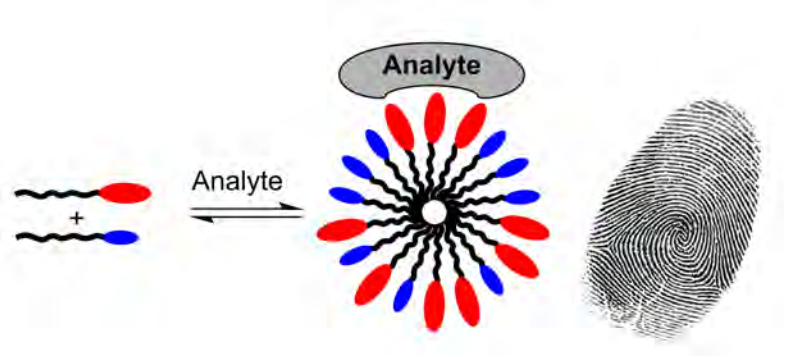
Aminoglycosidesare antibacterialtherapeutic agents that inhibit protein synthesis and contain an amino-modifiedglycoside(sugar) as a portion of the molecule.[1] .Here, we describe a conceptually new 'one-cuvette' sensing system for the pattern-based analysis of aminoglycosides.[2] A mixture of two amphiphiles with fluorescent head groups can be used as a sensing ensemble for the pattern-based analysis of aminoglycoside antibiotics. In buffered aqueous solution, the amphiphiles form a dynamic mixture of micellar aggregates. In the presence of aminoglycosides, the relative amount and the composition of the micelles are modified. The re-equilibration of the system is analyte-specific, and characteristic fluorescence spectra are obtained for different aminoglycosides. Accurate differentiation in the low micromolar concentration range can be achieved by a principal component analysis of the spectral data



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Probing localized chemical phases in thin film solar cells

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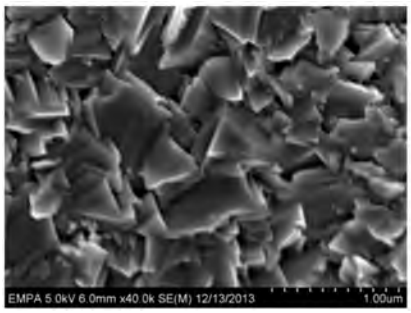
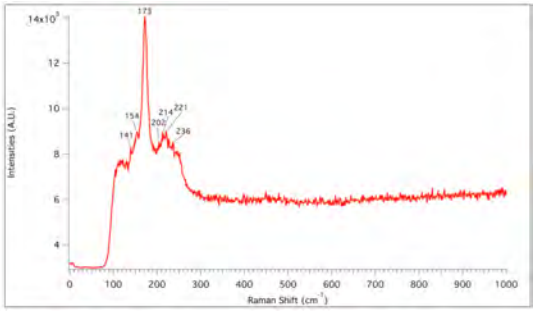
The efficiency of solar cells is of paramount importance for becoming cost-effective alternatives for electricity production. Thin film solar cells are particularly interesting due to much less amount of materials required for bulk solar cell designs, comparable efficiency to polycrystalline silicon (up to 20%)[1] and the possibility to be deposited onto flexible substrates. Among the most recently developed thin film solar cells, it was shown that chemical modification of the absorber layer led to new record efficiency devices. Hence CdTe devices with controlled Cu doping on metal foil (13.6%)[2] and CIGS devices with addition of potassium after the CIGS layer growth (20.4%)[3] are the focus of our study.

Solar cells are fabricated by depositing several thin layers of material onto a substrate. For better performance of the solar cells, one has to learn more about the defects within the layers, especially in the absorber material and at its interfaces. Since understanding the compositional inhomogeneity is crucial for high conversion efficiency, the challenges arise in analyzing these materials on the nanometer to micrometer scale.

We are using tip-enhanced Raman spectroscopy (TERS) to investigate the nanoscale spatial distribution of various phases within the layers. In CdTe solar cells, we probe diffusion and distribution of dopants and the chemical formula of resulting phases after Cu treatment that form at grain boundaries and/or at the interface between layers. Besides, potassium-induced surface modification of CIGS leads to a significant reduction in Na content in the bulk of the absorber layer and a strong depletion of Cu and Ga in the CIGS surface region[3]. We will examine the potential presence of the known ordered vacancy compounds (OVCs)[4] confined in the interface regions of CIGS and at the grain boundaries. With TERS, we cannot only obtain the spatial topography (fig. 1), but also chemical compound information at the same time (fig. 2)[5].





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Scanning Carbonate Samples for Radiocarbon Content with Laser Ablation Coupled to Accelerator Mass Spectrometry

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Laser ablation (LA) is a powerful sampling technique, which allows the removal of small quantities of material from a solid sample and its subsequent online analysis [1]. High intensity laser pulses focused on carbonate samples generate carbon dioxide that can directly be introduced into the gas ion source (GIS) of an Accelerator Mass Spectrometer (AMS) [2]. This new technique [3] allows rapid radiocarbon analyses at high spatial resolution. This is especially useful in the case of carbonate records, such as speleothems and corals, as conventional measurements are laborious and the achievable spatial resolution may not be sufficient.

A dedicated LA unit for feeding the gas ion source of an AMS was developed. An ArF-excimer laser (λ = 193 nm) is guided to the sample surface in an ablation cell and allows for ablation at a scale of less than 100 µm. Large samples (up to 150 x 25 x 15 mm3) are hosted in the ablation cell and can be moved precisely relative to the laser beam.

The applicability of this sampling technique has been tested on pressed carbonate powder reference materials and marble. Measurements of natural samples such as corals and stalagmites will be presented.

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RASPPberry, an automated sample preparation platform

Inken Plitzko[1]

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The development of automated laboratory workflows steadily replacing (semi-) manual methods generates several benefits - among them increasing throughput, cost savings, and less exposition to potentially harmful substances. Sample preparation with respect to NMR, even though being the most tedious and error prone part of analytical procedures, unfortunately has so far not been in the focus of this trend.

A major impediment in the past was to load third party devices, such as readers and centrifuges etc. on one deck, controlled by one operating software.

We here present RASPPberry (**R**oche **A**utomated **S**ample preparation **P**latform), an in house developed system to prepare three analytical samples (NMR, GC-MS, LC-MS) from one delivery sample.

Common challenges as solubility issues, liquid handling and (de-)capping of different container types are resolved by a setup employing third party components as well as in house developed elements, running on a SILA-based operating software. Perfectly integrated in the information flow from the electronic lab notebook of the sample provider to the instrument software and to the AWM LIMS system, in which submissions and finally the results are filed, this system ensures reliable tracking of samples and data at all time and reduces the intervention needed by the operator to a minimum.

Quantification of bufadienolides in Bryophyllum pinnatum leaves and manufactured products by UHPLC-ESI-MS/MS

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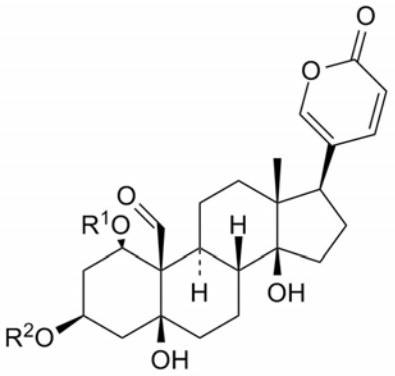
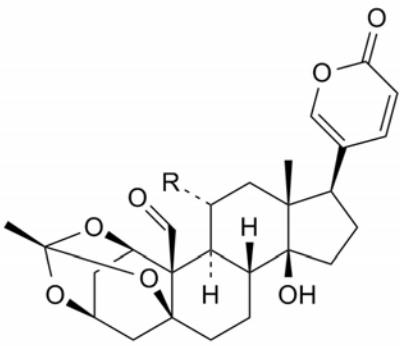
Bryophyllum pinnatum (Crassulaceae) is a succulent perennial plant native to Madagascar. It is used in anthroposophical medicine to treat psychiatric disorders, and as a tocolytic agent to prevent premature labour [1]. Besides flavonoids, the plant is known to contain bufadienolides, which reportedly possess sedative and positive inotropic properties, as well as central nervous system related activities [2].

In this context, a UHPLC-ESI-MS/MS method has been validated and used for quantification of the main bufadienolides, bersaldegenin-1-acetate (**1**), bersaldegenin-3-acetate (**2**), bryophyllin A (**3**) and bersaldegenin-1,3,5-orthoacetate (**4**) in leaves and manufactured products. Separation was performed on a Kinetex XB-C18 column with a gradient of MeCN (0.05% HCOOH) in H_2O containing 10 mM ammonium formate and 0.05% HCOOH. Leaves were extracted with EtOH by accelerated solvent extraction (ASE). For press juice used as active ingredient in anthroposophical products liquid/liquid extraction with EtOAc was applied. The contents of **1**-**4** were 1.45, 4.89, 7.75, and 5.17 mg/100 g dry weight (DW), resp., in leaves from plants grown in Brazil. The contents of these four bufadienolides were significantly lower in plants grown in Germany (0.99, 2.08, 1.18, and 0.94 mg/100 g DW, resp.). The total amount of bufadienolides was 1.71 and 0.59 mg/100 ml in press juices obtained from plants cultivated in Brazil and Germany, resp. This study provides for the first time reliable data on the content of bufadienolides in *B. pinnatum*. Data from ongoing studies designed to investigate the influence of harvest time on the bufadienolide content will be also presented.



1 R^1 = Ac, R^2 = H
2 R^1 = H, R^2 = Ac



3 R = OH
4 R = H

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At-line quantitative monitoring of the production of recombinant his-tagged proteins using fluorescence polarization

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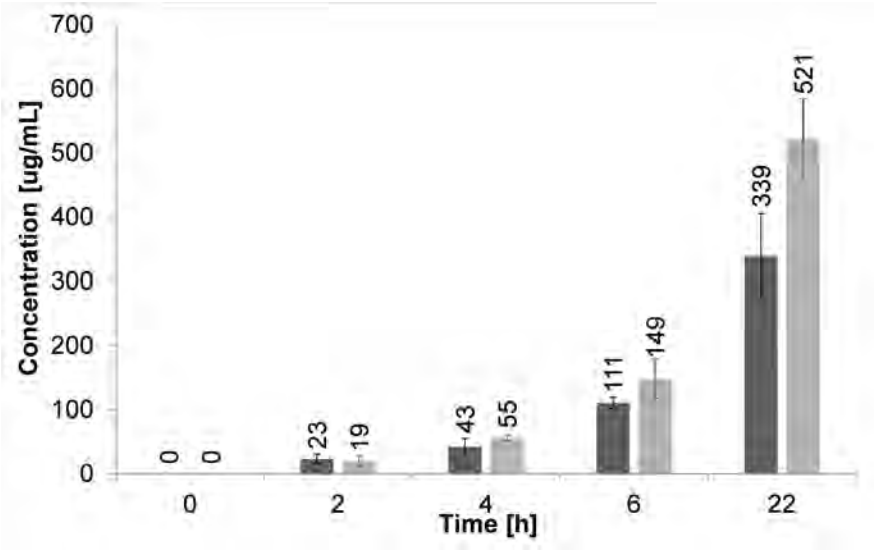
[1]University of Applied Sciences Western Switzerland Valais, [2]School of Engineering and Architecture of Fribourg

Nowadays, biotechnological productions are monitored using indirect parameters such as the optical density. The recombinant protein product itself is usually not quantified because current assays such as ELISA or Western Blotting are time consuming and labor intensive. The availability of rapid quantification assays could offer new means for the optimization of bioprocesses and the continual monitoring of production and downstream processing.

Here we present a novel quantification method based on fluorescence polarization and characterized by its rapidity (typically less than 20 min.) and ease of use (it only requires mixing of solutions). It makes use of fluorescent Ni-NTA moieties which specifically bind to His-tags with concomitant enhancement of the fluorescence anisotropy. The assay offers a large quantification range of more than a decade, a low limit of detection of 0.3 mg/ml, high reproducibility and good correlation with Western Blotting.



Qualitative and quantitative monitoring of the production of His-tagged proteins during fermentations could be performed « at-line ». Furthermore, affinity purification of the recombinant products could be monitored allowing rapid determination of the product-containing fractions. Finally, a proof of principle that the assay could be automatized demonstrated the feasibility of online monitoring of bioprocesses.

Optimized strategy for an efficient Normal Phase MS-targeted isolation of natural products

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[1]University of Geneva

The improvements of analytical techniques and methodological tools play an important role for the characterization and isolation of bioactive secondary metabolites in natural product research. Reversed phase liquid chromatography MS (RP-LC-MS) is widely used for the metabolite profiling of complex natural extracts and start to be more and more used for targeted MS isolation of biomarkers. Normal phase chromatography (NP-LC) is well suited for the purification of apolar secondary metabolites offering also some advantages compared to RP like low operating pressures and cheapest stationary phases. The potential of NP-LC-APCI-MS for metabolite purification at preparative scale using generic separation methods has been investigated on medium pressure preparative chromatography system (PuriFlash® - MS). A mixture of representative apolar natural product standards was chosen and analyzed under normal phase conditions. All parameters were carefully optimized for both separation and detection (gradient system, split rate, flow rate, temperature, Inj. Volume, column length, ionization source parameters). A special care was taken to find ionization and splitting conditions and that provide good detection and preclude source contamination. A linear gradient was applied at the analytical scale to enhance the separation of the molecules considered. The analytical gradient was then geometrically transferred to flash chromatography following a gradient transfer method after the calibration of the chromatographic systems [1].
An efficient, fast and generic approach for the MS targeted isolation of apolar natural products was obtained. MS detection in complement to UV enabled the monitoring of NPs with weak chromophores and the selectivity of MS was of great helps for a precise collection of partially coeluting compounds. APCI-MS detection with optimized splitting and post-column elution of appropriate solvent was found robust and well-suited for this purification.

The LC method transfer from analytical to flash level represent an innovative strategy for a rational isolation of specific biomarkers or bio-active compounds based on metabolite profiling results. The MS-triggered fractionation in addition to standard UV detection is a powerful tool to precisely orient the isolation. This rational approach can be widely used for direct purifications and isolation of synthetic and natural mixtures moreover the separation performed at preparative scale allows to purify tens to hundreds mg of compounds for further structural identification of bioactivity characterization studies.

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Excess Electron Transfer Through Phenanthrenyl Base Pairs Within DNA

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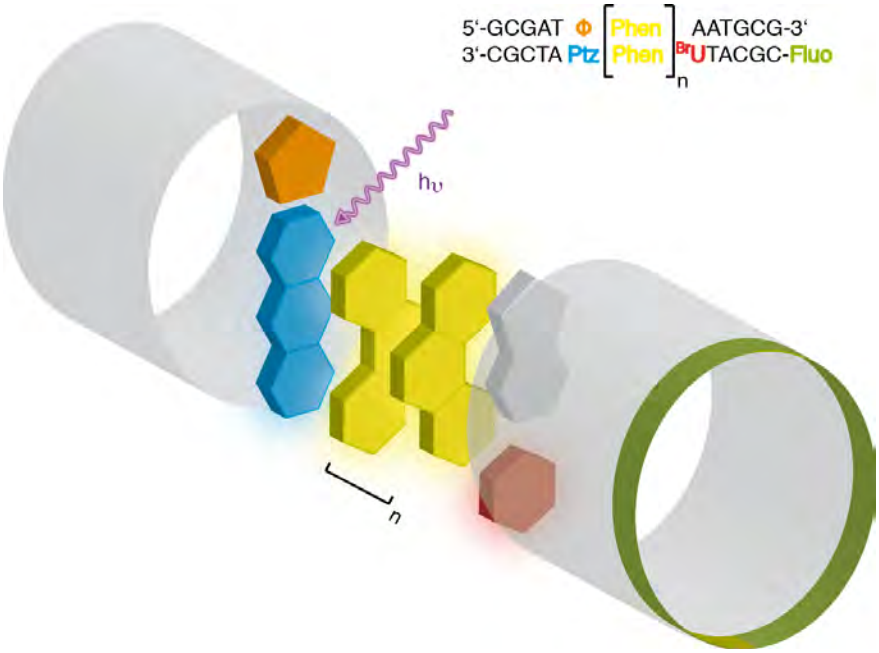
Excess electron transfer (EET) through DNA attracted considerable interest in fundamental research and found applications in biotechnology for nanoelectric sensors [1], [2]. We previously demonstrated successfully EET through a π-stacked phenanthrenyl (Phen) base pair in a DNA duplex containing 5-(pyren-1-yl)uridine (PyU) as an electron injector and BrU [3]. The weak redox potential of the injector compared to phenanthrene allowed only a superexchange electron transfer, which excludes an electron migration over a longer distance [4]. In order to test long range EET within DNA containing multiple phenanthrenyl residues, a donor with a suitable redox potential was used. The electron transfer showed enhanced efficiency by increasing the number of phenanthrenyl base-pairs [5].



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Nucleoside phosphate monitoring in mammalian cell fed-batch cultivation using quantitative matrix–assisted laser desorption/ionization time-of-flight mass spectrometry

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Monitoring intracellular metabolites in industrial fed batch cultures is used to document batch reproducibility and performance. Moreover metabolite profiles are very valid in screening experiments to investigate different fed batch conditions. We describe a novel high-throughput method based on matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (TOF-MS) for monitoring intracellular metabolite levels in fed-batch cultures. Currentmethods for monitoring multiple intracellular metabolite levels in parallel are limited in sample throughput capabilities and analyte selectivity. The MALDI-TOF-MS method presented here is based on a new microarray sample target and allows the detection of phosphorylated metabolites using stable isotope labeled internal standards. With short sample preparation steps and thus high sample throughput capabilities, the method is suitable for monitoring mammalian cell cultures in industrial environments. The method is capable of reducing the runtime of standard LC-UV methods to approximately 1 minute per sample (including 10 technical replicates). Its performance is demonstrated in an 8-day monitoring experiment of independently controlled fed-batches, containing an antibody producing mouse hybridoma cell culture. The monitoring profiles clearly confirmed differences between cultivation. Hypothermia and hyperosmolarity were studied in four bioreactors, where hypothermia was found to have a positive effect on the longevity of the cell culture, whereas hyperosmolarity lead to an arrest of cell proliferation. The results are in good agreement with HPLC-UV cross validation experiments. Subsequent principal component analysis (PCA) clearly separates the different bioreactor conditions based on the measured mass spectral profiles and highlights the usefulness of the dataset for statistical analysis.

Zoom feature for a chemical microscope based on tip-enhanced Raman spectroscopy

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We introduce the concept of a zoom feature for a chemical microscope based on tip-enhanced Raman spectroscopy (TERS), which combines scanning probe microscopy (SPM) and Raman spectroscopy. The proposed algorithm bridges the gap between micro- and nano-Raman imaging.

Confocal Raman spectroscopy allows mapping of the chemical composition of surfaces with a spatial resolution of approx. 500 nm, which is limited by diffraction. Tip-enhanced Raman mapping brings the resolution down to approx. 20 nm – yet the technique is restricted to small areas: the grid of pixels should be very dense, to avoid undersampling. Thus, large surface features may be investigated only with the diffraction-limited Raman microscopy, whereas small features can be studied only with the high-resolution, small-area Raman nanoscopy. Here, we propose an idea for bridging the gap between micro- and nano-Raman spectroscopies, in terms of the spatial resolution and the size of the investigated area.

In our approach, the investigated region is divided into an array of square pixels, and an averaged TER spectrum is acquired over each pixel. Every single TER spectrum is integrated while scanning the enhancing tip over the entire area of the pixel. The size of the pixel (and consequently, the image resolution) may be tuned in a broad range from 1 nm up to 10 mm. Thus, one may firstly identify a region with an elevated surface concentration of a compound of interest, and later on zoom on it, by reducing the scan range and the pixel size. In other words, one trades the spatial resolution for the scan range, like in optical microscopy or classical SPM.

The zoom feature is a keystone in the development of a convenient TERS-based chemical microscope for surface science.

^{1}H HR-MAS NMR based metabolic profiling of cells in response to treatment with a hexacationic Ruthenium complex

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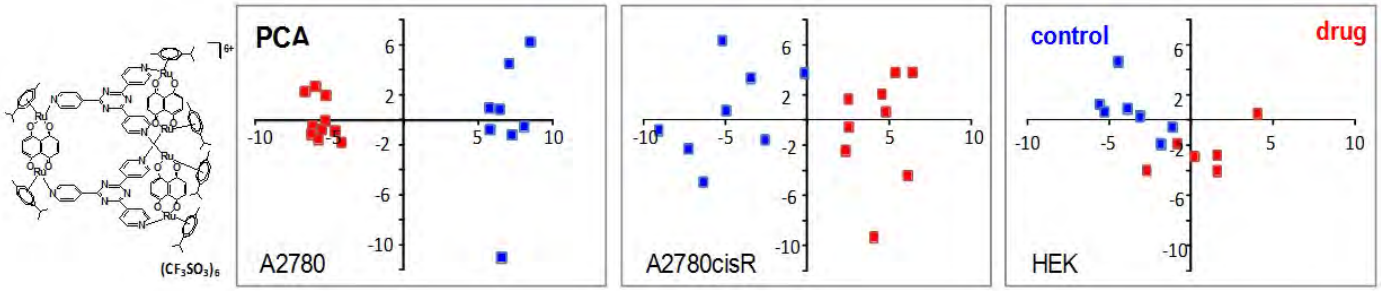
A water soluble hexacationic Ruthenium complex [(*p*-cymene)$_6$Ru$_6$(tpt)$_2$(dhnq)$_3$](CF$_3$SO$_3$)$_6$ with tri-pyridyl-triazine (tpt) and dihydroxy-naphthoquinone (dhnq) as bridging ligands was prepared and tested for its anticancer activity and interaction with potential biological targets [1]. The complex was found to be highly cytotoxic against human ovarian carcinoma cells (A2780) with an IC_{50} value of 0.45 mM.To learn more about the specificity and the mechanism of action, the effect of the complex on the metabolic profile of three different human cell lines was studied by High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy. HR-MAS NMR allows obtaining well resolved ^{1}H NMR spectra from living cell suspensions [2] well suited for chemometric analyses.
Cisplatin-sensitive and –resistant cancer cells (A2780 and A2780cisR) as well as human embryonic kidney cells (HEK-293) as healthy model cells were each incubated with the Ru-complex for 24 h and 72 h, respectively. The corresponding cell suspensions were submitted to HR-MAS NMR yielding a total of 104 ^{1}H NMR spectra of control and drug treated samples. Multivariate statistical analysis (PCA and PLS) of the spectra indicated clear metabolic changes between control and drug-treated cells for all 3 cell lines, as shown in the Figure for t_{incub} = 24h. The changes were most pronounced for A2780 cancer cells mainly due to lipids and choline containing compounds indicating potential drug-induced membrane breakdown. The single components responsible for the discrimination between all control and drug treated groups are discussed in more detail in this presentation.



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Fluorescent Sol based Optical Ammonia Gas Sensor

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This study focuses on the development of an optical ammonia gas sensor whose sensing mechanism is based on Förster resonance energy transfer (FRET) between selected dyes. As a model system for a fiber with an ammonia sensitive cladding, dye doped ormosil (organically modified silicates) sols were solvent casted on poly(methyl methacrylate) (PMMA) substrates, dried and exposed to gaseous ammonia. A logarithmic decrease of the coumarin donor fluorescence intensity with increasing gaseous ammonia concentrations was observed for FRET systems consisting of coumarin and fluorescein dye pairs (Fig. 1). This decrease was logarithmic from 40 – 40’000 ppm ammonia, which is a wide range. Such systems containing fluorescein as the donor dye and rhodamin B as the acceptor dye showed a logarithmic increase in the acceptor emission.

In both systems, the fluorescein operates as the main sensing dye species. The formation of the dianionic form lead to an increase of absorption and fluorescence intensity finally resulting in a stronger spectral overlap between involved dye pairs (Fig. 2). The enhanced FRET activity is responsible for the increase or decrease of the fluorescence emission intensity. The implementation of the FRET system resulted not only in a benefit regarding the logarithmic response but also in the signal change intensity in comparison to a single dye. Flow cell gas measurements demonstrated a sensitive and reversible response to gaseous ammonia. To improve porosity and response time, we are currently working on integrating FRET dye pairs in silicate particles. The ultimate goal is the development of a gas sensor system based on flexible polymer optical fibers, which finally can be stitched into textiles. This would significantly increase sensor wearability.

All Solid State Membrane Electrodes Based on Ferrocene Functionalized PVC

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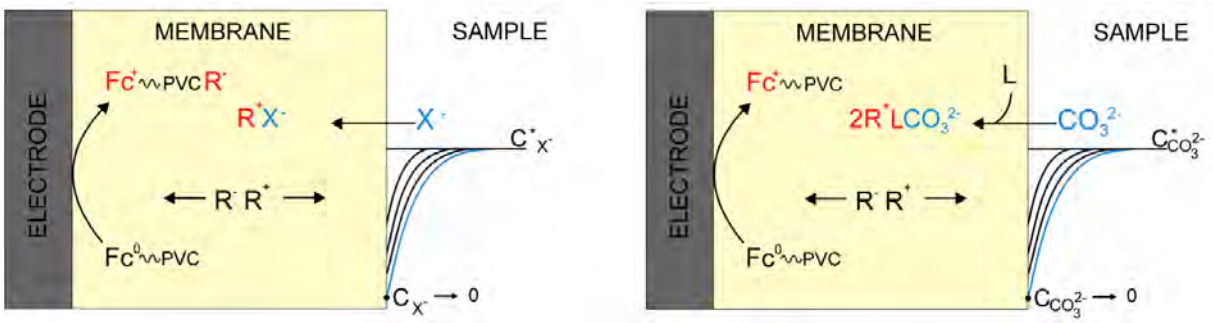
Solid contact ion-to-electron transducers have been extensively studied during the last two decades and most of them displayed excellent analytical characteristics in non-zero current measurements as well as in dynamic electrochemistry. Improvements were demonstrated when the inner solution was replaced by a solid transducer in ion-selective membrane electrodes. Solid contact technology has introduced a new generation of ion sensors for applications that cannot be easily achieved with traditional sensor designs.

The membranes based on Fc functionalized PVC allows one to operate the membrane in a chronopotentiometric sensing mode. The membrane contains reduced form of Fc-PVC, plasticizer DOS and ETH 500 (R^+R^-) and it is considered as initially non-perm-selective membrane. Fc plays role of the ion-to-electron transducer. Oxidation of Fc during galvanostatic pulse provokes a defined anion flux into the membrane and the ion depletion is observed at the membrane-sample interface (see Scheme 1). Different anions are depleted following Hofmeister selectivity pattern in absence of ionophore. If the membrane contains an ionophore, the Hofmeister selectivity pattern is modified, and the analyte of choice can be explored. [1-2] We provide here a comparative study between traditional ion-selective and all-solid-state membranes electrodes and also we demonstrate how the ionophore concentration levels modified the linear range of the sensor.



Scheme 1. Membrane mechanisms of initially non-permselective membrane.

Based on this work, we now aim to develop sensors for detection of polyions such as heparin (-70) and protamine (+21) for use in clinical analysis.[3]

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Influence of the target plate material and sample layer thickness on LDI ionization efficiency for C60

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Introduction

In order to establish the validity of models thus far proposed for matrix-assisted laser desorption/ionization (MALDI) models, such as disproportionation, the “lucky survivor” model, cluster formation and exciton pooling, a basic LDI approach, based on variable thickness electrospray deposition of C_{60} (stable and ionizable in both negative and positive mode) on a range of different target plate materials, was chosen as a starting point. The ion yield for pulsed ion extraction, at different time intervals after pulsed laser irradiation, was monitored by commercial MALDI-TOF MS instruments (Shimadzu AXIMA; Bruker Ultraflex III) for a range of different laser fluences. The presence of one or more ion yield maxima at different time intervals could be an indication for the number of ionization mechanisms contributing to LDI and the time frames in which they operate. Varying the target plate material and sample layer thickness can shed light on the sample influence itself and the interaction with the target plate material during ion formation in both negative and positive ionization mode.

Methods

An automated sample deposition setup, capable of high-throughput electrospray deposition, was developed. Standard MALDI target plates were milled out to be subsequently fitted with target insets made of a variety of different materials, among them a range of metals, alloys and insulators.

C_{60} was deposited on the MALDI target insets and was subsequently analyzed using a range of different laser fluences and delayed extraction times in positive and negative reflectron mode. The resulting spectra were processed in an automated fashion by means of a MATLAB script, yielding an intensity profile accompanied by a standard deviation plot.

Results

Preliminary results for the positive ionization mode suggest the presence of two maxima for copper and aluminum targets, whereas stainless steel insets just show a single maximum ion yield. Insulated sample insets provide higher ionization yields in positive mode and all targets tested thus far in negative mode have shown a lower ion yield compared with positive mode.

Conclusions

The possible presence of two ion yield maxima for copper and aluminum insets, seems to indicate more than one ionization step is involved. The results also suggest that the heat insulating properties of the insulating insets could affect the ion production efficiency in positive mode.

Ion-selective nanospheres as novel reagents in complexometric titrations

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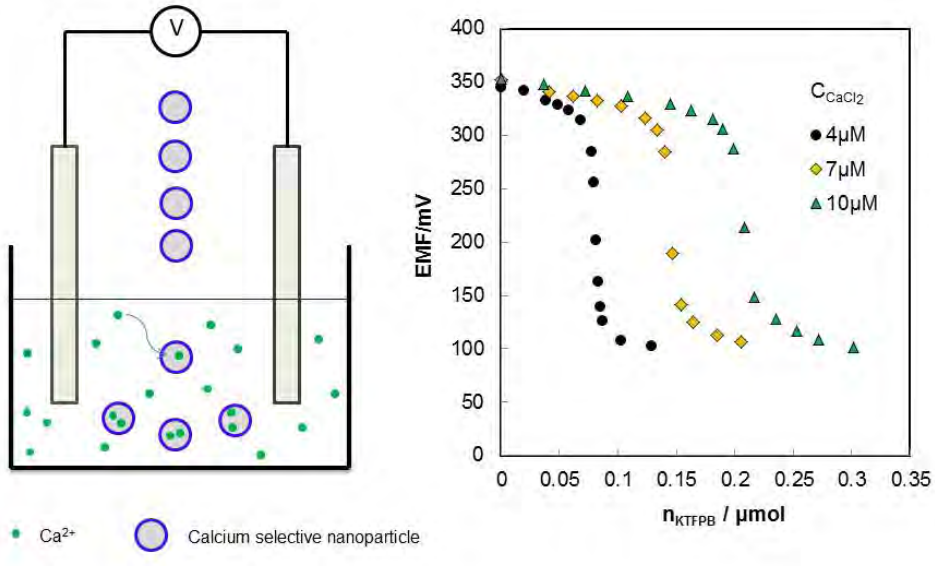
Conventional potentiometric titrations are performed with complexing agents to incrementally change the concentration of free metal ions in solution. In these applications, ion-selective electrodes are often used as endpoint detectors. The ability of the complexing agent to strongly interact with the analyte is of key importance. Most complexing agents are organic chelators, but their selectivity and working pH range are often rather limited. For example, calcium titrations with EDTA only work at a pH above 10.

We demonstrate here for the first time that a suspension of polymeric nanospheres loaded with ion selective *lipophilic* complexing agents (ionophores) can be used as a novel class of reagents in complexometric titrations. Such ionophores are routinely used in ion sensors and exhibit binding constants that are often very high, for divalent ions up to 25 orders of magnitude. Two examples are shown using Ca^{2+} and Pb^{2+} as the target ions. The ion-selective nanoparticles contain ion exchanger, plasticizer, surfactant Pluronic F-127, and ionophores selective for calcium or lead(II), are easy to prepare, and exhibit lifetimes of many weeks. The nanospheres work on the basis of ion exchange, where the initial counterion embedded in the particles is efficiently displaced by the target ion. Using such nanoparticles, one can titrate Ca^{2+} and Pb^{2+} accurately with errorrs better than ±3%. Compared to an EDTA titration of Ca^{2+}, sharper transition curves were obtained when calcium selective nanoparticles were used, and there is no need to buffer the pH. For the titration of lead(II), we demonstrate the titration of 10^{-7} M lead(II), which is of environmental relevance. The principle can easily be expanded to other ions by employing different ionophores.



Application of SWATH acquisition method to the mass spectrometry-based proteomics study of monocyte-derived dendritic cells

Ying Zhang[1], Dario Bottinelli[1], Aivett Bilbao[1], Bandar Alghanem[1], Caterina Strambio De Castillia[2], Emmanuel Varesio[1], Gérard Hopfgartner[1] *

[1]University of Geneva, [2]University of Massachusetts

The aim of mass spectrometry-based proteomics studies on monocyte-derived dendritic cells (MDDCs) is to support the understanding of their immune responses from a molecular point of view. Unlike the data-dependent acquisition (DDA) approach for MS/MS analysis which selects in real time precursors for fragmentation,Sequential Windowed acquisition of All THeoretical ions (SWATH) acquisition method collects fragment ion spectra for all the precursors contained in predetermined isolation windows over a defined *m/z* range and the whole chromatographic elution range. Preliminary data showed that the reproducibility of the quantitative result obtained by SWATH method is higher than DDA method with an average CV of 10% *v.s.* 21%, respectively,for five repeated measurements.

To apply SWATH method to the proteomics study of MDDCs, a spectral library containing 2312 protein groups and 18401 distinct peptides was constructed based on the result of the 2D-LC-MS/MS analysis of the pooled sample. According to the distribution of these peptide precursor ions within different *m/z* range, variable isolation window widths were designed for the subsequent SWATH analysis with the aim to equalize the number of precursor ions to be fragmented in each window, which meant smaller window width for more condensed *m/z* range and wider window for the opposite situation. Compared with the results obtained by SWATH analysis with fixed window width, this approach gave an average of more than 20% more confident peptide identifications. Quantitative proteomics analysis of the MDDCs after lipopolysaccharide (LPS) treatment revealed a total number of 84 proteins which were differentially expressed with more than two-fold difference compared to the control. Functional analysis of these proteins indicated that most of them were involved in the metabolic process, immune system process and response to stimulus.

Instrumentino: An open-source modular Python framework for controlling Arduino based experimental instruments

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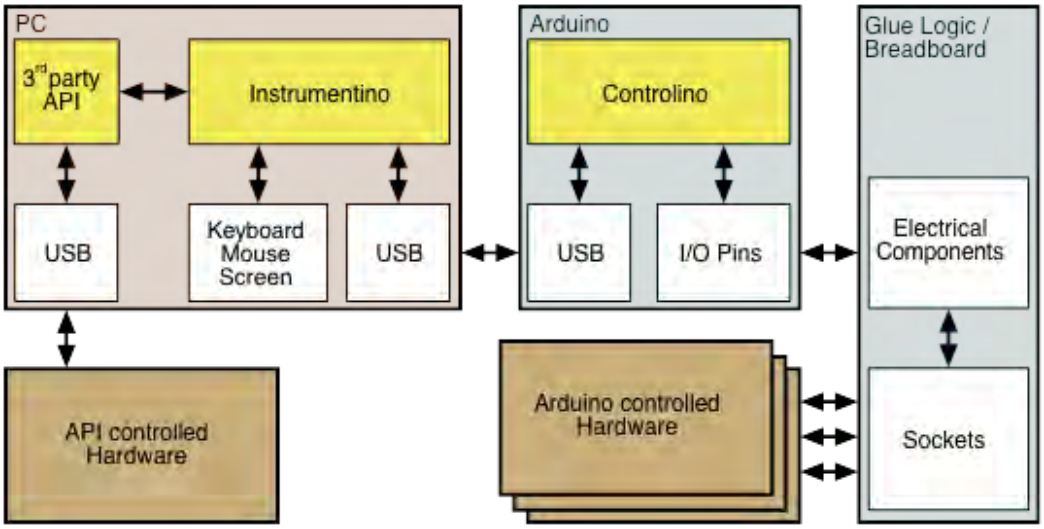
Instrumentino is an open-source modular graphical user interface framework for controlling Arduino based experimental instruments. It expands the control capability of Arduino by allowing instruments builders to easily create a custom user interface program running on an attached personal computer. It enables the definition of operation sequences and their automated running without user intervention. Acquired experimental data and a usage log are automatically saved on the computer for further processing. The use of the programming language Python also allows easy extension. Complex devices, which are difficult to control using an Arduino, may be integrated as well by incorporating third party application programming interfaces into the *Instrumentino* framework.



In this presentation, the *Instrumentino* [1] infrastructure will be explained and the benefits of its usage will be discussed. A series of example systems in the field of analytical chemistry, already using *Instrumentino*, will be presented as well.

The main goal is to expose the *Instrumentino* freamework for Swiss researchers and promote future collaborations.

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Evaluation of a high performance ion mobility-MS platform for structural measurements in different drift gases combined with computational strategies

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Introduction

Ion mobility spectrometry coupled to mass spectrometry (IM-MS) is becoming a valuable tool in analyzing complex mixtures as well as studying structures of biological compounds. Uniform-field drift tube instruments can be used to directly calculate collision cross section (CCS) values for gas-phase ions. A recently developed IM-QTOF instrument is modified to allow the use of different drift gasses in the system. The ability to use different drift gases in a low pressure uniform-field drift tube instrument adds another dimension to the capabilities of this instrument in complex sample analysis. This study will include the CCS calculations for different classes of molecules using different drift gases and a comparison between theoretical and experimental CCS data.

Methods

A modified high resolution IM-QTOF (Agilent Technologies, 6560 IM-QTOF) instrument was used to perform the experiments described here. Electrospray generated ions were introduce to the IM-QTOF system using an Agilent Jet Stream ESI source. Different drift gases were introduced to the drift tube using an inlet on the drift manifold towards the entrance of the drift cell. The instrument was modified to maintain a positive pressure in the drift tube to facilitate pure drift gas inside the drift cell. Pressure in the drift tube and high pressure funnel were regulated using flow regulators. Pressure measurements in the IM system were done using capacitance manometers. The structural interpretation of CCS values measured were examined using refined molecular dynamics simulation strategies.

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