CHIMIA

CHIMIA 2019, Volume 73
ISSN 0009-4293
www. chimia.ch
Supplementa to Issue 7-8/2019

SCS
Swiss Chemical
Society

SCS Fall Meeting 2019
Poster Abstracts

Session of Analytical Sciences

September 6, 2019
Irchel Campus, University of Zürich, Switzerland
http://scg.ch/fallmeeting/2019

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Ultrahigh ion-mobility separation of CID fragments for glycan analysis

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Glycan analysis is a major challenge due to their isomeric heterogeneity. Ion mobility spectrometry (IMS) combined with cryogenic vibrational spectroscopy has been implemented for identification and characterization of glycans, but it does not allow the separation of glycan isomers with subtle differences [1]. A promising approach to overcome this limitation combines ultrahigh-resolution IMS using structure for lossless ion manipulation (SLIM) with cryogenic IR spectroscopy [2]. In the present work, a SLIM board containing a series of on-board traps has been designed, based on the work by Smith and coworkers [3-4]. We have demonstrated the use of these traps for turning and confining ions and simulated ion trajectories using SIMION 8.1 to evaluate their potential application for on-board collision induced dissociation (CID). We will present the latest experimental results using this approach for glycan analysis.

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Efficient Tip Recycling for AFM-based Tip-Enhanced Raman Spectroscopy (TERS)

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Tip-enhanced Raman spectroscopy (TERS) is an analytical technique which combines scanning probe microscopy (such as Atomic Force Microscopy - AFM) and Raman spectroscopy with the aim of obtaining a vibrational spectrum from very small spots of the sample, well below the optical diffraction limit. It was pioneered in our lab in early 2000s and has proven to be very versatile for nanoscale characterization of 2D materials and biological samples [1]. The heart of every AFM-TERS experiment is an enhancing plasmonic probe; commercially available AFM cantilevers, usually made of Si, need to be metalized to become suitable as a TERS probe and the most common functionalization method is the deposition of a thin layer of metal (generally Ag) via physical vapor deposition (PVD). Despite being widespread and effective, the PVD coating process still requires several hours [2]. Conversely, the average operational lifetime of a plasmonic tip is less than 5 hours in ambient conditions and, for every experimental measurement, 3 to 5 tips need to be prepared as a backup. At the end of their life cycle, whether used or not, metalized tips have to be discarded as they are no longer enhancing and possibly contaminated with carbonaceous species from the environment. Moreover, since the metal coating swells the apex of the tip, their spatial resolution is compromised. Due to their high cost (up to hundreds of francs each) and the long time it takes to prepare them, the current procedure is considered neither efficient nor economical. The possibility to recycle tips is thus interesting from two main points of view. Firstly, reusing probes represents an obvious economic advantage. In addition, defining a consistent cleaning routine would possibly help tackling another major problem of TERS, i.e. the environmental contamination leading to the inconsistency of experimental results. In fact, even though the technique developed dramatically in the last years, contamination from the environment is a key issue the TERS community has been facing from the earliest times and is yet to be fully solved [3]. In this project, several approaches were tried with the goal of establishing an effective workflow for the recycling of AFM-TERS tips. Selective removal of the metal coating was achieved, without compromising the underlying silicon structure. Recycled tips were investigated using SEM and AFM and showed no significant difference in structural features and spatial resolution with respect to brand new ones. Preliminary TERS experiments on test samples exhibited no deviation from reference spectra, confirming the reliability of the approach. These results pave the way to a cheaper and more efficient probe preparation protocol that could easily be implemented in other laboratories.

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Mass spectrometric investigation of neutral soluble dietary fiber interactions with small molecules

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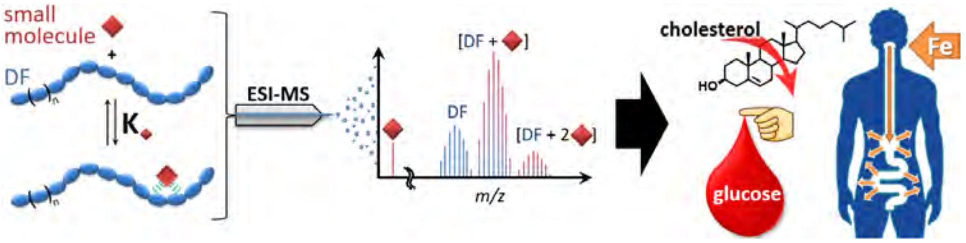
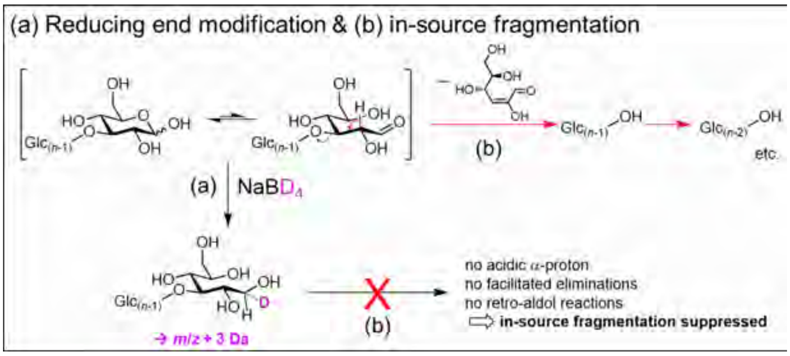
Regular intake of soluble dietary fibers (DF) is an effective way of reducing the risks of cardiovascular diseases related to high blood cholesterol levels and diabetes type 2. In addition, the bioavailability of nutritionally relevant minerals like iron highly depends on the food matrix. Thereby, fibers and their associated phytochemicals play an important role on mineral absorption. While some interactions of metal cations with negatively charged soluble fibers are well characterized in the literature, knowledge on binding of e.g. glucose, bile acids, and minerals with neutral dietary fibers in order to understand the mode of action for their health benefits is scarce. To study interactions, mass spectrometry (MS) has been used in the past for well-structured protein-ligand complexes, but has never been attempted for neutral, native polysaccharides.



Hence, the overall aim of this study is the development of a mass spectrometric method in order to investigate the structure-function relationship of bindings between dietary fibers and nutritionally relevant small molecules & minerals. For this purpose, a selection of linear and branched soluble dietary fibers such as mixed-linkage cereal β-glucan and guar gum galactomannan were partially hydrolyzed to produce polysaccharides of suitable length and fully characterized for the monosaccharide composition and molecular weight distribution by ion exchange and size exclusion chromatography, respectively.



Electrospray ionization (ESI) with quadrupol time-of-flight (qTOF) mass spectrometry (MS) was used for the detection of individual polymer chains, with successful ionization of glucans and galactomannans of up to 45 monomer units. For an enhanced ionization of polysaccharides, nano-flow electrospray ionization (nanoESI) is compared to conventional ESI. Reducing end modifications allowed for the ionization of the intact polysaccharide chains without in-source fragmentation occurring, an important prerequisite for the study of polysaccharide interactions with small molecules. The suppressed fragmentation can be explained by the lack of a carbonyl that would facilitate eliminations & retro-aldol reactions (see scheme above). Both in positive and negative mode MS, the observed distribution and average chain length of polysaccharide was in agreement with the known molecular weight of the used polysaccharide standard, making this direct-infusion ESI-qTOF-MS method a valuable tool for characterization of labelled polysaccharides to assess derivatization efficiencies. Finally, the proof-of-concept results for the MS study are presented for the interaction of neutral soluble dietary fibers with known binding partners such as calcofluor white, congo red, or borate/boronate esters as positive controls.

Host-guest interaction to improve the bioavailability of bent metallocenes? A mass spectrometric investigation

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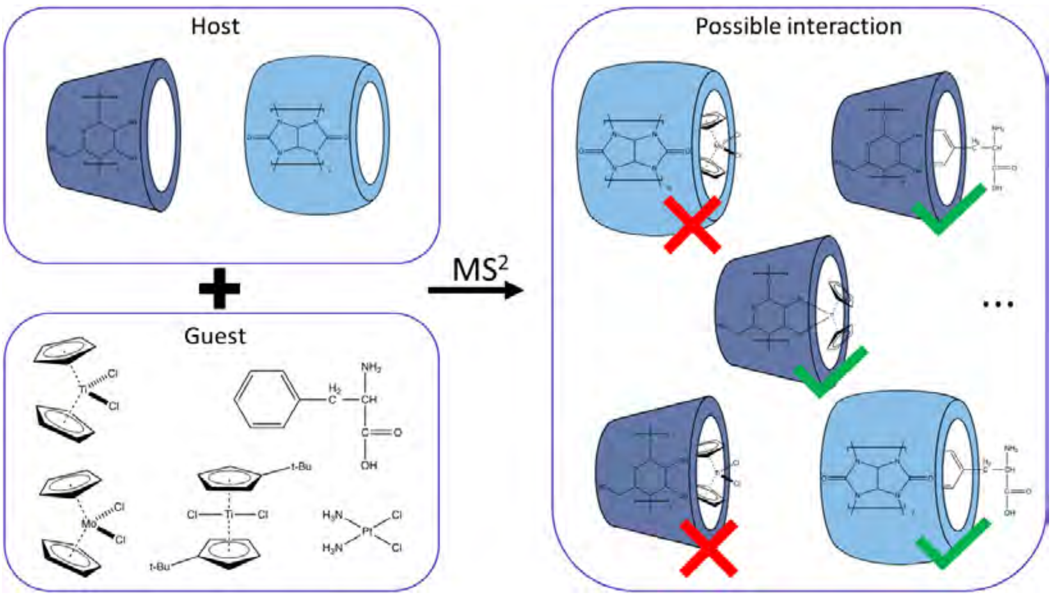
Transition metal complexes, such as the bent metallocenes (Cp_2MCl_2), have found widespread interest as anticancer agents in recent years. The active species of metallocenes are formed by exchange of the chloride ligands in aqueous environment, resulting in a low pH of the solution. Prior to administration of the drugs, the pH of the solution should therefore be increased to physiological condition. Unfortunately, this results in immediate inactivation of the metallocene due to degradation or extensive dimerization. Past studies examined the encapsulation of metallocenes in cyclodextrins and cucurbiturils by NMR, X-ray diffraction, and molecular modelling, and suggested the incorporation of Cp_2MCl_2 in the hydrophobic cavities. This encapsulation is claimed to result in a stabilization of the prodrug against inactivation at physiological pH.

Our study focuses on the investigation of these host-guest structures by tandem mass spectrometry in order to gain greater insight into the nature of the interaction. The study comprises four cyclodextrins and two cucurbiturils as host-molecules and a variety of guest-structures.



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A laser ablation ionization mass spectrometry approach for compositional analysis of Sn/Ag solder bumps

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Electrochemical deposition of Sn/Ag solder bumps, the technologically most relevant solder material of today[1], requires assistance of different organic additives in the plating bath to account for the highly different electrochemical properties of the two metals and assure their efficient co-deposition. However, it is very important that there is as little embedment of these organic additives into the alloy matrix as possible. This is because voids, inhomogeneities and non-metallic contaminations within the alloy bumps significantly reduce functionality and lifetime of the solder contacts formed from them.

To obtain more detailed understanding of the processes taking place during electrodeposition and to thus be able to optimize plating bath compositions towards achieving pure alloy deposits, chemical analysis approaches providing lateral and vertical information on the solder composition are required. Our femtosecond laser ablation mass spectrometer, the LMS instrument[2, 3], has previously been shown to provide such finely resolved data[4, 5]. In this contribution we present fundamental studies on laser-matter interaction on Sn/Ag alloy investigating the effect of different wavelengths. We furthermore show how the findings from these studies combined with novel measurement approaches allow to significantly improve compositional analysis results on Sn/Ag bump structures.

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The Transfer Rate of PCBs from Feed to Adipose Tissue Depends on Body Fatness in Growing Cattle

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs). Although they have been banned worldwide in 2004, it is estimated that around 80% of PCBs ever produced still remain in our environment. Their lipophilic property is responsible for their bioaccumulative nature, so that more than 90% of human exposure is through consumption of food of animal origin, of which 18% comes from bovine meat. In order to ensure chemical safety of animal products and further reduce the human exposure, it is important to understand the transfer of PCBs in the food chain. The aim of the present study was to better understand the transfer of PCBs in growing cattle by investigating the effect of body fatness and growing rate on the biotransfer factor (BTF) and the bioconcentration factor (BCF), indicators often used to assess and manage chemical risks in animal farming systems. Indeed, studies in monogastrics demonstrated that BTF and BCF are influenced by animal physiology, which deserve further study in ruminants. Eleven bulls were fed three different diets composed of maize, grass silage and concentrate, having PCB background concentrations. After a fattening period of 293 (±29) days, the animals were slaughtered at a body weight of 530 (n=4) or 600 (n=7) kg. Perirenal adipose tissue was sampled for PCB analyses, and carcass weight and the adipose tissue proportion of the 11th rib were determined for further estimation of carcass lipid content. In order to test the effect of body fatness and growing rate, the animals were classified based on low, medium or high carcass lipid content, as well as on slow, medium or fast growing rate (> 0.5 SD below, ± 0.5 SD from and > 0.5 SD above the carcass lipid content mean or growing rate mean, resp.). Effect of classes on BTF and BCF were further assessed using a mixed model in SAS 9.3. For all PCB congeners tested decreasing trends in both BTF and BCF could be observed from low to high carcass lipid content, which suggests that a dilution process occurred in the case of fatter cattle. Growing rate, however, had none effect on neither BTF nor BCF. Besides the animal physiology, also the PCB chlorination degree affected the transfer factors, as both BTF and BCF increased with chlorination degree, presumably because low-chlorinated PCBs are better metabolized than high-chlorinated ones. These results underpin the complex interaction between POP physiological properties and animal physiology, which leads to difficulties in interpreting average transfer factors to support chemical risk assessment and management in livestock farming systems.

Chemical Characterization of Proteo-lipidic Membranes with Atomic Force Microscopy and Tip-Enhanced Raman Spectroscopy

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The main components of biological membranes are primarily phospholipids and proteins, which form together a two-dimensional physical functional barrier, selectively connecting the inner milieu of the cell with the outside world. Membranes usually contain several different highly-functionalized structures that help regulating the basic functions of the cell. Functionalized domains in membranes play a major role in cell signaling as they may act as regulators of the protein activity; they are thus of key significance for biomedical sciences and drug design [1].

Bacterial purple membranes (BPM) from *Halobacterium salinarum* have been used as a model system to study the structure and function of biological membranes. Atomic force microscopy (AFM) and AFM based force spectroscopy (FS) are invaluable when studying proteins in biological membranes [2]. These techniques yield subnanometer spatial resolution and rich information on protein structure and organization. However, the spatial distribution of lipids around proteins, at the macro- and nanoscale, is yet to be ascertained. Confocal fluorescence microscopy has been combined with AFM and FS to differentiate cytoplasmic and extracellular membrane surfaces[3] but did not provide information on the lipids in the membrane.

Tip-enhanced Raman spectroscopy (TERS) was pioneered in our lab in early 2000s and combines scanning probe microscopy (such as AFM), using a plasmonically resonant tip, with Raman spectroscopy to obtain rich chemical information at the nanoscale [4]. In this work, we present the study of BPM patches, deposited on a flat metal substrate suited for plasmonically enhanced spectroscopy. We performed AFM imaging and TERS to correlate topographic and spectroscopic information at the nanoscale. The sensitivity of Raman spectroscopy to both lipid and protein domains allows us to complement previous studies and to improve our understanding of lipids and proteins' organization in biological membranes.

This work demonstrates the usefulness of TERS for the study of supramolecular domains in proteo-lipidic membranes.

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Novel strategies using UV and IR fs-LIMS for chemical depth profiling in laboratory space application

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To understand the origin, evolution and present state of our Solar System and its local interstellar environment it is mandatory to know the element and isotope composition of the primitive pre-solar nebula from which our planetary system formed. The comparison of the initial and current composition of various planetary objects gives insights to possible material transformation processes that occur in time. The composition of the Sun can thereby be used as the reference source for elemental abundances within the Solar System since it represents 99.86% of the total Solar System mass.

The Genesis Discovery Sample Return Mission launched by the National Aeronautics and Space Administration (NASA) was designed to capture solar wind ions in ultra-pure solid collectors (e.g. Si wafers) for laboratory analysis of the solar bulk composition.[1] The solar wind impacted with velocities between 400 and 800 km/s in the collectors leaving implanted particles (neutrals or ions) in some tens of nm depths of the substrate surface. It is estimated that about 10^{20} atoms (0.4 mg) of solar wind is returned to the Earth for detailed chemical analysis. Depending on the solar wind regimes and on the chemical property of the particles various implantation distribution with abundance maxima around 20-40 nm depths are expected. The shallow implantation depth and the low density of implanted particles rend the chemical analysis particularly challenging.

The Laser Ablation Ionization Mass Spectrometry (LIMS) system from the University of Bern provides promising measurement capabilities to tackle such particularly demanding measurements because it allows for chemical analysis with high sensitivity (ppb) and high spatial resolution in the micrometre (lateral) and nanometre (vertical) range.[2] The instrument consists of a miniature RTOF-mass spectrometer and an integrated femtosecond laser system that operates at 1 kHz and 775 nm wavelength. Combined with harmonic generators the laser system provides additionally output radiation at the second (387 nm) and the third harmonic (258 nm) of fundamental laser beam. Novel measurement strategies are however still required to successfully enable the chemical analysis of trace elements and isotopes in only 20-40 nm substrate depths.

In this contribution, new approaches dedicated to the improvement of laser-induced chemical depth profiling of thin solid layers will be presented on various samples, including semiconductor materials and certified standard reference materials (e.g. alternating thin Cr/Ni films from NIST 2135c). These strategies will involve the application of different wavelengths (from IR to UV) to elucidate the influence on the ablation (crater size and shape, heat affected zone), the usage of particular raster methods consisting of a 2D binning procedure[3] along the substrate surface to increase the sensitivity of the measured species (about 2 decades and more), or the employment of a double laser pulse system[4] to increase the signal intensity (2 decades) at the ablation threshold providing the possibility to reduce further the uptake rate of material in the ablation process. All together, these strategies will allow to provide accurate chemical insights of the Genesis samples, and therefore a better understanding of our Solar System.

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The A2[3]G1 and A2[6]G1 N-glycan structures by ion mobility mass spectrometry

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Introduction

N-glycans are composed of a few types of monosaccharide building blocks, but the large number of possible branching and linkage patterns results in an enormous diversity of structures. Glycan chains may exhibit identical atomic composition and mass, posing an analytical challenge for one-dimensional mass spectrometric analysis. Nonetheless, there has been increasing evidence that certain isomeric species can be distinguished when MS is combined with ion mobility separation. To investigate this hypothesis our research is focused on characterizing terminal α-linked galactose isomers, the main N-glycans present on the vast majority of proteins produced in mammalian cells.

Methods

Gas-phase separation of sodiated afucoslyated standards A2 (G0), A2G1 (G1) and A2G2 (G2) are analysed using traveling-wave ion mobility mass spectrometry (Synapt G2-S HDMS, Waters). To generate a database of selected N-glycans, the extracted arrival time distribution (ATD) are fitted with a Gaussian function and converted into their collision cross section (CCS) values. Subsequently, the terminal α-linked galactose structures are examined by gas-phase hydrogen/deuterium exchange (HDX).

Results

A clear difference in drift times and CCSs for the G1 N-glycan, a mixture of galactose α1,3 and α1,6, suggests the presence of two distinct conformers. An ATD with components at of 5.5 and 6.9 ms is observed. On the contrary, the intact ions of the non-isomeric species from the A2 family, which are structurally and chemically similar but differ in the last galactose (G0 – lack of terminal galactose and G2 – two terminal galactose), show only a single component ATD.

Conclusions

Our data highlight the potential of IMS as a complementary method in structural characterization of N-glycans. The approach does not require derivatization and is generally applicable for identifying galactose isomers from recombinant pharmaceuticals.

Capabilities of Single Particle Inductively Coupled Plasma Mass Spectrometry using different sample introduction strategies

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The use of nanoparticles (NPs) in different applications are growing, but various types may have a negative impact on the environment and human being, therefore it is required study and define their specific nanomaterial toxicity. [1] Risk assessment requires the development of validated methods and protocols in order to ensure reproducible characterization of NP characteristics and its relation to biological safety. In this respect, various international projects are on-going which has the aim to develop and evaluate different analytical techniques for NPs assessment (e.g. AceNano within Horizon2020[2]). NPs characterization includes the determination of mass, size, particle number concentration (PNC), morphology and elemental composition. Still, to detect and quantify NPs in the environmental samples in range of a few nanometers to several hundreds of nanometers, could be a challenge.[3,4] In order to detect as small as possible metal containing particles (<10nm, containing 5 The low acquisition speed however prevents acquisition of multi-isotope data, due to the short duration of NP signals (c.a. 0.5ms) [6]. For a performance study, gold NPs were used, as they are stable and reference materials are readily available. Besides gold, different types and sizes of NPs are included in this study. Different sample introduction setups are compared with respect to sensitivity and reproducibility: microdroplet generation (MDG), desolvation nebulizer system (DSN), or pneumatic nebulization. In particular, the difference between “wet” and “dry” plasma conditions, carrier gas composition and the use of a “Jet” interface was investigated. Additionally, different matrices are investigated (various concentrations of citrate buffer and water), in order to investigate agglomeration versus time, and transport and detection efficiencies are compared and discussed.

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Characterizing iron loading pattern of ferritin through native MS

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Ferritin is a principle iron storage protein in most living organisms, usually formed by a 24-subunit protein shell and an iron core. The concentration of ferritin in serum has long been considered as a primary measure of iron status and can be easily quantified with immunochemical methods. The iron loading pattern of ferritin was suggested to give a more comprehensive view than measuring only ferritin protein concentration, however the iron loading status of ferritin is less studied due to the lack of well-established method. Recent advances of mass spectrometry (MS) have expanded the observable m/z range, making the direct measurement of ferritin possible with MS.

In this study, a MALDI-TOF-MS coupled with a high-mass detector was employed to measure the iron loading distribution of ferritin. An average 2773 ±1584 iron atoms were determined for a commercial holoferritin sample, a coefficient of variation below 5 % was calculated from 12 technical replicates. To provide a guideline for sample preparation, the stability of holoferritin and apoferritin were assessed under different conditions, including various buffer pH, cross-linking agents and MALDI laser intensities. This work provides a MS-based toolbox for measuring ferritin stoichiometry, which is meaningful for analysis of iron loading status. By integrating the iron loading status data into iron metabolism studies, interesting biological findings are expected.

Nanoparticles tethered polysaccharide probes for potential investigation of supramolecular interactions between water soluble dietary fiber and target compounds.

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During the last 20 years, the combination of inorganic nanoparticles and carbohydrates known as glyconanoparticles (GlycoNps), has aroused great interest for both carbohydrate-carbohydrate and carbohydrate-protein interaction. However, only few studies on carbohydrate-small molecules interaction have been reported [1]. It is well recognized that consumption of complex carbohydrates (dietary fiber) reduces the risk for developing several chronic diseases. The health promoting property of dietary fiber is mainly due to a physical mechanism during which small molecules (*e.g.* sugar, toxins or cholesterol) are removed from the intestine through a gel formation [2]. However, the non-specificity of this mechanism may decrease the bioavailability of desire nutrients (*e.g.* minerals and vitamins) or modulate the biological activity of relevant molecule like bile acids. Therefore, it is relevant to understand which type of dietary fibers may be beneficial to human health and which kind of supramolecular interaction between fibers and target compounds takes place. Here, we create GlycoNps as an experimental tool require to understand, on the basis of chemical structure, the health promoting properties of neutral soluble dietary fibers. Spherical-like gold nanoparticles (AuNps) have been synthesized starting from metallic gold. UV-visible (SPR), Zeta Potential (ZP) and transmission electron microscopy (TEM) have been used to characterize the particles (SPR = 539 nm, ZP = -48.9±1.85 mV, diameter =12.5±1.6 nm). A controlled acid hydrolysis was used to degrade the native polymers (galactomannan, arabinoxylan and xyloglucan) to a lower degree of polymerization. In addition, fractional precipitation with isopropanol of the partially hydrolyzed dietary fiber (PHDF) has been used to obtain fiber fractions with lower polydispersity (PDI). The reduction of M_W, measured by size exclusion chromatography, was accompanied by an increase in PDI with typical value of a medium width distribution ($1.6 \leq \mathrm{PDI} \leq 2$). After precipitation, the PDI decreased in a range between $1.2 \leq \mathrm{PDI} \leq 1.3$. In addition, the monosaccharides composition of the hydrolyzed samples was evaluated by ion exchange chromatography. Based on the results, no significant difference was observed on the monosaccharide composition between native and PHDF. Thereafter, a one-step reductive amination of the reducing end of PHDF with a disulfide linker will be performed. Due to the high affinity of gold for sulfides, the immobilization, which lead to the GlycoNps formation, should be done efficiently. Overall, this methodology will lead to excellent nanoparticles-tethered polysaccharide probe that can be exposed to small molecules in order to study the supramolecular interaction using TEM. The supramolecular interaction will be provided by monitoring the space arrangement of the GlycoNps before and after addition of the target compound.

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Pesticide multi residue LCMS analysis for soil monitoring within the action plan plant protection products

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Modern agriculture depends on the use of pesticides and several 100 000 tons are applied in Europe each year. Recent studies put a focus on the fate and effects of plant protection products (PPP) in soil as processes in this compartment are complex and not well studied so far. Also a growing public awareness on the topic can be observed. Therefore, the Federal Council in cooperation with the Swiss soil monitoring network (NABO) and Agroscope want to establish a comprehensive soil monitoring for PPP in the years to come (action plan PPP).

Analytical basis for the monitoring is a multi-residue LCMS method which is comprehensive, sensitive and robust to be able to deal with a large number of diverse analytes on the one hand, on the other hand it must be high-throughput compatible for a potentially high number of samples gathered from the monitoring.

Prior to separation and detection, pesticides were extracted from soil by accelerated solvent extraction (ASE; Dionex ASE 350). The ASE method was adapted from Chiaia-Hernandez (2017). Dried soil (6 g) was transferred to an 11 mL extraction cell, which was equipped with glass fiber filters (Whatman GF/F). Two extraction steps were carried out. The first one with a mixture of acetone (Ac), methanol (MeOH) and acetonitrile (ACN) at a ratio of 65:10:25 (2 x 5 min, 65 °C, 110 bar) and a second one with a mixture of Ac and 1 % phosphoric acid at a ratio of 70:30 (2 x 5 min, 120 °C, 130 bar) to release acidic compounds from the soil. Both extracts were combined and spiked with a mixture containing 28 isotope labelled internal standards.

For chromatographic separation a Phenomenex Kinetex column (5 µm particle size, 100 x 4.6 mm) was used and a chromatographic run included a solvent gradient starting with a H2O:MeOH ratio of 90:10 and was ramped up to a ratio of 0:100 over 12 min. Two transitions for each analyte were measured on a Sciex QTrap 5500 in Multiple Reaction Monitoring (MRM) mode. 40 PPP and 5 of their transformation products were quantified using reference standards for the calibration and 23 isotope labelled internal standards for the compensation of matrix effects. Six standard mixture dilutions ranging from 0.25µg/L to 25 µg/L were used for calibration.

Method validation was carried out with three representative air-dried soils (silty to sandy loam, OC content: 0.9 to 4.9 %). Based on the raw extract, a tenfold dilution, a cleanup-step including subsample and spiked versions of the above-mentioned raw- and subsamples were prepared.

The method showed a high reproducibility (relative standard deviation < 15 %) as well as a good sensitivity (LOQ of 0.25 µg/kg for most investigated compounds). The measurement of an external reference standard (Ehrenstorfer Pesticide-Mix 1584) helped as a quality control and showed good accuracy during several measurement sequences (within the range of 80-120% recovery for the supported compounds). The poster will present and discuss these findings in more detail.

Concurrency analysis: a tool for discovery of multi-elemental nanoparticles measured with single-particle ICP-TOFMS

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Environmental risk assessments of anthropogenic nanoparticles (both engineered and incidental nanoparticles produced by human activity) require characterization and quantification of nanoparticles (NPs). To date, single-particle inductively coupled plasma mass spectrometry (*sp*-ICP-MS) offers the best possibility to count metal-containing NPs in environmental samples at relevant concentrations. However, even with *sp*-ICP-MS, the presence of the highly abundant naturally occurring NPs in real samples can mask signals from the other subpopulation of NPs and lead to inaccurate characterization of the anthropogenic NP fraction. One way we're trying to source NPs (as anthropogenic or natural) is based on the measurement of single-particle multi-elemental compositions using ICP time-of-flight mass spectrometry (ICP-TOFMS). In *sp*-ICP-TOFMS, multi-elemental particle signals either originate from multiple NPs with different composition being analyzed at the same time or from truly multi-element NPs. We have developed a concurrency analysis protocol that allows us to assign the likelihood of multi-element signals that arise from multiple NPs that happen to be analyzed at the same time bin. This approach helps us identify sub-classes of the NPs that are measured with statistically-relevant multi-element compositions. As case studies, we discuss concurrency analysis for characterization of heteroaggregation of engineered NPs (Ag, Pt, and Au NPs) and for the study of low-abundance anthropogenic Rh-containing CeO_2 particles found in the waste water treatment plant effluent.

Conformational studies on desolvated biomolecules

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Mass spectrometry offers many attractive ways to study complex biomolecules. Due to the high specificity and sensitive analysis, low sample quantities can be accessed and highly relevant information, such as mass, complex stoichiometry[1], binding constants[2] and even kinetic constants[3] can be obtained. In addition to the information directly accessible from mass spectrometry, a coupling of MS with structural probes[4] is possible. Ion mobility spectrometry is most often employed and enables the monitoring of structures and structural transitions[5-6] by collision cross sections. However, despite the great advantages of mass spectrometric techniques it remains unclear whether the desolvation process induces structural changes within the biomolecules. This has important implications for the transferability of data obtained by mass spectrometric techniques to a solution phase environment. Fluorescence resonance energy transfer is a well-known structural probe for solution phase measurements, providing the information on the distance between two or more labelling sites.[7] We employ fluorescence resonance energy transfer[8] to directly compare solution and gas phase structures. Measurements within the plume of an electrospray source[9] enable the direct observation of structural changes upon desolvation. A DNA duplex and α-helical as well as β-sheet model peptides were studied.

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Modelling archaeological wood degradation and validation by chemometry

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During burial in anoxic conditions, waterlogged wood is slowly degraded by erosion and/or tunneling bacteria and then accumulate sulfur species due to the presence of sulfur-reducing bacteria. However once excavated, wood items present severe conservation issues. Indeed, sulfur species, especially iron sulfides FeS_x, are sensitive to oxygen and convert to sulfuric acid and salts efflorescence that lead to irreversible physical and chemical damages within the wood structure.

For the development of an innovative and green extraction of these harmful S species, the preparation of proper model samples appears to be an important step. In particular, these model samples have to be as close as possible from waterlogged wood archaeological artefacts in terms of degradation state and presence of S species. To this purpose, two treatments steps are necessary: 1) the production of wood samples with a low carbohydrates content and 2) an artificial contamination of these artificially degraded samples with S species precipitated within the wood structure. Fresh balsa and oak wood were selected for our purpose. Balsa is a light and porous wood, similar in structure to degraded archaeological wood while oak is one of the wood species most commonly found in archaeological artefacts.

The degradation of carbohydrates content within wood was investigated after the immersion of samples of 2 x 2 x 2 cm^3 (168h in deionized water or acetic acid 1M). The degradation was evaluated by ATR-FTIR spectroscopy and spectra were compared with reference spectra of balsa and oak wood before immersion. The degradation was also evaluated through the ratio carbohydrates/lignin. For comparison, Neolithic oak wood samples were analyzed in parallel.

The ratios obtained suggest that the degradation is more important on water-immersed balsa wood. To validate these observations, Principal Component analysis (PCA) and Hierarchical Clustering Analysis (HCA) were operated on the whole set of ATR-FTIR spectra collected. Spectra were first corrected with the R studio software (opensource statistic software). In contrast to that observed, PCA plots show that immersed- and fresh balsa confident ellipses overlap suggesting that the degradation of balsa wood is not as significant as it seems at a first observation. On the contrary, water- and acid-immersed oak PCA confident ellipses are clustered and far from fresh oak confident ellipses. More interesting, PCA confident ellipses of water-immersed and Neolithic oak overlap and HCA dendrogram show a smaller distance among them. These results indicate that the carbohydrates degradation is significantly more important for oak wood and that this latter is more representative of degraded archaeological wood.

However, water-immersed oak samples still present a compact structure compared to Neolithic wood samples. Further degradation processes will be performed to obtain proper model samples as representative as possible of degraded archaeological wood. Hence, accelerated ageing procedures will be carried out in climatic chamber or in presence of brown-rot fungi. The most efficient process in terms of carbohydrates content degradation will then be selected at first treatment step before the impregnation to form FeS_x will be applied. Once these two-steps degradation protocol defined, a set of model samples can be used to evaluate the efficiency of the biological extraction method actually developed.

Methods to improve resolution in ^{1}H solid state NMR at ultra-fast MAS

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^{1}H, due to its high sensitivity, chemical ubiquity and richness in structural information, is the most usually detected nucleus in liquid state NMR. However, in solid state NMR the strong ^{1}H-^{1}H dipolar coupling network of organic solids leads to broad and highly unresolved spectra from which very little information can be extracted.

Magic angle spinning (MAS) [1] can greatly improve the resolution of solid-state spectra. Faster spinning rates lead to narrower ^{1}H linewidths and recently, newly developed technology allows the acquisition of spectra at spinning rates greater than 100 kHz. However, even in this spinning regime, ^{1}H linewidths are on the order of a few hundred Hz, which is still not sufficient for many applications.

Here we explore new methods to further improve spectral resolution in this ultra-fast MAS rate, and we show how to obtain experimental linewidths up to a factor of two narrower that those achieved with a conventional experiment at the same MAS rate.

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Tip-enhanced Raman Spectroscopy on Two-Dimensional Polymers and Chemically Functionalized Surfaces

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Tip-enhanced Raman spectroscopy (TERS) is a high-resolution nanoscopy method with chemical selectivity, which has been continuously developed since its invention in our research group at ETH Zurich.[1] Its spatial resolution below the limit of diffraction as well as its sensitivity, which allows for the detection of single molecules and nanodefects in extended structures, nowadays qualify TERS as method of choice for investigations on structured surfaces and thin films.[2-4]

Of particular interest to us are two-dimensional polymers, the chemical synthesis of which from their monomers and under mild conditions has only succeeded a few years ago.[5] Large areas of these novel materials can be produced, which exhibit a particularly uniform pore size distribution and high mechanical stability while being molecularly thin, thus making them promising candidates for membrane based separations.[3] A high enough spatial resolution and chemical specificity to identify and characterize defect sites that may negatively impact the material's properties is achieved by TERS imaging, a suitable analytical method for quality and production control of two-dimensional polymers.[2,4]

The work presented here not only employs TERS for the investigation of 2D-polymers at the nanoscale but also characterizes new covalently functionalized glass surfaces at ambient temperatures with TERS, comparing them with conventional coordinatively functionalized gold surfaces.[2] We investigate which underlying mechanisms dominate molecular transformations under the tip,[6] how they correlate with measurement conditions and the analyte's chemical environment, and whether degradation products can be predicted merely from the analyte's structural composition. An understanding of the dominating processes during sample degradation shall lead to the development of new highly defined and stable reference materials for TERS.

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Microwave Inductively Coupled Nitrogen Plasma Quadrupole Mass Spectrometry

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The Argon-sustained Inductively Coupled Plasma (Ar-ICP) is among the most used ion sources for element and isotope mass spectrometry (MS). Despite many advantages for precise and sensitive analysis, it suffers from several drawbacks, like the need for sophisticated RF-power generators, high running costs due to Ar consumption, and abundant Ar-based polyatomic interferences. A cost-effective, comparably simple alternative is the nitrogen-based Microwave Inductively Coupled Atmospheric-Pressure Plasma (N_2-MICAP), which has been introduced recently [1]. Initial studies showed it to be a feasible source in atomic spectroscopy, both in optical emission spectroscopy (OES) [2] and time-of-flight mass spectrometry (TOFMS) [3].

Here, we investigated the performance and characteristics of the N_2-MICAP ion source (Radom Corp, U.S.A.) in comparison to Ar-based ICPMS using a quadrupole mass spectrometer (ELAN 6100 DRC, Perkin Elmer/Sciex).

The different characteristics of the nitrogen plasma play a key role with respect to analyte sensitivities. Similar abundance ratios of molecular and atomic ions were observed, which indicates that similar gas temperatures [4] can be achieved. The higher expansion velocity of N_2, on the other hand, causes a larger spread of kinetic energies that needs to be compensated for by the ion optics system. Furthermore, the influence of geometry and pressure in the vacuum interface were investigated by changing sampler-skimmer separation, orifice sizes and evacuation speed.

Overall the performance of the source was found to be comparable to the Ar-ICP while the background spectrum for m/z>56 did not reveal substantial contributions from the source and the majority of background species observed were N_X^+ and $N_xO_y^+$ (x=1-4, y=1-2)).

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Metabolism During Sleep Monitored by Real-Time Breath Analysis

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Both circadian misalignment and sleep deficiency disrupt normal metabolism, increasing risk of metabolic disorders.[1] Therefore, not only in fundamental research but also from a medical perspective, there is a great interest in obtaining a better understanding of the underlying molecular mechanisms. It has been well established that a circadian clock directly regulates many metabolic pathways, and the biological pathways underlying this control have been studied extensively.[2,3] Effects of sleep restriction and sleep deprivation on human metabolism have also been studied recently.[4, 5] In contrast, metabolism during sleep and the impact of different stages of sleep on metabolism are poorly understood. The main limitation is a lack of sufficient sampling rates for biofluids such as cerebrospinal fluid, urine, saliva or blood during sleep. This limitation can be overcome by analyzing exhaled breath. Breath analysis provides real-time information within seconds, and it is a non-invasive technique. Secondary electrospray ionization coupled to a high-resolution mass spectrometer (SESI-HRMS) offers soft ionization capabilities in positive and negative ion mode, allows molecular formula assignment, covers a broad m/z range and achieves high sensitivity.[6] Hundreds of circadian metabolites have been reported from breath including hormones, fatty acids, and amino acids.[7]

In this study, we measured the exhaled breath of 14 healthy volunteers during sleep in two consecutive nights (one night in each MS polarity). For uninterrupted breath sampling, we coupled a modified continuous positive airway pressure (CPAP) mask to the SESI source. In parallel, a complete polysomnography (PSG) was performed to identify sleep stages (wake, N1-N3 slow-wave sleep, and REM sleep). 1996 discrete features were identified, and their variation across time was deconvoluted using empirical mode decomposition[8] and then correlated with the different sleep stages. To our surprise, although compounds showing acute stage-correlated variation were identified in all stages of sleep and wake, the largest signature by far was obtained for REM (rapid eye movement) sleep, with over a hundred discrete metabolites changing rapidly and significantly upon initiation and termination of this stage. Compound identification is ongoing in order to find out which metabolic pathways are affected. Moreover, preliminary results suggest a direct impact of sleep pressure on normally circadian compounds in breath. Therefore, it is likely that metabolism is controlled by output pathways not only of the circadian clock, but also of the complex neuronal regulation underlying sleep and wake.

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In situ Tip-Enhanced Raman Spectroscopy of 2D materials

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Tip-Enhanced Raman Spectroscopy (TERS) exploits the highly localized, extremely short ranged near-field electromagnetic field enhancement (hotspot) between a sharp scanning probe microscopy tip and a sample, to amplify Raman scattering. The extreme localization and small area of the hotspot are responsible for the high spatial resolution of the technique[1]. It is especially useful for studying 2D samples (biological lipid membranes, 2D polymers) and other molecules (e.g., electrocatalysts, structure and conformations of proteins, peptides, etc.), with a high resolution in their native environment. Since TERS is a label-free technique and does not require tagging the biomolecules of interest with any fluorescent probes, which can, for example, induce changes in the phase behavior of lipids [2,3], it is a promising nanoscale investigation tool for such biological membranes.

However, most of the TERS studies are performed in air or ultrahigh vacuum which is not an ideal environment to study such samples. They have a different stabilities, conformation, phase and activity in air/vacuum compared to their properties in water. Furthermore, performing TERS in air presents additional problems compared to water such as, poor heat dissipation causing tip/sample damage leading to sample desorption, or interference from molecules adsorbed on the tip from the air.[5] Also, oxygen in the air can cause oxidation of the tip (especially Ag TERS tips) leading to a loss of plasmonic enhancement and subsequently of the TERS signal.

By developing an electrochemical TERS (EC-TERS) system with a potentiostat in an electrochemical cell, the potential of the sample and tip can be precisely controlled, which further improves the stability of the system by providing lower and more stable background current resulting in higher measurement lifetimes of Ag tips, and also prevents the oxidation of Ag tips (if the tip is kept at a suitable redox potential). Lastly, by tuning the potential of the sample and the tip we can control the electronic density of states (DOS) in the sample and the tip, which can help in mitigating sample damage due to hot electron generation[4]. We can also tune the surface properties to enhance the sample substrate interaction compared to normal TERS[5].

We will present first results from such an *in situ* electrochemical TERS system, which has been used to study biological samples in phosphate buffer at a physiological pH of 7.0. We have studied the effect of this environment on the TERS spectra of the samples compared to that in air. Additionally, we are also performing studies on a water oxidation catalyst (WOC) using TERS, to investigate the catalytic mechanism. Further efforts have been made to improve the TERS spectra and to reduce the sample damage, bringing TERS once step closer to be an ideal nanoscale spectroscopic analysis tool for *operando* and *in situ* studies.

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Thermal Denaturation of G-quadruplexes and Their Effects on the Stability of Adjacent DNA Duplexes Studied by Temperature-Controlled nanoESI-MS

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G-quadruplexes (GQs) are guanine-rich helical structures of nucleic acids forming non-canonical G-quartets stabilized by incorporated cations. G-quadruplexes are important in many essential cellular processes such as DNA replication, transcription, and translation, and have therefore been increasingly studied.[1] Remarkably, these structures can have both downregulating and upregulating effects in dependence on their location in the gene.[2] Despite the great importance of the G-quadruplexes, the exact principle of their effects is not fully understood. Interaction between G-quadruplexes and adjacent duplexes can lead to a change in the stability of individual domains and thus influence some of the processes mentioned above.[3] These interactions may have a significant impact on unwinding of the DNA and consequently on gene expression.

Here, we present a laboratory-built, temperature-controlled nanoelectrospray ion source (TCnESI), which is designed such as the nESI emitter is placed between two copper blocks, guaranteeing uniformly distributed heat.[4] A Peltier element is used to precisely control the temperature, which is ramped at a rate of 2 ˚C/min in the range 15-85 ˚C. This TCnESI was used to investigate the thermodynamic stability of model DNA duplexes in the vicinity of *T95* G-quadruplex.

First, we performed temperature-dependent experiments of a folded duplex (*fD*) sequence placed on both the 5’or 3’end of a potentially GQ forming sequence *T95*, but in the absence of KCl, when no GQ is formed. The melting temperature (T_m) diminished compared to that of the duplex alone. Once the GQ was formed, the stability of the duplex dropped even more. On the other hand, the stability of *T95* remained almost identical in these experiments. Second, complexes with AT- and GC-rich duplexes revealed significant differences. The AT-rich duplex showed a substantially higher drop of the stability in the presence of *T95* on the 5’end whereas the GC-rich duplex evinced slight stabilization in the presence of the GQ at the same site. Our results indicate an additional unknown interaction between the GQ domain and adjacent GC-rich DNA structures.

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Direct Laser Desorption Mass Spectrometry of Amino Acids on Ocean Worlds

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Reliable in situ detection of signatures of life on Solar System bodies other than Earth is extremely challenging and depends on various parameters, including e.g. the selection of the appropriate field site for material sampling and the application of robust and sensitive measurement techniques for the detection of biological signatures, if present. The Galileo and Cassini-Huygens space missions determined the presence of oceans underneath the ice shells of Europa (Jupiter system) and Enceladus (Saturn system), thus called "Ocean worlds", which may harbor life. Through observed cracks in the ice shells, water is released from the oceans, containing possible biosignatures, which are deposited on the icy surface of these moons and may become detectable [1]. New measurement concepts and measurement techniques are required to enable the successful detection of biosignatures in such an environment. In our contribution, we demonstrate a novel laser desorption mass spectrometry (LDMS) technique based on an instrument built at the University of Bern, which has the capabilities to detect amino acids that represent prominent signatures of life at sub-µM concentrations.

LDMS measurements are conducted using a laser ablation ionization mass spectrometric (LIMS) system that consists of a miniature reflectron-type time-of-flight mass analyser (160 x Ø 60 mm) designed for space exploration missions and a nanosecond pulsed laser system (wavelength λ = 266 nm, pulse repetition rate of 20 Hz, pulse width of $\tau \sim 3$ ns) [2-3]. Samples from various amino acid stock solutions and mixtures thereof were prepared by drop casting (1 µL) of the analyte at various concentrations (100 µM – 1 µM) in small cavities (0.2 x Ø 3 mm) of a stainless steel holder. For the desorption studies, the sample holder was placed outside the laser focus to guarantee gentle laser desorption conditions. Each sample was rastered spot-wise by up to 40 positions, where each position was probed by 100 laser shots (resulting in up to 4’000 laser shots in total). Laser fluence campaigns on single amino acids were conducted to elaborate optimal laser desorption conditions and to identify for each amino acid the specific measurable biosignatures. The high detection sensitivity of the LIMS system allows to trace these biosignatures down do sub-µM concentration levels, which is currently limited by the background of the sample substrate. The measurement protocol and the results will be presented in detail during our contribution. The current measurement capabilities are highly promising for future space missions dedicated to the exploration of these ocean worlds and represent an alternative measurement technique to those currently applied, such as GC-MS, in space exploration missions.

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Investigation of Corneal Crosslinks in Keratoconus Treatment

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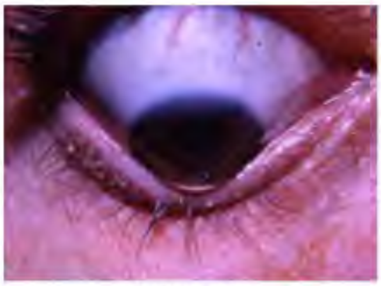
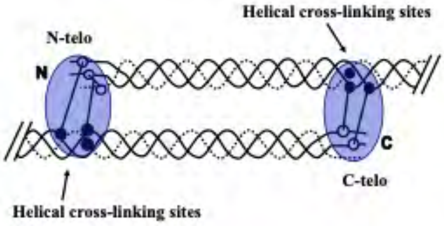
Collagens belong to the most common proteins in vertebrates and are made out of heterotrimeric molecules, each crosslinked at the amino (N)- and carboxy (C)-terminus. By the enzymatic activity of lysyl oxidase (LOX), telopeptide lysines and hydroxylysines are deaminated and undergo spontaneous reaction with a lysin or hydroxylysine of the helical domain to form natural crosslinks. Collagen molecules are, among others, present in cornea and are essential for its stability.

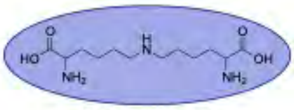
Keratoconus (KC) is the most common ectasia of the cornea and results in a thinner and weaker cornea. This transformation reduces the visual acuity and is responsible for myopia. To restrict the progression of the disease, new crosslinks are induced by the promising corneal cross-linking (CXL) therapy. The CXL therapy makes use of riboflavin (vitamin B), which generates reactive oxygen species upon irradiation with ultraviolet (UV) A-light, thus, providing the cornea with new biomechanical stability.

In this study, differences between the amount and type of crosslinks in treated and non-treated cornea are investigated. The analysis of the crosslinks is performed by HPLC-MS on a QTOF instrument.



Keratoconus eye





Lysinonorleucine

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POPs in microplastics in the South Atlantic gyre and their environmental risks

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Plastic materials can enter the aquatic environment and disintegrate over time into smaller particles, so called secondary microplastics (MP, < 5 mm). MP poses risks to aquatic organisms when being ingested. In addition, MP can act as a Trojan horse transporting persistent organic pollutants (POPs) and other toxic organic compounds into an organism. Original plastic items can already contain such chemicals used as additives (e.g., flame retardants and plasticizers). Furthermore, MP can act as a passive sampler for lipophilic pollutants present in water. In this project, we investigate relations between POPs and MP in the South Atlantic gyre and assess their environmental risks. For this, we sampled microplastics, larger plastic items, sea water, sediment and biomass during a sampling period of three weeks covering a distance of about 5000 km. POPs will be extracted from the samples and analyzed with gas chromatography coupled to mass spectrometry (GC-MS). Using this data, the risk of exposure to POPs will be determined for marine organisms. The relevance of microplastics as a carrier for POPs to remote areas will be assessed.

Deep UV Raman spectroscopy for online water analysis

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During the last decades the scientific interest in the waste water analysis has been growing due to the persistent presence of contaminants in treatment plants and due to the degradation of groundwater quality. Agriculture-derived inorganic compounds as ammonium, nitrate and nitrite, and phosphate ions have been reported with typical concentrations of tens mg/l or lower [1, 2]. Raman spectroscopy in the visible range is a promising online technique capable of detecting most of the pollutants in one measuring step. Due to low Raman cross section and overlap of fluorescence and Raman emission the limits of detection (LOD) of visible Raman spectroscopy is above the threshold required to be used as an online monitor in waste water analytics [3, 4]. However, by changing the excitation energy is theoretically possible to vary the relative intensity of the Raman and fluorescence signal.

We demonstrated the proof of this concept using an Optical Parametric Oscillator (OPO) as excitation source for Deep UV radiation. We achieve LOD for nitrate better by a factor of ten than 25 mg/l as required for an online water analysis method [5, 6]. In addition to its use as a practical tool, we are able to study fundamental photochemical processes taking place upon UV-illumination. The nitrate - nitrite photolysis process upon deep UV radiation has been investigated in detail [7].

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Standardization procedure for exhaled breath analysis using secondary electrospray ionization mass spectrometry

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Secondary electrospray ionization mass spectrometry (SESI-MS) enables screening for a broad range of breath derived compounds, with high sensitivity as well as high mass resolution. Accordingly, this non-invasive technology has already been applied in different clinical trials to discover disease specific biomarkers in exhaled breath. While its applicability seems promising as potential sets of biomarkers were found e.g. for obstructive sleep apnoea[1], standardization procedures for this fairly young method are currently missing. To enable comparability of data generated at different time points, sites or on different mass spectrometers, methodological standardization is necessary. We are implementing a gaseous standard mixture that is stably delivered into the system and tailored for SESI-MS. The reference gas mixture must contain low volatility compounds of various chemical classes, with a high molecular weight (50 to 300 Da) and at low molar fraction (ppbv or lower) to optimally cover the method´s range of detectability. Especially compounds that are detected in breath by SESI-MS are of interest. We propose to introduce such a mixture in gas cylinders into an instrumental set-up where the mixture is diluted and humidified for optimally simulating exhaled breath. Both, the instrumentation as well as the gas mixture composition are evaluated for their suitability. Such a reference gas mixture, however, finally allows for data comparability and precise monitoring of instrumental stability, which is urgently needed for long-term clinical trials. Especially identifying technical variations will be fundamental for robust data analysis and could improve reliable identification of biomarkers in breath.

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LC-APCI(-)-MS analysis of 1-chloro-2,4-dinitrobenzene, a model substrate for glutathione S-transferases

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The electrophilic compound 1-chloro-2,4-dinitrobenzene (CDNB) is used in a variety of fields, such as an algaecide or as medication in warts treatment. It is also well known as a substrate for glutathione S-transferases (GSTs), an enzyme family involved in phase II metabolism. GSTs catalyze a conjugation reaction of CDNB with glutathione (GSH) resulting in the formation of a GSH-conjugate (DNP-SG). The parent compound and the metabolite have different spectrophotometric properties, which leads to a change in the absorbance spectra from λ_{max} = 280 nm (CDNB) to λ_{max} = 340 nm (DNP-SG). This shift in absorbance can be precisely analyzed with high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detector and be correlated to GST activity. However, HPLC-UV only allows measurements of CDNB concentrations above 1.0 µM (LLOQ). Considering the frequent use of CDNB in biochemical assays, it is necessary to be able to cover the whole concentration range within a toxicity study. Taking into consideration that the median lethal concentration (LC_{50}) for CDNB is in the range of 3.3 to 1.0 µM for aquatic organisms, a more sensitive technique is required to assess low toxicity concentrations.

Here we established a LC-APCI(-)-MS method for CDNB analysis at and below the nontoxic concentration of the model organism *Danio rerio*. This method was successfully applied to verify the whole range of exposure concentrations within a common zebrafish embryo toxicity study.

Comparison of SIM Stitch and full mass scan direct infusion metabolomics for the assessment of the molecular stress response in Chlamydomonas reinhardtii exposed to Ag^{2+}

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Aquatic organisms are exposed to an ever increasing variety of chemicals. In the area of chemical risk assessment and molecular ecotoxicology it is therefore crucial to identify and understand the underlying molecular mechanisms of their stress response. Metabolism reflects the current biochemical state of an organism and hence can be used to capture the dynamics of the metabolic network in a stress response, which happen on a smaller timescale than the resulting physiological changes. In order to obtain insights into these metabolic adaptations we use accurate mass detection high resolution mass spectrometry.

Here we compare direct infusion and spectral stitched direct infusion mass spectrometry, two high throughput techniques, in regard to sensitivity and metabolite coverage, temporal resolution and applicability to investigations in the model organism *Chlamydomonas reinhardtii* under Ag^{2+} stress.

Gas-phase fluorescence from trapped biomolecular ions: instrumentation and photophysical studies

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With the advent of mass spectrometric studies of biomolecules and their complexes, there has been a growing interest to investigate if their native structure and function is preserved in the gas phase. Structural studies in the gas phase not only reveal intrinsic protein properties (i.e., those in the absence of solvent) but also provide useful benchmarks for theoretical modeling. Fluorescence-based techniques are one of the most popular methods to investigate the structure and dynamics of biomolecules in solution. Given their high sensitivity and ability to probe a variety of chemical and physical environments, analogous explorations in the gas phase have also started recently to gain sensitive structural information for structure elucidation of gaseous biomolecules.

We report a setup to perform laser-induced fluorescence experiments on trapped biomolecular ions. It consists of a quadrupole ion trap (QIT) mass spectrometer, modified in-house to enable optical access to trapped ions for excitation with fs-laser pulses and fluorescence collection from them. This apparatus allows both wavelength- and time-resolved fluorescence measurements. The light source in the experiments is a pulsed (~100 fs pulses), tunable (690-1040 nm) titanium:sapphire laser, which is frequency doubled to access the UV-Vis wavelength range (345-520 nm) with an output power ranging from 0.1 – 1 W. The tunability of laser light in this wavelength lets us probe the spectroscopic properties of biologically relevant chromophores. Additionally, the ultra-short pulses are crucial to correctly interpret complex fluorescence signals.

Our goal is to study the gas-phase photophysical properties of fluorescent dyes and their non-covalent complexes with biomolecules commonly used for probing the biomolecular structure. One such class of systems is non-covalent complexes of benzothiazole-based dyes (like thioflavin T and thiazole orange) and DNA quadruplexes. The bare dye (thiazole orange) does not show any detectable fluorescence in the gas phase, but upon binding with G-quadruplex DNAs, it can show selective and sensitive emission features with specific structures. For example, the binding of thiazole orange with parallel quadruplexes of human telomeric DNA (22AG) show ~1000 times fluorescence enhancement in solution[1]. It is envisioned that comparing the solution-phase measurements with the gas-phase measurements of thiazole orange binding with parallel and anti-parallel quadruplexes of DNAs, can help probe any structural changes in the DNA quadruplex in the gas phase. The difference in Stokes shift (of the order of ~100 cm^{-1}), increased fluorescence lifetime along with the fluorescence enhancement can help in disentangling and understanding the role of geometrical confinement and protection from the solvent.

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Quantitative microanalysis of porous materials by means of Laser Ablation Plasma Mass Spectrometry

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Porous materials are a challenge for direct quantitative microanalysis. Traditionally, the sample materials had to be dissolved with elaborate digestion methods and quantified by inductively coupled plasma mass spectrometry (ICP-MS). Spatially resolved information was however lost, such that laser ablation as a micro-sampling technique filled the gap, with elemental information at as low as few tens of microns. Clearly, the ablation of porous materials generates fluctuating signals, such that only qualitative information is easily accessible. Alternatively, because of the lack of suitable standard reference materials only semi-quantitative results are often produced.

Elemental microanalysis of wood samples, as one important class of porous materials, is investigated to study effective calibration strategies. Suitable LA-ICP-MS methods for quantification were compared considering the density, brittleness and porosity of the ablated material. Original strategies for quantifications are investigated, according to two different approaches: (i) *sample preparation techniques*, e.g. impregnation, hole-filling, spiking, etc; (ii) *data reduction strategies*, i.e. signal processing with the aim of unravelling the elemental and structural information.

The physical properties showed direct influence on the microsampling as well as on the amount of the transported analyte to the ICP-MS. In addition, both laser and material's parameters have a direct influence on the particle size distribution and thus again on the ablated amount of the sample.

The production of artificial reference samples is a method for the quantification of porous materials. However, these reference standards must not only correspond to the samples in the composition of the matrix, but should also have the same physical properties as possible.

Investigation of neutral soluble dietary fibre interactions with nutritional molecules and mineral cations by electron paramagnetic resonance spectroscopy

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Dietary fibre (DF) is the portion of plant-derived food that is not completely broken down by human digestive enzymes. During the digestion process, DF could bind and remove a variety of nutritional elements such as metal ions, toxins, sugars and bile acids. The consequence is twofold: the adsorption of harmful molecules like toxins and reduction of blood cholesterol is a benefit of fibre, while chelation of metal ions might result in mineral deficiency. Whereas there are no doubts in the interaction between charged fibres and nutritional elements due to electrostatic interactions, studying their binding by weak non-covalent interactions with neutral fibres is challenging for most analytical techniques.

We present a new strategy in the characterization of nutrient-fibre interactions based on Electron Paramagnetic Resonance (EPR) spectroscopy. In EPR experiments, minute changes in the environmental structure of paramagnetic species – metal ions or small molecules with unpaired electron – can be investigated. Three parallel methodologies are under development: the first is an application of spin-labelled nutrients (commercially available spin-labelled molecules; metal ions like Cu(II) or Fe(III)) with native fibres. The second method is the reverse – natural nutrients and spin-labelled fibres. Finally, the spectroscopically orthogonal labelling application (metal ion + spin-labelled fibre) is planned to unambiguously define fibre-nutrient interactions. However, currently existing approaches for the DF labelling are sparse.

Herein, novel DF labelling strategies are tested by modification of the reducing end or the versatile and ubiquitous hydroxyl group in polysaccharides. The first one is based on the selective modification of polysaccharides through their reducing end by oxime formation between DF and a TEMPO-derivative. This pathway is faster and provides higher labelling efficiency than standard direct reductive amination of the reducing end [1]. The second strategy is a random chemical modification of DF by applying a general coupling agent in the presence of 4-carboxy-2,2,6,6-tetramethylpiperidinyloxy (4-carboxy-TEMPO) free radical, which provides several spin labels randomly attached along the fibre chain through esterifications.

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Trapped ions accelerate the crystallization of amorphous calcium carbonate in air

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Through the exquisite control of the early stages of nucleation, a variety of organisms have mastered the art of utilizing calcium and carbonate ions to form functional materials ranging from eye lenses to protective shells. Amorphous calcium carbonate(ACC) is the first solid phase that forms during the formation of calcium carbonate from solution. The impeccable ability of these organisms to form functional calcium carbonate-based materials is associated with their ability to control the crystallization of this amorphous phase. However, for man, this is still a huge challenge particularly because the mechanisms governing amorphous calcium carbonate crystallization are still evasive. In air, amorphous calcium carbonate gradually crystallizes when exposed to humidity, however, the full understanding of this process is still unclear. Herein, we show that impurity ions play a critical role in this process. Most salts involved in the synthesis of amorphous calcium carbonate are deliquescent and are often trapped in the amorphous calcium carbonates owing to their very fast formation. These trapped salts (sodium or calcium chloride or ammonium) when exposed to certain levels of humidity absorb water and dissolve, resulting in a localized solution that facilitates the dissolution of the amorphous calcium carbonates and their subsequent crystallization. Hence, by controlling the type and amount of impurity, control over the crystallization can be achieved. This new insight opens up new opportunities for the fabrication of biomimetic materials with tuneable structures and mechanical properties.

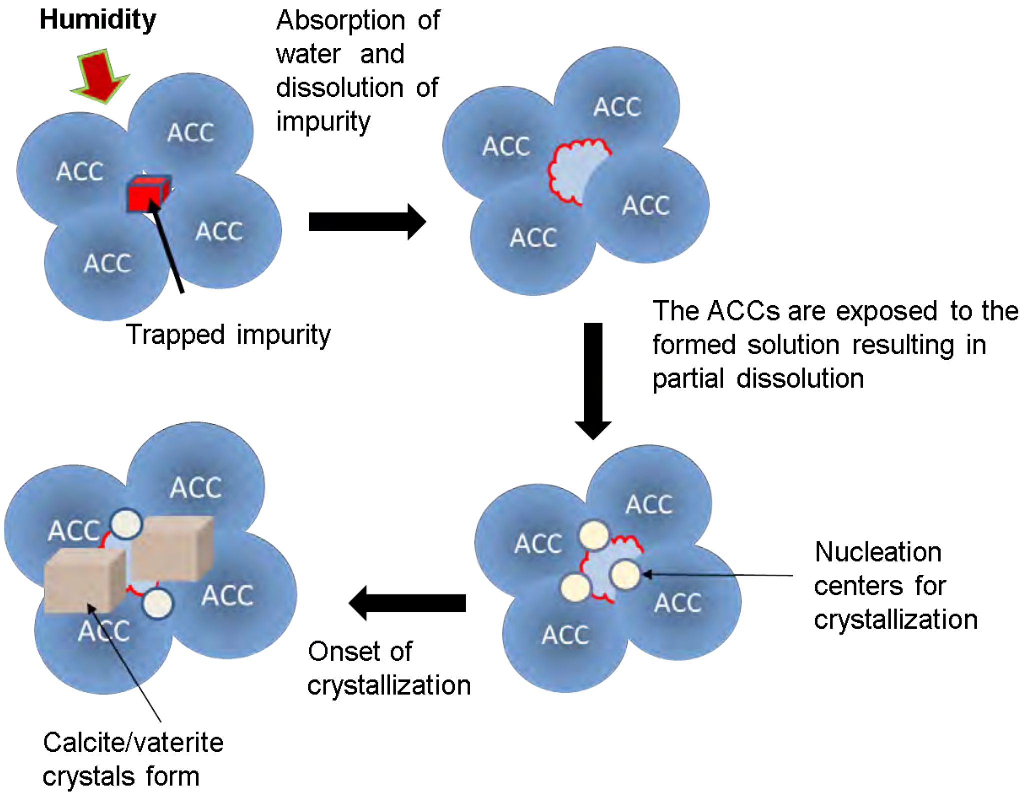

Figure 1: Figure 1: Proposed mechanism of amorphous calcium carbonate crystallization(ACC) in air

A Disassembly Approach for Selective Detection of Pyrophosphate using Fe^{III}-Salen Complexes

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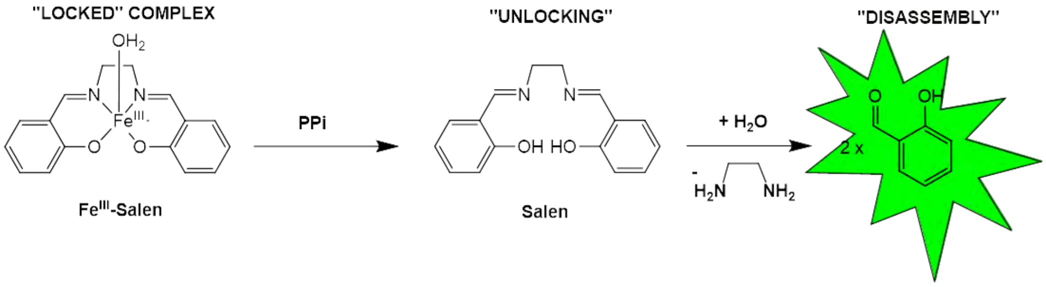
A stimulus-induced disassembly approach is presented for detection of analytes in living cells [1]. Particularly, we demonstrate that Fe^{III}-salen complexes fluorometrically detect endogenous pyrophosphate (PPi), an important diagnostic marker for cancer and many other diseases [1-3]. In our approach, PPi sequesters the Fe^{III} centre from the salen-complex and the “unlocked”, metal-free ligand then subsequently hydrolyses into its molecular subunits ethylenediamine and salicyaldehyde [3]. Initially, the intrinsic fluorescence of the salicyaldehyde subunit is quenched by the paramagnetic metal ion, but turns on back during the disassembly of the complex. Our group studies show that the selectivity and reactivity of the Fe^{III} complexes can be affected by incorporating modifications in the salen ligand structure.



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N-glycan analysis with ultrahigh-resolution ion mobility spectrometry and cryogenic ion spectroscopy

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N-glycosylation of therapeutic proteins is one of the most common post-translational modifications. Glycosylation plays an essential role in various biological processes, such as inflammation, immune recognition, and other cellular processes[1]. Characterization of N-glycans from glycoproteins is crucial, because their functions are strongly dependent on their glycosylation pattern. However, the analysis of N-glycans is particularly challenging because of their isomeric complexity and branched structure. Many approaches currently exist, each with their own advantages and limitations, but no one technique can fully characterize glycan primary structure[2,3].

In this work, we demonstrate that we can characterize N-glycans cleaved from glycoproteins using a multidimensional approach that combines ultrahigh-resolution ion mobility spectrometry (IMS) and cryogenic, messenger-tagging, vibrational spectroscopy.

We first released N-glycans from Rnase B using PNgase F and performed an intact mass analysis to monitor the efficiency of the enzymatic digestion using an ACQUITY H-Class PLUS UPLC (Waters) coupled to Micromass Q-TOF Premier. Due to the hydrophilic properties of the released glycans, their extraction was done using HILIC cartridges. We then used a homebuilt instrument in which we combine ultrahigh-resolution ion mobility spectrometry (IMS) based on SLIM technology with cryogenic gas-phase IR spectroscopy for analysis of the cleaved, unlabelled glycans in their sodiated form. The released glycans are identified by comparison to a database containing values of mass, drift time and a vibrational fingerprint spectrum.

We tested our new multidimensional IMS-IR spectroscopic approach on the three most abundant N-glycans from Rnase B: Man5, Man6 and Man9 by measuring the mass, drift time, and IR fingerprint spectrum of each of them. The vibrational spectra we obtain show well-resolved transitions in a wavenumber region where free and weakly hydrogen-bonded OH oscillators occur, and this structure is extremely sensitive to the subtlest difference between glycan isomers. We compare these spectra to a targeted database that we created based on standards of the above mentioned glycans and demonstrate that our IMS-IR approach can be used as a new tool for the analysis of released glycans.

We are currently expanding our database and performing experiments to determine the glycosylation pattern of different glycoproteins using our new technique. Considering the widespread use and importance of therapeutic proteins, our approach should provide an import addition to the toolbox for glycan analysis.

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Time trends of persistent organic pollutants in Swiss human milk samples

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Monitoring the environmental impacts and time trends of persistent organic pollutants (POPs) listed and banned under the Stockholm Convention requires advanced analytical methods that support governmental actions. Since 1987, the World Health Organization (WHO) has coordinated global biomonitoring programmes for the analysis of POPs in human milk. Fat-rich human milk was found to be an excellent bioindicator for this purpose, easily accessible and its sampling is non-invasive. The scientific efforts are oriented to address the following challenges: (i) collecting representative samples for the Swiss population according to the WHO-protocol, (ii) accurate quantitative determination of ultra-trace level of POPs by the use of isotopically labelled standards, (iii) proper exposure risk assessment of humans with spotlight on the sensitive breastfed infant, (iv) adopting further measures to reduce the exposure to POPs.

Switzerland has recognized the significant hazard connected with POPs in breast milk, and a concerted action between Government and Empa has generated remarkable benefits. Between July 2015 and July 2016, 54 human milk samples were collected in five Swiss hospitals and subsequently analysed for POPs. In addition, a pool sample of the collected milk samples was prepared and sent to the EU reference laboratory in Freiburg i.B. (Germany) for analysis and inclusion in the global survey. The list of compounds, analysed by gas chromatography high resolution mass spectrometry (GC/HRMS), included polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), including the dioxin-like PCBs (dl-PCBs), polybrominated diphenyl ethers (PBDEs), used as flame retardants, as well as legacy pesticides such as DDT and its metabolites DDE and DDD.

Results indicated that while most of the Stockholm Convention pesticides are present in Swiss human milk samples in the low or sub ng/g lipid range, the main metabolite of p,p'-DDT, namely p,p'-DDE, can still be observed at remarkable concentrations of around 100 ng/g lipid, although the use of DDT has been restricted in many countries, including Switzerland, since the beginning of the 1970ies. Fortunately, since the last participation in a WHO coordinated study in 2009, a significant decrease of 40% of the WHO toxicity equivalent (WHO-TEQ) of dioxins (PCDD/Fs) and dioxin-like PCBs could be observed.

Compared to results from an independent Swiss human milk study from 2002, the decrease of PCDD/Fs and PCBs, two highly toxic and persistent compound classes, over the period of approximately 14 years was as high as 67%. Calculated on the basis of the results from the years 2002, 2009 and 2016 a reduction of the PCDD/F and dl-PCB WHO-TEQ of 50% was accomplished after 7.3 and 10.3 years, respectively. Despite these positive time trends, partly observable as well on a global scale, further efforts must be undertaken to reduce the human exposure to dioxin-like compounds and other emerging persistent organic pollutants arising on the horizon, e.g. chlorinated paraffins.

Modern X-ray spectroscopy: XAFS and XES in the Laboratory

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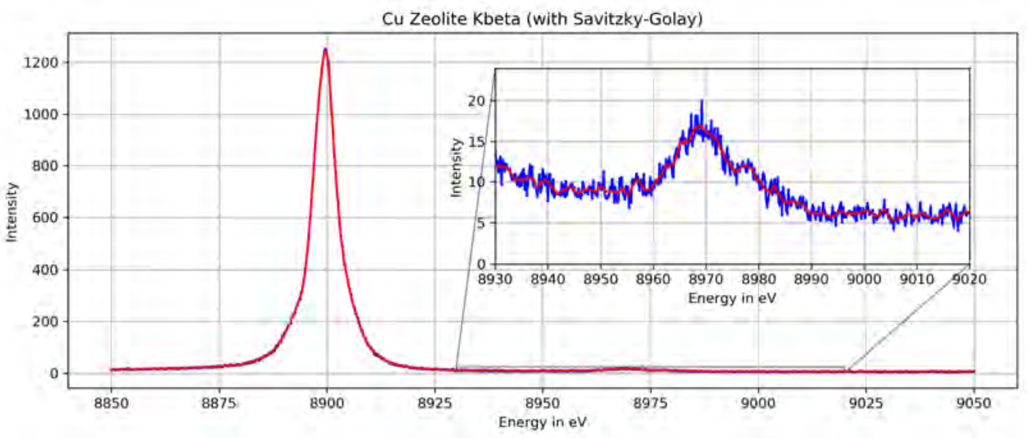
We will give a general overview of the possibilities with modern X-ray spectroscopy in the laboratory. While high-resolution wavelength dispersive X-ray Absorption Spectroscopy (XAS) and X-ray Emission Spectroscopy(XES)[1] is usually performed at large scale research facilities, such as synchrotron X-ray light sources, the technological advances in the recent past have made it possible to perform those experiments (with some limitations) in the laboratory.[2]

We are currently commissioning a new laboratory X-ray spectrometer for the hard X-ray energy range covering the K absorption edges and K emission lines of the 3d transition metal series. We'll shortly introduce the used experimental setup and its configuration. This is then followed by an overview of the possible detection techniques of our laboratory setup which will be used for X-ray Absorption Fine Structure (XAFS) and XES measurements.



One goal is to implement an in-situ reactor for routine measurements which will be used for the study of systems in heterogeneous catalysis. We will then conclude with some examples of the possible applications and an outlook for future developments such as the addition of a mass spectrometer.

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Analytical Development at Siegfried – Expect More

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- 2 LC-MS, 1 GC-MS, 1 ICP-MS
- NMR, XRPD
- UV, FT-IR, NIR
- Various instruments for wet chemistry
- Helos, Malvern, microscopy, sieving (Particle size determination)

INTERACTION and COMMUNICATION also plays an important role in daily business. Within the company, employees down to laboratory assistant level are required to COOPERATE and communicate across departmental boundaries. At analytical project manager level, this also includes external interfacing with customers and authorities.

CHIMIA

CHIMIA 2019, Volume 73
ISSN 0009-4293
www. chimia.ch
Supplementa to Issue 7-8/2019

SCS
Swiss Chemical
Society

SCS Fall Meeting 2019
Oral Presentation Abstracts

Session of Analytical Sciences

September 6, 2019
Irchel Campus, University of Zürich, Switzerland
http://scg.ch/fallmeeting/2019

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Pushing the limits of de novo genome assembly for complex prokaryotic genomes and establishing a basis to utilize microbiome isolates for plant protection

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My genomics and bioinformatics team closely collaborates with experimental groups that have established functional assays to isolate individual, functionally relevant microorganisms involved e.g. in biocontrol, biostimulation, or antibiotics resistance from complex microbiomes such as those present on plant surfaces, fermented foods or in the soil. Such interdisciplinary collaborations are essential for Agroscope's aim to bring microbiome research into applied practice.

We subsequently *de novo* assemble complete genomes of such strains using the latest Next Generation Sequencing (NGS) technologies from Pacific Biosciences and Oxford Nanopore Technologies (ONT) and state of the art assembly algorithms. Generating a complete, *de novo* genome assembly for prokaryotes is generally considered a solved problem because of their relatively small size and low complexity. However, the analysis of 9300 complete prokaryotic genomes indicated that a sizable fraction (10%) either harbored several hundred repeats of up to 7 kb in length (so-called class II genomes), or very long near identical repeats up to over 100 kb (so-called class III genomes), both of which impede complete genome assembly.

By using long PacBio reads, we could recently show that repeat-rich class II genomes of Lactobacillus strains could be readily assembled into complete genomes, and that this approach represents a distinctive advantage over the fragmented assemblies generated by short read Illumina data alone. Using very long reads from ONT, we could even completely assemble the genome of a highly complex *Pseudomonas koreensis* strain that harbored nearly identical repeats of 70 kilobase pairs. As accurate genome annotation is critical to exploit the deluge of completely sequenced genomes, a publicly available proteogenomics solution for improved genome annotation was developed with funding from the Swiss National Science Foundation.

In this talk, I will highlight some of the lessons learnt over the past four years and present an outlook on how we plan to use functional genomics technologies with the ultimate aim to use naturally occurring microorganisms for plant protection.

Bioanalytical workflows based on non-target high-resolution mass spectrometry and bioassays for the identification of toxicants in complex water samples

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The identification of unknown toxicants in complex water samples such as wastewater cannot be achieved with target chemical analysis alone. Tools and technologies that reduce the complexity of the sample and identify the chemicals responsible for toxic effects are necessary. The present study focused on developing a diagnostic bioanalytical strategy that integrates toxicological and chemical analyses based on effect-directed analysis (EDA) in order to identify toxicants in complex wastewater samples. The workflow is based on combining targeted high-resolution mass spectrometry (HRMS) analysis, fractionation, and bioassays to elucidate the structure of toxicants. Several fractionation strategies were investigated including thin-layer chromatrography, and preparative HPLC. Also, a chip-based nano-ESI source coupled to Orbitrap analyzer was used in the LC/MS fraction collector mode. This study highlights the importance of effect-directed analysis as an approach for the identification of unknown toxicants in complex water samples.

Determination of the primary structure of glycans by enzymatic cleavage and cryogenic IR-spectroscopy

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Glycans, or oligosaccharides, play critical roles in numerous physiological processes and are involved in some way or another in most major diseases. In addition, their characterization is essential for monitoring the quality of biotherapeutics. Because the function of glycans is directly related to their structure, glycan structural characterisation has become an exponentially growing field. Despite their importance, glycan characterization is challenging because of their isomeric complexity.

One of the most powerful techniques for glycan investigation is the combination of liquid chromatography (LC) with mass spectrometry (MS), however even this method cannot distinguish all the various forms of isomerism. In our laboratory, we have recently demonstrated that cryogenic messenger-tagging IR-spectroscopy provides unique vibrational fingerprints of glycans that are extremely sensitive to the slightest structural differences.

In the present work, we monitor the step-wise enzymatic degradation of glycans using liquid chromatography and then measure cryogenic IR spectra of the resulting smaller glycans. This approach will be applied for creating and then using a glycan database. The main principle of the identification of an unknown glycan involves the repetition of these two steps until the spectra that we measure are those of glycans already present in our database. By knowing the isomeric specificity of the applied enzymes, we hope to reconstruct the primary structure of the unknown glycan and then add its spectrum to our database. In this way, we define the structure of the unknown oligosaccharide and expand our database.

We chose the N-linked glycans Man-1, Man-3, NGA2 and NA2 as starting examples and performed two different approaches of glycosidase digestion. Protocols of single exoenzyme cleavage for Man-3, NGA2, NA2 and multi-exoenzyme cleavage for NGA2 and NA2 were developed. After digestion, we studied and cleaned all samples by hydrophilic interaction liquid chromatography (HILIC) using an AQUITY UPLC H-Class Plus System (Waters) with a Q-TOF mass spectrometer (Waters Premier) as a detector. The glycan fragments were separately collected and prepared for spectroscopy. We used a home-built, cryogenic, tandem mass spectrometer together with an IR OPO to measure the infrared spectra of the glycan reference compounds and the glycan fragments after enzymatic cleavage.

The results we have obtained confirm the possibility of combining these two complementary analytical techniques and serve as a starting point for constructing a glycan database. In the future, this approach will be extended to more complex unknown sugars as the database expands.

Magneto-optical Kerr effect set-up for in situ probing of oxygen evolution electrodes.

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Operando surface-sensitive analysis of electrodes is a great challenge, as most surface-sensitive techniques are not compatible with in situ measurements (e.g. high vacuum requirement for XPS). Here, we present an all-optical approach that allows for *in situ* probing of transformations that occur within top 10 nm of magnetically active electrodes (archetypically: Co, Ni or Fe). The spin carriers information on the electronic structure [1]. Although the interpretation is not straightforward, the advantage of the method lies in the compatibility of optics with an electrochemical setup. Typical electrode materials used in alkaline electrolysis are Ni and Fe, and their combination. A fundamental understanding, why iron impurities improve the electrocatalytic performance of Ni electrodes [2] is lacking, partially due to the fact that the surface oxide structure under operation conditions is unknown.

The measurement principle is based on non-linear electro- and magneto-optical Kerr effect. The polarisation plane of linearly polarised light rotates after it interacts with the birefringent matter (e.g. electrode) exposed to a magnetic field. The degree of rotation depends on intrinsic properties of the matter (e.g. electronic structure) and is directly related to the intensity difference between the incident and reflected beams. The polypropylene electrochemical cell is designed for optical probing with minimal interference from non-sample components (e.g. 0.5 mm optical window and 2 mm probing depth of electrolyte). The integrated CCD-camera allows for spatially resolved imaging over the whole sample surface.

The electrodes are prepared by sputtering on a gold-covered sapphire supports. Gradient-coated samples are used to differentiate between the bulk-influenced spectral response and the chemical changes of the catalytically active surface. Here, we demonstrate the applicability of the technique for determining the thickness oxide layers as a function of applied overpotential using the electrochemical MOKE setup.

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Methane, ammonia and volatile organic compound emissions at herd level in an experimental housing for dairy cows

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Agricultural activities, such as dairy farming, significantly contributes to the Swiss methane (CH_4) and ammonia (NH_3) emissions [1, 2]. Furthermore, dairy farming seems to be also a relevant source for non-methane volatile organic compounds (NMVOCs) [3].

We will present CH_4, NH_3 and NMVOC emission data for silage-based versus silage-free (hay) feeding collected at the experimental dairy housing for comparative emission measurements at Agroscope, Switzerland. The housing consists of two identical naturally ventilated compartments, which are spatially separated, to provide an opportunity for comparative quantification of emissions using different diets, housing concepts or management. Mass flow emissions in the naturally ventilated housing were determined using a dual tracer ratio technique, which is suitable for both areal and point emission sources and achieves an uncertainty of less than 10 % [4]. Applying different artificial tracer gases in both compartments, sulfurhexafluoride (SF_6) and trifluoromethylsulfur pentafluoride (SF_5CF_3), cross-contamination events can be detected. Tracer gas mole fractions were analysed by GC-ECD. NMVOC concentrations were determined using different analytical techniques, such as GC-FID and a total hydrocarbon monitor (THC). CH_4 and NH_3 concentrations were quantified using cavity ring down spectroscopy.

In summary, this study provides currently unavailable information about the emission of CH_4, NH_3 and NMVOCs emitted from Swiss dairy farming using silage-based vs. silage-free diets. NMVOC emissions in the compartment where silage-based diet was applied were dominated by ethanol (EtOH) followed by ethyl acetate (EtOAc) and methanol (MeOH). These components are produced by bacteria for example during fermentation and storage of silage. In the second compartment, receiving a silage-free diet, NMVOC emissions were substantially lower and concentration differences between EtOH, EtOAc, MeOH and acetone were less pronounced.

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The use of UV/VIS spectroscopy in process monitoring and diagnostics

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UV/VIS spectroscopy can be used as a simple and versatile tool for a diversity of applications in various industry segments. Here recent developments in bioprocess monitoring (A) and diagnostic (B) applications based on UV/VIS spectroscopy are presented and discussed with respect to increasing efficiency, safety and regulatory compliance.

A. Real-time analytical tools for bioprocess/fermentation monitoring are considered as effective alternatives to traditional laboratory-based analyses. This is mainly due to changing guidelines and the aim to augment the effectiveness of process control in order to save costs.
B. The analysis of cerebrospinal fluid for bilirubin in suspected subarachnoid haemorrhage is an application where errors in assessing the results can have a critical impact. A high performance UV spectrophotometer is required for accurate low-level determination. The decision tree for assessing the results is highly complex. Training staff to follow the decision tree without making errors is an involved process. The sensitivity and necessity of correct results interpretation demands for an automated analytical workflow support, whose results follow data-integrity compliance regulations.

Structure elucidation of low molecular weight compounds in spider venom

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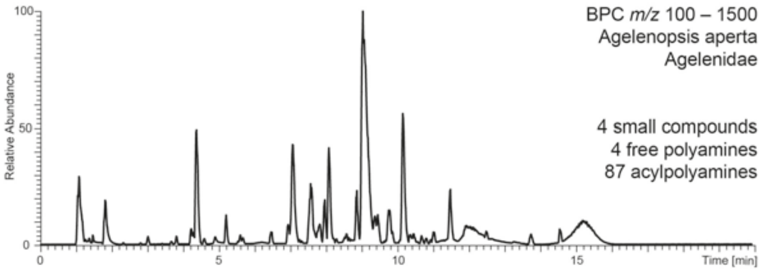
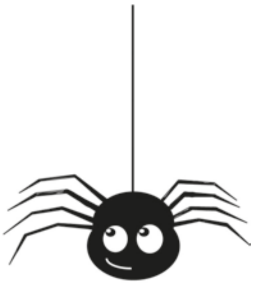
The venom of spiders is a mixture of proteins, peptides and low molecular weight compounds (< 1000 Da) such as nucleosides, amines and (acyl)polyamines. Several of these compounds were found to be promising lead compounds to treat cancer or neurodegenerative diseases like Alzheimer’s disease, epilepsy or stroke. [1] Yet, the knowledge about arachnotoxins is very limited asless than 1% of the venoms of the known spider species have been analysed up to date. Low available sample amounts, co-eluting isomers and use of trivial names in literature make the structure elucidation of low molecular weight compounds in spider venom demanding.

The spider venoms were analysed by UHPLC-HR-ESI-MS and MS/MS. Polyamines follow characteristic fragmentation rules; therefore MS/MS is ideal for their structure elucidation. [2] In addition, the number of acidic protons was determined in an on-line H/D exchange experiment (HDX). Moreover, *venoMS*, a freely accessible database for low molecular weight compounds in spider venoms, was created. The database provides fast access to related literature and acquired MS/MS spectra. Therefore, already known structures are identified easily and time can be used to focus on the structure elucidation of unknown lead compounds.





The fragmentation rules deduced from synthetic polyamine derivatives were used to develop and implement a fragment ion calculator allowing the structure elucidation of known and unknown acylpolyamines. Likely fragment ions are easily calculated by assembling the structure from simple building blocks.

The optimized analytical method in combination with *venoMS* enables a routinely annotation of known low molecular weight compounds and the structure elucidation of new acylpolyamines, even from limited sample amounts like spider venoms.

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An efficient Autosampler for the Screening of Protein-Ligand Interactions by native ESI-MS

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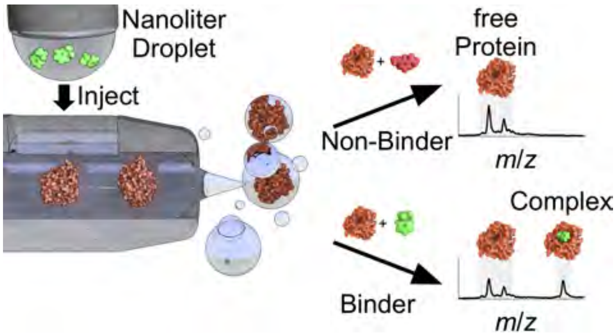
In modern drug discovery the screening of drug candidates against a biological target is often required. One technique suited for this task is native electrospray ionization mass spectrometry (ESI-MS). There, small amounts of molecule-target complexes can be detected in a label-free fashion. Since ESI-MS is often performed manually, Roche and our group collaborated to develop an ESI autosampler to speed up this analysis.[1]Recent modifications[2]allow the analysis of native molecule-target complexes. Based on those latest improvements, we developed a method to rapidly screen compounds against a target. First, a solution of a protein target is fed through an ESI capillary containing a hole. Next, a nanoliter droplet of a compound is picked up from a well plate and injected into this hole. Finally, the compound and the target are mixed in the capillary and binding can be observed via MS. Quantification of the injected ligand is achieved by in-line addition of an internal standard which is both in the droplet and the capillary. The capability of our method is demonstrated by screening 33 test compounds against carbonic anhydrase II as a model protein in 15 min. As a result, we found an excellent screening figure of merit[3]: *Z'* = 0.82. In Addition, binding constants can be determined either directly by our quantification strategy or by injecting the compound in different concentrations and fitting a titration curve. Compared to commercial autosamplers, our setup requires significantly less amount of compound (≈0.6 pmol/l) while operating at a similar throughput.



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A New ICP-MS Configuration for Single Cell Analysis

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For most of the ICP-MS-based techniques, the sample introduction is still considered as Achilles heel due to low or incomplete sample transport. [1] Highly efficient micro-flow nebulizer and microdroplet dispenser have been developed showing acceptable performance for a multiplicity of applications, although efficiency and throughput still need to be improved. Especially in the field of single cell analysis, the introduction efficiencies of the aforementioned sample introduction systems tend to decrease significantly with the cell size. [2][3] Low total masses and small sample sizes (ranging between several microns down to several nanometers) face high uptake rates and low transport efficiencies, which makes single particle and cell analyses challenging. Since every cell line shows a certain average size and size distribution, any type of sample loss goes hand in hand with the loss of information.

In this work, two sample introduction approaches shall be presented both featuring the highest transport efficiencies, i.e. 100%. Chinese Hamster Ovary (CHO) cells (13 µm) and other eukaryotic cells (10-20 µm) were successfully transported via monodisperse droplets and analyzed using time-resolved single cell ICP-MS. Every sampled cell was embedded into a droplet with the help of a so-called Autodrop Pipette (microdrop Technologies GmbH, Germany) emitting monodisperse droplets at selectable size in the range from 50 to 90 µm. The droplets were directly introduced into a vertically oriented low-temperature desolvation system, a so-called falling tube using an Ar-He mixture as a carrier and drying gas. [4] In the first approach, the low-temperature desolvation system was directly coupled with a downwards-pointing vertical ICP enabling a gravitation-assisted sample introduction for ICP-MS. In the second approach, additional make-up gas (up to 0.8 L/min of additional Ar) was used to transport the evaporated droplets horizontally into a state-of-the-art ICP. In comparison, the downwards-pointing vertical ICP did not require additional make-up gas and the droplets reached the plasma independent of the initial droplet size, the droplet evaporation or the cell size. The presented in-house developed prototype shows great promise for future single cell analysis studies.

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