CHIMIA

CHIMIA 2015, Volume 69
ISSN 0009-4293
www. chimia.ch

SCS
Swiss Chemical Society

Supplementa to Issue 7-8/2015

SCS Fall Meeting 2015

Poster Abstracts

Session of Analytical Sciences

September 4, 2015
Ecole Polytechnique Fédérale de Lausanne (EPFL)
http://scg.ch/fallmeeting/2015

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Investigating the binding sites of RAPTA-C and RAPTA-EA to a 50 amino acid peptide using ETD fragmentation and ChemInfo algorithms

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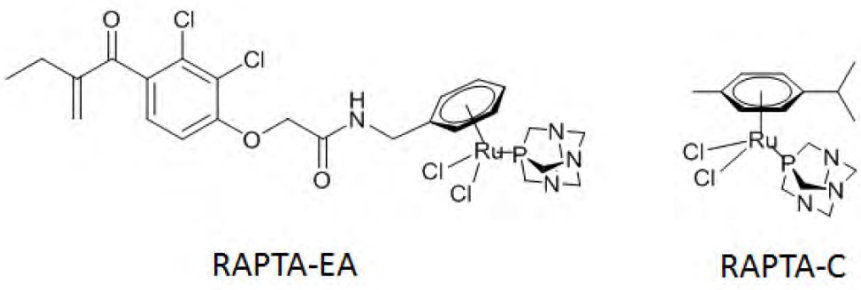
Tandem mass spectrometry (MS/MS) particularly using electron transfer dissociation (ETD) have shown tremendous utility in studying drug-protein interactions, especially in probing binding sites and protein conformation changes [1,2]. However, studying the protein binding of metal drugs is difficult, as the isotopic pattern of the metal coupled with the labile nature of the ligands surrounding the metal center greatly increases the complexity of the mass spectra obtained. Here, the binding of two ruthenium (II) organometallic anti-cancer complexes, RAPTA-C and RAPTA-EA (Graphics 1) to a model 50 amino acid peptide was investigated.



High resolution ETD MS/MS data was obtained on a hybrid linear ion trap (LTQ) Orbitrap Elite FT mass spectrometer coupled to a Triversa chip-based electrospray ionization system. Putative drug binding sites were determined with an in-house script [3] based on http://www.chemcalc.org which matched experimental to theoretical MS/MS data and assigned probability scores to different adducts formed with their corresponding c and z ions. Through simultaneous variation of the elemental composition of the ligands including theoretical losses of labile moieties during fragmentation and considering a number of proton and charge additions or defects, theoretical fragments were simulated alongside scores based on the full isotopic distribution similarities. ESI-FTMS mass spectra exhibited many different metal complex:peptide adducts that could be assigned with high similarity. Analysis of the ETD mass spectra of such complexes is challenging and the script developed has been of great help to interpret such complex data and assign the fragment ions observed to deduce its binding sites.

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Nano-SIMS to study the distribution of metal based anti-cancer compounds in vitro

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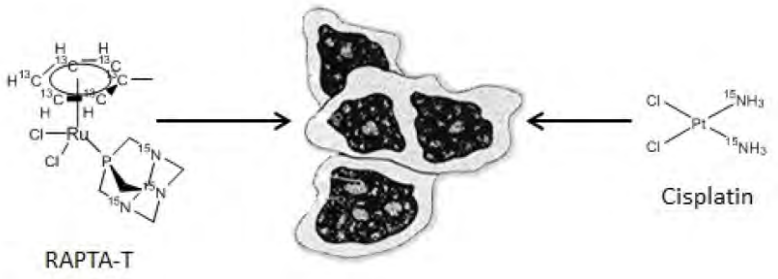
A nano secondary ion mass spectrometry (Nano-SIMS) method for the analysis of platinum and ruthenium based metal anti-cancer compounds was developed and utilized to study the distribution of cisplatin (a DNA targetting platinum anti-cancer drug) and RAPTA-T (an anti-metastatic ruthenium anti-cancer drug) in ovarian cancer cells. This method allows label free detection for metal drugs, which could be augmented by isotopic labelling of ligands to study the ligand binding state in biological systems. Here we discuss some recent findings and highlight future directions to improve upon the technique.



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A Universal Low-Flow Secondary ElectroSpray Ionizer: High Sensitivity Volatile Analysis on Pre-existing MS Instruments

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Secondary ElectroSpray Ionization (SESI) in tandem with Atmospheric Pressure ionization Mass Spectrometry (API MS) has achieved sensitivities in the sub-ppt range for polar vapors. Low-Flow SESI (LF-SESI), was developed in tandem with a Differential Mobility Analyzer, and in explosive detection applications reached sensitivities at the sub-ppq range. However, it is only available for dedicated applications. We therefore developed an add-on Universal Low-Flow SESI which can be coupled to a pre-existing MS.

The Universal LF-SESI was compared against a classical SESI. Both were coupled to an Orbitrap mass spectrometer. A constant amount of diethylamine (DEA) was injected through a vapor generator into the N_2 sample flow which fed the ionizers. Signal levels were recorded varying the N_2 sample flow rate.

The efficiency of the new ionizer was found to be one order of magnitude higher compared to the classical SESI source and the sample flow could be reduced by a factor of five (Figure 1a). We also characterized the performance of the Universal LF-SESI for some drugs of interest (Figure 1b).

We conclude that the Universal LF-SESI has superior performance compared to the classical SESI, allowing to improve sensitivity in volatile analysis.

We also present preliminary results from volatile analysis by using the new ionizer, showing the versatility of this development.

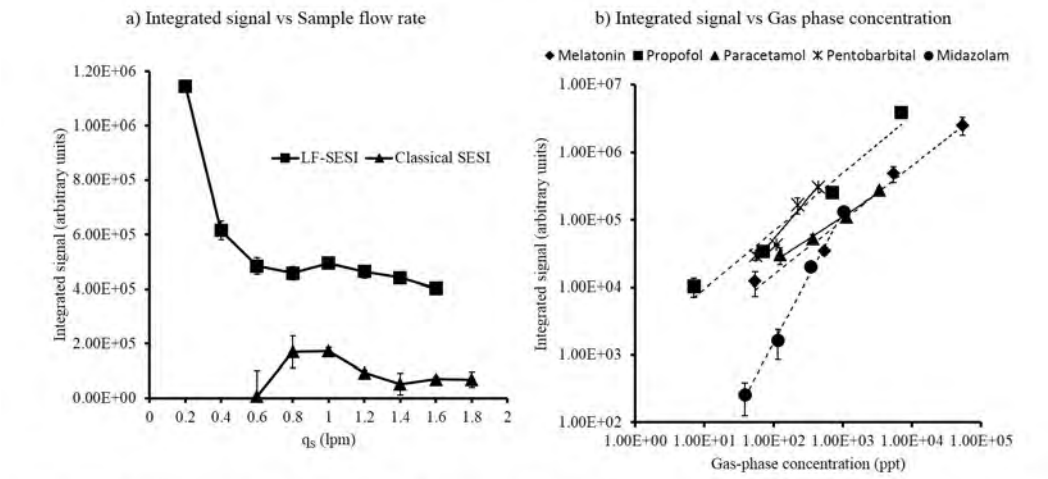

Figure 1. a) Experimental performance of the LF-SESI and the Classical SESI varying N_2 sample flow rate; b) Examples of drugs detected with the Universal LF-SESI

Characterization of a 2D Polymer Monolayer with Tip-Enhanced Raman Spectroscopy

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A 2D polymer (2DP) is a monomolecular covalent network of periodically linked monomers [1]. 2DPs are particularly interesting in surface and materials science, owing to their intriguing properties (separation, coating, sensing), which are distinct from those of bulk polymeric materials.

Powerful analytical methods such as high-resolution transmission electron microscopy, scanning probe microscopy, X-ray diffraction, solid-state nuclear magnetic resonance spectroscopy, infrared spectroscopy, and Raman spectroscopy are being used for analysis of periodic (crystalline) materials. However, they are normally not suitable for the analysis of monolayer [2], because they do not provide enough contrast.

Tip-enhanced Raman spectroscopy (TERS) combines scanning probe microscopy (SPM) with Raman spectroscopy, and allows one to simultaneously record the topography and a chemical fingerprint of samples with nanoscale resolution [2, 3]. Moreover, TERS has been shown to have a sensitivity down to the single molecule level [4], which is vital for obtaining Raman spectra from monolayer polymer sheets.
Here, we investigate covalent monolayers deposited on atomically flat gold by using TERS with scanning tunneling microscope (STM) feedback to establish whether or not they qualify as 2DP. The sheets are synthesized from rigid aromatic amine and aldehyde building blocks through dynamic imine chemistry [5] at a water/air interface by the Langmuir–Blodgett method. Taking advantage of the high sensitivity and high spatial resolution of STM-TERS, we explore formation and dissociation of chemical bonds within the monolayer at the nanoscale. Furthermore, utilizing density functional theory (DFT) calculations, we simulate theoretical Raman spectra of both monomers and the target 2DP.

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Target plate material resistivity influences LDI efficiency

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In order to establish the validity of models thus far proposed for matrix-assisted laser desorption/ionization (MALDI), such as disproportionation ionization[1], the “lucky survivor” model[2], cluster formation[3] and exciton pooling[4], a basic LDI approach, based on electrospray deposition of C_{60} (stable, clear fragmentation pattern and ionizable in both negative and positive mode) on a wide range of target materials, with respect to their heat conductivity and electrical resistivity, was chosen as a starting point. The ion yield was monitored in both positive and negative polarity on a commercial MALDI-TOF MS instrument (Bruker Autoflex, nitrogen laser, λ: 337 nm) varying both the laser fluence (0 – 3.53 Jcm^{-2}, Δ 10%) and ion extraction delay time (0 – 950 ns, Δ 50 ns).
$C_{60}$was electrosprayed with an automated, custom-made setup with sliding Teflon® masks, preventing cross contamination. 10 x 10 spots with a 3 mm Ø were made on the insets to be tested, which were subsequently fitted into a milled-out MALDI sample target plate, whilst ensuring equal surface height. Per sample spot and condition, 3 x 250 spectra were averaged and processed in an automated fashion (MATLAB): integrating the spectra over a 360-725 m/z range, averaging the resulting areas under the curve for repeating experiments and finally generating a signal intensity profile (see Figure 1) accompanied by a standard deviation plot.
Our results indicate that there is an ion yield difference between positive and negative mode, but this difference becomes smaller for materials with increasing electrical resistivity(copper ‹ stainless steel ‹ inconel 625®), especially during the first couple of hundred nanoseconds. This in turn seems to suggest that the resistivity is a key factor in the ion formation step. The initial charge separation is either facilitated, or the generated ions are not effectively neutralized at the target surface. Using more resistive target plate materials for (MA)LDI would therefore significantly increase the ion yield and therewith its analytical capacity.

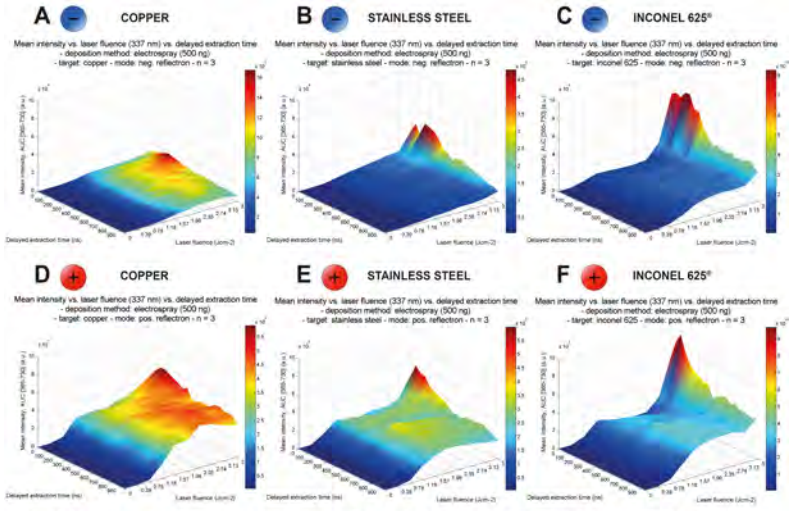

Figure 1. Ion yield vs. extraction delay time and laser fluence in negative reflectron mode for: **A)** copper, **B)** stainless steel, used as standard (MA)LDI target plate material, and **C)** inconel 625® (Ni61/Cr22/Mo9/Fe5). Ion yield vs. extraction delay time and laser fluence in positive reflectron mode for. **D)** copper, **E)** stainless steel **F)** inconel 625®. Note: each colorbar ranges from zero to the maximum ion yield measured for the corresponding graph.

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Sr isotope ratios and Rb-Sr ages by LA-ICPMS with isobar separation by on-line electrothermal vaporization

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High-precision Sr isotope ratio determination of solids by laser ablation multiple collector inductively coupled plasma mass spectrometry (LA-MCICPMS) is of importance for a variety of applications. The accuracy of the $^{87}Sr/^{86}Sr$ determination is however limited by the isobaric interference of ^{87}Rb on ^{87}Sr, which cannot be separated with the resolution offered by today's instrumentation. Unlike in solution based methods, where the separation of Rb and Sr is possible [1], in LA all elements in the aerosol reach the plasma, leading to the mentioned interference. By heating the aerosol in an electrothermal vaporization (ETV) unit, changes in its chemical composition can be initiated. R. Brogioli used an HGA-600 ETV, which contains a graphite furnace, to investigate the behaviour of heated laser-generated aerosols [2]. A partial suppression of the Rb-signal was observed, leading to improved $^{87}Sr/^{86}Sr$ accuracies. Selective Rb-evaporation and condensation was suggested to cause this effect, based on the different vaporization temperatures of Rb (1'000° C) and Sr (SrO ≈ 3'000° C). The goal of this work is to develop a standalone ETV unit and to further increase the Rb-suppression level and to improve the stability and the reproducibility of the set-up. First, NIST 610 and BCR-2G standards were analysed by coupling an ns-laser (213 nm) or fs-laser (257 nm) to the HGA-600. This set-up was compared to a first prototype system based on a tungsten wire heater inline the aerosol stream. With the HGA-600 the Rb-signal suppression was found to start at 1'600° C, while the Sr-suppression occurred at 2'400° C. The new ETV prototype was also found to induce signal suppression, whereas no selective suppression of Rb occurred. This lack of efficiency is most likely due to the limited temperature that was achieved, as well as some air leakage into the system, causing W to evaporate. In order to solve these problems, a second ETV prototype was built, consisting of an aluminium oxide cap and bottom, containing electrical contacts, two quartz glass tubes, a liquid cooling system between the tubes and a coiled tungsten wire heater. The usability and stability of the set-up was investigated, as well as the Rb-elimination efficiency and extent of signal suppression for other elements.

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Full-metal AFM probes for tip-enhanced Raman spectroscopy

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Tip-enhanced Raman spectroscopy (TERS) allows nanoscale chemical imaging of surfaces, by combining the chemical sensitivity of Raman spectroscopy and the nanoscale resolution of scanning probe microscopy, including atomic force microscopy (AFM). By now, the scope of systems studied by means of TERS has been limited due to the low stability of AFM-TERS probes and their vulnerability to contaminations.

We introduce a new generation of AFM probes for TERS, which overcome the two aforementioned limitations. The design of the newly developed probes consists of an etched silver wire, in contrary to the predominantly used metal coated AFM probes. By mechanical processing of the wire, a full-metal AFM probe is obtained whose stability and enhancement resemble STM-TERS probes.

The full-metal design of these probes overcomes the issues of mechanical wear of the metallized tips, because there is no fragile Ag-Si/SiO_2 interface. Furthermore, the roughness of the probes' sides is much lower than in the case of metallized probes, where contaminations adsorbed to the probe's sides contribute significantly to the collected enhanced Raman spectra.

We demonstrate the advantages of TERS imaging with the developed probes, and compare their performance in AFM- and STM-feedback.

MALDI-MS for Population Profiling with Single-Cell Resolution

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Introduction

Advances in single-cell analytical technologies allow the detection and characterization of heterogeneities in cellular populations. Addressing this level of information is necessary to understand and tackle important scientific phenomena such as persistence to biocides. In recent years, tools for the analysis of genomes, RNA-transcripts or proteins on the single-cell level became state of the art. However, the analysis of lower molecular weight compounds on a single cell level is still not covered. We are currently developing a new approach for profiling of microbial populations using a high-density microarray for matrix-assisted laser-desorption ionization – mass spectrometry(MALDI-MS).

Methods

Chlamydomonas reinhardtii cells are spotted onto a high-density microarray. Confocal fluorescence scanning locates the auto-fluorescent chlorophyll in the cells on the array. Cell lysis is achieved by fast freezing using liquid nitrogen and matrix crystallization from an organic solvent mixture. The photosynthetic pigments help to visualize the analytes during the sample preparation procedure.

Results

An array with 1430 wells gave approximately approx. 870 single cell events due to the stochastic distribution of the cells in the wells. 95 % of single-cell spots give a spectrum. The contactless piezo-spotting device prevents cross-contamination. The spectra obtained can be used to identify different strains of Chlamydomonas reinhardtii and give insight into the lipid composition of single cells in the population. Chlorophyll fluorescence is orthogonally detected with fluorescence spectroscopy to cross-validate the mass spectrometric data.

Conclusions

The system developed shows the potential of MALDI-MS for high-throughput screening of single cells. The new protocol can be used to characterize microbial populations and extract biological information such as lipid profiles that are not accessible by conventional fluorescence based methods.

Observing gas-phase proton transfer reactions inside the MALDI plume

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Although matrix-assisted laser desorption/ionization (MALDI) is widely used for routine and research applications in the field of analytical chemistry and biochemistry, the ionization mechanisms are still not fully understood. The UV-MALDI process is initiated by a laser pulse, usually with a duration of 3-5ns. After this period of time, a complex and partially unknown series of events take place, with a total time scale of several microseconds. Photoionization, thermionic processes, exiton-pooling phenomena are believed to contribute to the initial formation of the ions. Secondary reactions in the expanding plume are thought to be largely responsible to the formation of the (analyte) ions that are eventually detected. By using a simple experiment we are evaluating the contribution of gas-phase in-plume proton transfer reactions to the formation of protonated and deprotonated molecules in the MALDI process. This is possible only when proton transfer happening in the condensed phase can be separated from proton transfer inside the plume.

The experiments were performed on a commercial MALDI-ToF instrument (Bruker UltraFlex II). A new state-of-the-art internal MALDI source FTICR system will also be used for some experiments. A split sample holder is used to separately desorb/ionize two different samples by the same laser pulse. In this fashion, the two samples brought very close to each other, but mixing during sample preparation is avoided. By using a combination of deuterated and non-deuterated matrices and analytes it is possible to isolate the plume proton transfer processes from the ones that are happening in the condensed phase.

The expanding plume is believed to contain different reactive species, including hydrogen radicals, which can react with the desorbed analytes in a chemical ionization type of reaction. We are acquiring an extensive set of spectra to obtain statistically significant information. These results could be used to evaluate the relevance of already proposed (and partially conflicting) ionization model for MALDI.

Quantification of Trace Elements in Brass and Silicate Glasses by Portable Laser Ablation Sampling and Subsequent ICPMS

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Laser ablation inductively coupled plasma mass spectrometry LA-ICPMS is a powerful analytical method which enables the elemental and isotopic fingerprinting of solid samples. This method is highly attractive to the field of archaeometric research, wherein information concerning quantitative elemental composition and isotopic ratios is of intrinsic importance for characterizing objects regarding provenance, age, and authenticity. However, many objects in archaeometric research must not or cannot be transported easily into the laboratory because of their physical dimensions and/or regulatory restrictions. Therefore, an off-line portable (p)LA sampling method has been developed [1, 2] that uses a diode-pumped solid state (DPSS) laser and fiber optics. Subsequent coupling to ICPMS allows circumventing the aforementioned problems while providing spatially resolved analysis with the analytical performance of laboratory-based ICPMS.

In this project, brass and silicate glass reference samples were ablated by pLA applying different laser parameters in order to investigate and to adjust the sampling performance and to find optimal conditions for accurate quantification. The ablated material was sampled on polycarbonate membrane filters which were subsequently digested in nitric acid. In the final step, the elemental composition of the digested filters was measured either by ICP optical emission spectrometry (OES) or ICPMS using solution-based sample introduction. First results of these analyses are presented and discussed.

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Nucleoside phosphate monitoring in cell cultures using MALDI TOF MS

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The analysis of different intracellular metabolite classes ultimately opens up a variety of options to control and understand bioprocesses. Methods for routine biotechnological process monitoring are thus of high interest. Traditionally, metabolite levels are followed using liquid chromatography (LC) combined with UV detection. However the long LC-UV runtimes compromise the possibilities to regulate a running process *via* feedback. We are presenting a novel MALDI-MS method that enormously reduces the time to analyze intracellular metabolites, and provides excellent robustness.

Using this method, we have followed metabolite production in a Chinese hamster ovary monoclonal antibody producing cell line, which was cultured in a controlled and constant culture environment. A commercial MALDI TOF instrument (5800, ABSciex, Germany) and a microarray sample target for mass spectrometry were used to determine the metabolite profile. Adenosine-5´triphosphate was detected and quantified using an isotopically labeled internal standard (13C15N-Adenosine-5´-triphosphate). The di- and monophosphates of adenosine as well as guanisine, cytidine, uridine were also monitored. Any methodologically induced analyte fragmentation or hydrolysis was corrected for by the internal standard.

The appearance of ATP is in good agreement with literature data. Using principal component analysis the measured datasets were analysed to explain differences in the process history. The presented method has high-throughput capabilities as the effective measuring time per sample with 10 technical replicates is less than a minute and therefore competes with HPLC-UV approaches. The detected metabolite profiles were cross-validated using HPLC-UV.

Gap-mode TERS spectra of small organic amides and small peptides: is the amide I mode present?

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The position of the amide I band in protein samples with vibrational spectroscopy gives information about their secondary structures.[1] In particular, α-helical secondary structures have a peak around 1650-1655cm^{-1}, β sheets in the range of 1665-1670cm^{-1} and random coils ›1680cm^{-1} in their both Raman and IR spectra.[2,3]

Tip-enhanced Raman Spectroscopy (TERS) is a very powerful technique to get simultaneous chemical and local information from molecules on surfaces, with less than 20nm spatial resolution. TERS has great potential for the structural characterization of proteins and their conformation in complex environments. However, it is still unclear whether the amide I band is present in TERS spectra or not. In some of the literature, the amide I mode was not visible in gap-mode TERS spectra of a number of polypeptides.[1] Other references claim that the amide I mode is visible, depending on which type of side chains the examined proteins have. According to the latter results, if the amino acid side chains are small (like H or CH_3), TERS spectra show an intense amide I band, whereas if the amino acids side chains are bulky, the amide I band is visible only in half of the spectra acquired.[4] Possible explanations for the absence of the amide I band in TERS spectra were: (i) a too large distance of the peptide bonds from the metallic tip; especially for large proteins, (ii) a perpendicular orientation of the peptide bonds relative to the main axis of the TERS tip, (iii) a strong direct interaction of the protein backbone with the gold substrate, which may cause a change in the amide I mode, (iv) an influence of the tunneling current between the tip and the gold surface that influences the corresponding spectra, and (v) that the aromatic ring vibrations dominate the TERS spectra such that the amide I mode disappears in the noise. To study this puzzle in more detail gap-mode TERS spectra of small organic amides and small peptides, which were chosen because they can form self-assembled monolayers on gold, were examined in detail. The results obtained show that the amide I band is absent for all analyte molecules studied.

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Novel fluorescence assays for monitoring recombinant proteins during biotechnological production and purification

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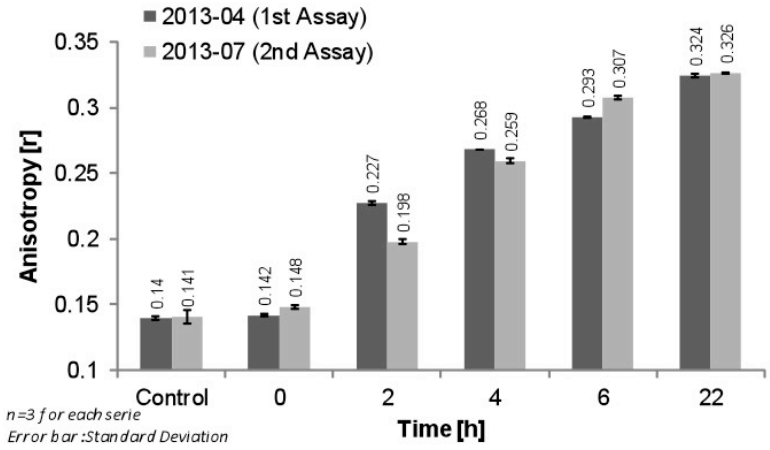
Nowadays, biotechnological productions are monitored using indirect parameters such as the optical density. The recombinant protein product itself is usually not quantified because current assays such as ELISA or Western Blotting are time consuming and labor intensive. The availability of rapid sensing methods 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 probes which specifically bind to the recombinant protein with concomitant enhancement of the fluorescence anisotropy. The assay offers a large quantification range of more than an order of magnitude, 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 antibodies and His-tagged proteins could be performed « at-line ». Furthermore, affinity purification of the recombinant products could be monitored allowing rapid determination of the product-containing fractions. Finally, preliminary results showed the possibility to integrate these assays within automatic devices and demonstrated thereby the feasibility of online monitoring of bioprocesses.



Defensin Levels in Spider Hemolymph

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Spiders, like all arthropods, exclusively rely on an innate immune system localized in the hemocytes to protect against pathogen invasion. In the hemocytes of the wandering spider *Cupiennius salei (C. salei)*, defensin expression was found to be constitutive.[1] Defensins belong to the group of antimicrobial peptides, which appear in most taxonomic groups, and play an essential role in innate immunity. It has further been reported that during the primary immune answer of *C. salei*, the peptide content of hemocytes changes markedly,[2] which may indicate the release of defensins from the hemocytes. However, no data on the peptide levels in *C. salei* hemolymph has so far been published.

Formerly, the involvement in the primary immune answer was considered the only function of defensins. However, recent findings strongly suggest that the importance of defensins goes far beyond. There is evidence for defensins contributing to the adaptive immune response, to angiogenesis, and furthermore to tissue repair, i.e. to a variety of essential processes in living organisms.

To date, only very little is known about the identity of *C. salei* defensins and their detailed mode of action. The goal of the work presented herein is the identification of hitherto unknown *C. salei* defensins in hemocytes and the hemolymph. Moreover, the levels of defensin expression under differential conditions are compared by the means of liquid chromatography-tandem mass spectrometry (LC-MS/MS).

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Binding of Metallocenes to Short Oligonucleotides

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Cancer is one of the most severe and widespread diseases and an ideal treatment has not yet been found. In the last decades, cisplatinum was commonly applied in cancer therapy with very good results. However, serious side effects and resistant tumors necessitated the development of new antineoplastic agents, such as metallocenes dihalides. These are metal-based compounds exhibiting two cyclopentadienyl ligands and a *cis*-dihalide motif. They resemble the *cis*-chloro configuration of cisplatinum, which propounds a similar mode of action. Metallocenes comprising one of the transition metals titanium, molybdenum, vanadium, niobium, and zirconium as the metal center have been shown to be effective against several cancer cell lines. Evidence for the accumulation of metallocenes in the nucleus implied that DNA is one of the major targets [1]. Although several studies reported adduct formation of metallocenes with nuclear DNA, as yet substantial information about the general binding pattern and the binding to higher-order structures is lacking.

Mass spectrometry can fill this gap as it constitutes a powerful technique to investigate the formation of organometallic adducts. Presented data demonstrate that the two agents titanocene dichloride and molybdenocene dichloride bind to single-stranded DNA and RNA. Distinct fragment ions formed upon collision-induced dissociation help to unravel preferential binding sites within the oligonucleotides. Moreover, adducts with duplexes and quadruplexes shed light on the molecular mechanism of action.

[1] Köpf, H., Köpf-Maier, P.: Titanocene Dichloride - The First Metallocene with Cancerostatic Activity. *Angewandte Chemie* **18**, 477-478 (1979)

Elucidation of the gas-phase structure of a sugar-modified DNA analogue

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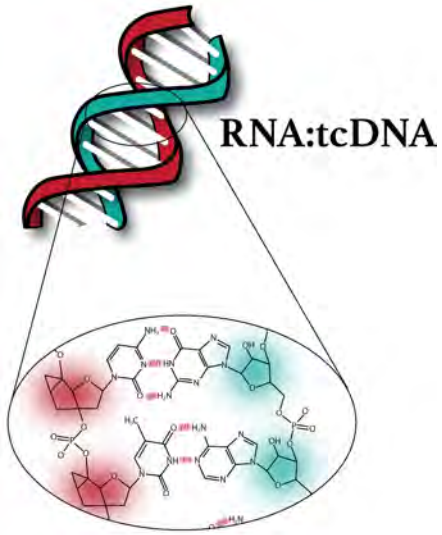
Antisense oligonucleotides are medical agents for the treatment of genetic diseases that are designed to interact specifically with mRNA. This interaction either induces enzymatic degradation of the targeted RNA or modifies processing pathways, e.g. by inducing alternative splicing of the pre-mRNA. The latter mechanism applies to the treatment of Duchenne muscular dystrophy with a sugar-modified DNA analogue called tricyclo-DNA (tcDNA). In tcDNA, the ribose sugar-moiety is extended to a three-membered ring system, which augments the binding affinity and the selectivity of the antisense oligonucleotide for its target[1].

The advent of chemically modified nucleic acids for antisense therapy presents a challenge to diagnostic tools, which must be able to cope with a variety of structural analogues. Mass spectrometry meets this demand for non-enzyme based sequencing methods ideally, because the technique is largely unaffected by structural modifications of the analyte. Sequence coverage of a fully modified tcDNA 15mer can be obtained in a single tandem mass spectrometric experiment.



Beyond sequencing experiments, tandem mass spectrometry was applied to elucidate the gas-phase structure and stability of tcDNA:DNA and tcDNA:RNA hybrid duplexes. Most remarkable is the formation of truncated duplexes upon collision-induced dissociation of these structures. Our data suggest that the cleavage site within the duplex is directed by the modified sugar-moiety. Moreover, the formation of truncated duplexes manifests the exceptional stability of the hybrid duplexes in the gas-phase. This stability arises from the modified sugar-moiety, which locks the tcDNA single strand into a conformation that is similar to RNA in A-form duplexes. The conformational particularity of tcDNA in the gas-phase was confirmed by ion mobility-mass spectrometry experiments on tcDNA, DNA, and RNA.

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Carbonate-Selective Potentiometric Solid Contact Electrode

D. J. Yuan[1], A. H. Anthis[1], M. G. Afshar[1], G. A. Crespo[1], E. Bakker[1]*

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We report on the development of all-solid-state carbonate selective electrode based on highly lipophilic multi-walled carbon nanotubes (f-MWCNTs), which are conveniently dispersed in tetrahydrofuran (THF). This advantage is important to develop uniform films without the need of using surfactants that might deteriorate the performance of the electrode. This solid contact carbonate sensor has comparable analytical characteristics than inner liquid electrodes. A Nernstian slope of 27.2±0.8 mV.dec^{-1}, a LOD=2.3μM, a response time of 1-s, a linear range of four logarithmic units and a long-term stability of 0.04 mV.h^{-1}were obtained in a buffered solution (0.1 M Tris-H_2SO_4, pH=8.6). Water layer test, reversibility, selectivity (chloride, nitrate and hydroxide) are also reported. The excellent properties of f-MWCNTs as a transducer are contrasted to the deficient performance of poly(3-octyl-thiophene) (POT) for anion detection. This is evidenced both with the significant drift in the potentiometric measures and the enormous sensitivity to light (sunlight or artificial light), which may be problematic for environmental *in-situ* measurements (night/day cycles). Finally, the concentration (strictly activity) of carbonate is determined in a river sample (the Arve river) and compared to a reference method (automatic titrator with potentiometric pH detection). We believe that nanostructured materials such as f-MWCNTs are an attractive platform as universal ion-to-electron transducer for potentiometric membrane electrodes.

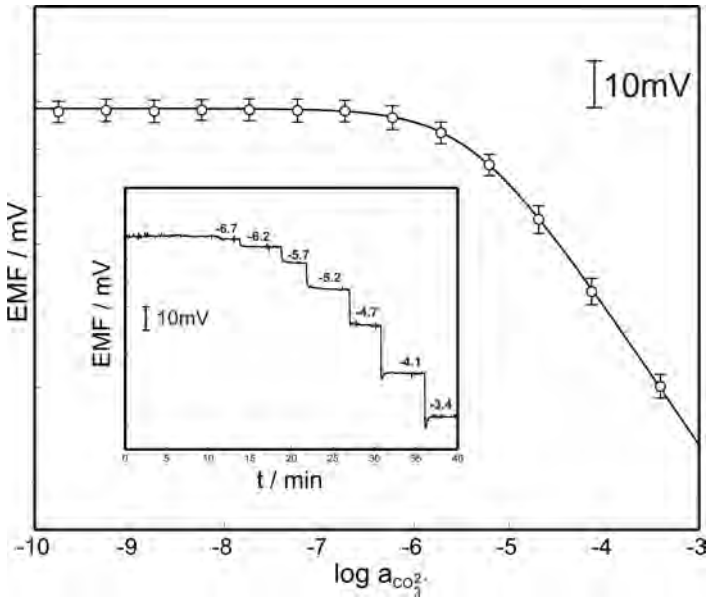

Fig.1. Calibration curve of the all-solid-state carbonate electrode

Efficient normal phase MS directed purification of natural products at the preparative scale

D. Righi[1], A. Azzollini[1], E. F. Queiroz[1], J.-L. Wolfender[1]*

[1]University of Geneva

Introduction: 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 complementary usage of both of stationary phases for metabolite purification at the preparative scale using generic separation methods has been investigated on medium pressure preparative chromatography system (PuriFlash® - MS).

Methods: A mixture of representative natural product standards was chosen and analysed under both normal phase/reverse 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 MS ionization and splitting conditions and that provide good detection and preclude source contamination. The HPLC analytical gradient was transferred to flash chromatography following a geometric gradient transfer method after calibration of the chromatographic systems [1].

Results: An efficient rational approach for the MS targeted isolation of natural products was obtained. MS in complement to UV detection enabled the monitoring of NPs with weak and strong 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 purifications in both NP and RP modes. The LC transfer method from analytical to flash levels 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 used for a rapid and efficient isolation of natural products in complex mixtures. Separation performed at the preparative scale allows to purify tens to hundreds mg of compounds for further structural identification of bioactivity characterization studies.

Novel Aspect: simultaneous UV APCI/MS, flash-chromatography, normal/reverse phase conditions, Analytical to flash chromatography method transfer.

[1] Davy Guillarme, Dao T.T. Nguyen, Serge Rudaz, Jean-Luc Veuthey, *Eur. J. Pharma.Biopharma. 2008, 68, 430*

Ion-selective Nanospheres as Heterogeneous Indicator Reagents in Complexometric Titrations

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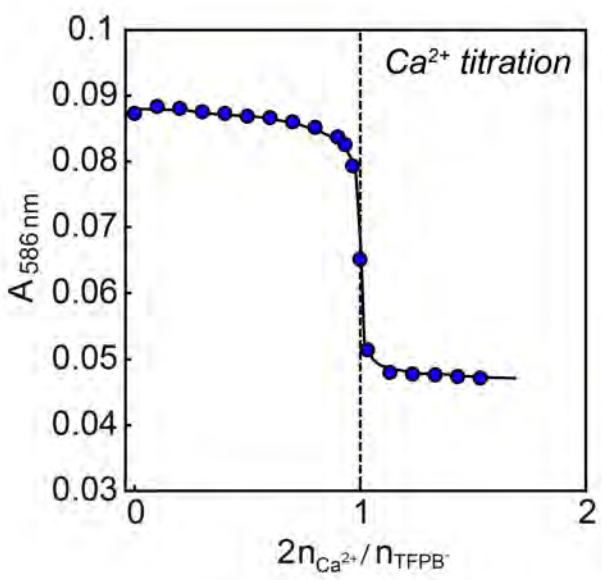
[1]University of Geneva

Complexometric titration is a mature analytical technique that is being used and taught all over the world in analytical science. Titrations are routinely used to determine ion concentration, speciation as well as complexation reactions in various fields such as environmental, clinical and bioanalytical chemistry.

Traditionally, optical titrations of inorganic ions are based on a rapid and visible colour change at the endpoint with water soluble organic dyes as indicators. However, their pH dependent complexing ability and rather rigid selectivity remains problematic.

The endpoint indication may be performed with an additional lipophilic pH indicator. The indicating nanospheres rely on a weaker extraction of the analyte of interest by ion-exchange, owing to the additional incorporation of a lipophilic pH indicator in the nanosphere core. Ca^{2+} titration is demonstrated as a proof-of-concept.

In addition, we present a novel approach where the indicating nanospheres contain a polarity sensitive dye. The complete displacement of the original counter at the endpoint by the analyte gives rise to a drastic potential change at the nanosphere–water interface that results in an altered partitioning of the polarity sensitive dye. These dyes are not pH sensitive and provide optical titration endpoints that are largely independent of the sample pH.



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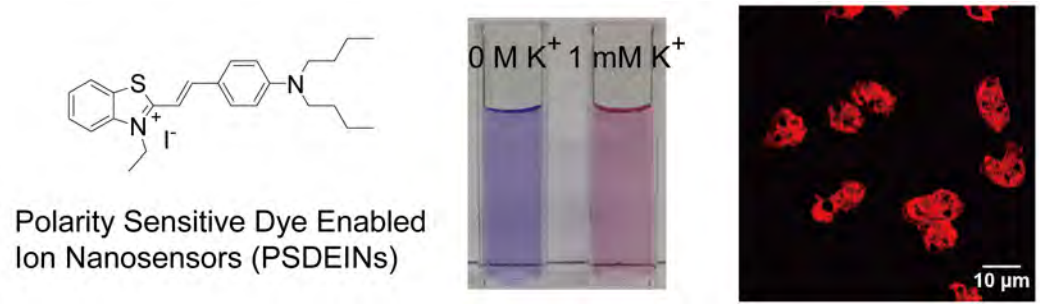
Charged Solvatochromic Dyes as Signal Transducers in Fluorescent and Colorimetric Ion Selective Nanosensors

X. Xie[1], A. Gutiérrez[1], V. Trofimov[1], I. Szilgyi[1], T. Soldati[1], E. Bakker[1]*

[1]University of Geneva

Ionophore-based ion-selective electrodes (ISEs) have been widely applied in clinical and environmental monitoring during the past few decades. ISEs require the implementation of conducting wires for signal transduction, which unfortunately, is impractical for the readout of individual nanoparticles. It has been demonstrated recently that the potentiometric response of ion-selective nanospheres can be observed with voltage-sensitive dyes, thereby converting nanoscale electrochemical signals into an optical readout.

Electrically charged solvatochromic dyes are introduced here as a new family of signal transducers to form polarity-sensitive dye-enabled ion nanosensors (PSDEINs). A series of dye molecules with a D-π-A structure were synthesized and characterized in various solvents, ion selective nanospheres for K^+, Na^+ and H^+, and *Dictyostelium discoideum* cells. A theoretical model was developed for the response of PSDEINs. The nanosensors exhibited a tunable response range, high sensitivity, and improved thermal and photostability. Dye leakage was greatly suppressed (in vitro and in vivo) when the polarity-sensitive dyes (PSDs) were encapsulated in the nanosphere core, rendering PSDEINs potentially valuable bioimaging tools.



1. Xiaojiang Xie, Jingying Zhai, and Eric Bakker, J. Am. Chem. Soc., **2014**, *136* (47), pp 16465–16468
2. Xiaojiang Xie, Agustín Gutiérrez, Valentin Trofimov,Istvan Szilagyi, Thierry Soldati, and Eric Bakker, *Angew. Chem. Int. Ed*, **submitted**

Quantifying the detection capabilities of LA-ICPMS

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) is frequently employed for the analysis of minute isotope contents in the presence of a background noise. Distinguishing between the sample signal and the background noise at a given confidence level thus represents a routine challenge. For count numbers N_s and N_b collected during the sample and the background analysis, respectively, the statistical significance of their difference, N_s-N_b, is usually considered: how probable is it to obtain this difference by just subtracting two randomly selected background replicates? If it is probable, the signal is statistically indistinguishable from the background and the analysed isotope is not detected. If the corresponding probability is below some threshold, the isotope is detected. The value on the divide between the above alternatives, given in net counts or mass (content) units, is called critical level; optionally, it can be complemented by the computation of the detection limit; such values are often reported in the literature. Less discussed is the appropriateness of computational methods used to estimate these values in the current LA-ICPMS practice. Troubles arise from the attempts to apply Gaussian confidence intervals to small, discretely distributed count numbers contained in real LA-ICPMS background acquisitions, and from a biased estimation of the background activity in some of the detection capability quantification methods based on paired measurements. Combined, these factors result in uncontrolled, excessively high rates of false detections (background reported as detection of analyte in the sample). We provide a review of methods available for the critical level and detection limit estimation and discuss how to evaluate the performances of these methods to enable an educated computation of LA-ICPMS detection capabilities, including the case of very small Poisson-distributed count numbers.

[1] Jozef Przyborowski, Henry Wilenski, *Biometrika*, **1940**, 31, 313-323.
[2] Bernard Altshuler, Bernard Pasternak, *Health Physics*, **1963**, 9, 293-298.
[3] Daniel Strom, Jay MacLellan, *Health Physics*, **2001**, 81, 27-34.
[4] MARLAP Multi-Agency Radiological Laboratory Analytical Protocol Manual, **2004**,19-20.
[5] Lloyd Currie, *Journal of Radioanalytical and Nuclear Chemistry*, **2008**, 276, 285-297.

The ICPMS signal as a doubly stochastic Poisson process

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Understanding the structure of noise associated with a measurement process is interesting theoretically and has practical applications related to the quantification of detection capability, signal uncertainty and dead time. Here, we present and analyse arguments explaining the appearance of the Poisson process in the distribution of count numbers in inductively coupled plasma mass spectrometry (ICPMS) signals. We consider the Poisson distribution as a special case of the binomial distribution constrained by inefficient ion transmission from the ICP ion source to the detector. The universal form of the relevant Poisson process is doubly stochastic: the random nature of count numbers acquired per time unit is defined not only by the probabilistic selection of ions during their transport through the interface and ion channel, but also by fluctuations of the ion contents sampled by the spectrometer from the plasma and, more generally, by fluctuations of the rate of the Poisson process itself. Compared to an ordinary Poisson process, the doubly stochastic Poisson process has an excess variance that increases at higher analyte contents. The excess variance in the uncertainty of ICPMS signals is also known as flicker noise; it is an integral part of the doubly stochastic Poisson process and not a fully individual noise component. We review processes pertinent to its origin and formalisms used to describe it.

[1] Alex Ulianov, Othmar Müntener, Urs Schaltegger,*Journal of Analytical Atomic Spectrometry*, **2015**, doi: 10.1039/C4JA00319E

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Online analysis of mass spectra of hydrocarbons

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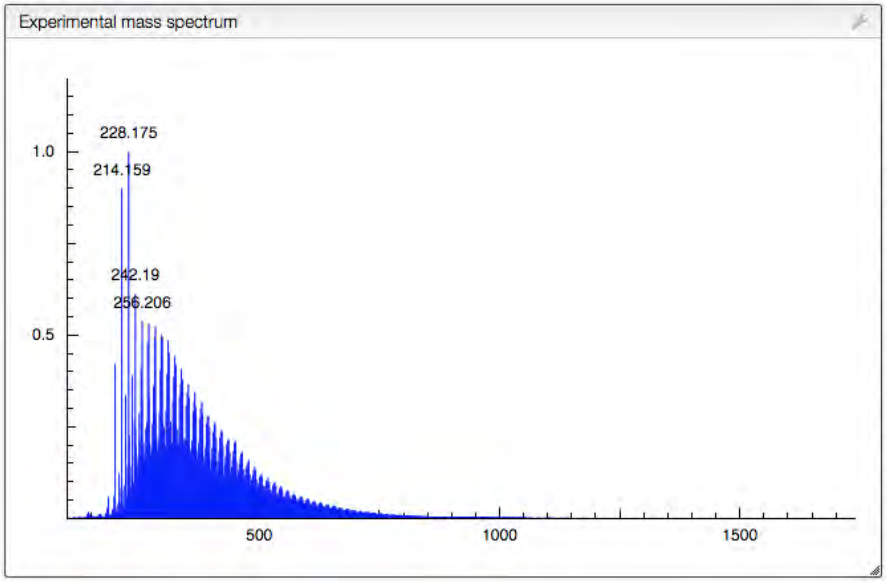
With continuous progress in computational resources and increasing availability of high-resolution mass spectrometers, web-based analysis of complex analytical mixtures becomes a feasible task. Here, we expand the capabilities of the data analysis web-tools at www.cheminfo.org by implementing online analysis of mass spectra of petroleum-derived analytes – one of the more challenging natural complex mixtures typically containing upwards of hundreds, and usually thousands, of molecules. The concept involves inputting raw mass spectra and performing elemental composition assignment on the fly based on the previously described core library ChemCalc [1] . The process is done in 4 steps:

1. Noise reduction and peak picking
2. Selection of molecular formula candidates from monoisotopic masses
3. Confirmation of the candidates is based on
    1. isotopic distribution
    2. presence of homologous series
4. Report generation and interactive visualisation of the results

In order to easily compare various samples we have integrated the hexagonal class representation [2] of all the constituent heteroatom classes found.



Target mass	Best match	Δ ppm	Number results
218.190308688	$C_{15}H_{24}N^{+1}$	0.0802000000...	6
219.104148085	$C_3H_{140}N_3^{+1}$	0.1362	6
219.107564035	$H_{144}N_3S^{+1}$	-0.0684	5
219.174310683	$C_{15}H_{23}O^{+1}$	0.1419	6
219.193588715	$C_2H_{33}N_4NaO_2...$	-0.2609	5
220.112077577	$C_{16}H_{14}N^{+1}$	-0.007699999...	7
220.115423536	$C_{13}H_{18}NS^{+1}$	0.1066	6
220.169511085	$CH_{149}N_3O^{+1}$	0.0306000000...	4
220.205994766	$C_{15}H_{26}N^{+1}$	-0.083999999...	6
221.041784136	$C_2H_{136}N_2S^{+1}$	0.4101	5
221.115345594	$C_3H_{23}N_4NaO_2...$	-0.2912	6
221.118803657	$C_3H_{148}NaO^{+1}$	-0.175	5
221.119893291	$C_{16}H_{15}N^{+1}$	0.0344	7
221.153456208	$CH_{148}N_2O_2^{+1}$	0.3491	4
222.111277662	$C_5H_{27}NaOS_3^{+1}$	1.5601999999...	4

References

1. Patiny, L., Borel, A. (2013). ChemCalc: a building block for tomorrow's chemical infrastructure. *Journal of chemical information and modeling*, 53(5), 1223-1228.
2. Zhurov, K. O., Kozhinov, A. N., Tsybin, Y. O. (2013). Hexagonal class representation for fingerprinting and facile comparison of petroleomic samples. *Analytical chemistry*, 85(11), 5311-5315.

Lipid droplets and large unilamellar lipid vesicles investigated by asymmetric-flow field-flow fractionation in combination with multi-angle light-scattering

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[1]University of Ljubljana, [2]NIC Slovenia, [3]NIB Slovenia

Asymmetric-flow field-flow fractionation technique coupled to a multi-angle light-scattering (AF4-MALS) together with dynamic light-scattering (DLS) and transmission electron microscopy (TEM) were used to study the size characteristics of the trioleoylglycerol lipid droplets (LD) covered by a monolayer of sphingomyelin and cholesterol, as well as the large unilamellar lipid vesicles (LUV) composed of sphingomyelin and cholesterol. Sonicated LD or extruded LUV were prepared at two different molar ratios (1/1, 4/1) of sphingomyelin and cholesterol. In AF4-MALS, various cross-flow conditions and mobile phase compositions were tested to optimize the separation of LD or LUV particles. By coupling AF4 with MALS the average geometric radius, R_{geom}, as well as the average root mean square radius, rms, of lipid particles were determined, whereas DLS in batch-mode gave the average hydrodynamic radius, R_h. From the rms/R_h shape factor, the solid sphere structure of non-uniform density was determined for the LD (0.81-0.89), whereas the shape factor of the LUV was close to one due to shifting of the center of mass towards the particle periphery. The globular shape of LD and LUV was confirmed by particle visualization using TEM, however, their size appeared larger from the values determined by AF4-MALS and DLS, which could be ascribed to flattening of lipid particles on the surface.

Native nano-ESI-MS Applied to Fragment Based Drug Discovery.

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[1]ETH Zurich, [2]Astex Pharmaceuticals

Native nano-ESI-MS has successfully been applied to investigate noncovalent interactions of biomolecules that are potential drug targets. In this study, it was applied as a quick method for ranking and measuring K_D values for antagonists of a protein-protein interaction. The investigated ligands were generated by fragment-based drug discovery (FBDD) which is an established approach for generating hit compounds but despite the advantages of native nano-ESI-MS, this approach has not been widely used in FBDD.

Here, the binding affinities to XIAP (X-linked inhibitor of apoptosis) of a number of compounds of different molecular weight and binding affinity were determined by single-point measurements. The experimentally determined dissociation constants (K_D's) were in good agreement with data from solution phase techniques. ESI spectra were acquired with a hybrid quadrupole time-of-flight mass spectrometer (Q-TOF ULTIMA, Waters/Micromass, Manchester, UK). Very gentle MS source conditions were used to ensure minimum in-source dissociation of analytes, and MS parameters were also carefully optimized in order to reduce nonspecific binding and to minimize adduct formation.

Native nano-ESI-MS was used to quantify the binding affinities of hit compounds generated by FBDD to XIAP. Information regarding the specificity, stoichiometry and relative strength of the observed interactions was obtained. XIAP interactions with the inhibitors are prone to dissociation in the gas phase even after carefully controlling the instrument parameters and in-source dissociation can lead to artificially low binding constants based on the reduced abundance of the complex ions. Therefore, imidazole was used as a stabilizing agent to minimize dissociation of the complex ions and optimize the abundance of weakly bound complexes [1]. In order to gain information about the stability of the complexes in the gas phase, collision-induced dissociation (CID) experiments were performed and a goodcorrelation between the gas-phase stability and the binding affinity in solution was shown.

[1] Sun, Jiangxiao, Elena N. Kitova, and John S. Klassen. 2007. "Method for Stabilizing Protein-Ligand Complexes in Nanoelectrospray Ionization Mass Spectrometry." *Analytical Chemistry* 79(2):416–425.

Unraveling the requirements for immortality – Phenotype characterization of the Δtlc1Type II ALT survivors.

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The Alternative lengthening of telomeres (ALT) mechanism is responsible to achieve unlimited cellular proliferation by overcoming the shortening of telomeres associated with DNA replication, and accounted for 10-15% of cancer phenotypes.[1]

Today, there is no complete understanding on how the ALT mechanism is maintained, thus a metabolomic analysis can provide new insights not available with any other existing analytical technique. Although cancer biology is hard to study in *Saccharomyces cerevisiae*, the development of the ALT phenotype is one of the rare systems where yeast can be directly compared to cancer cells.[2]

Here we used a combination of single-cell level techniques such as microarrays for mass spectrometry (MAMS), fluorescence activated cell sorting (FACS), and traditional metabolomics platforms such as mass spectrometry to establish mechanistic links between cellular central metabolism and the requirements for telomere maintenance based on an ALT mechanism.

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UV-fs-LA-ICPMS of $La_{0.4}Ca_{0.6}MnO_3$ PLD thin Films

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[1]ETH Zurich

The perovskite-structured compound $La_{1-x}Ca_xMnO_3$ exhibits interesting magnetoresistant properties [1], depending on what exact stoichiometry it has when formed into thin films via pulsed laser deposition (PLD). Up until now, the PLD films' composition has been arduously quantified via Rutherford Backscattering (RBS) [2]. Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) would qualify as a much more efficient and faster technique for the quantification of the raw material and any thin film derivatives thereof. However, LA-ICPMS of $La_{1-x}Ca_xMnO_3$ PLD thin films deposited on silicon wafers, applying a near infrared (NIR) femtosecond (fs) laser emitting at a wavelength of 800 nm in combination with single point calibration using NIST silicate glass reference materials proved challenging mainly due to differing LA thresholds and optical penetration depths.

Now, the usage of a novel UV-fs-LA system emitting at 257 and 206 nm allowed lowering the optical penetration depth, thus significantly decreasing material up-take rates per laser shot when operating at low fluences (‹ 0.5 J/cm^2). This allowed for hitherto unmatched signal quality, making these PLD films accessible to spatially resolved analysis via LA-ICPMS.

Results of the LA-ICPMS analyses performed on the aforementioned films, such as a comparison of the different UV wavelengths, quantification data (including bulk analysis depth profiling), and studies of crater morphology and ablation rate using confocal and electron microscopy will be presented and discussed.

[1]: Y. Moritomo, A. Asamitsu, H. Kuwahara, Y. Tokura, *Nature*, **1996**,380, 141-144.
[2]: M. Döbeli*, J. Phys. Condens. Mat*, **2008**,20, 1.

Dopant-induced conformational changes of proteins in the gas phase evaluated by Transversal Modulation Ion Mobility Spectrometry.

N. A. Meyer[1], K. Root[1], R. Zenobi[1]*, G. Vidal-de-Miguel[1,2]*

[1]ETH Zurich, [2]SEADM S.L.

Tandem IMS-IMS-MS analysis with added dopants provides mobility measurements in dry and doped gases, increasing the separation capacity. The mobility of proteins depends strongly on their conformational stage. Our goal is to evaluate the effect of different dopants on a well characterized protein (Cytochrome c), and to evaluate whether dopant-induced conformational changes can be observed in the gas phase. We found that these changes can indeed be produced and measured.

We employed a Transversal Modulation IMS (TMIMS) with two stages coupled to a linear ion trap (LTQ XL, Thermo) mass spectrometer. TMIMS utilizes an axial electric field, which pushes the ions forward, and an oscillating transversal field that deflects their trajectories. Ions are transferred only when the period of the oscillating electric field equates their time of residence. The first TMIMS stage transmits only ions in a narrow mobility range. In the second stage, gaseous dopant molecules are introduced. The interaction between ions and dopants results in a mobility shift which is also measured. We produced native and denatured proteins with a nanoelectrospray of ammonium acetate buffer and ammonia to adjust the pH, and a nanoelectrospray of methanol and formic acid (pH=2), respectively.

The mobility of native cytochrome c (charge state +7) shifted strongly depending on the specific dopant utilized. Ammonia, water and hexane produced the weakest effects. Formic acid, methanol, 2-propanol, and tert-pentyl alcohol produced much stronger shifts (with the strongest shift observed for tert-pentyl alcohol). The denatured spray produced more charge states. The charge state +7 behaved as the native protein. Charge states +9 to +14 showed lower mobilities (because the protein is stretched and has a larger cross sections), which shifted similarly with the addition of the dopants. Interestingly, without dopants, the cross section of charge state +8 is very similar to the native state. However, as the dopant is added to the gas, its mobility falls to the levels of the stretched protein. This major mobility shift shows that the +8 charge state undergoes a dopant-induced conformational change: it starts out with a compact, folded structure that unfold when the dopant is added. This outlying feature enables to pre-filter +8 cytochrome c ions in the TMIMS with an improved separation capacity.

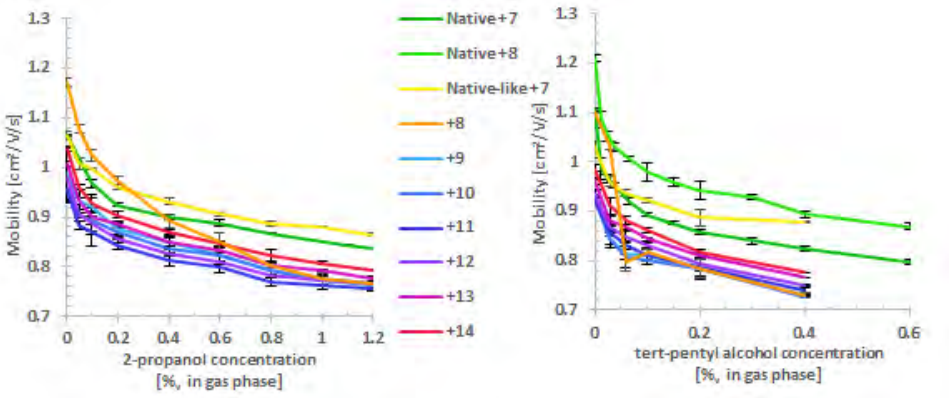


Figure: Measured mobility of denatured (charge states +7 to +14) and native (charge state native +7 and +8) cytochrome c dependent on 2-propanol and tert-pentyl alcohol concentration in the gas phase.

Comprehensive detection of obstructive sleep apnea in humans and drug monitoring in mice by real-time breath analysis

P. M. Sinues[1], E. Schwarz[2], R. Dallmann[3], X. Li[1,4], L. Bregy[1], D. Garcia Gomez[1], S. Brown[3], M. Kohler[2], R. Zenobi[1]

[1]ETH Zurich, [2]University Hospital Zurich, [3]University of Zurich, [4]Jinan University

Introduction

Exhaled breath contains relevant metabolites that may reflect the biochemical activity within a subject. However, in contrast to other biofluids (e.g. plasma), the analysis of breath remains far less explored. Here we present some recent examples of how real-time breath analysis may contribute to the fields of disease diagnosis and drug monitoring.

Methods

We modified the entrance of a commercial quadrupole time-of-flight (Qtof) mass spectrometer to allow for the real-time analysis of breath via secondary electrospray ionization-mass spectrometry. We have studied i) obstructive sleep apnea (OSA) in a randomized controlled trial; ii) breath levels of ketamine and other drugs in mice injected with these substances.

Results

Diagnosis of OSA. We found a panel of breath metabolites that were significantly enhanced in breath after treatment withdrawal. The figure (a) shows one such example (pentanal). Further identification of the compounds enabled gaining insights into OSA.

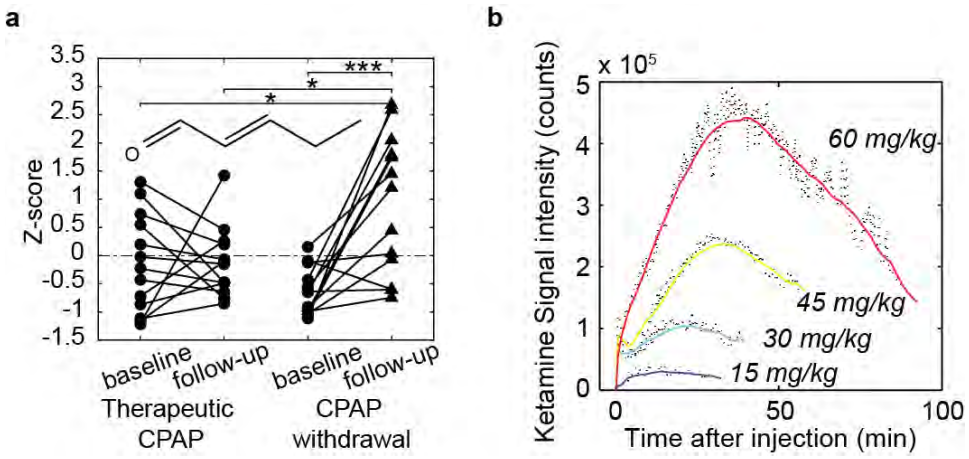
Drug monitoring. We could trace pharmacokinetic curves of ketamine in real-time. The figure (b) shows time-dependent ketamine signal for 4 different doses: 15, 30, 45, and 60 mg/kg. Each dose was injected in different mice (n=4).

Conclusions

We conclude that the real-time mass spectrometric analysis of exhaled metabolites may contribute to address some of the most relevant clinical and pharmacological problems, which are currently investigated through the analysis of body fluids other than breath.

Novel aspect

In vivo monitoring of exhaled compounds related to OSA and drugs.



Studying the 3-(2-Furoyl)quinoline-2-Carboxaldehyde (FQ) protein labelling reaction to improve accuracy of quantification in capillary electrophoresis-sodium dodecyl sulfate with laser induced fluorescence detection (CE-SDS-LIF)

M. Arrell[1], F. Kálmán[1]

[1]HES-SO Valais

Capillary electrophoresis-sodium dodecyl sulfate with laser induced fluorescence detection (CE-SDS-LIF) of proteins following derivatization with a fluorescent tag, such as 3-(2-Furoyl)quinoline-2-Carboxaldehyde (FQ), can achieve limits of detection as low as 5 ng/ml, providing a high performance equivalent of SDS-PAGE with silver staining sensitivity.[1] Silver staining is problematic for quantification due to limited linear dynamic range and uneven staining, with proteins varying in their 'stainability' by silver over several orders of magnitude.[2] Quantification using CE-SDS-LIF with FQ labelling offers advantages in terms of ease and accuracy of integration and quantification, but is poorly defined in terms of inter-protein variability due to differences in lysine labelling efficiency.

In this study, 14 proteins with molecular weights between 6 kDa and 70 kDa and containing between 1 and 59 lysines were labelled with FQ. The number of labels bound was studied using mass spectrometry (MS) and the fluorescence signal studied. Using these techniques, protein to protein variation in labelling is assessed and an estimate provided of the accuracy of CE-SDS-LIF with FQ labelling for the quantification of ‘real’ protein samples where a purified standard does not exist.

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Ion-exchange nanosphere doped hydro-gel as buffer for electrochemical AS(III) detection in weakly buffered environmental media

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[1]University of Geneva

Environmental electrochemical sensors are attractive because they are reagentless, low cost, and well-suited for the miniaturization and automation for *in situ* trace metals measurements with minimal sample manipulation. Nevertheless, in complex media, the analyte signal may experience interferences from the presence of other species or from a modification of environmental parameters such as pH. Indeed, alkalinity may vary both temporally and spatially within a lake and the pH needs to be buffered or adjust manually prior to each electrochemical experiment to obtain reliable results.

For this purpose, we present here a heterogeneous nanoscale pH buffer originally developed for ion sensing purposes [1].The nanospheres exhibit a size of around 100 nm and are composed of the cation-exchanger potassium tetrakis(4-chlorophenyl) borate (KTClPB), the H^+-ionophore tridodecylamine (TDA) and a triblock copolymer Pluronic® F-127, which also functions as a surfactant to stabilize the nanoparticles. The presence of these nanospheres in the hydrogel can compensate for variations of pH occurring in the sample on the basis of ion-exchange. A variation of pH in the sample will result in the release or uptake of H^+ from the nanospheres, thereby compensating for the local H^+ concentration change close to the electrode surface where the electrochemical measurement takes place.

These nanospheres were incorporated and trapped in an antifouling membrane (1.5% LGL agarose) deposited on a gold plated Ir-based microelectrode [2].SWASV measurement were performed to detect spiked As(III) at 50 nM. With unmodified gel coatings, the As(III) peak current was found to decrease at pH less than 7.5. With nanosphere doped gels, the As(III) peak current remains unchanged between pH 6.5 and 8.7, which corresponds to the pH range found in natural waters.

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Ion-selective fluorescent and pH independent nanosensors based on functionalized polyether macrocycles

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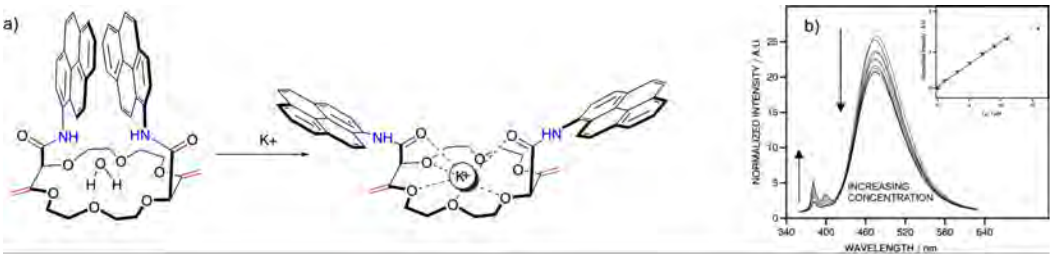
Ion sensors are important research tools in fundamental chemical science, as well in applied work such as clinical medicine or environmental analysis. During the last decades, ionophore-based ion selective sensors that are interrogated under electrochemical or optical conditions have been developed for various analytes. A major disadvantage of so-called ion-selective bulk optical sensors is the dependence of the sensor readout on sample pH since it is based on a competition between the analyte ion and hydrogen ions, which are required for an optical readout with a hydrogen ion-selective chromoionophore. [1]

We present here a new family of conventional polymeric ion-selective membrane electrodes and optical sensor of emulsified nanospheres based on densely - functionalized 16- and 18-membered pyreneamide derivatives. These optode nanoparticles exhibit a strong affinity to the potassium cation over other metal ions and may work in an exhaustive detection mode where the analyte is completely consumed by the sensor probe. The potassium cation interacts with the amide and crown-ether oxygens and this interaction results a conformational changes and switching of the amide groups after binding (Scheme 1). The logarithmic complex formation constant was determined using the segmented sandwich membrane method and was found to be 6.5. The nanosensors were characterized in broad pH range from 3 to 8.50 and the same linear calibration was obtained in the concentration range from 10^{-7} M to 10^{-5} M and thus the pH dependent response was overcome. These nanosensors are simple to prepare, stable and exhibit a rapid response.



Scheme 1. *Sensing mechanism of the ligand with potassium cation (a) and fluorescence spectra of the K^+ -selective nanooptodes operated exhaustively with various K^+ concentrations (b).*

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Solve complex and challenging mass spectrometry problems directly from the browser

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[1]EPF Lausanne

Challenging research problems can be solved when interpreting correctly High-Resolution (HR) mass spectra but those are often too complex to be manually analysed so that the information hidden in the experimental data cannot be fully exploited. We describe here new applications of the core ChemCalc library previously published for solving real case research problems. Applications involving metallic contaminant identification, non-natural peptides sequencing and complex mixtures analysis will be presented.

Methods

From a target monoisotopic mass and a range of possible molecular formula, ChemCalc quickly displays all the possible molecular formulas. However for large molecules too many candidates are obtained even for ~ 1 ppm accuracy mass spectra and the use of the isotopic distribution is essential. In order to compare spectra, we implemented a fast algorithm to evaluate the similarity between the theoretical isotopic distribution and the observed spectrum. The algorithm compares a theoretical isotopic distribution of infinite resolution to the experimental data by trying to fit all the peaks based on an estimated experimental error, resulting in the end an interactive on-line tools for high-resolution mass spectra analysis

Results

We developed ChemCalc as a very general core library to deal with calculation of the isotopic distribution from a molecular formula. This library is available on-line on www.chemcalc.org. Among the provided tools, any isotopic distribution containing several hundreds of amino acids or elements having complex isotopic pattern like ruthenium can be simulated and all possible molecular formula found from an experimental monoisotopic mass. While this tool has been already used by many mass spectrometrists and cited in various papers, it was necessary to implement an efficient way to calculate the similarity between theoretical and experimental spectra to sort results and release all it’s power.

In order to measure the similarity between 2 mass spectra, the following steps have been implemented:

1. Define a comparison *window*
2. Normalize the theoretical and experimental mass spectra
3. Fit each peak between the 2 spectra considering an isosceles trapezoid for which the top width and bottom width (defined by the user) are related to the experimental resolution and precision of the spectrometer

Online tools implementing this approach have already allowed to successfully elucidate unresolved problems. Practical examples applied to chemistry and biochemistry will be presented here.

Chemical Composition and Biological Activities of Essential Oils Extracted from Pittosporum Mannii Hook (Pittosporaceae)

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[1]University of Nairobi, Kenya

In Cameroon, *Callosobruchus maculatus* F. and *Sitophilus zeamais* Motsch causes major losses during storage of Cowpea (*Vigna unguiculata*) and maize (*Zeamays*) respectively. The present work aimed to analyse stem bark, leaves and fruits essential oils from *Pittosporum mannii*. Hook (Pittosporaceae), to evaluate their antioxidant activity and to verify their insecticidal effects. Essential oils were extracted by hydrodistillation and analysed by GC-MS equipment.The evaluation for their antioxidant activity by using two complementary test systems namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and β-carotene/linoleic acid test systems was assessed. Insects (*S. zeamais and C.maculatus*) were reared in darkness at room temperature on maize and cowpea grains. Chemical composition determination indicated that α-pinène was the major component from stem bark (23.25%), leaves (16.53%) and fruits (11.64%) essential oils. Stem bark essential oil (SBEO) and leaves essentialoil (LEO) were more effective in DPPH free radical scavenging, recording IC_{50} values of 35.54 ± 0.52 µg / ml and 44.72 ± 0.13 µg / ml respectively. While, oxidation of the linoleic acid was strongly inhibited after 2 hours by the leaves essential oils (LEO), indicating its highest percentage antioxidant activity (AA_{120min} = 68.89 ± 0.59%), relatively compared to the positive control Butylated hydroxitoluene (BHT). Results from insecticidal assays were compared to the synthetic conventional insecticide malathion 5% and SBEO was the most repellent (*PR* = 85.62 ± 1.42%) against *C.maculatus* at the concentration of 0.4 µg / cm^2. While, fruit essential oil (FEO) was the most repellent (PR=47.37±2.48%) against *S. zeamais* at the same concentration. In all trials, mortalities among adult of *C. maculatus* and *S. zeamais,* tested respectively with SBEO (LD_{50}= 0.17 ± 0.01 µl/g) and LEO (LD_{50} = 0.07 ± 0.01 µl/g), were observed to be hight (100% mortalies after 72h). All the essential oils of *P. mannii* were found to be significantly (P≤0.05) affecting the survival of the two insect species, where SBEO was the most effective, reducing 93.1 ± 0.1% and 87.87 ± 0.50% *S. zeamais* F1 progeny and *C. maculatus* F1 progeny emergence respectively. Results also revealed that activities of the essential oils were concentration dependent.

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CHIMIA

CHIMIA 2015, Volume 69
ISSN 0009-4293
www. chimia.ch

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MIR Spectroscopy beyond trace levels - environmental and industrial applications

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[1]Empa, [2]University of Tromsø - UiT , [3]FHNW

Advances in mid-IR quantum cascade laser (QCL) technology have triggered an impressive progress in instrumental developments. This progress is in line with a continuous quest for high-precision and selective measurements of a large variety of molecular species, as well as for compact, robust and field deployable gas analyzers. Correspondingly, the applications cover very diverse areas, including environmental sciences and industrial process control. Recent trends include the miniaturization of QCL spectrometers and multi-component instruments that are based on multi-wavelength QCLs [1].

Nitrogen dioxide (NO_2) is one of the most prominent examples. QC laser based trace gas analysis is well suited for remote locations with very low NO2 mixing ratios. Using an astigmatic Herriott sample cell with 200 m optical path length, a precision of 3 ppt and 10 ppt for $NO_2$and NO, respectively was obtained for continuous, on-site measurements under predominantly free tropospheric air conditions at the high-alpine station Jungfraujoch [2].

The fact that isotope ratios of atmospheric trace gases contain highly valuable information about their sources and sinks triggered numerous instrumental developments based on QCL spectroscopy, because laser based methods can deliver real-time data with high temporal resolution and precision. This is illustrated by 6 years *in-situ* isotope ratio measurements of CO_2at Jungfraujoch [3]. Furthermore, laser spectroscopy is inherently specific to structural isomers having the same mass, and thus capable to perform site-specific isotopomer N_2O measurements [4,5].

Fast detection is illustrated by the development of a patented analyzer for leak detection of aerosol can propellants. The instrument allows detecting small leaks with high speed (‹ 10 ms) and sensitivity (1 ppm), allowing the industrial testing of up to 750 cans per minute [6].

The concept of “multi-color” spectroscopy has been explored based on a dual-wavelength QCL [7]. The active region of this laser consists of two different active layers stacked on top of each other, optimized for a broadband emission at 1600 $cm^{-1}$and 1900 cm^{-1}, while single-mode emission at the desired wavelengths is ensured by a succession of two distributed-feedback (DFB) gratings with different periodicities. A prototype spectrometer allows fast (10 Hz) operation during automotive exhaust emission measurement and ambient air monitoring in a suburban environment [8].

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Detection and identification of non-covalent interactions with SPRi-MALDI MS

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[1]ETH Zurich, [2]HORIBA Jobin Yvon, [3]University of Zurich, [4]Bruker Daltonics

In pharma and life science research, there is a lot of interest for fast and accurate screening methods. The coupling of SPRi and MALDI MS technologies meet the strong demand for high-throughput analysis and identification of biomolecular interactions. In addition it provides information on binding kinetics and binding affinity in real time.

SPR measurements were performed on the SPR Plex II (Horiba, Palaiseau, France) working with polyoxyethylene functionalized gold slides including NHS-esters for immobilizing ligands. Mass spectrometric detection was done with a commercial MALDI TOF mass spectrometer (Ultraflex II TOF, Bruker Daltonics, Bremen, Germany) and the gold slide was mounted to an adapter target. The measurements were performed in the linear positive ion mode with standard settings and a “smartbeam” laser with proper energy. Each mass spectrum was the average of 2000 laser shots acquired at random sample position.

We will show an investigation of off-target binding of various DARPins. DARPins (designed ankyrin repeat proteins) rival antibodies for target binding, but are genetically engineered and more robust. It is known that the DARPin off7 interacts specifically with maltose binding protein (MBP) [1]. MBP is part of the maltose/maltodextrin system, which is a sophisticated regulatory and transport system involving many proteins. The binding specificity of DARPins with a mixture of important proteins will be determined while DARPins are immobilized. In an inverse experiment, MBP (0.4 mg/ml) was immobilized and a mixture of DARPins (off7 and a non-interacting DARPin, E3_5) was injected.

The K_D was determined based on the measured SPR data and resulted in 4.39 nM (± 0.03), which fits very well with the theoretical value of 4.40 nM [1]. A MALDI MS measurement on the SPRi-chip directly after the SPRi experiment verifies the specific interaction between off7 and MBP due to the mass spectrometric identification of off7 and no other protein.

The coupling of surface plasmon resonance and mass spectrometry enables the multiplexed investigation of specific interactions out of a crude mixture with no need of purification, separation steps or labeling in real time, as shown for the interacting partners off7 and MBP.

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Quick and easy NMR titration using slice-selective experiments to study concentration gradients in agarose gels

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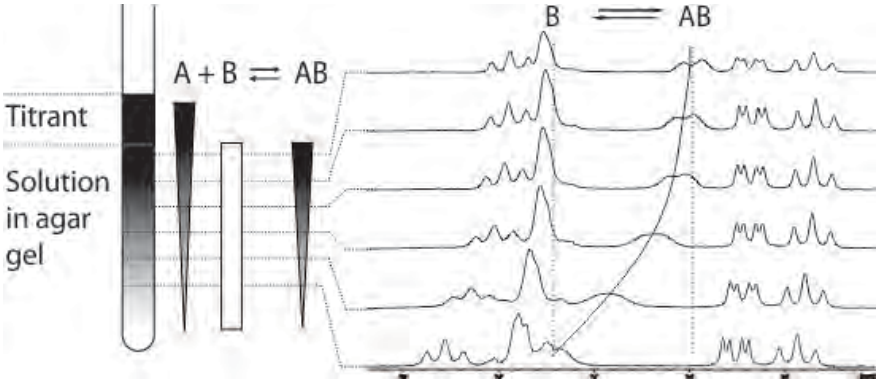
[1]University of Geneva

NMR titration is a long recognised method for determination of equilibrium constants or thermodynamic parameters of reactions, but suffers from the drawback of being time consuming. Recently, spatial-selective NMR spectroscopy for reaction monitoring was proposed[1]. The method relies on slow diffusion of one of the reaction components into a polystyrene gel, containing the other, thus resulting in a spatially dependent sample composition along the NMR tube. Following a similar approach we present the use of agarose gels as the medium for single-experiment NMR titrations in water. It was used to study the inclusion of paracetamol in cyclodextrine macrocycle as a model reaction. The agarose gels benefits from a very simple and reliable sample preparation. Moreover, we observed no interaction between the matrix and the compounds under study and obtained high-resolution spectra, identical to those obtained from solution samples.



One of the main advantages of the proposed method is its speed achieved by performing the slice selective experiments in an interleaved manner, thus affording the acquisition of quantitative spectra in 1-10 minutes. In addition to the room temperatures measurements, the variable temperature NMR titration has been also investigated, as the agarose gels possess the attractive property to lower the freezing point of water making it possible to study water solutions up to -8 C.

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High-speed, high-resolution, multi-elemental imaging of geological samples

M. Burger[1], A. Gundlach-Graham[1], S. Allner[1], L. Gyr[1], H. Wang[1], G. Schwarz[1], B. Hattendorf[1], D. Grolimund[2], D. Günther[1]*

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Elemental imaging has recently gained a lot of attention in the field of geology, biology and medicine, where the characterization of micro-structures and the evaluation of elemental distributions across heterogeneous samples are of major relevance.
Until now, most instrumental approaches applied in either two or three dimensional imaging studies were comprised of a laser ablation system coupled to a scanning (quadrupole or sector-field) mass analyzer. The fundamental operating principle of those systems did not allow simultaneous multi-element detection. In order to enable shot by shot detection, the system had to be operated at low laser repetition rates which made it suffer from spectral intensity skew errors and accounted for an extended data acquisition time. Laser spot sizes of a few 10s of microns were commonly applied impairing high lateral resolution.
Here we report the coupling of an ArF excimer laser ablation system (λ=193 nm) to a prototype ICP-TOF mass analyzer enabling high-speed, high-resolution, multi-elemental imaging of geological specimens.
Operating a low-dispersion laser ablation tube cell, minimum signal widths of 9 ms were obtained when ablating NIST 610 (full width at 1% peak maximum). The system's capability to run at 100 Hz laser repetition rate while maintaining baseline separation of individual transient signals was demonstrated. Limits of detection in the low µg/g range were observed for most trace elements. Full mass spectra can be acquired at a speed of up to 33 kHz.
Following the thorough characterization of the new setup performing different imaging experiments on standard reference materials, this LA-ICP-TOFMS setup was applied in quantitative two and three dimensional imaging studies dealing with various geological specimens including a cesium infiltrated Opalinus clay thin section with pyrite inclusions and a meteorite sample. Laser spot sizes varying from 1.5 to 5 µm and a laser repetition rate of 20 Hz were applied. The results of these studies will be compared to the findings made by synchrotron based X-ray tomography. Advantages and shortcomings of both techniques will be discussed.

Electromembrane extraction: a new technical development.

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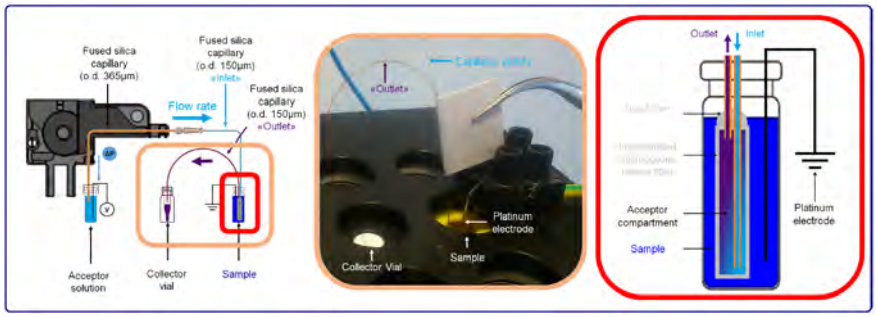
[1]University of Geneva

Introduction

Electromembrane extraction (EME) is a relatively recent electro-assisted extraction method [1]. EME consists in the migration of charged compounds under an electrical field, through a supported liquid membrane (SLM) impregnated on a microporous hollow-fiber. This method has already shown high potential for the extraction of low molecular weight basic compounds of medium polarity. In this study, a new set-up of EME device was developed. The main attribute of this original device, directly inspired from microdialysis, relies in the continuous refreshment of the acceptor compartment, enabling higher preconcentration factors compared to the conventional static set-up, and good recoveries.



Methods

Standards solutions (50 ng/mL) of 15 neuropeptides were extracted with the new EME set-up. For the first time to our knowledge, a polypropylene hollow-fiber with a 50 µm wall thickness was used. Pressure and voltage were applied thanks to an Agilent Electrophoresis 7100 CE system. Several parameters such as the SLM composition (nature of the organic solvent and carrier), the voltage, the extraction time, and the flow rate of the acceptor phase were evaluated towards preconcentration factor and recovery. Extracts were analyzed by RP-UHPLC-MS/MS with SRM using an Agilent QqQ 6490 MS system. Preconcentration factors and recoveries were estimated by comparison with a standard solution injected in the same conditions.

Results

Although alcohols have been described the most suitable solvents for peptide extraction, the addition of decanone to a nonanol SLM (1:1, v/v) stabilized the extraction process and offered better results for a few peptides. The addition of DEHP as a carrier to the SLM was found to enhance the selectivity for polar peptides. The high electrical field (ca. 200/cm) significantly influenced the extraction process. High extraction times (up to 45 min.) had no significant influence on the preconcentration factor, but greatly improved recovery (up to 70%). Decreasing the flow rate of the acceptor phase enabled a high preconcentration factor (up to 50-fold).

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Drug quantification in blood within microstructures for Point-of-Care Therapeutic Drug Monitoring

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Many modern therapeutics involved for instance in the treatment of infections, cancer or in post-transplant therapies require Therapeutic Drug Monitoring (TDM) owing to their narrow therapeutic range. Currently this process is demanding for the patients, as several milliliters of blood are required, slow and costly, as the sample need to be transferred to a central laboratory, and suffer of limited efficacy, as the results are difficult to interpret for a non-specialist. To overcome these problems, we aim at providing a simple, rapid and sensitive solution by develop a compact and cost-effective Point-Of-Care drug quantification device based on miniaturized competition assay. Preliminary results have demonstrated the feasibility of downsizing FPIA and shown that two prototypical drugs: Tobramycin and Tacrolimus, an antibiotic and an immunosuppressant can be quantified using minute amounts (only 20 µl) of human blood. For Tobramycin, the assay could be further miniaturized down to just one µl of human serum while preserving its performance. Moreover, the assays could be transposed into different microstructures using a custom-made Fluorescence Polarization instrument, as a first step towards a Point-Of-Care Therapeutic Drug Monitoring device.

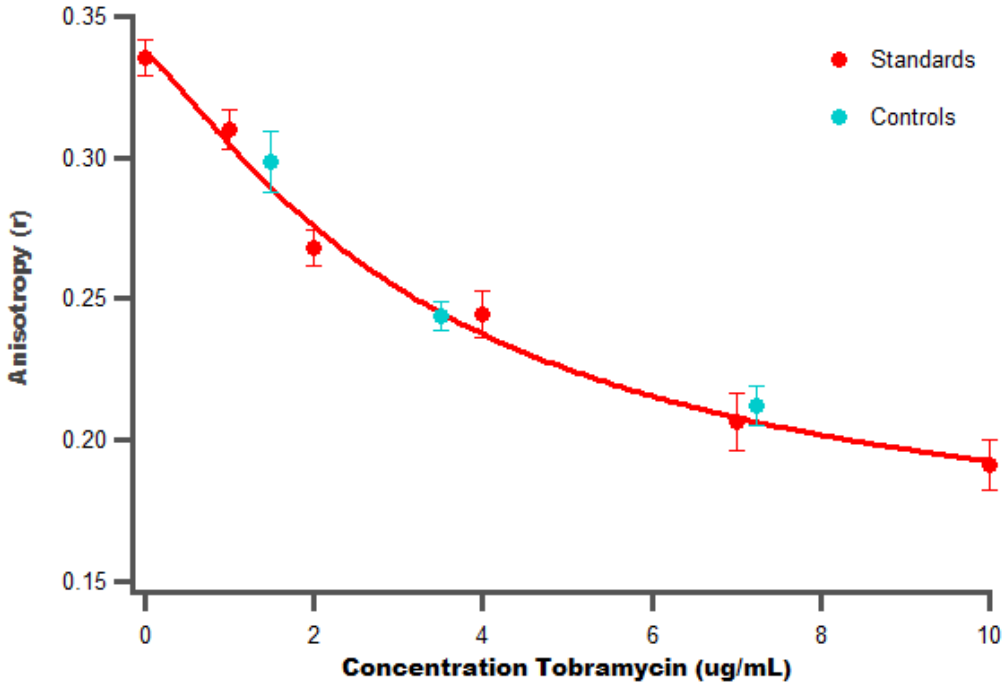

Fig.: Fluorescence Polarization Immunoassay (FPIA) calibration curve with a novel Tobramycin derivative for Tobramycin quantification using minutes amount of blood

Sanavio B and Krol S (2015) On the slow diffusion of point-of-care systems in therapeutic drug monitoring. *Front. Bioeng. Biotechnol.* **3**:20.

Thin Layer Ionophore-Based Membranes for Multianalyte Detection

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A novel concept is introduced here that allows one to detect multiple ions simultaneously using a single ion-selective membrane. This is demonstrated with thin plasticized polymeric membranes (less than 300 nm in thickness) containing up to two ionophores in addition to a lipophilic cation-exchanger that is back side contacted with a film of electropolymerized poly(3-octylthiophene) (POT) as an ion-to-electron transducer.

The operating principle is shown in Figure 1a, illustrating a membrane that contains lithium and calcium ionophores simultaneously. An anodic scan partially oxidizes the POT underlayer, which results in the expulsion of cations from the membrane at an appropriate potential. Distinct ion transfer waves appear in the obtained voltammogram owing to the different standard ion transfer potential of the cations involved in the process (Figure 1b). Moreover, each peak shifts to more positive potentials with increasing activity of the corresponding cation according to the Nernst equation. In other words, calibration curves analogous to potentiometric sensors are found for lithium and calcium, independent of the concentration of the other cation.

The basis of the developed concept is established for lithium and calcium accompanied by a response model that agrees well with the experimental results.[1] This is the first time that a selective potentiometric multianalyte analysis is achieved with an ion-transfer electrochemical technique. Current efforts in our laboratory aim to explore different membranes containing multiple ionophores to analyze several cations in the same sample using a single electrode.

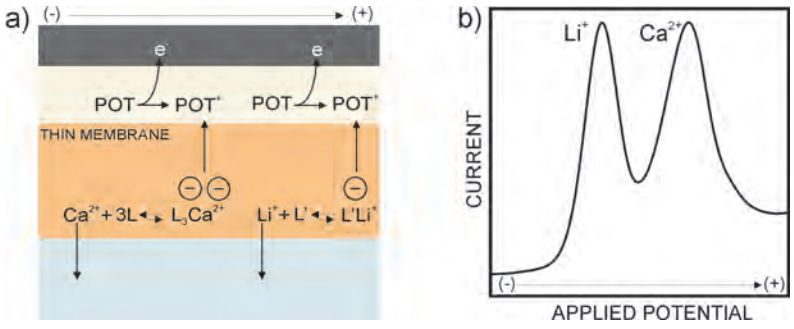

Fig. 1a) Scheme of the electrochemical process (L=calcium ionophore and L'=lithium ionophore). **b)** Voltammograms obtained for equimolar concentrations of lithium and calcium.

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Multistage Transversal Modulation Ion Mobility Spectrometry: Reducing the Voltage Required for High Resolution IMS for pre-existing mass spectrometers.

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Commonly used IMS techniques provide a pulsed output of ions, which requires interfacing of these instruments to very fast Mass Spectrometers (such as ToF). Our goal is to develop an Ion Mobility Spectrometer (IMS) that can be coupled with any pre-existing MS, and which does not require a fast IMS-to-MS synchronization.
Transversal Modulation IMS (TMIMS) combines an axial electric field and a transversal-oscillating electric field that provides a continuous output of mobility selected ions. It can also be operated in full transmission mode, and at atmospheric pressure. These features make it a very versatile IMS solution for pre-existing mass spectrometers. We have developed two prototypes that demonstrate the possibilities of the instrument. However, these systems still require further improvements to meet final user requirements: (i) the transmission must be increased, (ii) the voltage of the instrument inlet must be reduced to manageable levels, (iii) the oscillating voltages must be reduced.

Here we present a new instrument that uses a ladder of six small TMIMS stages, each powered with a square wave with a voltage of 1KV. This voltage is about six times smaller than the one used in previous prototypes, and it can be easily handled with low-cost electronic components. According to simulations (COMSOL), selected ions are sequentially focused to the center of each slit of the ladder, while non-selected ions accumulate lateral displacements as they traverse the TMIMS ladder. As a result, despite the low deflection voltages used, ions are deflected away from the TMIMS outlet as if a much higher deflection voltage was used, providing high resolving powers with low voltages.

The inlet of the new instrument was grounded, thus facilitating coupling with standard ions sources. The outlet was coupled through a resistive capillary with a LTQ mass spectrometer (Thermo). The resistive capillary transfers the ions from the outlet electrode of the TMIMS (which was powered at 8kV) to the inlet of the MS. We used two different sources to test the performances of TMIMS ladder. Our experiments with the new prototype show that, as expected, although the resolving power of each isolated TMIMS stage is poor, the resolving power of the ladder is comparably much better. Additionally, by comparing the shape of the spectra when only some stages of the ladder are powered, we could show that the ladder also improves the robustness against over-current limitations: when too large currents are inputted through the TMIMS inlet, space charge spreads the ions beam, thus broadening the peaks in the spectra. In the ladder, space charge effects are restricted to the first stage, allowing the rest of stages to function normally.

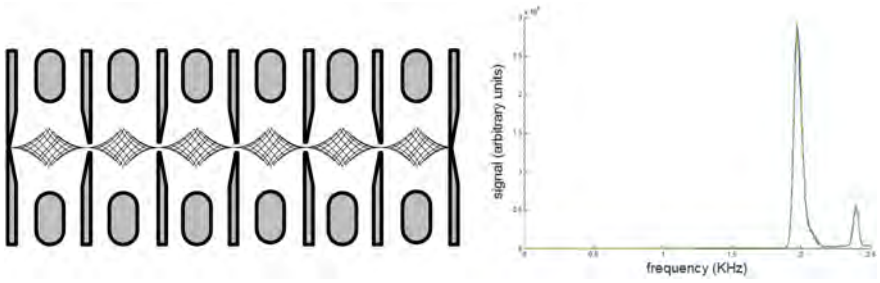

Figure: (left) Scheme of the TMIMS ladder, and simulated trajectories of the selected ions through the TMIMS ladder; (right): Tetraheptylammonium signal measured at the MS as a function of the frequency of the oscillating field.

Fourier optical beam shaping of femtosecond laser pulses for high resolution depth profile analyses by LA-ICPMS

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Element-selective depth profile analyses of high-tech materials, including thin films and multilayer systems, have gained importance over the past years in order to keep up with the ever growing standards concerning control and optimization of manufacturing processes. Here, technologies utilizing ion beam probes such as secondary ion mass spectrometry (SIMS) and Rutherford backscattering (RBS) [1] are most frequently chosen due to their high depth resolution, sensitivity, and selectivity. By comparison, laser ablation (LA)-based photonic probes in combination with, *e.g.*, inductively-coupled plasma (ICP)MS have rarely been applied in this context, even though they offer the feasibility of atmospheric sampling and, therefore, a high throughput of specimen. Furthermore, up-take rates of ‹ 10 nm per shot have been reported [2] when femtosecond (fs) laser sources are employed, which potentially allows to perform depth profile analyses with resolutions in a range of 100 nm and below. For this, a homogenization of the laser radiation delivered to the sample surface is required to ensure the formation of craters with steep walls and flat bottoms and, in this way, to suppress mixing of material originating from different layers in the course of analyses. However, ahigh-grade beam homogenization has remained challenging since most of the design concepts applicable are accompanied by either beam distortion and/or stretching of the laser pulse duration.

In this study, a Fourier transformation (FT)-based two-step optical processing scheme for the generation of homogenized laser intensity profiles in an objective`s focal plane was conceived, which rests on the delivery of diffracted beams. Initially, the laser radiation is truncated by a circular aperture to let pass only the inner region of the beam where intensity gradients are smallest and uniformity is highest. Downstream the aperture, the beam diffracts along its path to form an Airy disk in the far field (1^{st} FT). Finally, the diffracted radiation is focused by an aberration-corrected objective lens to re-transform the Airy disk into a homogenous spot at the sample surface (2^{nd}/reverse FT). The performance of this scheme concerning shape, morphology, and general appearance of craters formed in borosilicate glass by fs-LA @ 400 nm wavelength and 150 fs pulse duration was tested under both, helium atmosphere and ambient air. Crater shapes, morphologies, and up-take rates per shot were examined using optical and confocal microscopy as well as scanning electron microscopy.

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High resolution laser ablation depth-profiling mass spectrometry

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Accurate investigations of chemical heterogeneity of solid materials is a highly demanding task in various fields of applications, ranging from purity control of high performance material in industrial application, tissue analysis in medical diagnostic or investigation of surface weathering in space research. Laser depth-profiling combined with mass spectrometric analysis is one of the most powerful methods which offers high vertical resolution together with highly sensitive chemical analysis.

In our recent studies we analysed the chemical composition of various solid materials by means of depth profiling method. The chemical analysis were conducted by combining a fs-laser ablation/ionisation ion source (~190fs, λ = 775nm, laser spot diameter Ø ~15µm) with a miniature reflectron time-of-flight mass spectrometer (LMS).[1,2] Electrochemically deposited copper samples bearing spatially-confined impurity layers were used as platform for the development of a novel quantitative depth-profiling technique. These samples were fabricated under galvanostatic conditions by means of an additive-assisted plating procedure, which is used in the semiconductor industry for the fabrication of small sized copper interconnect architecture of Si-based logic and memory devices.[3,4] This plating procedure, however, suffers from incorporation of additives, which causes the formation of defects within the embedding copper material decreasing the performance and life time of the integrated circuits. The understanding of the embedding conditions and the quantitative chemical analysis of these impurity layers becomes mandatory to improve the filling procedure within this industrial sector.

The quantification of the mean ablation rate by means of optimising the laser irradiance and single shot experiments allowed for optimal depth profiling conditions with unprecedented vertical resolution in the sub-nm range. The actual instrument performance, which includes the high vertical and lateral resolution, the high detection sensitivity reaching ~10ppb concentration (atomic fraction) of elements within a sample material, and the high dynamic range of more than eight orders of magnitude enables high precision and quantitative chemical analysis of the grain boundary sites where the impurities within the copper deposit are incorporated. Our present capabilities in laser ablation mass spectrometry complement and improve significantly upon previous SIMS measurements providing a better understanding of the incorporation of distinct additives.

[1] A. Riedo et al., *J. Anal. At. Spectrom.,* **2013**, 28, 1256-1269.
[2] M. Tulej et al., *Geostand. Geoanal. Res.,* **2014**, 38, 441-446.
[3] V. Grimaudo et al., *Anal. Chem.,* **2015**, 87, 2037-2041.
[4] P. Moreno-García et al. to be submitted to *Electrochemistry Communication*.

Single-walled Carbon Nanotubes (SWCNTs) for Bioanalyte Sensing

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Nanomaterials offer distinct chemical and optical properties that are advantageous in a breadth of bioanalytical applications, including the development of highly selective and sensitive sensors [1]. Polymer-wrapped single-walled carbon nanotubes (SWCNTs), in particular, have been used to develop optical sensors selective towards a variety of biomolecules, including nitric oxide (NO) [2], riboflavin (vitamin B2), and the hormone, estradiol. These sensors use SWCNT fluorescence to quantitatively monitor analyte concentration. The selectivity of these sensors is tuned by engineering the polymer wrapping, which imparts the nanotube with molecular recognition capabilities. Because SWCNT fluorescence is strongly affected by the nanotube environment, perturbations in the environment caused by analyte adsorption and desorption result in fluorescence changes. Previous studies have shown that analyte concentration modulates SWCNT fluorescence with sensitivities that can even extend down to the single-molecule limit.

SWCNT-based optical sensors have been used in the quantitative, spatiotemporal measurement of biomolecules in several biological systems, including mammalian cells, *in vivo* models [3], photosynthetic organelles [4] and, most recently, whole plant leaves [5]. This presentation summarizes the recent developments and applications of the field, as well as our ongoing endeavors in engineering a new generation of optical nanosensors.

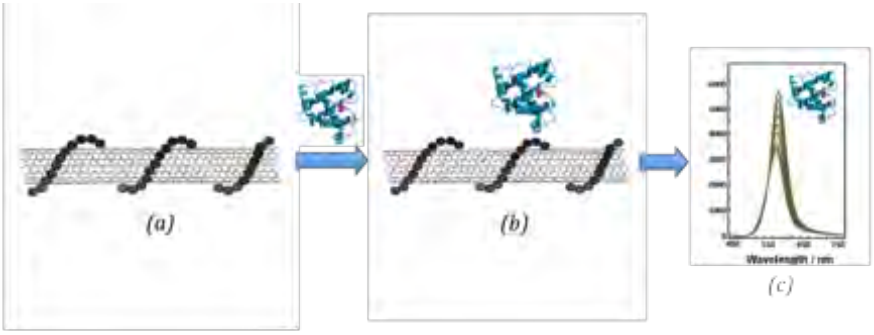

Figure 1. Schematic representation of SWCNT-based optical sensors for bioanalyte detection:
(a) SWCNT wrapped with surfactant, polymeric, or biological coatings.
(b) Binding of the bioanalyte to the SWCNT (blue molecule).
(c) Change in SWCNT optical signal (fluorescence emission).

[1] A. A. Boghossian et al., *Chem. Sus. Chem*. **4**, 848 (2011).
[2] Z. W. Ulissi et al., *Nano Letters* **14**, (2014).
[3] J.H. Kim et al., *Nature Chemistry* **1**, 473 (2009).
[4] A. A. Boghossian et al., *Advanced Energy Materials* **3**, 881 (2013).
[5] J. P. Giraldo et al., *Nature Materials* **13**, 400 (2014).

Variable Q1 windows for MS acquisition and predicted LC retention time ranges for LC-SWATH-MS analysis

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The analysis of metabolomics samples by LC-MS commonly allows the detection of hundreds of compounds. Precursor ion intensities can span over several orders of magnitudes. Data dependent acquisition (DDA) methods often miss the acquisition of MS/MS spectra for low intensity compounds which cannot be used for quantification. Data independent acquisition (DIA) methods like SWATH are emerging to obtain all MS/MS spectra for qualitative and quantitative analysis.

SWATH uses Q1 windows with a certain width to obtain all MS/MS spectra within a *m/z* range. It is important to note that ramped collision energies (CE) are commonly applied. As such reference MS libraries with composite MS/MS spectra which cover the whole expected CE range from 10 – 100 eV are needed for improved MS/MS matching. We developed such a MS metabolomics library which includes 528 compounds from all the major human metabolite classes.

Depending on sample complexity SWATH data acquisition can lead to multiplexed MS/MS spectra when several co-eluting precursor ions are acquired in the same Q1 window. To address this issue, we propose to use variable Q1 windows. We developed the in-house software SwathTuner to calculate optimal window widths based on the *m/z* and TIC distribution of the LC-MS features detected in the sample.

For many compounds and especially isomers a good MS/MS matching is not always sufficient for unambiguous identification. In these cases, we propose to use predicted LC retention time ranges based on a linear regression between the retention times for a set of isotopic labeled metabolites and their log D values.