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NMR Studies of Hierarchical Protein Dynamics

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A fundamental challenge in biology is to understand the complex interaction between protein motion and function. Due to the complexity of this interaction and the wide range of timescales on which protein motion occurs, this task remains hard or even impossible. Recently, Lewandowski and coworkers have shown that temperature dependent magic angle spinning multinuclear solid state NMR relaxation measurements, at temperatures ranging from 105 to 280K, can provide a window into the hierarchy of dynamic processes in proteins.1 Other available methods often focus only on a specific transition and are limited. In contrast, solid- state NMR allows simultaneous access to a wide range of observables (here we observe sixteen different probes (4 relaxations parameter for 4 different nuclei) within one protein)

We have reproduced those results1 with a high accuracy, validated the previously proposed model and extended the method of this studies to a different system to conclude on the universalism of those dynamics.

The reproduced experiments allowed us to map the energies related to protein dynamics. Similar transitions in the relaxation pattern can be observed for different probes within the protein and the solvent. We propose that internal motion can be model as a two-component system, where the higher energy motion (20-30 KJ.mol-1) dominates the lower energy motion (5-10 KJ.mol-1) with rising temperature.

Quantitative description of motions occurring in the protein and the solvent are dependent on the applied magnetic fields. Thus, we have validated our previous model using different fields strength, in order to obtain field-independent data: 9.4, 11.7, 14.1 and 18.8 T (400, 500, 600 and 800 MHz). Our model accurately predicts the results found for all the used fields strength.

Different proteins (SH3, OmpG, Sendai virus Large protein IDP domain) have also been studied, in order to see if the proposed model can predict fundamental properties shared by all peptides. Our results show similar (but not identical) behavior for those proteins. We thus conclude, that protein motions show several similar dynamical properties, which can be accurately described by a two motional model.

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Characterization of Biomimetic Phospholipid Membranes with Atomic Force Microscopy (AFM) and Tip-Enhanced Raman Spectroscopy (TERS)

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Cell membranes (CMs) are typically composed of a phospholipid bilayer and they represent the physical barrier and interface between the cell and the outside world. Membranes usually contain several different highly-functionalized structures (e.g. trans-membrane proteins, glycoproteins) that help regulating the basic functions of the cell. The detailed study of CMs, and especially of their functionalized domains is important because they play a major role in cell signaling and may act as regulators of the cell activity, and are thus of key significance for biomedical sciences and drug design [1].

Supported lipid bilayers (SLBs), prepared by the vesicles fusion technique, are widespread models for biological membranes and are widely used in the research community. They are easy to prepare, do not require particular instrumentation and offer a good and reliable “workbench” for preliminary studies [2].The technique of choice to study such samples is atomic force microscopy (AFM), especially because of its very high resolving power (down to <1 nm) and because it does not require samples to be conductive [3]. Despite the very high spatial resolution, though, the AFM is "chemically blind", i.e., it can only give some tentative characterization of chemical properties, e.g. by means of phase or friction force imaging.

Tip-enhanced Raman spectroscopy (TERS) was pioneered in our lab in early 2000s and combines scanning probe microscopy (such as AFM) with Raman spectroscopy with the aim of obtaining a vibrational spectrum from very small spots of the sample, well below the optical diffraction limit. Recently, a spatial resolution down to 1.7 nm has been demonstrated in ambient conditions [4].In this work, the formation of reliable SLBs through vesicles fusion on different substrates has been evaluated, with the goal to achieve stable and reproducible model samples for chemical imaging studies. Preliminary TER spectroscopy has been carried out for feasibility studies on nanoscale chemical characterization of membranes. Promising results were obtained in terms of chemical differentiation of domains. This work shows interesting possibilities for further investigations, and highlights in particular the capabilities of performing TERS on delicate samples.

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Short-wavelength photo-ionization mass spectrometry overcoming the “LOD vs. space resolution” trade-off

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Counting statistics dictates a degradation of the detection power as a consequence of reduction in sample amount. Microanalysis of e.g. nano-scale application profited from the sensitivity of ICP-MS, achieving limits of detections (LOD) in the low ng/g across the mid and high mass range. Combined with laser ablation, the reduction of the laser wavelength helped improving the spatial resolution at the cost of LOD in the ppm. Downscaling of the space resolution poses thus challenges to keep the LOD at trace level.

Further challenges are the non-stoichiometric (“fractionation”) and destructive laser sampling, and the lack of accessibility to important elements such as HCNO and related molecular species. The issue of fractionation has been mitigated using short-pulse lasers. An alternative approach proposed here is that of using short-wavelengths to irradiate the sample. The main advantages are: (i) a direct photoionization of the sample thanks to the >25eV photons; (ii) a drastic reduction of the spot size thanks to the <50nm wavelength; (iii) the access to HCNO and related molecular species thanks to the in-mass-spec micro-sampling; (iv) enabling a full-field (non-scanning) chemical imaging.

XUV using laser-produced or discharge-produced hot/dense plasmas is demonstrating suitable to close such gaps. XUV laser-action has been also accomplished on table-top systems, which in the time of large beamlines with limited access, helps bridging the gap between the user and the tools.

Aim of this talk is present latest results of a few specific applications in materials science.

Enhanced Sensitivity of GC-FT (Orbitrap) – MS Enabling Trace-Level Persistent Organic Pollutant Analysis

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A hyphenation of gas chromatography (GC) with the high-resolution mass spectrometry offered by Orbitrap Fourier transform mass spectrometry (FTMS) is a recent addition to the palette of the analytical methods available for qualitative and quantitative volatile compounds analysis. However, a single measurement of a low concentration sample may be not sensitive enough to detect the compounds of interests or to accurately quantify their levels. The fundamental nature of FTMS suggests a possible way of increasing the sensitivity of targeted and untargeted analysis for both isolated compounds and those embedded into a complex matrix, by summation of time-domain unprocessed data (transients) across a number of technical replicates from GC-MS measurements, followed by Fourier transformation. A principal obstacle to realize this approach is the absence of an access to the transient signals from Orbitrap FTMS instruments. Here, we describe an implementation of a transient-recording capability on the GC Orbitrap FTMS and further method development and application for increasing the sensitivity of the trace level persistent organic pollutant analysis.

Samples containing organic pollutants, such as dioxins, were prepared in different concentrations and combined with an addition of heavy isotopically labelled compounds for relative quantitation. Additional samples were prepared by embedding molecules of interest into complex matrices. A commercial Q Exactive GC Orbitrap FTMS (Thermo Scientific) equipped with electron impact ion source was interfaced with a high-performance data acquisition system (FTMS Booster from Spectroswiss) to enable acquisition of unprocessed data in the form of time-domain transients. Data processing was performed using both custom-made Peak-by-Peak software for FTMS data processing (Spectroswiss) and commercial XCalibur data analysis software (Thermo Scientific). Transient and spectral summation of GC FTMS data from multiple GC-MS runs have been performed. Areas under the curve for selected ion chromatograms were calculated to compare workflow performance.

Preliminary results demonstrate that a multiplexed GC-MS approach is indeed highly beneficial for the increased sensitivity and improved quantitation accuracy in the quantitative analysis of low abundant dioxins.

Transgenerational Fate Modeling of Polychlorinated Biphenyls in Cattle

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Polychlorinated biphenyls (PCBs), classified as persistent organic pollutants (POPs), are hardly degradable contaminants and bioaccumulate in our food chain due to their hydrophobic characteristics. Although the use of PCBs has been restricted since the 1970s and has been banned worldwide by the Stockholm Convention on POPs in 2004, PCBs are still ubiquitous in our environment. More than 90% of human exposure is attributed to food of animal origin, where frequently elevated levels of PCBs are detected. This can not only lead to long-term toxic effects, but is also accompanied with depreciation of the products at the expense of the farmer. In this doctoral thesis, the input pathways of PCBs into the food chain, in particular the uptake of PCBs by cattle via feed, will be investigated with the focus on the transfer of PCBs via milk to the suckling calves. Therefore, an animal experiment will be performed, where the mother cows will be exposed to a long-term intake of silage containing soil with usual PCB background concentrations present in the environment, while feeding their calves. These experimental data will be used to validate and improve a new physiologically based toxicokinetic (PBTK) model [1], which incorporates the uptake of soil while grazing and the transfer of PCBs from the mother cow to their suckling calf. Both, experimental data and modeling, will help to understand the accumulation process of PCBs in cattle, thereby deriving recommendations for best agricultural practices to further reduce contamination by PCBs and improving food quality. Besides, the findings of this thesis will also contribute to our understanding of other POPs, making it internationally important given the global relevance of POPs.

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Targeted on-line breath analysis supports altered collagen metabolism in idiopathic pulmonary fibrosis

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Background: On-line breath analysis is a powerful technique to obtain insights into the metabolism of a person. Idiopathic pulmonary fibrosis (IPF) is a chronic and poorly understood lung disease whose diagnosis often requires CT-scans and lung biopsies. A recent study identified increased levels of collagen related amino acids in lung tissue of IPF patients using GC-MS profiling. [1] Given that some of these metabolites are detectable in breath via secondary electrospray ionization-high resolution mass spectrometry (SESI-HRMS), we hypothesized that these altered amino acid levels might be mirrored in exhaled breath, which would allow for a non-invasive screening of IPF.

Methods: Breath analysis was performed on-line using SESI-HRMS including 21 IPF patients and 21 healthy controls. Their exhaled breath pattern was analyzed for the previously reported target compounds. For all compounds where a signal was detected at the accurate mass in real-time, UHPLC-MS measurements of exhaled breath condensate (EBC) were performed, to confirm their identity. Their classification performance was estimated by performing 1 million leave-one-out cross-validations using correct and random labels.

Results: We could detect robust signals for proline, 4-hydroxyproline, alanine, valine, leucine/isoleucine, allysine, phenylalanine and pyroglutamic acid, most of which could also be confirmed in EBC. Six of the compounds showed a significant increase ($p<0.05$) in exhaled breath of IPF patients. Additionally, we observed a signal correlation across subjects, and those amino acids with a higher abundance in collagen or elastin showed the most pronounced effect. This is consistent with altered collagen and elastin turnover being the underlying metabolic processes. Using the signals of all detected amino acids, we were able to obtain a cross-validated area under the receiver operating characteristic curve of 0.86.

Conclusions: Using targeted on-line breath analysis with SESI-HRMS, we could detect increased amino acid levels in IPF patients, which allowed for a good discrimination from healthy controls. This is consistent with previous metabolomic findings from lung biopsies, however we could capture this information in a non-invasive and rapid fashion, underlining the strength of real-time breath analysis.

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Enhanced extraction of small molecule-drug conjugate targeting carbonic anhydrase in cancer chemotherapy by automated SPME coupled to ESI-MS

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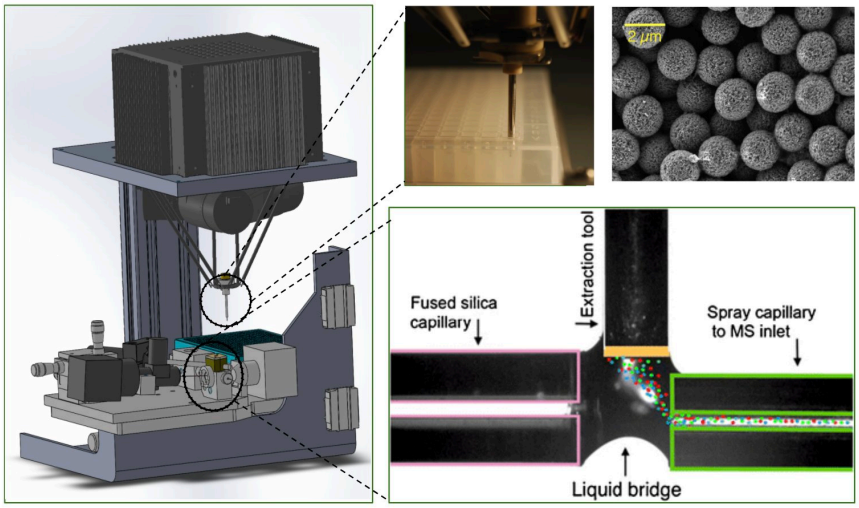
Introduction: In order to avoid dose limiting toxicity, targeted delivery of cytotoxic agents into tissues, especially malignant cells is an attractive strategy [1]. One of the common used target moiety for selective delivery of cytotoxic agent is acetazolamide. In this area, there is a pressing need to develop rapid techniques to quantify the amount of drug in the tissue in order to investigate the tumor targeting performance of the different ligands. So-called the "capillary gap sampler" is capable of performing automated and site-specific extraction of very small sample amounts.

Method: The capillary gap sampler, a miniaturized sampling device hyphenated with ESI-MS is capable of automated low-volume sample handling [2]. Sample extraction is done by a coated stainless steel pin. Later sample desorption is performed in a liquid bridge of several nanoliters between two capillaries, where one acts as the ESI-MS spray needle. Enhanced drug extraction is possible by covalently immobilizing carbonic anhydrase (CA) on the pin tip.

Preliminary Data: The development started with optimization of the coating procedure, desorption solution, etc. ACN 60% in water was found to be the optimum desorption solution. Extraction of the drug from PBS was performed by dipping the CA modified extraction tool inside the solution. After a quick washing step with water, it enters into the liquid bridge, where the analyte desorbs and is sprayed in the ESI source. Peaks corresponding to the drug ($drug^+$), ($drug+Na^+ + H^+$), ($drug+2H^+$), (therapeutic warhead = cytotoxic agent coupled to the targeting agent + H^+) were observed in the spectrum. Finally, repeatability of the extraction using CA modified beads are evaluated by performing thirteen 5-minute extractions of 500nM acetazolamide in PBS solution. The relative standard deviation is below 10%.



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Insights into the challenges regarding the quantification of geological silicate samples by LA-ICPMS

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The upper continental crust, being the most accessible part of the Earth, has long been the target of geochemical investigations [1]. Its rich geological history helps to understand the origin and differentiation of the Earth. Many geologically important rock types are based on silicate phases, as Si is the second most abundant element of the continental crust. To reveal the elemental and isotopic composition of such rock samples laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) is a very powerful analytical technique commonly applied. Two key components to this success are 1) the possibility for the direct analysis of solid samples and hence the circumvention of digestion based analysis and 2) the ability to quantify matrix and trace elemental composition during one course of analyses. The major challenges concerning the accuracy of analysis are due material-dependent laser ablation processes, which can result in separation of the elements into different phases formed during the LA process, causing material-dependent elemental fractionation. [2] For example, previous studies have shown significant material-dependent laser induced fractionation between Si and Ca [3]. Consequently, certified reference materials (CRMs) are required to enable matrix-matched calibrations.

The aim of this paper is to resume investigations of the phenomenon of elemental fractionation occurring during state-of-the-art UV-ns-LA-ICPMS for Si. An extensive comparison between different natural silicate CRMs processed into two different phases - glass, and pressed powders by [4] - will be presented. These CRMs are based on various igneous (basalt, granite, diorite and gabbro) and metamorphic rocks (serpentinite). In this study we will compare the different CRM matrices and also the different phases with identical matrices. The fundamental mechanism behind the observed fractionation between Si and Ca for all different kind of CRM will be discussed. Finally, these CRMs are used to quantify different mineral samples, such as Topaz (30 wt-% SiO_2), Wollastonite (50 wt- % SiO_2), Anorthite (60 wt-% SiO_2), Olivine (40 wt-% SiO_2), Plagioclase (65 wt-% SiO_2), Clinochlore (30 wt-% SiO_2) and Clintonite (20 wt-% SiO_2).

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A Versatile Software for Control of Advanced Laser Ablation ICP-MS Element Imaging

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) showed significant improvement in providing valuable insights to the elemental distribution within solid materials in terms of lateral resolution, data acquisition speed. Furthermore, the use of time-of-flight (TOF) mass analyzers and their capabilities of fast and multi-element detection made this approach more versatile. We think that mere line scans will soon be outdated by more advanced modes of operation which require control systems which tightly integrate the different components (translation stage, laser, and ICPMS).

In this work we present a control software, specifically developed for experiments for LA-ICPMS, with extensibility and ease of use in mind. In allowing for custom ablation area masks to be imaged, laser pulse patterns, and MS data acquisition trigger the measurement sequence can be customarily fitted to a sample’s specific structures for 2D and 3D scans. The initial implementation includes a communication interfaces to an ArF excimer laser ablation system (193 nm, GeoLas C, Lambda Physik, Goettingen, Germany) triggered by a separate microcontroller (Arduino Uno). The firmware for the microcontroller was also developed in-house. Positioning controls were implemented for the translation stage (SLC-24 series and MCS-3 controller, SmarAct GmbH, Oldenburg, Germany) used in the setup. New communication interfaces can be easily added due to the modular design of the software allowing inclusion of different hardware setups. As programming language Python 3 was chosen to profit from its large library support as well its popularity in the scientific community.

As further work we are aiming for streamlining the process from data acquisition, data processing to data analysis and evaluation.

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Insights into the rare earth element pattern of Jade objects for provenancing

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We report on the current state of our research towards into the rare earth element (REE) concentration patterns in Jade (jadeite and nephrite) for provenacing. Jade has been used during the Neolithic period (5500-2000 BCE) to produce axe heads, which have been exchanged on a large part of Europe (from Scotland to the Black Sea) [1]. One of the project's aim is to check whether the samples from the supposed outcrops, the Italian Alps, bear some patterns which could help to relate them with prehistoric artefacts containing jade. The analysis of REEs in Jade usually require highly sensitive analytical techniques since most of these elements have been shown to occur in traces at the ng/g level and below [2]. One of the methods of choice for the determination of the (ultra-)trace element composition of solids is laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). It provides small sampling areas (10's of μm^2) with minute amounts of ablated material in combination with the high sensitivity and the multi-element capabilities of ICPMS, achieving quasi-non-destructive analysis with low limits of detection (ng/g) [3]. However, the instrumental and infrastructural requirements for the ICPMS hinder the application of this method for the analysis of immobile or large samples on site, which are not possible or allowed to be transported or removed. The portable Laser Ablation system, developed in our laboratory [4], is the combination of the advantages by sampling like in conventional laser ablation, the almost unlimited sampling performance of a portable analytical device, as e.g. a portable XRF, and the analytical power of (LA)-ICPMS for the analysis back in the laboratory. A set of Jade samples (jadeite and nephrite) collected from two different geographical sites of the Italian Alps were analysed. The focus was set on their general elemental composition and especially their REE-pattern. The measurements were performed by conventional LA-ICPMS (λ = 193 nm) and the quantification was based on external calibration using matrix-matched standards. Provided that sufficient material can be collected to ensure detection of the REE the results could be reproduced by the pLA-system (λ = 532 nm) and a subsequent analysis of the ablated material by re-ablation under conventional LA-ICPMS conditions (λ = 193 nm). The relative REE-pattern showed no discrepancies in-between the two approaches. These findings indicate the pLA to be fit-for-purpose for a minimally invasive analysis of trace element composition of minerals. The direct comparison with conventional LA-ICPMS demonstrates the capabilities and ensures the quality of the measurements but shows also the limitations of the approach.

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Investigations on cation-adduct formation in MALDI mass spectrometry

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Cation adducts have been shown to dominate MALDI mass spectra for certain analytes with a high cation affinity and low basicity, such as polymers.[1-2] Several mechanisms of ionization have been proposed, gas-phase attachment of cations being the most widely accepted.[3] However, experimental results are sparse and clear evidence for any of the proposed pathways is still absent. We employ a MALDI target plate consisting of two metal plates separated by a small gap[4] to enable co-desorption of individually deposited polymers and salts. Deposition of polystyrene and copper trifluoroacetate on separate plates results in ionization of the polystyrene by copper adduct formation. The exclusive observation of ions upon ablation at the conjunction of the two plates provides clear evidence for cation transfer in the MALDI plume.

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Artificial impregnation for modelling waterlogged wood contaminated with iron sulfides

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Archaeological waterlogged wood containing iron sulfides, are very stable in anaerobic environment. Once exposed to oxygen, an acidification and salts precipitation occurs, leading to irreversible chemical and physical damages[1]. We propose a pioneering extraction method of the Fe and S species by the use of harmless microorganisms. To select them, test on model samples have to be carried out. The model samples were prepared on freshbalsa wood with three different impregnation protocols. Round robin test was performed in parallel in LATHEMA and Arc'Antique to evaluate the efficiency and repeatability of the protocols. After being fully immersed for 168 hours in deionized water, the samples were immersed in an equimolar solution of Fe^{2+} and S^{2-} under vacuum (IP1); in a solution of SO_4^{2-} containing corroded terrestrial archeological nails (IP2); under inert atmosphere by a neutral solution of Fe^{2+} and S^{2-} (IP3)[2,3,4]. The samples were characterized by non-invasive and non-destructive analyses.

A change to a darker hue was observed for IP1 and IP3, while IP2 presented a brown-orange hue. The degradation of the wood was evaluated by Fourier Transformed Infrared (FTIR) spectroscopy using an Attenuated Total Reflectance (ATR) accessory. A decrease in the lignin content was observed, suggesting lignin was degraded but not carbohydrates. . Finally, Raman spectroscopy allowed to identify iron sulfides for IP1 and IP3 and only iron oxides for IP2. These results showed the efficiency of using IP to prepare model samples from fresh wood and hence to simulate waterlogged wood contaminated with iron sulfides. In particular it has been demonstrated that the IP should be carried out on raw balsa wood to induce the carbohydrates degradation. Next steps will include the validation of the selected protocol on archaeological wood before testing selected bacteria using these model samples.

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Identification of biomarkers from pathogens of patients with cystic fibrosis via secondary electrospray ionization-mass spectrometry

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Persons suffering from cystic fibrosis (CF) are prone to chronic bacterial airway infection. Early recognition of airway colonization is important to initiate appropriate treatment to prevent airway damage resulting in a progressive lung disease. Exhaled breath contains biochemical information and thus can give insight into the health status of a person. In this context, the analysis of breath using secondary electrospray ionization-mass spectrometry (SESI-MS) would be a powerful and promising tool to profile infections in CF patients since it is fast, precise, and non-invasive.

Our aim is to investigate the ability of SESI-MS to differentiate between important bacterial pathogens for CF lung disease in the headspace of liquid bacterial cultures. The following biological replicates were investigated: B. cepacia, E. coli, H. influenzae, P. aeruginosa, S. aureus, S. maltophilia and S. pneumoniae. Subsequently, we performed analysis of variance (ANOVA) on 130 peaks in the mass spectra with intensities over 1000 cps. 61 peaks showed significant differences in peak intensity between the bacteria strains. Based on these preliminary results, we are expanding this study to 30 biological replicates and will then start with the compound identification by comparing fragment spectra of the peaks that characterize particular pathogens with those of proposed reference compounds.

In conclusion, we identified differences in the spectra of bacterial pathogens that frequently infect CF patients using SESI-MS . With a set of unique volatile organic markers for each investigated bacteria strain, it would be possible to differentiate between the individual strains via SESI-MS. With this and other studies, we will try to establish a fast and non-invasive diagnosis of airway infections in CF patients using real-time breath analysis.

Transformation of chloroparaffins to chloroolefins during metal drilling

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Chloroparaffins (CPs) are high production volume chemicals (>1 Mio t a^{-1}) produced as complex mixtures and widely applied as flame retardants, plasticizers and metal working fluids. We showed that HCl is eliminated when CPs are exposed to heat, resulting in chloroolefin (CO) formation. When applied as additives in metal working fluids, CPs are exposed to high temperatures. In this study, we show that metal drilling too induces the elimination of HCl and thus the formation of COs [1]. Recorded mass spectra show that isotopic clusters of CPs and COs of the same chain length and chlorination degree strongly overlap. Thus investigating CP/CO mixtures is a complex task that requires soft ionization mass spectrometry (MS) and mathematical deconvolution of interfered mass spectra [2].

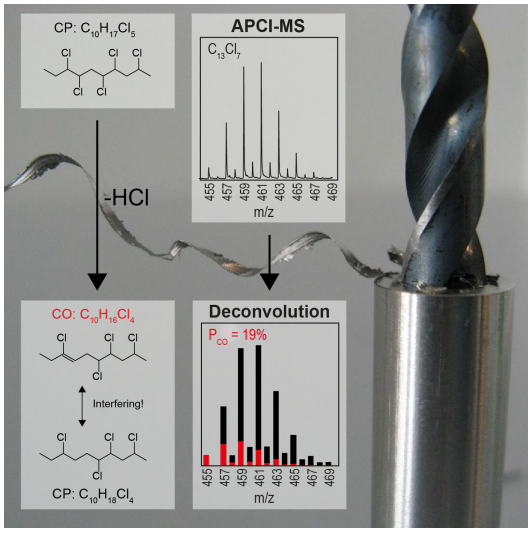

Figure: Mass interferences of CPs & COs and respective deconvolution.

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Characterization of gold nanoparticles using inductively coupled plasma mass spectrometry (ICPMS)

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The growth of nanoparticles (NPs) application in various fields such as textile industry, cosmetics, food packaging, pesticides, sporting goods, paint, optics, and medical devices have driven the industrial production reaching nowadays kg/h. [1]

Even though this technology is expected to have major benefits, little is known about their impact on environment and human health.

In order to asset the potential risks that they pose, a characterization of nanoparticles (NPs) is needed. NPs characterization includes the determination of mass, size, particle number concentration (PNC), morphology and elemental composition. Currently there is no exist method, which could be used on a routine basis for obtaining simultaneously all the above mentioned characteristics in reliable and reproducible way. [2, 3] Inductively coupled plasma mass spectrometry (ICPMS) is a promising approach which allows fast and sensitive determination of most elements, and can be used for analysis of NPs' mass, composition and number concentration. [4]

In this work we focus on the use of state of the art sector-field ICPMS, which is offering highest detection efficiency and low instrumental background. However, the inherent sequential detection of the instrument and the fast signal arising from a nanoparticle, limits the detection to only one isotope per NP. Gold NPs are analyzed with different sample introduction configurations: either microdroplet generation (MDG) or single particle with conventional nebulization. Moreover, the influence of different instrumental configurations will be presented. More precisely: wet and dry plasma condition, nitrogen addition to the carrier gas and the use of Thermo's "Jet" interface. The stability of NPs and their change in size and agglomeration state versus time in a different medium will be investigated. This data are important in order to ensure that the NP distribution at the point of sampling can be preserved until the analysis or to what extent a transformation must be considered.

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Understanding electrospray ionization mechanisms of biomolecules using laser-induced fluorescence

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Electrospray ionization (ESI) is one of the most common ways to generate gaseous ions for mass spectrometry (MS) analysis. However, the mechanisms which govern ion production remain difficult to study. Mechanistic insights promise not only to further our fundamental understanding of the ESI process, but will facilitate optimization of ion formation as well. Towards these ends, several groups have devised different experimental and theoretical schemes. In particular, it has been shown that laser-induced fluorescence experiments in an electrospray plume, at different distances along the electrospray axis, provide snapshots of the conformations, electronic structures, and dynamics of electrosprayed analytes both within charged droplets, and also once completely desolvated.

We have developed a setup to study laser-induced fluorescence at different distances along the electrospray axis. A unique aspect of this apparatus is also that it facilitates both wavelength and time resolved fluorescence measurements from a particular spot in the plume. 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). Tunability of laser light in this wavelength lets us probe the spectroscopic properties of biologically relevant chromophores.

Laser-induced fluorescence of biomolecules along the electrospray axis is being studied to understand different electrospray ionization mechanisms. Proteins like apo-myoglobin are labelled with fluorescent dyes and their fluorescence is monitored as they traverse from the droplet phase to the gas phase. This gives us valuable information about structural changes in the protein and its surrounding, which in turn can be used as a proxy for the mechanism governing ion production. For example, small organic ions, such as the rhodamine family of dyes, all exhibit dramatic (~1400 cm^{-1}) blue-shifts in their emission maxima as one moves only a few mm away from the ESI emitter. Conversely, globular proteins including myoglobin and bovine serum albumin, both of which have been covalently tagged with these same dyes, show no measurable change in spectroscopic properties under the same conditions. This suggests a much longer timescale for ion production in the case of the latter, larger analyte ions.

Current efforts are focused on the study of how different classes of molecules follow different ionization mechanisms, and to probe the influence of solvent and spray conditions on the ion yield and ionization mechanism. As a next step, Fluorescence Resonance Energy Transfer (FRET) experiments are expected to give distance sensitive information that can be used to track conformational changes of electrosprayed protein along the electrospray axis. Details of the developed setup will be presented, along with results from laser-induced fluorescence on biomolecules along the electrospray axis.

Space resolved laser microanalysis of Potassium & Iodine in Laccase-catalysed woods

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Laccase-catalysed grafting of bioactive molecules onto the lignocellulose of wood represents an environmentally friendly method for wood protection [1]. Laccase-catalysed iodide oxidation in the presence of lignocellulose leads to a non-leachable antimicrobial wood surface which is highly resistant against colonization by different classes of heterotrophic microorganisms. Such pre-treated wood surfaces in combination with a suitable coating system are expected to show an improved weathering performance.

To gain more information regarding the effectiveness of the treatment, different analytical tools are necessary. Key factors are the determination of the penetration depth in freshly prepared samples and the distribution of Iodine after a leaching process. In the past this task was covered by Microprobe elemental mappings (SEM-WDX). Since the region of interest has to be limited and the measurement is fairly costly other analysis techniques are needed. LA-ICP-MS / LIBS are potential methods to fulfil the requirement for a space resolved elemental (i.e. K and I) detection with a huge dynamic elementary detection range from 0.1ppm to % (LA-ICP-MS) and fast sampling speed <1s (LIBS). In a first step based on two different sample preparation techniques the detection limits, the RSD and the signal stability were investigated. The first results illustrating the leaching process and indicate the main behaviour of the process.

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Microdroplet-assisted conveyance of Single Cells for time-resolved Analysis by ICP-TOFMS

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The capability of analyzing biological single cells has gained high interest during the last years and gave molecular biologists new insights into cell-to-cell differences in a cell population that seem to be identical at 1st glance. [1] Single cell analysis enables us not only to identify cell sub-populations, it also allows conclusion of whether discovered sub-populations show a biological function or can be ignored. An analytical technique that combines single cell detection capability, simultaneous measurement of multiple cellular features in a single cell at high sample throughput is Mass Cytometry (MC). [2] In MC, antigens are tagged with a polymer moiety that are marked with enriched stable rare-earth element (REE) isotopes. While MC focusses on the detection of REE-labeled antibodies, elemental MS, *i.e.* time-resolved ICP-MS may be used to acquire full mass spectra across the entire periodic table simultaneously with rates of 33 kHz. Therefore, MS allows to quantitatively analyze the elemental composition of biological cells to, *i.e.,* determine the uptake of metals into cells. For the most of the ICP-MS based techniques the sample introduction system is still considered as a weak spot. Highly efficient micro-flow nebulizer and micro droplet dispenser have been developed showing acceptable performance for multiplicity of applications, although efficiency and throughput still need to be improved. Especially in the field of single cell analysis, the introduction efficiencies tend to decrease significantly with the cell size. [3] In this study, a microdroplet generator attached to a low-temperature desolvator tube was used in order to measure aqueous cell suspensions. [4] Droplets with an average diameter of 50 µm were generated, mobilized and transported into the Plasma. The droplet signal was monitored and highest transport efficiencies at a selected frequency between 25-100 Hz were obtained. This microdroplet-assisted conveyance setup was coupled to an all-commercial ICPTOF-MS (icpTOF, TOFWERK AG) for comprehensive analysis of individual cells. The acquisition of a full elemental spectrum at high time resolution enables us to detect cell constituents at a frequency of 22 kHz, corresponding to a time resolution of 46 µs. Preliminary results indicate that we are able to detect single cell events. In addition, a complete workflow including the cultivation of Chinese hamster ovary cells, counting procedures and the preparation steps for a quantitative elemental analysis will be demonstrated as possible procedure to study the metal uptake of single cells.

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The study of non-covalent interactions between G protein-coupled receptors and their partners by MALDI mass spectroscopy

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G protein-coupled receptors (GPCRs) are a family of important membrane proteins. Noncovalent interactions of GPCRs complexes and their underlying mechanisms are not easy to study, because of the requirement of a lipid or detergent environment to maintain the GPCR structure and functionality. Nanodiscs have become a leading technology to solubilize membrane proteins, mimicking a biological environment and protecting their biological functions [1]. Here, noncovalent binding efficiencies between nanodiscs loaded with GPCRs and their partners, including different subunits of G proteins, a G protein mimicking nanobody Nb80, and an engineered G protein ($miniG_s$)[2,3] are investigated by chemical crosslinking and high-mass Matrix-Assisted Laser Desorption/ Ionization (MALDI) Time-of-Flight Mass Spectrometry. The agonist isoprenaline and inverse agonist S32212 are applied to adjust the conformational states of GPCRs to its active and inactive states, respectively, which affects the noncovalent interactions between GPCRs and their partners [3]. These different conformational states are reflected by changes in the mass-to-charge ratio, which aids in uncovering the presence of active and inactive GPCR forms. Our approach provides a fast, convenient, and sensitive way to explore the biological functions and formation conditions of GPCR-complexes, and may, in the future, help for selecting GRCRs targeting drugs.

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Xeno Nucleic Acid Nanosensors for Enhanced Stability

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The omnipresence of salts in biofluids creates a pervasive challenge in designing sensors suitable for *in vivo*applications. Fluctuations in ion concentrations have been shown to affect the sensitivity and selectivity of optical sensors based on single-walled carbon nanotubes wrapped with single-stranded DNA (ssDNA-SWCNTs). We herein observe fluorescence wavelength shifting for ssDNA-SWCNT-based optical sensors in the presence of divalent cations at concentrations above 3.5 mM. In contrast, no shifting was observed for concentrations up to 350 mM for sensors bioengineered with increased rigidity using xeno nucleic acids (XNAs). Transient fluorescence measurements reveal distinct optical transitions for ssDNA- and XNA-based wrappings during ion-induced conformation changes, with XNA-based sensors showing increased permanence in conformational and signal stability. This demonstration introduces synthetic biology as a complementary means for enhancing nanotube optoelectronic behaviour, unlocking previously unexplored possibilities for developing nano-bioengineered sensors with augmented capabilities.

Characterization of Double-stranded DNA on Single-walled Carbon Nanotubes (SWCNTs)

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DNA has been extensively studied due to its versatility as a dispersant of SWCNTs. The specificity and binding affinity of DNA to various analytes has been shown to be strongly sequence dependent. As a result, through modifications of the nucleotide sequence, the DNA conformation on the SWCNT surface can be tailored to suit specific needs for techniques such as single-molecule detection, *in vivo*imaging, and chirality separation. To date, the majority of research has focused on the interaction between single-stranded DNA and SWCNT surface, with less focus on the nature of the interaction between double-stranded DNA (dsDNA) and SWCNTs. As a result, the exact interaction mechanism governing this type of complex remains strongly debated and largely unknown.

In this study, we employ various biochemical methods to infer the conformation of dsDNA on the surface of SWCNTs. Our methods are based on imaging techniques for identifying DNA-modifying enzyme activity in the presence of dsDNA-SWCNT complexes. Our findings suggest that dsDNA can partially retain its native conformation on the SWCNT surface, and the degree of dsDNA accessibility is strongly sequence dependent. These findings offer new possibilities for SWCNT sensing applications that employ dsDNA, such as the optical detection of DNA-protein interactions.

Mediatorless, Reversible Optical Nanosensor Enabled through Enzymatic Pocket Doping

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Single-walled carbon nanotubes (SWCNTs) exhibit intrinsic near-infrared fluorescence that benefits from indefinite photostability and tissue transparency, offering a promising basis for *in vivo*biosensing. Existing SWCNT optical sensors that rely on charge transfer for signal transduction often require exogenous mediators that compromise the stability and biocompatibility of the sensors. This study presents a reversible, mediatorless, near-infrared glucose sensor based on glucose oxidase-wrapped SWCNTs (GOx-SWCNTs). GOx-SWCNTs undergo a selective fluorescence increase in the presence of aldohexoses, with the strongest response toward glucose. When incorporated into a custom-built membrane device, the sensor demonstrates a monotonic increase in initial response rates with increasing glucose concentrations between $3 \times 10^{-3}$and 30×10^{-3}M and an apparent Michaelis–Menten constant of $K_{M(app)} \approx 13.9 \times 10^{-3}$M. A combination of fluorescence, absorption, and Raman spectroscopy measurements suggests a fluorescence enhancement mechanism based on localized enzymatic doping of SWCNT defect sites that does not rely on added mediators. Removal of glucose reverses the doping effects, resulting in full recovery of the fluorescence intensity. The cyclic addition and removal of glucose is shown to successively enhance and recover fluorescence, demonstrating reversibility that serves as a prerequisite for continuous glucose monitoring.

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A Multi-Detector Set-Up Comprising of UV/Vis Detection, Charged Aerosol Detection and Single Quadrupole Mass Spectrometric Detection for Comprehensive Quantitative Sample Analysis

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This presentation shows the reliable verification of the presence of additional compounds in a sample, e.g., of impurities, degradation products of the analyte or extractables from containers.

A multi-detector HPLC set-up comprising UV/Vis, charged aerosol and mass spectrometric detection was employed. The first two detectors were used for quantitative detection, and the mass spectrometer was employed for m/z-based confirmation of the analyte identity.

Extracts from single-use cell culture bags were analyzed. 18 known extractables and 19 unknown extractables could be quantified. The charged aerosol detector was used for quantification of all unknowns and for eleven of the known analytes. The UV detector was used for quantification of seven of the known analytes. The mass spectrometer was used for identity confirmation of the detected analytes.

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Quality by Design in pharmaceutical industry as trigger for production process improvement and its extension to the analytical method lifecycle

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The delivery of pre-defined and fully understood, constantly complying quality is a must in pharmaceutical industry during development and commercial production of medicines in order to meet the stringent demands to ensure patient safety. The routine application of Quality by Design (QbD) principles in industry started off after the Draft Process Analytical Technology (PAT) FDA guidance was published in 2004 and after the International Conference on Harmonization (ICH) guideline Q8 was published in 2006.

QbD principles will not just help to reduce variance and variability and enhance process capability and also provide significant benefits during analytical method development: To allow for an earlier and deeper understanding and identification of potential variables affecting method robustness and performance. During product lifecycle, QbD principles will also support the improvement of the performance of analytical methods during development and operational application used to assess the overall product quality.

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[2] ICH Quality guidelines
[3] Guideline on Real Time Release Testing, European Medical Agency
[4] Stimuli article: Lifecycle Management of Analytical Procedures: Method Development, Procedure Performance Qualification, and Procedure Performance Verification, G Martin, 2013

Real time release and on-line analytics in the Pharma Industry: A reality check based on cases studies?

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Pharmaceutical manufacturing is known to be a conservative business with a low readiness and appetite for changes, assuming risks or to embark on new technological strategies and approaches. Within the last decade however, new on-line analytical technologies have been introduced into pharmaceutical manufacturing supported and encouraged by regulatory initiatives and new guidance.

In this presentation, case examples will be presented how process spectrometry, new automation concepts and multivariate modeling tools have been used to establish new approaches to control strategies compared to the classical end product testing.

The examples will encompass chemical API manufacturing and secondary manufacturing of tablets.

[1] FDA Guidance for Industry PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance
[2] ICH Quality guidelines 8 -11
[3] Guideline on Real Time Release Testing, European Medical Agency
[4] FDA Guidance for Industry: Process Validation – General principles and Practices

Photon energy and time-dependent Raman study of aqueous organic compounds

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During the last decades the scientific interest in the waste water analysis has been growing due to the appearance of new harmful contaminants in treatment plants. Pharmaceutical ingredients (APIs) such as Benzotriazole, Aminusulpride and Carbamazepine have been reported with typical concentrations of ug/l or lower [1, 2, 3]. The standard chemical analysis is commonly performed in analytical chemistry laboratories by means of gas and liquid chromatography (GC and LC) and Mass spectroscopy (MS) [4]. However, at present an alternative online technique to perform quantitative analysis and detection is missing. Raman spectroscopy in the visible range is a promising online technique, but its maximum sensitivity is still limited [4,5] due to overlap of fluorescence and Raman emission. By changing the excitation energy is theoretically possible to vary the relative intensity of the Raman and fluorescence signal but the final result strongly depends on the sample under investigation. For that reason a specific characterization is crucial for a deeper understanding of such phenomena. By using different laser sources we performed a photon energy dependent Raman investigation of main contaminants as Benzotriazole, Aminusulpride, Carbamazepine, Citolapram, Hydrobromide, Clarithromycin and Hydrichlorothiszide in water based solutions and by varying their concentration. Our study focused on the temporal dependence of the fluorescence relaxation mechanism with respect to the transient Raman interaction. The former occurs indeed on a picoseconds time scale (nanoseconds) while the latter occurs on a picoseconds time scale. We carried out a time-resolved study in order to disentangle these two different processes in the context of spectroscopic detection of micropollutants.

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Comparison of genotoxic potentials of current diesel and gasoline vehicle exhausts and impact of filters

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Gasoline direct injection (GDI) vehicle and multipoint fuel injection vehicles (MPI) are replacing traditional port fuel injection vehicles. However, high emissions of particles exceeding the Euro 6 limit of $6x10^{11}$ particles/km and emissions of genotoxic pollutants like polycyclic aromatic hydrocarbons (PAHs) are produced. Solutions to reduce these toxic emissions are required. Particle filters are most promising technology. In this study, exhaust from from 7 GDI vehicles and MPI vehicle have been sampled, representing different technologies and a Euro-5 diesel vehicle with a particle filter (D-DPF) at the chassis dynamometer of the UASB. Vehicles were driven following the worldwide harmonized light-duty vehicles test cycle under hot (hWLTC) and cold start conditions (cWLTC). Four different prototype gasoline particle filters, two non-coated and two coated (GPFs), were mounted after the TWC and tested with one vehicle (GDI4-Euro-5). Moreover, a coated and a non-coated filter were tested in the MPFI vehicle. Samples were processed following extraction and clean-up procedures and analysed by HRGC-HRMS and concentrations of PAHs and alkyl-PAHs were determined. Concentrations in ng TEQ/m^3 of 8 genotoxic PAHs are shown in Figure 1 in the cWLTC. On average, GDI vehicles emit up to 17-fold more genotoxic PAHs than the bench mark diesel vehicle with DPF. The MPI vehicle emits similar levels as the mean GDI fleet. It was found that PAH emissions were reduced with GPFs except for one non-coated, where a 2-fold higher emission was observed. Nevertheless, emissions are still higher than the diesel with DPF (2-18 times). It is obvious that GPFs are needed to lower genotoxic PAH and particle emissions of GDI vehicles.

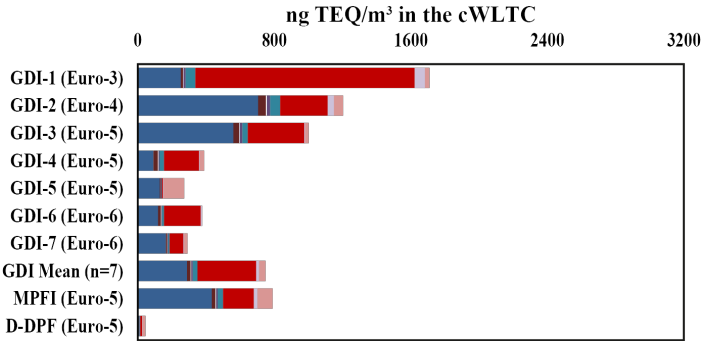

Figure 1. Concentrations of the genotoxic PAHs (ng TEQ/m^3) of GDI vehicles, MPFI and diesel vehicles equipped with DPF (D-DPF).

A Validation Study for Real-Time Diagnosis of Obstuctive Sleep Apnoea by Analysis of Exhaled Breath Using Secondary Electrospray Ionization Mass Spectrometry

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Since breath analysis shows a high potential as a non-invasive diagnostic tool, many explorative case-control studies investigating different diseases have been carried out in the recent years. However, only very few results have been validated in a larger cohort later on. Here, we present a validation study for the diagnosis of obstructive sleep apnoea (OSA) by analyzing exhaled breath with secondary electrospray ionization mass spectrometry (SESI-MS). In a pilot study carried out in our group [1], a panel of potential biomarkers was found defining a specific pattern in exhaled breath of OSA patients. The predictors identified in that study provided an AUC = 0.87 of the receiver operating characteristic curve. This study aims at validating these biomarkers in a blinded study with a larger group of patients.

Subjects with suspected OSA (n=150) were diagnosed by conventional in-laboratory respiratory polygraphy. In addition, they underwent exhaled breath analysis using SESI-MS. Metabolic breath patterns were analyzed and the diagnosis of OSA was predicted for the subjects using the predictors found in our previous study. The person analysing the data was blinded regarding clinical diagnosis.

Between the pilot study and the validation study, some technical improvements on our SESI-MS setup were made. We upgraded to a commercial SESI source making the data challenging to compare. In a preliminary data analysis of 130 subjects measured so far, we were able to render the old and the new dataset comparable using empirical Bayes methods [2]. Thus, the data from the pilot study can be used for training the classification model for predicting the validation cohort. A positive outcome of this study will strengthen real-time breath analysis by SESI-MS as a diagnostic tool tremendously and bring it a step closer to its application in clinical routine.

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Clumped N_2O isotopes by mid-IR laser spectroscopy

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For many years, nitrous oxide (N_2O) has been a major focus of greenhouse gas accounting agreements. Understanding the mechanisms of its formation and clarifying its sources and sinks is highly important for mitigating N_2O emissions. Measuring the doubly substituted "clumped" isotopocules of N_2O will add new and unique opportunities to fingerprint and constrain the biogeochemical cycle of this important greenhouse gas.[1, 2]

Mid-IR spectroscopy is a highly attractive technique to analyze N_2O isotopocules based on their specific ro-vibrational absorption characteristics. We are developing an analytical technique for the selective and precise analysis of the most abundant clumped N_2O isotopic species $^{15}N^{14}N^{18}O$, $^{14}N^{15}N^{18}O$, and $^{15}N^{15}N^{16}O$. The measurement setup is based on a dual quantum cascade laser absorption spectrometer (QCLAS) with a multi-pass absorption cell. Under optimal measurement conditions, the instrument reaches precision levels of 0.1 ‰ for all isotope ratios.

As reference gases for clumped N_2O isotopes are not commercially available, we are currently elaborating strategies for a reference frame linking clumped N_2O measurements to stochastic distribution.[3] Equilibration of the N–O bond has been achieved by heating N_2O over activated Al_2O_3 at different temperatures (100 – 200°C). We demonstrate that QCLAS technique using this reference frame is a very promising alternative to currently emerging high-resolution mass spectrometric approaches[4] regarding ease-of-use, field deployability, sample throughput, precision, and its inherent selectivity for the clumped isotopomers $^{15}N^{14}N^{18}O$ and $^{14}N^{15}N^{18}O$. In summary, this novel technique can offer a broad range of prospective applications from the biogeochemical N_2O cycle to stratospheric chemistry or industrial catalytic processes.

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Efficiently Automated UV/VIS Spectroscopy

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Efficient automation of analytical techniques, such as optical spectroscopy, is an essential aspect in optimizing *time to result*. It requires not only a fast analytical technique but also automation of time-consuming, laborious and error-prone analytical workflow steps such as sample/standard preparation. Array-based spectrophotometers scan complete spectra within seconds, enabling fast and reliable measurements, including simultaneous identification of impurities. A cuvette changer takes a first step towards efficient automation of samples series and reference standards, yet steps, such as dissolution/dilution and the transfer of sample into cuvettes, are nevertheless carried out manually. Efficiency can be increased further with an analytical system that automates the preparative steps and enables accurate, direct, cuvette-avoiding optical measurements. In this presentation, new automation solutions in analytical spectroscopy are discussed and assessed with respect to efficient and reliable result generation.

Isolation and characterization of a spider venom protease responsible for maturing of neurotoxic peptide precursors

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With over 47’000 different species known today, spiders belong to the most diverse orders of organisms. Most spiders possess a highly potent venom, which is a rich source of neurotoxic and cytolytic acting peptides. These peptides are expressed as precursors, which are post-translationally cleaved by proteases to yield the mature peptides as present in the venom. The cleavage occurs at a specific processing motif also known as processing quadruplet motif (PQM). We have recently published the first report about the protease responsible for this specific cleavage. The protease was isolated from the venom of the spider *Cupiennius salei*in a three dimensional chromatographic approach. Using mass spectrometric analysis, we have investigated its amino acid sequence and the disulfide bridge pattern. The enzyme was found to be a heterodimeric chymotrypsin-like protease with optimum activity at venom’s pH of 6.0. Specific PQM cleavage was shown by LC-MS analysis of digested synthetic substrates. Finally, the high biological relevance of the isolated enzyme is demonstrated by identification of homolog venom gland transcripts in many other spider species.

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Combining Cryogenic Ion Mobility Spectrometry and Cryogenic Vibrational Spectroscopy for Use in Analytical Workflows

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The success of mass spectrometry (MS) in analytical applications is owed to its ability to accurately determine the mass-to-charge (*m/z*) ratio of even low-abundant compounds in complex mixtures. However, when it comes to the identification and characterization of isomers, MS often needs to be combined with further separation techniques such as liquid chromatography or ion mobility spectrometry (IMS). Isomers of carbohydrates pose a particular challenge to today's standard workflows and commercial instrumentation, since their complex stereochemistry and branched structures can lead to unresolved features and therefore ambiguous results. We recently reported a new approach for carbohydrate analysis [1] based on conventional MS and IMS analysis as a first step, complemented by cryogenic infrared spectroscopy. The resulting IR spectrum represents a unique fingerprint for a given carbohydrate isomer and can subsequently be screened through a database for unambiguous identification of the compounds. However, this spectroscopic investigation presents a bottleneck for high-throughput applications. Increasing the resolving power of the fast ion-mobility separation step can therefore potentially reduce the number of necessary IR laser scans and thereby reduce analysis time.

We have used recent developments in IMS technology that employ electrodes miniaturized on printed circuit boards (PCB). With these so-called Structures for Lossless Ion Manipulation (SLIM), drift paths can include corners and turns, which enables compact IMS devices with extremely long drift paths and therefore high IM resolution [2]. Furthermore, these types of IM electrodes can easily be cooled, which should increase the IMS resolution and potentially "freeze out" and separate different conformers of the same molecule that would otherwise interconvert on the timescale of the experiment.

Our new experimental setup consists of a commercial ESI ion source paired with a cryogenically cooled SLIM IMS device, which can reach temperatures of as low as 29 K. This instrument is coupled to a tandem MS section including a cryogenic ion trap. [3] This allows rare gas tagging spectroscopic investigation of *m/z*and ion-mobility selected species. Details of the instrument and first results of the cryogenic SLIM IMS device will be presented.

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Innovative approaches for the quantitative analysis of laser-induced craters

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Quantitative and spatially resolved chemical analysis of a solid sample by means of laser ablation techniques (e.g. LA-ICP-MS, LIBS, and LIMS) requires an accurate characterisation of the laser-induced cavities. In the course of the profiling experiment, it is of high importance to consider the change of ablation conditions, which affects the local ablation rate and therefore the assignment of a particular measured chemical information to a specific location inside the sample. Recently, we developed new and simple sample preparation approaches, which will be discussed in detail in our contribution, for the fast and complete characterisation of multiple laser-induced craters with variable ablated volumes of typically large depth-to-diameter ratios. In these studies, a fs- laser system ($\tau \sim 190$ fs, $\lambda = 775$ nm, laser spot diameter $Ø \sim 15$ µm) was used for the formation of 2D crater-arrays consisting of craters with various shapes and sizes.

The first approach is based on the combination of standard lithographic and deep reactive-ion etching (DRIE) techniques that promote an anisotropic cross-sectioning of the laser induced-craters. The second approach consists of a non-destructive casting procedure that applies a polydimetylsiloxane (PDMS) mould to the craters resulting in a 3D replica of their interior. The third method involves an anisotropic pre-etching step of the surrounding material and a subsequent high-resolution focused-ion beam (FIB) milling step for the precise cross-sectioning of the craters. Alternating slicing and imaging steps of the entire crater provide a tomographic representation of the induced cavity, which allows to study in detail the laser-matter interaction.

Last but not least, important laser ablation effects, including e.g. *i*) the re-deposition of ablated material at the inner side-walls of the craters, and *ii*) incubation effects affecting the bulk material around the cavity, could be identified and characterised and will be further discussed in this contribution.

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Characterization of a Dielectric Barrier Discharge Ionization Source for Mass Spectrometry

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In the last decade, ambient ionization coupled to mass spectrometry generated enormous attention due to the fast and sensitive analysis with nearly no sample preparation. However, the development of new sources dominated over the understanding of the ionization mechanism of already existing methods. In this work, the active capillary plasma ionization source was characterized to get a better understanding of the behavior of the reactive species. The active capillary plasma ionization source contains a stainless steel capillary (ground electrode) which is separated by a glass capillary from a copper ring (sine-modulated high voltage). Nitrogen or helium was used as discharge gas. The plasma source was directly connected to the mass spectrometer. All optical measurements were performed in direction of the cross section of the plasma source using either a UV-VIS spectrophotometer in the range of 200-850 nm or a iCCD camera.

The active capillary plasma ionization source based on nitrogen dielectric barrier discharge was characterized by optical emission spectroscopy when applying different plasma parameters. The two most abundant signals were the transitions of NO (g system) and N_2 (2^{nd} positive system) and in low abundance the transition of N_2^+ (1^{st} negative system) and O_2^+ (Schuhmann band) was obtained. It is known that N_2^+ is the reactive species for producing $(H_2O)H_3O^+$ or protonated solvent, which are mostly responsible for the protonation of the analyte (MH^+). However, N_2^+ was less abundant in the nitrogen plasma compared to a helium plasma, which is due to a different ionization pathway. The nitrogen plasma ignites as irregular filaments between the inner electrode and the glass, which was confirmed by using an iCCD camera. Analytes can therefore travel through the plasma-free regions inside the capillary without fragmentation. The properties of the filaments were studied by monitoring the transitions of NO (g system) and N_2 (2^{nd} positive system) when applying different plasma parameters (voltage, frequency, humidity, etc.). Characterization of the plasma not only helps to improve the understanding of the ionization mechanism but also allows to optimize the performance of the plasma source when coupled with the mass spectrometer.

Anion formation in MALDI depends on matrix and target plate material choice

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In matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, the initial charge separation and subsequent charge transfer between the species in the plume remain elusive. To improve analyte anion signal intensity, to enhance sensitivity, and to gain more insight in the role of the target plate material and the matrix in MALDI anion formation, a systematic study was carried out.

The influence of different metals (Au, Ag, Al, Cr, Cu, Fe, Ga, Mo, Ni, Pb, Sn, Ta, W, Zn) and alloys (beryllium copper, brass, phosphor bronze, stainless steel 1.4301, $Ti_{90}Al_6V_4$, inconel 625, invar) as target plate materials, and a range of matrices (fullerene-C_{60}, 9-aminoacridine, 9-nitroanthracene, 9-cyanoanthracene, anthraquinone and the novel matrix benzo[1,2-b:4,5-b']dithiophene-4,8-dione) on anion yield, in the absence and presence of diphenyl phosphate (DPP) as a model analyte, was studied. The molecular anion signal intensities of the ablated species were systematically analyzed and compared by varying the laser fluence (0-3.53 J cm^{-2}) on the 21 different materials with varying melting points, thermal conductivity, electrical resistivity and work function. A comparison of DPP molecular anion signal intensity was made, based on the matrix's molar absorption coefficient (λ: 337.1 nm), its electron affinity and the crystallization upon sample deposition. An attempt was made to take as many factors, considered to be of importance for MALDI, into account to explain the observed anion signal intensity differences.

Metals with oxide layers that, upon surface ablation, show an intrinsically high anion formation in absence of a deposited sample, provide the highest DPP anion signal intensity. Using Ag as target plate material generally increases the analyte signal intensity 10-fold relative to the conventionally used stainless steel. Metals with high melting points (such as Cr, W, Mo, Ta) tend to underperform. For most metals, the homogeneously deposited fullerene-C_{60} performed best as a matrix, though anthraquinone and 9-cyanoanthracene performed best on Ag at the highest tested laser fluence.

We conclude that when matrix and target plate material are chosen carefully, this can significantly enhance the ion signal intensity of an added analyte in MALDI. Hence, the commonly used stainless-steel target plates should be reconsidered. That the target plate material might act as a catalyst for charge-transfer reactions, causing the observed anion signal intensities, should be taken into consideration and, hence, MALDI might be used in future to predict catalysts for (photo-)chemical reactions.