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Kinetics and identification of non-covalently interacting partners in a multiplex way with SPRi-MALDI MS

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By coupling SPRi and MALDI MS investigations of non-covalently interacting biomolecules in a multiplexed and high-throughput fashion with high sensitivity (low fmol range) become feasible. It provides information on binding kinetics, binding affinity in real time and identifies the interaction partners and without any need for purification or labelling.

SPR imaging measurements were performed on the SPR Plex II (Horiba, France) working with polyoxyethylene functionalized gold slides that are carrying NHS-ester groups for immobilizing ligands. Mass spectrometric detection was done with a commercial MALDI TOF mass spectrometer (Ultraflex II TOF, Bruker Daltonics, Germany). The gold slide was mounted in an adapter. MS measurements were performed in the positive ion reflectron mode with standard settings and a 532 nm pulsed laser. Each mass spectrum was the average of 2500 laser shots acquired at random sample positions. One of the problems when solutions (e.g. trypsin or matrix) are spotted by pipets is the low reproducibility due to spreading and therefore the dilution of the sample. To overcome this, we developed a protocol to spray trypsin or matrix solution. This works in a short time and preserves the multiplexing SPRi measurements.

As a proof of concept we will show an investigation of specific binding of various designed ankyrin repeat proteins (DARPins) against different proteins, like maltose-binding protein (MBP), ribosomal protein S6 kinase 2 (RPS6KA2), Kirsten rat sarcoma protein (K-Ras), mitogen-activated protein kinase 8 (MAPK8) and green fluorescent protein (GFP). Most of these proteins fulfill important functions within cellular processes. RPS6KA2 was recently identified as a potential drug target and seems to be suitable for the development of novel inhibitors for pancreatic cancer therapy. K-Ras works as a regulating molecular switch. Mutations can lead to the production of permanently activated K-Ras (GTP bound) followed by a permanent growth stimulating signal in the cell. MAPK8 is involved in processes such as proliferation, differentiation and programmed cell death. Stress stimulates the MAP kinase signaling pathway where MAPK8 is activated and in turn phosphorylates a number of transcription factors. The two other proteins are commonly applied as a protein expression tag (MBP) or fluorescent label (GFP). DARPins are a very promising class of non-immunoglobulin binders that rival antibodies for target recognition. Importantly and in contrast to antibody-derived binders, DARPins predominantly bind to structural rather than linear epitopes and thus allow recognition of their respective targets with very high specificities and sensitivities.

In this study, DARPins were immobilized, dissolved pure proteins were injected, and the K_Ds were determined based on the measured SPR data (K_Ds between 0.4 and 27.7 nM were found). When injecting a mixture of all proteins, a MALDI MS measurement on the SPRi-chip directly after the SPRi experiment analyzed peptides resulting from an on-chip tryptic digest. This verifies the specific non-covalent interactions even in the low fmol range.

Towards fluorescence-based probes of gas-phase protein structure

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Introduction:
Given the newfound prevalence of the soft electrospray ionization (ESI) method for analyzing biological molecules by mass spectrometry (MS), an important goal that has been placed at the forefront of the field has been on the development of new structural probes for studying protein structure in the gas phase [1-5]. MS analysis is ultimately done in vacuum, an environment which differs significantly from the crowded aqueous environment of a cell, or even the hydrophobic environment of a cell membrane. Thus, a necessary prerequisite to fully exploiting the very useful mass information provided by a mass spectrum is to have a solid understanding of the solvation–desolvation link. In this work, we extend efforts towards the development of an instrument which enables the use of fluorescence-based probes [5-7] for studying gas-phase protein structure.

Experimental:
A commercial 4.7 Tesla FT-ICR mass spectrometer (IonSpec Inc.) is currently being modified for fluorescence spectroscopy, using commercially available parts, and using components made in-house.

Results:
Instrument design considerations will be illustrated in depth, including those specific to robust laser beam alignment, fluorescence collection alignment, and maximization of measurement duty cycle. Gas-phase fluorescence experiments, including those on the bright class of rhodamine dyes, and their noncovalent complexes with host molecules, will be shown. For these model systems, we will show that gas-phase fluorescence measurements can be used to delineate the modulating photophysical effects of solvation. Current efforts are focused on improving the collection efficiency of the set-up, using state-of-the-art sensitive detectors, and an improved fluorescence collection alignment procedure. Time permitting, novel measurements of fluorescent molecules non-covalently bound to model proteins will be demonstrated. By comparison with measurements done in solution, gas-phase fluorescence measurements will aid in assessing the integrity of the dye’s binding site in the gas phase, thus shedding light on the structure of the noncovalent biomolecular complex in the gas phase.

[1] Breuker, K.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, 105 (47), 18145.
[2] Jurneczko, E.; Barran, P. E. *Analyst* **2011**, 136 (1), 20.
[3] Hall, Z.; Robinson, C. V. *J. Am. Soc. Mass Spectrom.* **2012**, 23 (7), 1161.
[4] Meyer, T. et al. *WIREs Comput. Mol. Sci.* **2013**, 3 (4), 408.
[5] Czar, M. F.; Jockusch, R. A. *Curr. Opin. Struct. Biol.* **2015**, 34, 123.
[6] Frankevich, V. et al. *Chemphyschem* **2013**, 14, 929.
[7] Czar, M. F. et al. *Anal. Chem.* **2015**, 87, 7559.

Bio-Oil Upgrading by Esterification; The Case of Guaiacol and Octanoic Acid over Heterogeneous Sulfonic Acid

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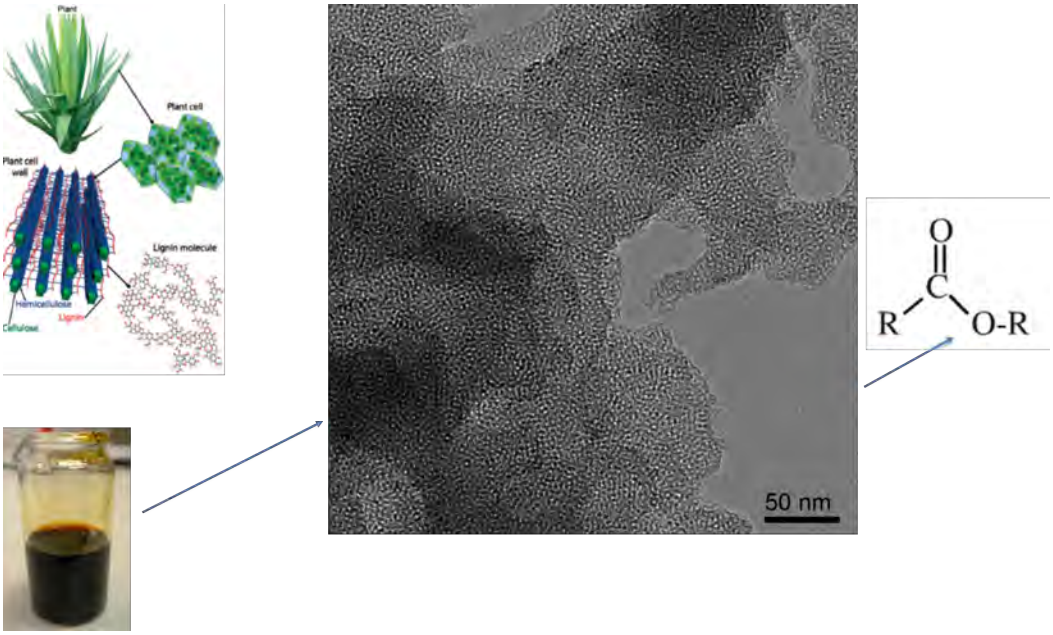
Esterification is an important reaction in the chemical industry. Biodiesel is produced by esterification of fatty acids with methanol using a solid catalyst and high temperature[1]. Lignin is a promising, renewable, and oxygen-rich resource for phenol and phenol-derivative compounds. Pyrolysis of lignin yields bio-oil and contains the monomers and dimers of the parent lignin. However, the properties of bio-oil are disadvantageous and contain too many compounds to afford separation[2]. Our idea is to chemically catch the oxygen-rich lignin monomers and react them to produce new chemicals. This can be done by esterification and acylation with another biomass resource, a long chain fatty acid.



MCM-41 functionalized with propyl-sulfonic acid moieties are efficient catalysts for guaiacol esterification with octanoic acid. The small pore MCM-41, MCM-41-$PrSO_3H$ (1.9) was found to be the best catalyst for the esterification reaction examined in this study and hence emerges as a potential catalyst which can be tested for upgrading of lignin pyrolysis bio-oils. Low sulfur leaching in the spent catalyst was a hallmark of the stability of the heterogeneous catalyst. The activity of the catalyst was about 3 times lower than that of sulfuric acid in terms of ester produced per octanoic acid. The octanoic acid conversion was similar with sulfuric acid and MCM-41-$PrSO_3H$ (1.9) catalyst.

Successfully synthesis of MCM-$PrSO_3H$ without destroying the structure can be used for bio-oil upgrading. Low sulfur leaching, high activity and easy separation makes its use unavoidable.

[1] Shih-Yuan C *et al*, Applied Catalysis B: Environmental **2007**, 148-149, 344-366.

Titanocene and Nucleic Acids: Analysis of a Fruitful Liaison

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Antitumor titanocene dichloride (Cp_2TiCl_2, Cp = cyclopentadienyl) and its derivatives are considered very promising in chemotherapy, attributable to their high activity in cancer cell studies and their low toxicity against healthy tissue. Though the precise mechanism of action has not been elucidated yet, the accumulation of the transition metal in the nucleus points towards DNA as one of the primary targets. Different analytical techniques and computational studies evidenced the interaction of metallocenes with oligonucleotides, but the exact ligand composition of the formed adducts and the underlying binding specificities remain unknown. Mass spectrometry constitutes an ideal tool to illuminate the ligand stoichiometries of the formed adduct. The ability to select and collisionally activate a certain metallocene-oligonucleotide complex further allows the specific localization of preferred binding sites.

In this study, positive nanoESI-MS/MS experiments were conducted on titanocene-nucleic acid adducts. Binding preferences were examined by competition experiments on DNA and RNA hexamers. Detailed elucidation of the binding pattern was performed by tandem mass spectrometric approaches comprising collisional activation and electron transfer. Furthermore, the influence of the transition metal coordination center on the gas-phase dissociation of the olignucleotides is discussed.

Identification of metabolite families in exhaled breath using secondary electrospray ionization MS and UHPLC-MS/MS

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Exhaled breath is a complex mixture of endogenous and exogenous compounds that reflect key information about the human metabolism. Therefore, knowledge of the chemical identity of metabolites in breath is crucial to establish a link between biochemical pathways and exhaled metabolites.
Secondary electrospray ionization coupled to high-resolution mass spectrometry (SESI-HRMS) is a rapid method, which enables the analysis of exhaled breath with excellent sensitivity. Clusters of correlating signals provide information on families of compounds that share a common metabolic origin. In this work, we used SESI-HRMS for the on-line analysis of exhaled breath of 171 healthy participants. Based on the data obtained, we performed correlation analysis to find respective families of exhaled metabolites.
However, unambiguous compound identification is difficult due to interferences with isobaric compounds and the occurrence of isomers. Thus, ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) of exhaled breath condensate (EBC) was utilized as complementary method to determine retention times and fragments for the confirmation of putative structures.

Real-time measurements were carried out using a home-built SESI source coupled to a high-resolution time-of-flight mass spectrometer recording from 50-450 Da in positive and negative ion mode. 171 healthy participants were asked to exhale into the ion source through a disposable mouthpiece. Correlation analysis was then carried out to find groups of correlating signals. Different online databases (HMDB, KEGG) were utilized for the determination of possible metabolite candidates. EBCs of 13 healthy participants (6 male, 7 female) were pooled and pre-concentrated by lyophylization. UHPLC-MS/MS experiments were then used to obtain retention times and fragments for the previously identified correlating families. Possible structures were then confirmed by comparison with a standard where available.

The correlation analysis revealed several strongly connected metabolite families, suggesting either transformation cascades through common biochemical pathways or homologous series. Compound identification is currently ongoing. Additionally, we were able to identify several structures of recently reported possible biomarkers of pulmonary fibrosis, offering an insight into the pathophysiology of the disease.

Effects of ^{1}H-^{1}H homonuclear couplings in ^{1}H-^{13}C HMBC spectra

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Heteronuclear long-range correlation experiments are crucial experiments to connect structural fragments via nonprotonated carbons or across heteroatoms, and, currently, there are a plethora of proton-detected methods available for long-range heteronuclear shift correlation [1].
The oldest and still, quite likely, most widely used long-range heteronuclear shift correlation experiment is the HMBC experiment described in 1986 by Bax and Summers [2]. Among the important issues associated with the HMBC experiment [3-4], it is commonly admitted that the $\Sigma\cos(\pi^{n}J_{HH}\Delta)$ term, originating from the homonuclear proton proton couplings can cause accidental cancellation of cross-peaks. However, as will be shown in this contribution, this assumption appears incorrect, and cross peaks in HMBC *only* vanish when the long-range coupling evolution delay, Δ, matches the long-range heteronuclear coupling constant, $\Delta = 0.5/^{n}J_{CH}$. As such, it appears that HMBC-based experiments are more robust than HSQC-based experiments optimized for long-range couplings (LR-HSQC or HSQMBC), because the possibility that long-range cross peaks are missing due to a particular combination long-range coupling evolution delay-long-range heteronuclear coupling constant-homonuclear coupling constant is much lower in HMBC-based experiments.

[1] G. E. Martin, C. E. Hadden, *J. Nat. Prod*. **2000**, 63, 543-585.
[2] A. Bax, M. F. Summers, *J. Am. Chem. Soc.* **1986**, 108, 2093-2094.
[3] J. Furrer, *Concepts Magn. Reson., Part A* **2012**, 40A, 101-127.
[4] J. Furrer, *Concepts Magn. Reson., Part A* **2012**, 40A, 146-169.

Capillary gap sampler: low-volume and fast sampling platform directly coupled to ESI-MS

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Among analytical tools for the early drug discovery process, direct hyphenation of miniaturized sampling devices to electrospray ionization mass spectrometry (ESI-MS) has attracted attention during the last decade. Reasons are that ESI-MS is compatible with microfluidics, and allows comprehensive sample analysis yielding information that is orthogonal to that available from optical methods, which are usually applied.[1] We present a “capillary gap sampler” as a platform for directly connecting microfluidics to ESI-MS. The sampler is robust, light and compact, and allows precise liquid handling and extraction of very low sample amounts in the range of 10 nanoliters.[2]

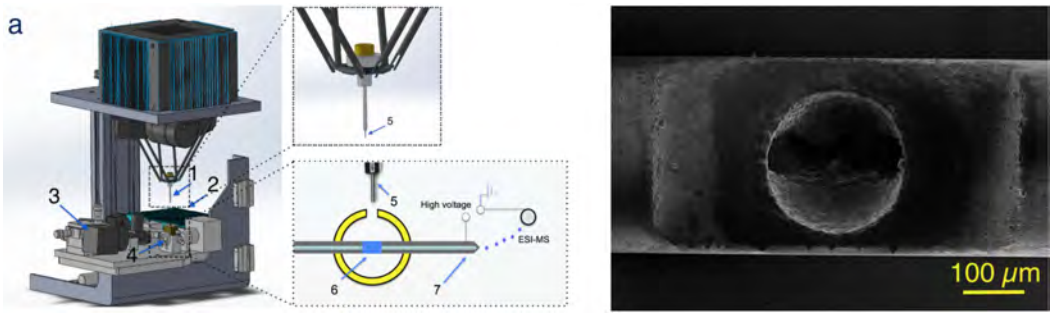

Figure left: Components of the capillary gap sampler: a) overall design of the capillary gap sampler which consists of different components including, 1) extraction tool and pin sleeve, holds by delta robot arms, 2) micro well plate, 3) camera for online monitoring of the gap, 4) pressure chamber, 5) solid stainless steel pin (229 µm diameter), 6) liquid bridge for sample desorption, 7) stainless steel capillary with a tapered tip which sprays the desorption solution to the orifice of MS by applying high voltage to the spray.

Important requirements for the sampler design come from an increasing interest in both site-specific sample pickup (e.g., for imaging applications) and for improved productivity in resource-saving screening procedures. The basic idea consists of creating an“ open” system for sample infusion, by forming a liquid bridge of several nanoliters within a micrometer-sized gap between two capillaries, which minimizes the system dead volume as well as sample adsorption/sticking on surfaces. One of the capillaries acts directly as the ESI-MS spray needle. This design allows for the system to be constantly ready for sample infusion. A solid pin is used for sample uptake and delivery, such that neither valves nor additional lines for sample introduction are needed. Other components include onboard micro robotics, optics, and a sophisticated pressure and liquid flow regulation system that enables robust sample infusion without destabilizing the liquid bridge in the gap. The weight of the entire device hanging in front of the mass spectrometer is less than 5 kg.

The system shows good performance characteristics such as symmetric peak shapes, low sample carryover (below 1%), and total injection cycle times of less than 15 s. This device thus has the potential for rapid analysis of biomedical and pharmaceutical samples with limited sample amounts in a high-throughput mode. Characterization of the liquid bridge as a new microfluidic element showed a miniaturization-friendly behavior based on self-stabilization, which opens the door for further reduction of injection volumes, gap dimensions, and capillary lengths, in order to further minimize the internal system volumes and sample dilution. Performance tests of the sampler revealed promising figures of merit in terms of sensitivity, response linearity, and robustness for multiple sample analysis.[2]

We present a monolithic design so called “semi open capillary” to address challenges for keeping the liquid bridge stable. The geometry of the semi open capillary is optimized in order to achieve minimum sample dilution, thus higher sensitivity. The scanning electron microscopy image of the optimum geometry is presented in the next figure. The liquid bridge stability is increased through coating the outer and inner surface of the capillaries. The relative standard deviation for twelve injections is less than 20 %, confirming the good repeatability of the system performance. Fewer

limitations in terms of chamber operating pressure and flow rate are the main advantages of the new design. Being quicker to operate and less sensitive to the fluctuations in flow rate and chamber pressure makes the system more robust, thus more useful for wider range of applications.
Finally an active feedback control of the system based on automated image acquisition and analysis was implemented, in order to allow for automated compensation of disturbing effects during operation to keep the liquid bridge stable.

[1] Koster, S.; Verpoorte, E. Lab Chip **2007**, 7, 1394-1412;
[2] Neu, V.; Steiner, R.; Müller, S., Fattinger, C.; Zenobi, R. Anal. Chem. **2013**, 85, 4628−4635.

Ablation Study for Depth Profiling of a Structured Multiphase System

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The striving for increased performance and miniaturization of logic and memory devices demands from the semiconductor industry the redesign of integrated circuit architectures towards three-dimensional (3-D) stacked wafer/chip systems. In this context, the so-called through-silicon via (TSV) technology provides a promising integrating concept, which allows for the further increase of the packing density of the circuit with lower power consumption.[1] However, these through wafer interconnects show high aspect ratios (e.g. channels of Ø = 5 µm and depth = 60 µm), which challenges the electrochemical filling process of Cu. One important drawback that the semiconductor industry is facing with this technology is the formation of defects within the interconnect material. The source of these defects is so far not yet understood and believed to be caused by impurity incorporation during the electrochemical deposition of Cu. To verify this hypotheses chemical composition analysis of the embedded material is required, which is highly demanding due to the geometrical structure of the feature and the rather distinct physical properties of the two main materials present in such structures.

The chemical composition was investigated by means of a top-down laser depth-profiling methodology on using a miniature reflectron-type time-of-flight mass spectrometer (LIMS technique) that is coupled with a fs- laser system ($\tau \sim 190$ fs, $\lambda = 775$ nm, laser spot diameter Ø ~ 15 µm) used for clean ablation and ionization of the analyte.[2-4] An empirical ablation study involving the laser irradiance and the number of applied single laser shots was conducted on the major phases, Si and Cu, providing information on the distinct ablation behavior of these two materials with remarkably different physical properties (semiconductor vs. metal). Compared to layered samples, the distinct materials form a complex 3-D multiphase structure that has to be eroded uniformly and measured simultaneously to preserve the quality of the chemical depth-profile. An alternative approach, in which the chemical composition was analyzed over the TSV cross-sections, is presented as well.

[1] Jeffrey P. Gambino et al., *Microelectronic Engineering*, **2015**, 135, 73-106.
[2] Andreas Riedo et al., *J. Anal. At. Spectrom.*, **2013**, 28, 1256-1269.
[3] Andreas Riedo et al., *J. Anal. At. Spectrom.*, **2015**, 30, 2371-2374.
[4] Valentine Grimaudo et al., *Anal. Chem.*, **2015**, 87, 2037-2041.

Instrumentation and methodology for comprehensive and quantitative inorganic nanoparticle measurements in real systems

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As more anthropogenic nanoparticles are included in consumer products and manufacturing processes, the transport of these particles into non-target biological and environmental systems is unavoidable. In the past decade, numerous researchers have sought to understand the fate and transport of nanoparticles within organisms and in other complex systems, such as wastewater processing, surface water, and soils. Clearly, understanding the speciation and presence of nanoparticles is vital to assess routes of exposure and potential human- and eco-toxicity of these particles. However, analytical measurements of the concentration and speciation of nanomaterials in real systems are still required to verify the relevance of many lab-scale nanoparticle exposure studies and of computer models of nanomaterial fate and transport. Importantly, the assessment of nanomaterials in real systems is lagging because most currently available analytical methods for nanoparticle detection have insufficient specificity and sensitivity.

Anthropogenic nanomaterials in biological or environmental systems are difficult to detect because they are small (hundreds to millions of atoms), dilute in terms of total mass concentration, and are present in complex matrices that may even contain native (naturally occurring) nanoparticles. Here, I will discuss an emerging method called single-particle inductively coupled plasma time-of-flight mass spectrometry (sp-ICP-TOFMS), which offers potential to provide high-throughput and sensitive determination of inorganic nanoparticles in real systems. In sp-ICP-TOFMS, a dilute nanoparticle-containing solution is introduced into a plasma that is hot enough to fully break down the nanoparticles into their component atoms and ionize those atoms. When measured by TOF mass spectrometry, the ion cloud from each nanoparticle produces a brief and intense transient signal at the mass channels specific to the elements present in the particle. The structure and frequency of these “single-particle” [1] events carries information about the nanoparticles in a sample, including the elemental and isotopic composition of particles, particle-number concentrations, and particle-mass distributions. Importantly, sp-ICP-TOFMS presents a direct route to measure very low (i.e. environmentally relevant) concentrations of diverse inorganic nanoparticles because the measured analytical signal does not depend on bulk concentration, but just the mass of analyte atoms present in each particle.

Here, I will present an overview of nanoparticle detection by sp-ICP-TOFMS, highlighting both the advantages and limitations of the instrumentation and current methodologies. I will emphasize our recent developments in sample introduction and calibration strategies to facilitate the high-throughput simultaneous quantification of diverse, multi-elemental nanoparticles from environmental samples. Additionally, I will explain how elemental “fingerprints” can be used to distinguish anthropogenic particles against a natural nanoparticle background.

[1] Degueldre, C.; Favarger, P. Y., *Colloids and Surfaces A,* **2003**, 217, 137-142.

Electronic and Optical Characterization of the Active Capillary Plasma Ionization Source

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Ambient ionization coupled with mass spectrometry has generated significant interest because it opens new ways for direct, fast and sensitive mass spectrometric detection of organic molecules. However, there are also limitations: the ionization takes place in an open environment, it is difficult to separate sample desorption from the ionization stel, and ion transport efficiency to the mass spectrometer is limited. Additionally, there are only few fundamental studies on plasma-based ionization. In our lab, we developed an active capillary plasma ionization source, which is directly connected to the MS. It provides an enclosed ionization volume, precisely controllable gas-phase conditions and a 100% transport efficiency of the ions to the MS.

In this work, this active capillary plasma ionization source, which is based on a dielectric barrier discharge, was characterized using optical emission spectroscopy and electrical current measurements of the discharge events. The source consists of a stainless steel capillary inserted into a glass capillary, working as one electrode, and a copper ring surrounding the glass capillary functioning as the counter-electrode. A cold plasma is generated by applying a sine or square modulated high voltage to the electrode, in nitrogen or air as discharge gas. All optical measurements were performed using UV-VIS spectrometry in the range of 200-850 nm facing the plasma source from the front.

We studied the influence of the applied wave form on the discharge. Square and sine waves were compared by measuring the current in the plasma. The sine wave produced more uniformely distrbuted discharge events than the square wave. However, the charge (integration of the current) over one period was the same for both wave forms, therefore the square-wave generated a higher charge carrier density. Additionally, the poperties of the filaments were studied by monitoring transitions of NO ($A^2\sum$ --> $^2\pi$) and N_2 (2^{nd} positive system and Meinel band) using optical emission spectroscopy. The intensity of the N_2 transition ($C^3\pi_u$ --> $X^1\sum_g$) could be modified by increasing the applied voltage, since the excited N_2 state $C^3\pi_u$ is mainly formed through a reaction of ground-state N_2 ($X^1\sum_g$) with electrons.

In conclusion, 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.

Extension of the linear dynamic range for nanoparticle sizing using single-particle ICPMS by matrix addition

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Single-particle inductively coupled plasma mass spectrometry (spICPMS) is currently the method of choice for analyzing metal nanoparticles by ICPMS, as it allows for particle sizing and determination of the particle number concentration. In spICPMS, ICPMS signals are recorded at high time resolution, such that individual particles ionized in the plasma produce a detectable burst of ICPMS signal; the frequency and intensities of single-particle signals are proportional to, respectively, the number and mass (i.e. size) of nanoparticles in a sample. Even though 6 to 12 orders of magnitude linear dynamic range are routinely achieved for ICPMS analysis of dissolved metals using conventional continuous liquid introduction, this linear calibration range is not currently accessible for the analysis of particulate samples directly introduced into the plasma. Efficient vaporization, ionization and atomization are key for a wide linear calibration range. However, because discrete particles vaporize at different positions along the plasma depending on their size, both the ionization efficiency of particles and the ion-collection efficiency of the mass analyzer are affected by particle size, which limits the linear calibration range. Large particles require long residence time in the ICP for complete vaporization and ionization; however, long residence time increases the diffusional losses of ions generated early in the plasma. For the analysis of small nanoparticles (< 20nm), highest sensitivity is obtained with the ICP pushed closed to the mass analyzer sampling orifice to avoid diffusional losses. However, when the system is optimized for analysis of small particles, larger particles may not spend enough time in the plasma to get efficiently ionized. For such an optimization, the calibration curve is non-linear, and potentially even double-valued, for high-mass particles due to incomplete vaporization and only possesses one order of magnitude linear dynamic range. Ideally, one would wish to achieve efficient ionization for all particles at one common sampling position along the ICP (i.e. one residence time in the ICP) while minimizing non-linear calibration of low-mass particles caused by diffusional losses. Here, we explore extending the dynamic range of NP calibration by adding excess matrix to all solutions: excess matrix salt engulfs each particle introduced into the ICP so that the mass of individual analyte particles —regardless of nanoparticle size— is insignificant compared to the total mass of the composite particulates. The addition of the matrix salt shifts the size-dependent optimal sampling position for analyte nanoparticles to a common position and extends the linearity of the single-particle calibration. A proof-of-principle study was conducted using microdroplets, which were used as proxy for other mass-limited discrete samples, in combination with an ICP-Time-of-Flight Mass Spectrometer. In this study, different gold concentrations in microdroplets represent variably sized nanoparticles and, without matrix addition, produce gold-concentration dependent optimum ICP sampling positions. When a matrix of highly concentrated lithium solution (500 mg/g) was added to the gold solutions, a shift in optimal ICP sampling position was observed and conserved for all gold concentrations. Thereby one single sampling position allowed for an increase of the linear calibration of the mass of Au in microdroplets from one to three orders of magnitude. The end goal is to extend the linear calibration range for discrete samples over 4 orders of magnitude to enable the measurement of different sizes of nanoparticles ranging from 20 nm up to 250 nm at one single sampling position.

Investigating the Polarity Range of Dielectric Barrier Discharge Ionization: Detection of Organic Microcontaminants in Water

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Ambient mass spectrometry enables a fast and direct analysis, requiring minimal or no sample preparation; these attributes have established it as the preferred technique for applications in numerous fields (e.g., biomedical, forensic, and environmental). A very efficient ambient ionization method that has been developed and optimized in our research group involves an active capillary plasma ionization source based on a dielectric barrier discharge (DBD) [1]. By directly coupling this source to solid-phase microextraction (SPME), a simultaneous clean-up and enrichment of compounds of interest can be achieved, further simplifying and accelerating the analysis. The versatility and sensitivity of our source and method were previously demonstrated, for example for the sub-ng/L detection of pesticides [2]. Nonetheless, poorer ionization efficiencies for low-polarity compounds (such as polycyclic aromatic hydrocarbons, PAHs) precluded their detection at comparably low levels. The goal of this study was, thus, to explore ionization efficiencies across a wide polarity range, under varying plasma conditions. Results show that the presence of different solvents (e.g., acetonitrile, chlorobenzene, acetone) greatly affects ionization efficiencies and mechanism. For example, depending on the presence of solvent vapors, the preferential formation of radical versus protonated cations from PAHs was observed. This behavior will be further investigated and exploited towards the potential use of solvent as “dopants” in order to increase ionization efficiencies.

Additionally, the findings obtained will aid in further developing a detection method of organic microcontaminants in different water matrices (such as tap, ground, and treated wastewater) via the direct coupling of SPME to the previously mentioned DBDI source. Preliminary results show that a high-throughput analysis, with detection limits in the low ng/L range for specific compounds (e.g., N,N-dimethyl toluamide), good repeatability, and excellent linearity on ≥ 3 orders of magnitude, can be achieved. These promising results show that this approach has the potential of competing with, and perhaps even replacing, more established techniques such as gas chromatography-mass spectrometry (GC-MS). An increased efficiency for a larger polarity range will further expand the range of possible applications.

[1] M. M. Nudnova, L. Zhu, R. Zenobi, *Rapid Communications in Mass Spectrometry,* **2012,** 26, 1447-1452.
[2] M. F. Mirabelli, J.-C. Wolf, and R. Zenobi, *Analytical Chemistry,* **2016,** 88, 7252-7258.

Characterization of the ABC-transporter PglK and its Complexes with Nanobodies using High-Mass Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry

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The structural investigation of membrane proteins is an important area of research due to their relevance in biochemical processes. These include the transport of ions and proteins, signaling, and cell-cell interactions. The characterization of the membrane proteins involved could lead to a better understanding of these processes, e.g., the development of more specific drugs or the prevention of drug resistance in cancer therapy (1).

Investigation of membrane proteins via mass spectrometric techniques (e.g. ESI MS) (2) often requires extensive sample preparation prior to analysis, due to high concentrations of salts and detergents in “protein-friendly” buffers. To minimize such sample preparation we apply matrix-assisted laser desorption ionization (MALDI) MS combined with a high-mass detector for accurate mass determination and characterization of membrane proteins and their complexes (3).

In this study we investigated a lipid-linked oligosaccharide flippase (PglK), known as an important biological actor for the translocation of lipid-linked oligosaccharides that serve as donors in N-linked protein glycosylation. PglK was in complex with different nanobodies, designed to stabilize its native structure, in order to obtain pure crystals for x-ray crystallography. We applied chemical cross-linking to investigate the stoichiometry of the nanobody-PglK complexes. Furthermore, we incubated ATP and ADP with the ABC-transporter to observe the effect of structural changes on nanobody-PglK interactions. Based on the different complex formation in the presence of nucleotides, we could locate the binding site of certain nanobodies. These results are in agreement with crystal structures obtained from PglK and NBs. In another experiment, we investigated different molar ratios of PglK and NBs to get insight into the binding behavior and classify the binding strength of the interacting proteins. These results could contribute to obtaining high quality crystals for x-ray experiments.

[1] Leonard GD, Fojo T, & Bates SE, *Oncologist,* **2003**, 8(5):411-424.
[2] Whitelegge JP, Gundersen CB, & Faull KF, *Protein Sci,* **1998**, 7(6):1423-1430.
[3] Chen F, *et al.*, *Analytical chemistry,* **2013,** 85(7):3483-3488.

Chemical kinetics and microfluidic approaches for the analysis of protein properties in bioprocessing

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Protein stability towards unfolding and aggregation plays a key role in the development of a range of important biotechnological products such as therapeutic proteins, vaccines, enzymes and food proteins. During storage and manufacturing, proteins can self-assemble via several possible multi-step processes, which are challenging to characterize and rationalize. Here we show the application of two emerging methods to address this challenge: a) a chemical kinetic platform to identify protein aggregation path-ways at the molecular level; b) novel microfluidic techniques that enable the measurement of sizes and viscosities of polydisperse protein samples under native conditions and on a timescale of few seconds.

We demonstrate the potential of these approaches by analyzing the aggregation mechanisms of different model proteins (IgGs, human insulin, peptides) and we discuss the implications of these methods for monitoring and designing product quality during bioprocessing.

[1] P. Arosio et al., *ACS Nano*, **2016**, 10, 333-341.
[2] P. Arosio, et al., *Analytical Chemistry*, **2016**, 3488-3493.
[3] P. Arosio, et al., *Nature Communications*, **2016**, 7:10948.

Breath Analysis Using Secondary Electrospray Ionization Mass Spectrometry - Steps Towards Absolute Gas-phase Concentrations of Metabolites

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Exhaled breath contains hundreds of volatile metabolites that provide biochemical information about the health or pathophysiological state of an individual. Therefore, chemical analysis of the molecular composition of breath is a promising tool for the non-invasive and rapid diagnosis of diseases. [1]

Previous studies have shown that respiratory diseases like chronic obstructive pulmonary disease or obstructive sleep apnea can be diagnosed by analyzing exhaled breath on-line using secondary electrospray ionization mass spectrometry (SESI-MS) [2,3]. SESI coupled to a high-resolution mass spectrometer is a highly sensitive and specific technique, which allows the detection of hundreds of metabolites within a broad mass range (up to 900 Da) [4]. In terms of sensitivity and compound coverage, it is able to overcome limitations of other techniques used in breath analysis [5]. However, one main drawback of SESI-MS is the difficulty to obtain absolute gas-phase concentrations of biomarkers in breath. To overcome this limitation, a sample delivery system was developed in this study and integrated into an existing SESI-MS setup. This allows for a stable gas-phase delivery of compounds at low mixing ratios (parts per billion range), thereby enabling quantification by external calibration or standard addition.

Our results indicate that the quantification of breath biomarkers is possible with SESI-MS. The ability of standardization and absolute quantification renders the measurements more robust and environmental influences can be eliminated. Furthermore, it allows for a better correlation between levels of metabolites found in exhaled breath and in blood.

[1] B. de Lacy Costello et al., *J. Breath Res.* **2014**, 8, 014001.
[2] P. Martínez-Lozano Sinues et al., *Respiration* **2014**, 87, 301-310.
[3] E.I. Schwarz et al., *Thorax* **2015,** 71, 110-117.
[4] M. T. Gaugg et al., *J. Breath Res.* **2016**, 10, 016010.
[5] P. Martínez-Lozano Sinues et al.,*J. Breath Res.* **2011**, 5, 016002.

Generalized Incremental Model Identification for Chemical Reaction Systems

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Identification of kinetic models and estimation of kinetic parameters in chemical reaction systems can be done using Incremental Model Identification (IMI). By using IMI, it is possible to separate the effect of the different reactions and thus investigate each reaction individually. In contrast, with simultaneous approaches, it is necessary to work with a complete model that includes a rate candidate for each reaction, which might lead to a large number of possible model combinations. Hence, IMI allows faster computation of the identified models and estimated parameters [1]. There exist essentially two main approaches for IMI: extent-based IMI and rate-based IMI. In extent-based IMI, reaction rates are integrated to yield extents, and the parameters are estimated via least squares by fitting these simulated extents to experimental extents obtained from measured concentrations [2]; in rate-based IMI, the parameters are estimated via least squares by fitting simulated rates to experimental rates obtained by differentiation of measured concentrations [3].

This contribution proposes a generalized IMI method that offers much more flexibility in the use of measurements, particularly in the way the various measurements are weighted. The parameters are estimated via weighted least squares by comparing simulated and experimental extents. The peculiarity consists in comparing extent values not only at the measurement points but for all possible time intervals between measurement points. Then, it can be shown that both the extent-based and rate-based IMI can be reformulated as particular cases of this generalized method. For example, the extent-based method would correspond to positive and equal weights for all time intervals that start at time zero, while the rate-based method would correspond to positive and equal weights for all time intervals with a length of one sampling period. This reformulation allows the investigation of new approaches by testing compromises between different methods, which can potentially result in a better IMI method.

With such a generalized method, it is also possible to test if there is an optimal weight distribution or, more generally, if there are important features in the weights to best perform model identification. The effect of the weight distribution on (i) the accuracy and precision of the parameters, and (ii) the model discrimination power can be investigated via different optimization methods, such as classic gradient-based algorithms or genetic algorithms. The different directions followed to find the best weight distribution are illustrated with simulated examples, and these results are compared to extent-based and rate-based IMI.

[1] Bhatt et al., *Chem. Eng. Sci.*, **2012**, 83, 24-38.
[2] Bhatt et al., *Ind. Eng. Chem. Res.*, **2011**, 50, 12960-12974.
[3] Brendel et al., *Chem. Eng. Sci.*, **2006**, 61, 5404-5420.

Nanospectral Imaging of a Two-dimensional Polymer Monolayer with Tip-enhanced Raman Spectroscopy

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Two-dimensional polymers (2DPs) are covalently linked monomolecular networks with periodic bonding and repeat units that show in-plan, large-range order [1]. Compared to graphene, 2DPs are expected to offer better flexibility in composition, porosity, modifiability and other physicochemical properties, which should enable applications in optoelectronic devices, separation membranes, surface catalysis, and molecular sensing [2]. Although diverse strategies have emerged to synthesize and characterize 2DPs, an in-depth understanding of the structure of 2DPs has proven challenging. To achieve this, it would be particularly useful to record chemical “fingerprints” down to an individual monolayer with sub-nanometer thickness [3].

Tip-enhanced Raman spectroscopy (TERS) integrates scan probe microscopy (SPM) for nanoscale spatial resolution with Raman spectroscopy for chemical characterization, and can simultaneously provide topography and chemical fingerprints of such samples.

Here, we synthesized a new 2DP monolayers from rigid aromatic amine and aldehyde building blocks through dynamic imine chemistry at a water/air interface by the Langmuir–Blodgett method. Taking advantage of the high sensitivity and high spatial resolution of TERS, we investigated a single 2DPs sheet by using TERS to obtain further understanding of planar network information of 2DPs, such as the used end groups, newly formed covalent bonds, molecular orientations, and nano-defect domains.

[1] W. Dai, F. Shao, J. Szczerbiński, R. McCaffrey, R. Zenobi, Y. Jin, A. D. Schlüter, W. Zhang, *Angew. Chem. Int. Ed.* **2016**, *55*, 213-217.
[2] Boott CE, Nazemi A, Manners I. *Angew. Chem. Int. Ed.* **2015**, 54(47): 13876-13894.
[3] Colson JW, Dichtel WR. *Nat. Chem.* **2013**, 5(6): 453-465.

Characterizing capabilities of a 213 nm high resolution laser ablation inductively coupled plasma mass spectrometry imaging system

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) is a method for direct solid sampling which can provide concentrations of major, minor, trace and ultra-trace elements in almost any solid sample. Of particular interest in research is high-resolution, high-speed, multi-elemental imaging by LA-ICPMS. The particular challenges in this approach related to crater sizes in the low µm-range for high spatial resolution, high data acquisition rate for high throughput, simultaneous detection of multi-elemental capabilities, and application to different sample types. [1-3]

The LA system consists of a solid state Nd:YAG-based 213 nm laser with beam homogenization and a low-dispersion ablation chamber. The optical system allows for ablation with spot sizes in a range of 1 to 5 µm in diameter with flat-bottom crater profiles. Laser ablation can be carried out with a repetition rate up to 20 Hz and pulse energy of ca. 1 mJ and ion signals from each individual laser pulse can be discretely detected due to the tube-cell arrangement, which provides a washout time < 20 ms. For compositional analysis it is coupled to an ICP-TOFMS (icpTOF, TOFWERK, Thun, CH) for simultaneous detection of elemental mass spectra. In order to reduce the plasma ion background, to avoid spectral interference and to improve spectral resolution, a reaction/collision cell with hydrogen as collision gas was used.

A characterization of the setup was carried out with the standard reference material NIST 610. Limits of detection were found to be similar to a conventional ArF eximer laser based ablation system (GeoLas C, Coherent, Göttingen, DE). Furthermore, we present imaging applications for both soft thin tissue and geological samples. In both cases, the elemental distribution is acquired at µm pixel resolution corresponding to single laser pulses. In order to address quantitative imaging of thin tissue sections, we investigated a multi-layer approach to correct for variations of material removal for each laser shot.

[1] Wang et al., *Anal Chem*., **2013**, *85*, 10107.
[2] Gundlach-Grahm et al., *Anal. Chem*., **2015**, *87*, 8250.
[3] Burger et al., *Anal Chem*., **2015**, *87*, 8259.

Understanding electrospray ionization mechanisms of biomolecules using laser-induced fluorescence

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Although Electrospray ionization (ESI) is a well-established technique for producing intact gaseous ions for mass spectrometric analysis, the mechanisms driving the formation of these gaseous ions are still illusive. A better mechanistic insight will enhance our understanding of ion formation process and will help us to increase the ion yields. Specifically, in the case of biomolecules, it will give clues about the structural evolution as the analyte passes from the droplet phase to the gas phase. Such studies have attracted attention of several groups and considerable attempts have been made from both experimental and theoretical sides. It has already been shown that laser-induced fluorescence experiments in the electrospray plume at different distances give snapshots of the electrospray process from the droplet phase to the desolvated gas phase.

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 studies simultaneously 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. Additionally, the ultra-short pulses are crucial to make sense from complex fluorescence signals.

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 is a reliable indicator of the mechanism of ESI taking place. Further, studying the fluorescence along the spray axis also provides us with time scale information, which is known to be different in different mechanisms. We have already developed a setup that simultaneously allows us to record the fluorescence spectra and lifetime from the same spot in the ESI plume. The initial optical alignment and calibration have also been completed. Now the main focus is not only to study how different classes of molecules follow different ionization mechanisms, but also to probe the influence of solvent/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 preliminary results from laser-induced fluorescence on biomolecules along the electrospray axis.

[1] Chingin, K., Frankevich, V., Balabin, Roman M., Barylyuk, K., Chen, H., Wang, R. and Zenobi, R., “Direct access to isolated biomolecules under ambient conditions.” Angew. Chem. Int. Ed., 2010, 49, 2358.

Is Asymmetrical flow field-flow fractionation more than a sizing technique?

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Flow field-flow fractionation (FlFFF) is well-suited state-of-the-art technique finding growing applications in the separation and size characterization of natural and engineered nanoparticles. The hyphenation of the FlFFF with a very sensitive elemental detection, such as inductively coupled plasma-mass spectrometry (ICP-MS) and single particle ICP-MS, opens novel avenues to explore the interactions of metal-containing forms, e.g. traces metals and engineered nanoparticles, with different abiotic and biotic components in the aquatic systems. Determining the speciation of dissolved trace metals in the complex environmental and biological systems is paramount for the assessment of their reactivity. Some of the recent advances with respect to the understanding of the trace metals and metallic nanoparticles behavior in the aquatic systems by using the asymmetrical FlFFF coupled to ICP-MS will be exposed. With the examples of our own research we will illustrate the capabilities of the AFlFFF-multidetection system (i) to explore metal association, size or molar mass distribution of metal complexes with dissolved and colloidal organic matter; (ii) to characterize engineered nanoparticles and their interactions dissolved and colloidal organic matter, (iii) to explore the interaction of manufactured nanomaterials with intracellular enzymes, (iv) to distinguish between the contaminant forms e.g. dissolved and nanoparticulate.

More than a sizing technique, and thanks to detectors available online, AFlFFF can be considered as a global and integrative tool that give important insights at the nanoscale about the behavior and bioreactivity of the various metal containing forms in complex environment.

Construction and initial characterization of an internal source matrix-assisted laser desorption/ionization Fourier-transform ion cyclotron resonance mass spectrometer

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The number of commercially available MALDI-FTICR mass spectrometers and their instrumental flexibility is limited. To attain more experimental possibilities a MALDI-FTICR-MS was constructed to enable fundamental research of the processes governing matrix-assisted laser desorption/ionization.

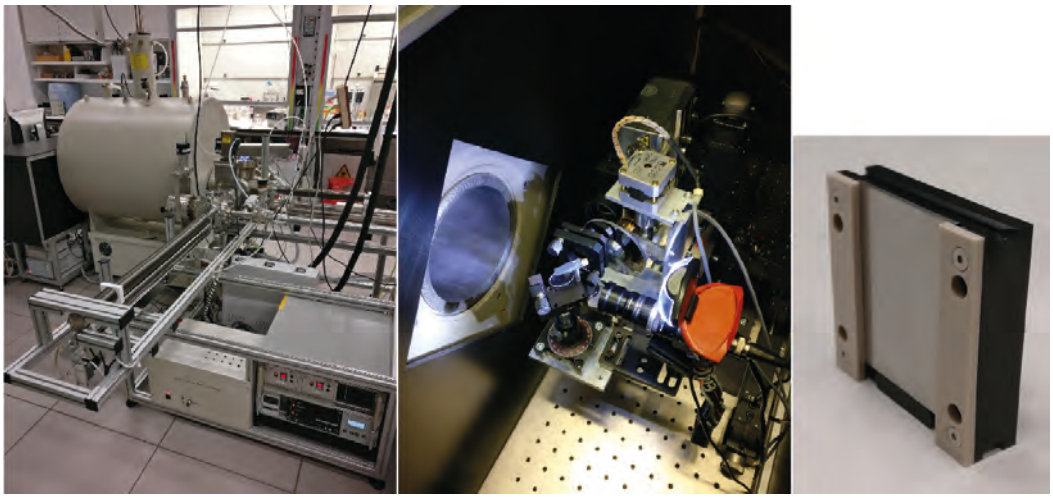

Figure 1: From left to right: ultrahigh vacuum interlock system with 4.7 T superconducting magnet; Nd:YLF laser (349 nm) setup with attenuator and camera on a 2D translation stage; target plate holder fitted with a 30.0 × 40.0 × 1.00 mm target plate (grounded with spring contacts on the back).

The system is constructed on a rail system, can be completely retracted from the magnet and allows easy modifications. Additionally, it allows for facile target introduction with an automated interlock system, simple exchange of the ICR-cell for dedicated experiments, exact positioning (with a 0.635 µm step size) of the MALDI target plate at varying distances from the cell inside the magnet, as well as a free choice of laser positioning by means of a 2D translation stage onto which the optical setup is mounted, where each laser pulse triggers the movement to next desired sampling point. The laser intensity can be controlled with an attenuator. The optical setup is controlled with LabView-based software. The target holder enables the user to study various different target plate materials, since the only requirement is the proper plate dimensions of 30.0 × 40.0 × 1.00 mm.

Currently, we are increasing the system's sensitivity by installing a new preamplifier to enable calibration over a wider *m/z* range. The instrument's design features and initial characterization will be shown. The authors would like to acknowledge the help of Christian Marro and Heinz Benz with the instrument part manufacture and the LabView programming, respectively.

Endotoxin separation and detection by SEC

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Endotoxins (ET) are an integral part of the outer membrane of gram-negative bacteria and thus may appear as a contaminant in biotechnological production streams. They are pyrogenic and can cause a septic shock with a few ng introduced into the human blood stream. Thus, products for parenteral administration must be controlled for as low as < 5 endotoxin units (EU)/dose and kg body weight.[1]

ETs form aggregates of different shapes and structures in a molecular weight (MW) range of < 10 to > 6000 kDa depending on solvent, salts, detergents, chelating agents and temperature. Furthermore, the aggregation depends on the type of ET monomer, which is highly variable with an average MW of 2 kDa for rough ET mutants and up to 42’000 kDa for smooth ETs. The biological response depends on ET aggregation and can therefore be different for the same concentration of ET in different formulations.[2]

When measuring ETs with the widely used biological Limulus Amebocyte Lysate (LAL) assay, samples are usually diluted in ET free water. This is believed to ensure equal aggregation and thus comparable ET measurements. However, the LER (Low Endotoxin Recovery) effect has recently received much attention because some formulations with spiked ET standard could not be recovered with LAL.[3]

ET aggregation in different formulations is of interest for ET testing and for potential purification strategies. *E.g.* separation of ET from a target molecule by size can be assessed. Thus, new simple and fast analytical tools for this purpose are needed. Accordingly, we analyzed ETs in common buffers such as PBS with SEC-UV/CAD, techniques available in most analytical laboratories. Polymer and silica based SEC columns in the MW range from <1000 Da to 10^6 Da were used. ET aggregation was reduced from large aggregates towards intermediate MW and monomeric forms using salts, detergents, elevated temperature, sonication and different aqueous/organic mobile phases. The effects were different for ET from different bacterial strains. Time dependent aggregation could also be observed. Apparent MW estimation is difficult due to a lack of suitable MW-standards. Thus, we aimed for the development of MS based MW determination of ETs after SEC separation. SEC eluents were adapted accordingly for SEC-qTOF analysis. Furthermore, the monomer MW of ETs was determined with MALDI-TOF-MS, using different matrices and additives.

[1] https://www.fda.gov/iceci/inspections/inspectionguides/inspectiontechnicalguides/ucm072918.htm (Accessed: 10.02.2017)
[2] D. Su, R.I. Roth, M. Yoshida, J. Levin, *Infect. Immun.*, **1997**, 65, 1258-1266.
[3] https://www.outsourcedpharma.com/doc/the-great-ler-debate-0001 (Accessed: 20.01.2017)