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Analytical Chemistry **AC 1**

Quantitative Microscopic Studies with High Spatial Resolution of Contaminant Diffusion into Opalinus clay rock

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Safe nuclear waste storage is gaining more and more significance in view of endorsing the sustainable development of modern civilizations. In terms of safety assessment, understanding reactive transport mechanism of radionuclides with respect to the natural barrier is therefore of critical importance. Opalinus clay rock, due to its low water content and low water permeability, has been considered and studied as a potential nuclear waste storage material. The samples investigated in this study originated from a unique field-scale multi tracer migration experiment conducted at the Mont Terri Underground Rock Laboratory, located in northwestern Switzerland.

Several microprobe techniques have been employed in this study to investigate the trace ^{133}Cs distribution pattern in heterogeneous media Opalinus clay rock. Synchrotron Radiation based micro X-Ray Fluorescence (SR-microXRF) provided ***semi-quantitative high spatial resolution*** (few micrometers) 2D elemental distribution patterns. A complementary Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) measurement generated ***fully quantitative moderate spatial resolution*** 2D elemental distribution patterns. The agreement of these two independent techniques not only enhanced the analytical robustness in general, but also yielded chemical images of the trace Cs distribution pattern with full quantification and high spatial resolution. Additionally, elemental correlations represent fundamental information towards an improved understanding of the relevant geochemical retention mechanisms limiting the mobility of Cs in the barrier material.

Analytical Chemistry **AC 2**

The Power of One: Microarrays for Mass Spectrometry and their application for Single-Cell Metabolomics

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Although mass spectrometry is a very successful approach for population-level metabolomic studies, little has been done for using it at the single-cell level. The need for a switch from population to single-cell level is based on recent discoveries in cellular behavior. In particular, those that suggest that specific cell metabolomic phenomena can only be properly studied if they are isolated from the averaging effects present during population-level studies (e.g. the display of multiple metabolic phenotypes within a single clonal population).

In 2008, our group made a first step toward this new direction by demonstrating that it is possible to monitor metabolites from single cells using mass spectrometry.[1] In 2010, we developed MAMS (microarrays for mass spectrometry), which provide a unique insight into the molecular machinery.[2] However, the first measurements using MAMS were focused on answering analytical questions. Thus it was unclear (at that time), if this new approach would successfully address the current interests and needs from the (systems) biology community.

Here, I will present our first results in our quest to improve and to validate MAMS technology for day-to-day use. For example, we would like to provide new insight to a well-known (but not completely understood) biological question,[3] which metabolic changes are to be expected from *S. cerevisiae* when challenged with 2-deoxy-D-glucose?.

[1] A. Amantonico, et al. *Angew. Chem.* **2008**, *120*, 5462-5465.
[2] P.L. Urban, et al. *Lab Chip* **2010**, *10*, 3206–3209.
[3] M. Ralser, et al. *P. Natl. Acad. Sci. USA* **2008**, *105*, 17807-17811.

Analytical Chemistry **AC 3**

A novel non-card based format for dried blood spots analysis for µcsLLE liquid chromatography mass spectrometric analysis

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Dried blood spots (DBS) have shown their usefulness for a large number of applications as sample collection and storage device, from their early days with newborn screening [1] to their recent use for pharmaco and toxicokinetics studies [2]. In bioanalytical applications, a portion or the DBS spot is usually punched out of the card, and analytes are then submitted to a generic solid-liquid extraction (SLE), using an organic solvent (or a mixture with water).

We present herein a novel, tube based format, which allows either sample collection on paper and analyte extraction, in a single device. This eliminates the need of an often tedious, punching step and prevents any related cross contamination. Moreover, our format in addition to SLE allows applying micro cellulose supported liquid-liquid extraction (µcsLLE) on filter paper in a simple an efficient way.

Extraction efficiencies were evaluated for both SLE and µcsLLE, using a mixture of 18 analytes, representative of 5 distinct chemical classes (amphetamines, cocaine and metabolites, tricyclic antidepressants, benzodiazepines, antiretroviral drugs). The selectivity of the extraction procedure was studied by assessing the removal of interferences and phospholipids, the latter having been demonstrated as one of the major cause of matrix effects in LC-MS/MS. Finally, the potential of our format for quantitative purpose is demonstrated for the therapeutic drug monitoring of saquinavir in blood.

[1] R. Guthrie, A. Susi, *Pediatrics.* **1963**, *32*, 338.
[2] N. Spooner, R. Lad, M. Barfield, *Anal. Chem.* **2009**, *81*, 1557.

Analytical Chemistry **AC 4**

Ion Funnel as a New Interface for ICPMS

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Despite of the popularity of ICPMS for trace and ultra trace analysis, the technique is limited by low overall ion transport efficiency. The main ion loss occurs due to the interface connecting the atmospheric pressure ICP to vacuum of the mass spectrometer. As a result, only 1 ion out of 5000 – 500000 atoms from a sample can currently be successfully detected [1].

Ion funnels (IF) are for example used in electrospray mass spectrometry to enhance ion transmission [2]. It consists of a stack of ring electrodes with decreasing inner diameter and a radiofrequency potential of alternating polarity. This RF field creates a potential barrier near the electrode walls to confine the ions at the funnel axis.

This study has investigated the characteristics of an ion funnel as an interface for ICPMS. Initially, ion transmission with the IF placed downstream of a common sampler-skimmer interface was studied. Ion confinement in the ion funnel was observed although the total ion current was lower than with an electrostatic ion lens. Placing the IF directly within the initial expansion stage of the plasma however lead to significant thermal effects at the electrodes. Additionally the electric conductivity of the plasma caused breakdown of the pseudo-potential inside the funnel. An additional expansion stage before the ion funnel again decreased current load to the funnel, but still no improvement by the applied RF field was observed. Measurements of the angular distribution of the ions at the IF exit showed that an RF field or a steep potential gradient along the funnel axis cause ion discrimination but also decrease the angular spread after exiting the IF. There was no focusing effect of RF field, indicating that the ion current at the funnel exit aperture is limited by space charge effects.

[1] S.Al Moussalami, et al., *Rev. Sci. Instrum.,* **2002**, *73*, 884.
[2] Kim, Tolmachev, *Anal. Chem.,* **2000**, *72*, 2247.

Analytical Chemistry **AC 5**

Tandem Mass Spectrometry of Platinated DNA Quadruplexes

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During the past decades, the interest in atypical nucleic acid structures has grown rapidly. Since the discovery of a coplanar arrangement of four guanine bases by *Gellert et al*. in 1962, the so-called DNA quadruplexes attracted notice to researchers. Enhanced sequencing techniques revealed a huge number of putative quadruplex-forming regions in various organisms such as yeasts, human, or ciliate protozoa. The formation of quadruplexes was found to play key roles in several biological processes, such as telomere regulation and organization, as well as chromosome replication and epigenetic gene regulation. In 2001, evidence for the *in vivo* existence of quadruplexes was found by help of a specific antibody which recognizes the folded intact quadruplex.

Herein we report data on mass spectrometric investigation of these higer order nucleic acid structures. Furthermore, the binding behavior of cis- and transplatin, and related organometallic drugs was investigated. High-resolution tandem mass spectrometry was used to elucidate the fragmentation patterns of adducted tetra-, bi-, and monomolecular quadruplexes. Data obtained provides fundamental information about the binding pattern of the drugs, thus, laying the groundwork for fast and accurate quadruplex diagnostics.

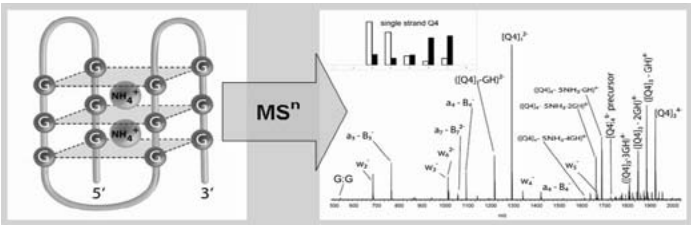

Figure 1: **Multistage mass spectrometry is used to investigate DNA quadruplexes.**

Analytical Chemistry **AC 6**

A Straightforward Approach to Study the Intrinsic Properties of Host-Guest Cyclodextrin Complexes in the Gas Phase

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Dissociation energies and structural assignments of cyclodextrin complexes were for the first time studied by experiments and theoretical calculations. Threshold collision-induced dissociation experiments were performed on a customized 24-pole Finnigan TSQ–700 tandem mass spectrometer to get accurate dissociation energy [1]. DFT calculation was carried out to further interpret interaction and structure within host-guest complexes. Hydrogen bonding interaction contributed mainly for the stability of gaseous complex, but inclusion geometry still favored according to the experimental and calculation results. The experimental dissociation energy (41.0 kcal mol^{-1}) was in agreement with DFT calculation results (38.9 kcal mol^{-1}).

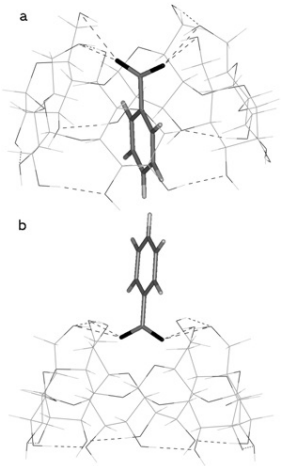

Figure 1. BLYP calculated global minimum geometries for head (a) and tail (b) orientation of [α-cyclodextrin + benzoic acid]$^-$. Dashed line presented as H-bond.

Table 1. Calculated and experimental dissociation energy (kcal mol^{-1})

	Head	Tail
[α-cyclodextrin + benzoic acid]$^-$		
M06-2X/6-31+G(d,p)//BLYP/6-31+G(d,p)	38.90	32.66
M06-2X/6-31+G(d,p)//M06-L/6-31+G(d,p)	46.94	34.79
Experimental dissociation energy	41.1 ± 0.8	

[1] Narancic, S.; Bach, A.; Chen, P. *J. Phys. Chem. A.* **2007**, *111*, 7006.

Analytical Chemistry **AC 7**

Calibration Free Reactive Dissolution Kinetic Modeling with in-situ FBRM, ATR-IR, and Calorimetry

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Modeling efficiently the kinetic of dissolving particulate systems, is a key step in several fields, such as for the controlled released of any active pharmaceutical ingredient. Since a decade the Focused Beam Reflectance Measurement (FBRM) technique became the most common method for the direct in-situ study of particulate systems. The FBRM modeling has been addressed by several authors[1]. A majority of these models aim to recover the Particle Size Distribution (PSD) from the Chord Length Distribution (CLD), i.e., the FBRM raw signal. The matrix problem formulation and its inversion has shown to be ill-posed, and several regularization techniques have been proposed. These techniques are often numerically complex and not suitable to be nested in within a calibration free kinetic analysis framework.

In this contribution, a solvent free reactive dissolution system is simultaneously monitored by in-situ ATR-IR, Calorimetry and an FBRM device. Two FBRM models, which provide quantitative information on the solid phase state are nested in within a multivariate calibration free kinetic hard modeling analyses. As validation, the solid phase kinetic modeling results are statistically compared to the well established kinetic analyses based on the calorimetry signals and the multivariate ATR-IR spectroscopy[2].

We acknowledge financial support by the SNF (grant no 200021-113473).

[1] Kail, N., et al. *Chem. Eng. Sci.*, **2009**, *69*, 984-1000.
[2] Maeder M. and Neuhold Y.-M., *Practical Data Analysis in Chemistry. Elsevier, Amsterdam* **2007**

Analytical Chemistry **AC 8**

Nanoscale Chemical Imaging of Segregated Lipid Domains using Tip-Enhanced Raman Spectroscopy

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According to the lipid raft concept, phase segregation into lipid domains is involved in processes like protein sorting and signaling in biological membranes.[1] Direct visualization of these small and highly dynamic domains in the complex environment of a cellular membrane is a very challenging task. Phase segregated giant vesicles as well as supported lipid mono- and bilayers with known chemical composition serve as a popular model to gain insight into the processes associated with the compartmentalization of biological membranes.

In this work, we present reproducible and strongly enhanced tip-enhanced Raman spectra originating from a very small number of molecules (250 on average) in a lipid monolayer on a gold surface, probed by the apex of a nanometer-sized silver tip in STM feedback. For the first time, we show large (128 x 128 pixels), high-resolution (< 50 nm) tip-enhanced Raman images of binary lipid mixtures with full spectral information at each pixel revealing the lateral distribution of the individual components in the monolayer with chemical selectivity.[2]

Future investigations using AFM feedback control and experiments in an aqueous environment would pave the way for detailed nanoscale chemical imaging on more complex membrane models or even real cell membranes.

[1] K. Simons, E. Ikonen, *Nature* **1997**, *387*, 567.
[2] L. Opilik, T. Bauer, T. Schmid, J. Stadler, R. Zenobi, *Phys. Chem. Chem. Phys.* **2011**, DOI: 10.1039/c0cp02832k

Analytical Chemistry **AC 9**

Acetaminophen toxicity: QUAL/QUAN liquid chromatography high resolution mass spectrometric approaches for drug metabolism and metabolomic investigations

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Acetaminophen (APAP) overdose has been described as the major cause fo acute liver failure in developed countries before viral hepatitis. Limited lite rature can be found describing acetaminophen metabolism of patients wit already impaired hepatic functions despite the large use of acetaminophe post-operation as pain reliever. Liquid chromatography-high resolutio mass spectrometry was applied to i) quantify changes of APAP and its me tabolites in plasma ii) profile exogenous metabolites iii) screen endogenou metabolites changes using metabolomics tools.
The quantification of APAP and two of its metabolites was performed on fast acquisition quadrupole-TOFMS (50-100 ms duty cyle, RP 30'000 compatible with UHPLC time constraints. High Resolution – Selected Reac tion Monitoring (HR-SRM) was applied to validate the assay with simila sensitivities compared to the classical triple quadrupole SRM mode. Addi tionally, identification of high resolution MS/MS spectra made possible th profiling of other metabolites of acetaminophen circulating in plasma. Fur ther metabolomics investigations were also pursued on the same instrumen using full scan approaches. In a first analysis, the principal component anal ysis outcome was dominated by the exogenous metabolites. As expected, reduced metabolism of acetaminophen in the hepatic surgery group wa observed but also an augmentation of acetaminophen-cysteine levels in th same group a day after ingestion. Exogenous metabolites were then re moved for a subsequent principal component analysis that aimed at the dis covery of potential new biomarkers with the combined power of accurat mass and MS/MS fragmentation for identification.

Analytical Chemistry **AC 10**

Investigation of strategies to introduce laser ablation aerosol into a ms-pulsed glow discharge time-of-flight mass spectrometer

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A setup combining laser ablation (LA) as a sampling technique to a ms-pulsed glow discharge time-of flight mass spectrometer (GD-TOFMS) was developed. Different LA-GD models were designed, constructed and coupled to a TOFMS. In a first model, one-cell design was used, whereas the LA takes place inside the GD cell. Ionization enhancement up to one order of magnitude compared to the LA ion signal was achieved when metallic samples were analyzed. For organic materials, elemental, fragment and molecular ions were detected in the plateau and the afterglow regime of GD.
In a second model, an "atmospheric" LA cell was coupled to the GD-TOFMS instrument by using a particle beam (PB) interface. When the LA-generated aerosol was introduced perpendicular to the anode-cathode axis, the sensitivity was two orders of magnitude lower than that obtained with the first setup. In order to enhance the sensitivity of this setup, an additional steel tube (1 mm i.d.) was coupled to the end of the PB interface and its outlet was placed in the front of the cathode surface, so that the aerosol has more time to interact with the NG ionizing species. With this setup, better temporal stability of the LA-GD signals than that of the first sample introduction setup was achieved. Similar results were obtained when the aerosol was introduced through a narrow channel made through the cathode material, but, in this case, higher intensities of elemental ions such as C^+ were observed. However, due to the material loss, particularly the small particles, inside the PB interface, the LAGD ion intensity remains lower than the first setup. To reach good reproducibility and higher sensitivity, a third model consisting of a reduced pressure LA cell directly connected to the GD chamber was built and tested and will be discussed.

Analytical Chemistry **AC 11**

Characterization of extracellular nanostructures of river water bacteria by tip-enhanced Raman spectroscopy

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Nanostructures play an important role in many biological systems. Bacteria, for example, use their pili to adhere to surfaces. Even though the pili architecture is well understood, only little is known about how the adhesion process works on the nanometer scale. Until now it was not possible to characterize these structures on such a scale with a method that also provides chemical information. Tip-enhanced Raman spectroscopy is a method that combines the advantages of two techniques: the high lateral resolution of atomic force microscopy and the chemical information that is provided by Raman spectroscopy. With this method chemical fingerprint data from spots as small as 15 nm in diameter can be collected.

The characterization of pili on the outer surface of bacteria will be presented. We used river-water bacteria for our experiments because they are easy to grow and adhere well on different substrates. Their pili are interesting samples for tip-enhanced Raman spectroscopy as they are typically only 2 nm thick, 1-1.5 nm long and are chemically heterogeneous. We grew river-water bacteria in a minimal medium and added a substrate in order to first investigate the adhesion of the bacteria to this surface. The conditions under which single bacteria on a substrate can be investigated by tip-enhanced Raman spectroscopy were explored. We plan to use tip enhanced Raman spectroscopy in the so called "gap mode" configuration to characterize individual pili as well as other extracellular nanostructures both topographically and chemically.

[1] T. Schmid et al., *SPIE* **2010**, 7586, 758603-1.
[2] T. Schmid et al., *Anal. Bioanal. Chem.* **2008**, 391, 1899.

Analytical Chemistry **AC 12**

NASCA-HMBC, a new NMR methodology for the resolution of overlapping signals. Application to Agathisflavone

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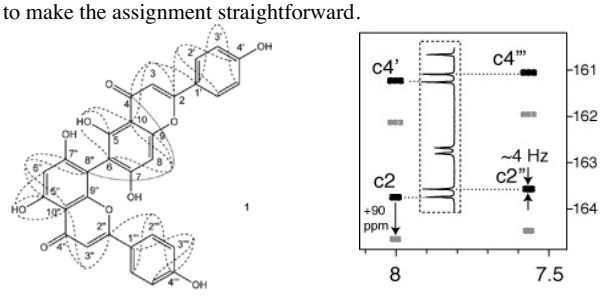
The low resolution of standard 2D HMBC spectra makes it very difficult to assign signals of close pairs of carbons. The NASCA-HMBC (Non-ambiguous Assignment by Superposition of Coupled Aliased HMBC) combines a pair of aliased HMBC spectra to provide one order of magnitude increase in the resolution. Application to agathisflavone, a biflavonoid found in *Ouratea gilgiana* resulted in spectra with a sufficiently high resolution to make the assignment straightforward.



[1] G. Bayiha Ba Njock et al, *Phytochem. Anal* **2011**, in press.

Analytical Chemistry **AC 13**

Tritium emissions of the river Rhine at Basel

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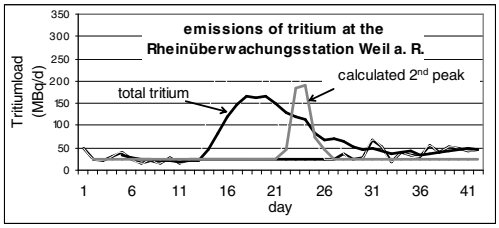
On the watershed of the river Rhine almost 75% of the Swiss surface water is collected and leaves the country at Basel. Therefore, since 1993 the river Rhine is under permanent control at the international monitoring station Weil am Rhein near Basel. Organic chemicals as pesticides and persistent chemicals are the major goal of the monitoring programme, but also heavy metals and geogenic components [1,2]. Radiochemical analyses of water and suspended matter is a part of this programme and also part of the Swiss monitoring programme for radioactivity and radiation doses.
Tritium emissions of the local chemical industry, nuclear power plants and tritium producing industry are under permanent control. The yearly inspection work at the nuclear power stations is based on permitted limits of emission and are announced in advance. Sporadically higher emissions of tritium are detected due to accidental incidents or waste disposals legalized by the federal ministry of health.



[1] N.A. Corfù und M. Zehringer: *AWBR*, **1995**, 171-183.
[2] http://www.aue.bs.ch/fachbereiche/gewaesser/rheinberichte/analysen und-ergebnisse.htm

Analytical Chemistry **AC 14**

Estimation of the formal redox potential of antioxidants thanks to ferrocene derivatives as redox mediators

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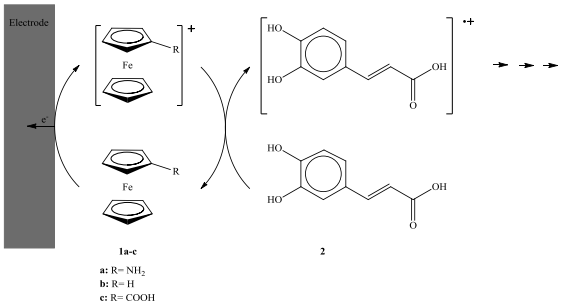
The determination of the formal redox potential of antioxidant species is of importance to predict their potential protecting capacity towards aggressive radicals such as ROS (Reactive Oxygen Species). Direct electrochemical studies of antioxidants do not provide any accessible formal redox potential as they often reveal an irreversible electrochemical behavior. In this study, cyclic voltammetry was employed to determine a formal redox potential scale for antioxidants thanks to ferrocene derivatives **1a-c** (aminoferrocene, ferrocene and ferrocenecarboxylic acid respectively) used as redox mediators. The formal redox potential of caffeic acid **2** was then evaluated using the proposed method.



Analytical Chemistry **AC 15**

Laser Ablation - Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) with simultaneous detection of the full elemental m/z range

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Laser Ablation - Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) is a versatile tool for the analysis of major, minor and trace elements in solids. Using Laser Ablation a high temporal resolution is required to follow transient signals by MS. Commonly ICPMS instruments were either limited in the temporal resolution, because of the sequential (quadrupole, sector-field) or discontinuous signal acquisition (time-of-flight), or the limited mass range (multi-collector) for the simultaneous detection. These limitations can be overcome by the Mattauch/Herzog MS geometry [1]. This geometry focuses all elemental isotopes simultaneously onto a focal plane. Coupled with a faraday-strip detector [2] this allows to read out the entire elemental mass range simultaneously (m/z 5 – 240).
In this study the first commercially available instrument (Spectro MS; Spectro Analytical Instruments, Germany) was coupled to LA sampling (LSX-213, CETAC Technologies, U.S.A.). The performance of the instrument was evaluated on trace element determination and isotope ratio precision using single pulses. Additionally, the capabilities and figures of merit of the instrument were investigated and two of the most recent glass references materials (BAM-S005A and BAM-S005B) were analyzed.

[1] J. Mattauch and R. Herzog, *Zeitschrift für Physik A Hadrons and Nuclei*, **1934**, *89*, 786-795.
[2]. J. A. Felton, et al., *J. Anal. At. Spectrom.*, **2011**, *26*, 300-304.

Analytical Chemistry **AC 16**

A miniaturized approach combining Zebrafish guided bioassays, UHPLC-TOF-MS and capNMR for the identification of anticonvulsants in the Pilippine medicinal plant *Solanum torvum*.

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The rapid acquisition of structural and bioactivity information on natural products (NPs) at the sub-milligram scale is key for performing efficient bioactivity-guided isolations. Zebrafish offer the possibility of rapid *in vivo* bioactivity analysis at the microgram scale [2], an attractive feature when combined with high-resolution fractionation technologies and analytical methods such as UHPLC-TOF-MS and microflow NMR [1]. In this respect we have evaluated the feasibility of a high resolution *in vivo* bioactivity profiling of *Solanum torvum* (Solanaceae) in a microtiter plate-based zebrafish seizure assay. The crude methanolic extract of the stem bark of *S. torvum* has exhibited a significant bioactivity. UHPLC-TOF-MS profiling revealed the presence of numerous steroid glycosides. The high resolution microfractionation of this mixture by semi-prep LC-MS enabled the resolution of most constituents that could be directly obtained in 96-well microtiter plate. The analysis of the wells by microflowNMR in combination with the LC-MS provided a good mean to dereplicate the compounds identified as spirostanol glycosides derivatives and estimate their microquantities for the anticonvulsant bioassay. The whole procedure enable a rapidly estimation of the bioactive potential of NPs directly in crude extracts.

[1] Glauser, G et al., *J. Agric. Food Chem*., **2009**, 57,
[2] Crawford, A.D. et al., *Planta Med*, **2008**, 6:624

Analytical Chemistry **AC 17**

Label-free determination of protein-ligand binding constants using electrospray mass spectrometry

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Electrospray (ESI) is a soft ionization technique that allows to transfer non-covalent complexes from solution into the gas phase and to study them by mass spectrometry (MS). Current research suggests that proteins in the gas phase are in a folded conformation which reflects the native conformation in solution, i.e. they still possess binding pockets and will bind ligands/inhibitors.

ESI-MS is becoming increasingly important in pharmaceutical research especially in drug discovery to investigate protein-ligand interactions and determination of binding affinities. We present a quantitative assessment of binding strengths of proteins interacting with small molecule ligands preformed with ESI-MS. The model systems studied for this purpose are well known serine proteases with well characterized inhibitors. The validation of such a known system allows us to study newly recognized type II transmembrane serine proteases (TTSPs).

TTSPs can be exploited as suitable diagnostic cancer markers and potential therapeutic targets, because they are involved in tissue homeostasis and a number of human disorders such as cancer. We plan to use TTSPs as drug target and a series of potential drugs and inhibitors against this target to determine the dissociation constants (K_{DS}) by MS.

The advantages of MS compared to other analytical methods (for example (micro) calorimetry, radioactivity-based assays, optical and fluorescence spectroscopy) are the lower sample consumption and the possibility to perform label-free measurements.

[1] M. Jecklin et al., *J. Mol. Recognit.* **2009**, 22, 319-329.
[2] F. Béliveau et al., *FEBS J.* **2009**, 276, 2213-2226.

Analytical Chemistry **AC 18**

Active role of water molecules in chromatographic system

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Besides its function as a solvent, water is known to play an active functional role in biological and chemical systems. However, complex systems including chromatographic systems are highly influenced by the amount of water present between the silica layers. The dynamics and ordering is different due to different interfacial regions and the intermolecular interactions at the surface[1], e.g. -OH groups of silica surface and the hydration sites in lipid bilayers[2]. Due to this reorganisation dynamics it becomes difficult to study such a system by standard structural techniques such as X-ray or NMR spectroscopy.

Owing to the great practical relevance of Reversed phase liquid chromatograpy (RPLC), a more detailed study of water dynamics, energetics and the morphology in the stationary phase and at interfaces is carried out by molecular dynamics (MD) simulations. In this work we investigate exchange dynamics of water between the surface-region and the bulk[3] and the H-bond structures, dynamics at water/silica interface[4,5], because it selectively captures the interfacial dynamics and contribute to their stability and functionality through hydrogen bond interaction[6,7]. Such a study reveals the temporal evolution of this complex system at an atomistic level.

[1] Scott R., *Faraday Symp. Chem. Soc.*, **1980**, *15*, 49.
[2] Finer E., Darke A., *Chem. Phys. Lipids*, **1974**, *12*, 1.
[3] M. Orzechowski and M. Meuwly *J. Phys. Chem. B*, **2010**, *114*, 12203.
[4] Nagata Y., and Mukamel S. *J. Am. Chem. Soc.*, **2010**, *132*, 6434.
[5] S. M. Melnikov et. al. *Analytical chemistry*, **2011**, *83*, 2569.
[6] J. Braun, A. Fouqueau, R. J. Bemish and M. Meuwly, *PCCP*, **2008**, *10*, 4765-4777.
[7] L. Zhang, S. Singh, et. al. *J. Chem. Phys.*, **2009**, *130*, 154702.

Analytical Chemistry **AC 19**

Fractionation Studies on the Pb-U-System in Zircons using LA-ICPMS

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) is routinely applied for age determination of geological samples in general and zircons in particular. As zircon incorporates U but not Pb during its crystallization, zircon minerals are widely used for age determination based on the U–Pb decay.

LA-ICPMS allows analysis of zircons with high spatial resolution and less sample consumption than required for solution nebulization ICPMS. On the other hand, to achieve correct ages, matrix matched standards are necessary and have been applied in form of natural zircon standards such as 91500, GJ-1, Plesovice and others. The use of these standard zircons yields a better agreement with reference ages than the silicate glass standard NIST 610. Nevertheless, there is evidence of laser induced elemental fractionation when ablating different zircon samples. As Kuhn *et al.*[1] pointed out, there is a selective loss of Pb in form of condensation and a retention of U within the crater due to the formation of baddeleyite[2].

We present evidence that even ages obtained with a series of natural zircon reference materials, particularly at small crater diameters, are not in agreement with their reference ages when considering the $^{206}Pb/^{238}U$ ratios. Possible strategies for matrix correction and its influence on the accuracy of the Pb/U ratio will be discussed.

[1] J. Kosler, M. Wiedenbeck, R. Wirth, J. Hovorka, P. Sylvester and J. Mikova, *J. Anal. At. Spectrom.*, **2005**, *20*, 402-409.
[2] B. K. Kuhn, K. Birbaum, Y. Luo, and D. Günther, *J. Anal. At. Spectrom.*, **2010**, *25*, 21-27.

Analytical Chemistry **AC 20**

Hydrogen Sensing Properties of Hexagonal MoO_3 Sensors

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We have developed synthetic approaches to transition metal oxides with different morphologies that are now investigated with respect to their gas sensing properties. Their nanoscale morphologies can be shaped and stabilized by introducing inorganic additives such as alkali and alkaline earth cations[1, 2] into the oxide frameworks. Here we report on hexagonal molybdenum oxides, which were synthesized via a one-pot hydrothermal preparation starting from the dissolution of Mo powders in 30% hydrogen peroxide at RT until the molybdenum was fully oxidized. An excess of sodium chloride was added and the mixture was transferred into a Telfon-lined stainless steel autoclave, followed by treatment at 180 °C for 48 h.

The obtained hexagonal molybdenum oxides display a high and reliable sensitivity towards different concentrations of hydrogen and ammonia. Their operating temperature was found to be 225 °C and a constant gas flow of 500 sccm was applied. Each H_2 gas sequence was introduced via flowmeters with synthetic air into the testing chamber and consisted of 50, 100, 200, 400 ppm and 5 ppm NH_3, respectively. As expected, the h-MoO_3 sensor behaved as an n-type semiconductor so that the resistance decreased upon exposure to H_2. The dynamic responses display reproducible performance at constant and variable concentrations of target gas. XRD and SEM investigations show that the annealing has no significant effect on the structure and morphology of the hexagonal molybdate sensors.

[1] A. Michailovski, R. Kiebach, W. Bensch, J.-D. Grunwaldt, A. Baiker, S. Komarneni, G.R. Patzke, *Chem. Mater.* **2007**, *19*, 185.
[2] Y. Zhou, N. Pienack, W. Bensch, G. R. Patzke, *Small* **2009**, *5*, 1978.

Analytical Chemistry **AC 21**

High-Resolution Chemical Imaging using TERS

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Chemical identification on the nanometer scale is one of the key questions analytical chemists face nowadays. The detection of small amounts of molecules on a surface is very important to control local functionalization (e.g., patterned surfaces on biochips or in sensorics) Also, the ability to distinguish molecules (even similar ones) is necessary for a localization of molecules by their chemical identity in heterogeneous environments.

Tip-enhanced Raman spectroscopy (TERS) delivers chemical information with a spatial resolution in the order of tens of nanometers, enhancing the Raman signal strength sufficiently to allow imaging. Using a special on-axis top-illumination and top-collection TERS setup we can combine high resolution with the ability to work on opaque samples[1]. By evaluating specific marker bands, Raman imaging (a map of Raman spectra) yields information on the spatial distribution of molecules, determined by their Raman fingerprint with highest possible resolution. Due to the enhancing nature of the TERS experiment, small coverages (monolayers) of weak Raman scatterers can be detected and localized on a conducting surface.
This has been demonstrated using a mixture of two non-resonant isomeric thiols on a gold surface. These thiols were patterned using micro contact printing to create a surface with known distribution (mimicing a biochip) and both isomers in the pattern were successfully detected, identified and localized using TERS imaging in a single experiment[2]. This molecular film recognition has until now been restricted to conducting surfaces, but nonetheless already represents an important step forward in chemical analysis.

[1] J. Stadler, T. Schmid, R. Zenobi, *Nano Lett.*, **2010**, *10*, 4541.
[2] J. Stadler, et. al *Beilstein J. of Nanotechn.*, **2011**, submitted

Analytical Chemistry **AC 22**

Study of UV irradiation effects on *Vitis* leaves at the metabolome level by UHPLC-TOFMS

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UV irradiation of plant leaves is known to results in cell damages including DNA lesions, protein degradation and cell membrane lipid peroxydation. In response to this abiotic stress, induction of several repair mechanisms and UV shielding compounds have been discovered [1]. In the case of *Vitis vinifera*, cultivars resistant to downy mildew specifically synthetize stilbene derivatives at the infection sites on the leaves [2]. On the other hand, these compounds are known to enhance UV protection by their radical scavenging and UV-absorbing activity. *Vitis* cultivars resistant and susceptible to mildew have been subjected to UV irradiation. A metabolomic study of the leave response based on UHPLC-TOFMS fingerprinting has shown a similar global metabolic response pattern to UV stress and mildew attack. Several biomarkers could be efficiently highlighted. These results open interesting perspective in the comprehension of the chemically mediated events that triggers *Vitis* UV response and search for novel bioactive stilbenes. Based on a larger screening of pathogen-induced and UV-induced responses this approach will also permit to better select resistant cultivars for further field assays based on favorable stress-induced metabolome profiles.

[1] Kunz, B. A., Cahill D. M., Mohr P.G., Osmond M.J and Vonarx E.J. *Int. Rev. Cyt.* **2006**, *255*, 1-40.
[2] Allonso-Villarde V., Voinesco F., Viret O., Spring J-L. and Gindro K. *Plant Phys. And Bioch.* **2011**, 49, 265-274

Analytical Chemistry **AC 23**

Nanoparticle analysis using a microdroplet dispenser head coupled to ICP-MS

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Recently, C.C. Garcia et al.[1] measured sub-micrometer silicon dioxide and gold particles by inductively coupled plasma optical emission spectrometry (ICP-OES) using a droplet generator. The main idea of this system was to introduce single droplets carrying single particle or dissolved analyte with a distinct temporal separation into the analyzer. Size-related detection limits of 200 nm for Au and 470 nm for SiO_2 particles were obtained. Changing to mass spectrometry (MS) instead of OES improves the sensitivity approximately by three orders of magnitude.
Several modifications done to the system enabled us to transport droplets (diameter approx. 40 µm) generated by a commercial microdroplet dispenser into the ICP with nearly 100% efficiency and improve time resolution of a quadrupole MS.[2] Single particle analysis was performed using both highly diluted nanoparticle suspensions and residues of dried single droplets doped with different standard solutions. Calibration can be accomplished by droplets doped with different concentrations of standard solution. Additionally, multi-element particles can be analyzed by spectrometers capable of simultaneous isotope detection. In this paper, system modifications, general performance features with respect to nanoparticle analysis, as well as current limitations and future strategies will be discussed.

[1] C. C. Garcia, A. Murtazin, S. Groh, V. Horvatic and K. Niemax, *J. Anal. At. Spectrom.* **2010**, *25*, 645 - 653.
[2] S.Gschwind, L.Flamigni, O.Borovinskaya, J.Koch, S.Groh, K.Niemax and D.Günther, *J. Anal. At. Spectrom.* **2010**, DOI:10.1039/C0JA00249F.

Analytical Chemistry **AC 24**

Enhanced sensitivity for extractive electrospray ionization mass spectrometry using an ion funnel under slightly reduced pressure

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Extractive electrospray ionization (EESI)[1] has proven to be a powerful ionization technique for a variety of applications e.g. the detection of trinitrotoluene or RDX on skin[2]. In comparison to ionization techniques such as proton transfer reaction mass spectrometry (PTR-MS)[3], EESI has higher limits of detection yet a broader mass range of substances that can be detected. Combining this higher mass range (> 200Da) with enhanced sensitivity would allow EESI to become an even more powerful technique, e.g. rendering the detection of anesthetics or doping agents in breath possible.
One of the main reasons for its limited sensitivity is the fact that the EESI ionization process as well as the delivery of generated ions into the mass spectrometer is very inefficient. We overcame this sensitivity bottleneck by building an ion funnel working at almost atmospheric pressure (~700 mbar). The EESI spray setup is located in this region of reduced pressure. The dispersing ions generated by the EESI setup are directly fed into the ion funnel lens system where a sine-wave radio frequency and a linear field of decreasing field strength that are applied to the lenses refocus and drive the ions through the funnel, respectively. The lens system focuses the ions from 1200 mm^2 to an area of 3 mm^2 before they are transferred into the mass spectrometer for mass analysis. First results show that an increase in sensitivity of more than two orders of magnitude can be achieved, making EESI comparable to PTR-MS.

[1] HW. Chen, A. Venter, RG Cooks, *Chem. Comm.* **2006**, *19*, 2042.
[2] HW. Chen, R. Zenobi, *Nature Protocols* **2008**, *3*, 1467.
[3] A. Hansel, A. Jordan, R. Holzinger, et al., *Int. J. Mass Spectrom.* **1995**, *149*, 609.

Analytical Chemistry **AC 25**

Direct detection of chitosan in toothpaste with HR-MAS NMR

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^{1}H High Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HR-MAS NMR) spectroscopy has been applied in several research areas where semi-solid like materials are involved, mainly in biomedical applications [1] and food analysis [2]. Toothpaste is a substance with a semi-solid consistency that seems to be a suitable sample for HR-MAS. Chitosan, a natural linear polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine units, is an interesting ingredient for toothpaste formulation because of its rheological properties, its tendency to adhere to negatively charged surfaces like dental enamel, and for its known beneficial tissue regenerating and wound healing properties [3].
To detect the presence of chitosan in toothpaste with liquid NMR, we have developed a sample preparation procedure consisting of 2 steps. A suspension prepared with toothpaste and acidic D_2O has been stirred overnight in order to extract the chitosan. Subsequent centrifugation permits to separate the liquid phase from the insoluble material. The ^{1}H-NMR spectra of the supernatant show a characteristic peak at ~ 2 ppm deriving from the acetyl-groups of chitosan. Here we present the results of the direct analysis of toothpaste using HR-MAS NMR, which permits to skip the sample preparations steps allowing for easier and faster detection of chitosan.

[1] Moestue S. et al, *Curr. Top. Med. Chem.,* **2011**, 11, 2.
[2] Nestor G. et al, *J. Agric. Food Chem.*, **2010**, 58, 10799.
[3] Ravi Kumar M.N.V et al, *Chem. Review*, **2004**, 104, 6017-6084

Analytical Chemistry **AC 26**

Analytical Chemistry

Induction of antifungal metabolites by fungal co-culture

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Microorganisms are a very rich source of antimicrobial secondary metabolites [1]. To produce original fungal metabolites, new approaches have to be developed to induce metabolic pathways that are often silent [2]. Recently, strategies were used to induce these orphan pathways through application of environmental stresses or using genetic engineering. Lately, we showed that fungal co-cultivation also results in new secondary metabolites' production [3]. In the course of a screen based on fungal confrontation, we identified intriguing morphological co-culture patterns where fungi stay at distance when grown on agar plates. In order to identify new fungal metabolites responsible for this long distance repulsion, confrontation zone and pure fungal strains were compared by UHPLC-TOF-MS fingerprinting using differential metabolomics. Data mining resulted in an efficient selection of stress induced molecules which were purified using preparative HPLC and identified by microflow NMR. Antifungal activity of these mycoalexins was assessed to verify their relation to the fungal repulsion. This innovative strategy can be used to identify new antifungal metabolites against fungi such as *Fusarium*.
*Acknowledgements:*This work was supported by Swiss National Science Foundation Sinergia Grant CRSII3_127187 (to J.-L. W. and K. G.)

[1] Berdy, J., *J Antibiot.***2005**, *58*, 1.
[2] Scherlach, K. et al., Org. *Biomol. Chem.*, **2009**, *7*, 1753.
[3] Glauser, G. et al.,*J. Agr. Food. Chem.*, **2009**,*57*, 1127.

Analytical Chemistry **AC 27**

Properties of a fast LIBD system operated at 100 Hz

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Laser induced breakdown detection (LIBD) offers an opportunity to detect nanoscale particles in aquatic systems. The breakdown probability (BDP) defined as the ratio of detected plasma events to the total number of laser shots depends on the size and the number density of nanoparticles in suspension. Recording the BDP as a function of the laser energy results in so-called energy curves which can be used to extract the size and the concentration of nanoparticles in aqueous matrices.
The plasma events can be detected in several ways as described in [1]. In our LIBD apparatus, the plasma is detected based on the energy ratios of the laser before and after passing through the measurement cell containing the particle suspension. Our laser system allows measurements at a repetition rate of 100Hz which is 5 times faster compared to other LIBD system.
Walther [2] described the energy-curves are by semi-empirical model with 2 free parameters relating to the concentration and breakdown threshold energy. At low pulse energies the BDP is proportional to E4 which can be explained with the generation of the "first electron" requiring four photons. The initial part of our energy curves, representing the E4 dependence can be fitted well using the Walther algorithm. However, at higher energies, our data cannot be described using the Walther algorithm anymore. This is most likely caused by a stronger dependence of the breakdown volume on the laser pulse energy. We therefore will present a new fitting algorithm accounting for the different properties of our LIBD system.
[1] C. Latkoczy, R. Kägi et al., Journal of Environmental Monitoring, 2010, 12, 1422 - 1429
[2] J.-I. Kim, C. Walther, Environmental colloids and particles, 2007, 12, 556 -612

Analytical Chemistry **AC 28**

Investigation of the precision and accuracy of isotope ratio measurements for atmospheric sampling for laser ablation MC-ICPMS

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Laser ablation-ICPMS (LA-ICPMS) is a powerful tool for solid sample measurements. However the ablation cell size is a limiting factor, as the sample has to fit into it. The coupling of a gas exchange device (GED)[1] to the ICPMS allows to allows ablation without an ablation cell. The aerosol is generated in air environment and is then aspirated into the GED by a membrane pump through a tube located directly at the ablation site. The air is exchanged to Argon in the GED and the aerosol is then transported to the ICP in an Argon atmosphere. The figures of merit for atmospheric sampling were investigated and similar accuracies as for conventional LA were found[2].
Coupling LA to multi collector-ICPMS (MC-ICPMS) allows precise isotopic information of a sample, used for e.g. age determination. These samples are mostly archeological and will not always fit into an ablation cell. The isotope ratio determination with LA-GED-MC-ICPMS would therefore represent a method of choice for large and precious samples.
The precision and accuracy of the atmospheric sampling for isotope ratio measurements were investigated with a Ti-Sapphire based femtosecond laser coupled to a GED and a MC-ICPMS. The samples of interest were lead, galena, brass and zircon. A loss in sensitivity is observed, however, similar precision and accuracy were found comparing atmospheric ablation with the conventional LA set up.

[1] Nishiguchi K., Utani K., Fujimori E., *J. Anal. At. Spectrom.*, **2008**, *23*, 1125.
[2] Kovacs R., Nishiguchi K., Utani K., Günther D., *J. Anal. At. Spectrom.*, **2010**, *25*, 142.

Analytical Chemistry **AC 29**

Bubble cell for magnetic bead trapping in a capillary

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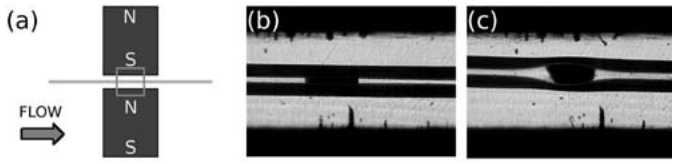
Magnetic beads are widely used in both research and biomedical applications. In microfluidics, they offer many advantages, like an increased specific surface available for molecule adsorption, a reduced diffusion pathway and easy manipulation by magnets [1].

A bubble cell capillary classically used to extend the optical path length for UV-Vis detection is employed here to trap magnetic beads. With this system, a large amount of beads can be captured without inducing a strong pressure drop, as it is the case with magnetic beads trapped in a standard capillary, thereby having less effect on the experimental conditions. Using numerical simulations and microscopic visualizations, the capture of beads inside a bubble cell was investigated with two magnet configurations and compared to trapping in a standard capillary (Figure).



Pressure-driven and electro-osmotic flow velocities were measured for different amounts of Protein-A coated beads or C18 functionalized beads (RPC-18). Solid phase extraction of an antibody on Protein-A beads and preconcentration of fluorescein on RPC-18 beads were performed as proof of concept experiments.

[1] Gijs M.A., *Microfluid Nanofluid*. **2004**, *1*, 22.

Analytical Chemistry **AC 30**

Optimization of *Conus consors* venom profiling using ultra-high-pressure liquid chromatography

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LC-MS has become the reference profiling technique in peptide analysis such as venomics, providing abundant valuable data [1]. Efforts have been mainly focused on the detection part while the chromatographic aspects were not thoroughly investigated. An optimized separation could provide more resolved peaks and thus, more valuable MS data. The new generation of analyzers able to acquire automatic fragmentation data with a higher acquisition rate, allows working with thinner peaks and thus higher peak capacity. Thus, there is a need for higher resolution in the LC dimension. In this study, the use of columns packed with sub-2µm particles and of a Ultra High Pressure LC (UHPLC) system were used in different chromatographic conditions. The goal was to obtain a higher peak capacity when analyzing venom of the marine snail species *Conus consors* and to model the separation as a function of chromatographic parameters. Theoretically, the use of smaller particles size impact extremely positively on the peak capacity of peptides separations [2]. In the best conditions, a peak capacity beyond 1000 was experimentally attained as well as an increase in the number of resolved peaks. The optimal separations were finally tested during a complete peptide sequencing process with a Q-TOF-MS analyzer. Since analyses were carried at high temperature (up to 90°C), tests were performed to verify peptide stability.

[1] D. Biass, S. Dutertre, A. Gerbault, J.L. Menou, R. Offord, P. Favreau, R. Stocklin, *J. Proteomics* **2009**, *72*, 210.
[2] J. Ruta, D. Guillarme, S. Rudaz, J.L. Veuthey, *J. Sep. Sci.* **2010**, *33*, 2465.

Analytical Chemistry **AC 31**

Determination of As in Stalagmite from Meghalaya (NE, India) by LA-ICPMS: chasing lower limits of detection

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The determination of trace elements in geological samples by LA-ICPMS has already been proved to be a suitable tool for direct solid samples analysis. The addition of small amounts of hydrogen or methane to the carrier gas of laser generated aerosols has been demonstrated to enhance sensitivity for ICPMS. For elements with high first ionization potential (P, As, Se, Te, Au) the sensitivity enhancement has been shown to be 5-7 fold when adding hydrogen [1]. In this study the addition of carbon using methane as source is compared to the addition of carbon released from an electrothermal vaporization (ETV) unit operated at temperatures between 1000 and 2650 °C. The signal enhancement was measured for a wide palette of elements using a synthetic glass standard (NIST SRM610). Especially the sensitivity for elements with high first ionization potential (As, Se and Te) was enhanced significantly by both methods. Methane, being a more effective carbon source, led to more pronounced signal enhancement than carbon from the ETV. While the carbon addition via ETV yielded a factor 2 sensitivity enhancement, with the methane addition, signal enhancement factors of ~10 were achieved for As. The applicability of both approaches for quantitative analysis was first tested by analyzing 4 different glass standards: NIST SRM612, BHVO 2G, BIR 1G and TB 1G. For the quantification NIST SRM610 was used as calibration standard and ^{42}Ca was chosen as internal standard. Furthermore the method was tested on a stalagmite sample from Meghalaya (NE, India). By adding methane (9 ml/min) to the laser generated aerosol, the limit of detection for As could be lowered by a factor of 2.

[1] M. Guillong, C.A. Heinrich, *J. Anal. At. Spectrom.* **2007**, 22, 1488

Analytical Chemistry **AC 32**

Production of micro- and nano-sized particles by drying solution droplets produced by a piezo-driven dispenser

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Production of monodisperse nano- and micrometer-sized particles of desired composition still represents a technical challenge, even for small quantities. Sol-gel methods lead to reasonably monodisperse particles, yet a control over their exact shape and size seems to be difficult, and the possibly achievable compositions are limited. The work presented here deals with a novel approach for the production of particles by drying solution droplets released by a commercial piezoelectric dispenser head. The device presented here has been already successfully coupled to ICP-OES [1] and ICP-MS [2] for the measurement of single micro- and nano-particles. By changing solute type and its concentration, particles of different size and composition can be produced. Furthermore, a solvent extraction process exploiting the higher diffusion rate in helium gas is implemented, which allows the defined generation of dry particles. Both theoretical considerations as well as experimental data on the approach will be shown, aiming for a better understanding of the processes involved in the fast solvent removal, which allows the generation of dry particles of any composition. The controlled particle generation will have significant implications on further ICP optimization strategies.

[1] Garcia, C.C., Murtazin, A., Groh, S., Becker, M. & Niemax, K, *Spectrochim. Acta, Part B* **2010**, *65*, 80-85.
[2] Gschwind, S., Flamigni, L., Koch, J., Borovinskaya, O., Groh, S., Niemax, K. & Gunther, D., *J. Anal. At. Spectrom.* **2011** DOI:10.1039/C0JA00249F.

Analytical Chemistry **AC 33**

Insights into the Mechanism of Electrospray, MALDI and Laserspray Ionization

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It was recently demonstrated that UV laser ablation of MALDI matrixes could produce multiply charged ions similar to ESI [1]. ESI like MALDI ions could be obtained using standard MALDI conditions but with transmission geometry of the ion source. This method was termed as laserspray ionization (LSI). But the mechanism of multiply charged ion formation in LSI is still undo investigation.

Fluorescence spectra, being sensitive to the solvent environment, can be used as an alternative to MS in order to detect gas-phase ions. Recently we describe the discovery that completely unsolvated ions are present in abundance inside an ESI plume already at ambient conditions [2]. This discovery in fact, provides a novel method to probe the properties of gas-phase molecular ions.

Our experimental data give us direct insight into the mechanisms of the gas-phase ion production by ESI and LSI. It was shown that gas-phase ions in ESI are preferentially formed directly from the liquid droplets (ion evaporation model) rather than by gradual solvent evaporation (charged residue model) while the charge residue model is mostly responsible for ion formation in LSI ionization.

[1] Trimpin, S., Inutan, E. D., Herath, T. N., McEwen, C. N. *Mol. Cell. Proteomics*, **2010**, 9, 362.
[2] Chingin K., Frankevich V., Balabin R. M., Barylyuk K., Chen H. M.,Wang R., and Zenobi R. *Angew. Chem. Int. Ed.* **2010**, 49, 13, 2358.

Analytical Chemistry **AC 34**

Development and validation of an analytical method for the quantification of CGP69669A, a Sialyl Lewis^x mimetic

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A simple, rapid, precise and specific isocratic HPAE-PAD method for quantification of CGP69669A was developed and validated. CGP69669A is a sialyl Lewis^x glycomimetic, antagonist of E-selectin with potential use for treating inflammatory skin diseases [1,2]. Quantification was performed using a Dionex CarboPac^{TM} PA-200 anion-exchange column (3 x 250 mm) with 100 mM NaOH solution as mobile phase, a flow rate of 0.50 ml/min and an injection volume of 10 µl. A carbohydrate quadruple potential waveform was used for detection. Rafinose was used as internal standard. This is the first validated direct method for CGP69669A quantification and in addition to its use in our skin permeation and pharmacokinetic experiments, it should be of use for other applications of this molecule.

[1] K.E. Norman, G.P. Anderson, H.C. Kolb, K. Ley, B. Ernst, *Blood.* **1998**, *91*, 475.
[2] A. Titz, J. Patton, M. Smiesko, Y. Radic, O. Schwardt, J.L. Magnani, B. Ernst. *Bioorg Med Chem*. **2010**, *18*, 19.

Analytical Chemistry **AC 35**

Physical Chemistry of Acridine Adsorption onto Gold Surface: The Influence of Nanostructure as Revealed by Near-Infrared and Tip-Enhanced Raman Spectroscopy (TERS)

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Here we report a detailed study of adsorption of acridine ($C_{13}H_9N$) and a number of its derivatives: benz[a]acridine, benz[c]acridine, etc. Thermodynamic and kinetic data, obtained by solution-phase near-infrared spectroscopy (NIRS), clearly show a great difference of adsorption behavior for flat and nanorough (~4 nm) gold surfaces. Gibbs free energies of -4.57±0.10 vs. -7.67±0.13 kcal mol^{-1} and adsorption kinetic constants of 26±3 vs. 4160±220 min^{-1} M^{-1} were determined for flat and SERS-type surfaces, respectively. This result could be interpreted in terms of the type of adsorption model: adsorption by the conjugated pi-electron system or by the lone pair of the nitrogen atom in the former and latter cases, respectively. The characterization of the polyaromatic monolayer directly at the Au surface, as measured by tip-enhanced Raman spectroscopy in scanning tunneling microscope mode (STM-TERS), has confirmed the dependence of the adsorption parameters on the (nano)roughness of the metal surface.[1] The shift of Raman active vibrations (by 2-8 cm^{-1}) of the molecules at the surface and the appearance of new bands were used as a direct indicator of the adsorption model type. Density functional theory calculations (DFT-B3LYP, DFT-M06) were used to confirm the assignment. A unique possibility of TERS to enhance the molecular Raman signal independent on the surface structure and type was fully realized. The importance of the findings for industry and nanotechnology are discussed.

[1] M. Turner, et al. *Nature* **2008**, *454*, 981.

Analytical Chemistry **AC 36**

Mass spectrometric analysis of single-stranded DNA-binding protein bound to DNA by chemical cross-linking

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Chemical cross-linking, combined with high mass MALDI-MS, shows great potential for understanding noncovalent interactions among large biomolecules. Protein-DNA complexes, one type of noncovalent complex, have been observed in many cellular processes including transcriprtion, translation and DNA duplication. In our previous study, a complex consisting of a single DNA strand (DR5) and a heterodimer of the retinoic acid receptor (RAR) and the 9-*cis* retinoic X receptor (RXR) was observed after cross-linking using N-hydroxysuccinimide (NHS) esters.[1] It is still unclear how an NHS ester, as a highly amine recative group, should bind to DNA. To further explore chemical cross-linking in protein-DNA complexes, single-stranded DNA-binding protein from Escherichia coli (*E. coli* SSB) was selected here. *E. coli* SSB forms a stable homotetramer in solution and preferentially binds to single stranded DNA following two major pathways.[2] The binding patten of *E. coli* SSB and DNA is strongly dependent on the salt concentration and the length of the DNA sequence. In preliminary results, the covalently linked homotetramer of *E. coli* SSB was obtained by using NHS esters. A series of heterobifunctional cross-linkers with different photoactivatable groups on one end and an NHS ester functionality on the other end will be applied. The reactivity of the different photoactivatable groups towards the nucleotides will be further investigated by comparing cross-linking efficiency.

References:
[1] Bich, C.; Baser S.; Jecklin M. C.; Rochel N.; Peluso-lltis C.; Panagiotidis A.; Nazabal A.; Moras D.; Zenobi R., *J. Am. Soc Mass Spectrom.*, **2010**, *21*, 635-645
[2] Lohman, T. M.; Ferrari, M. E., *Annu. Rev. Biochem.*, **1994**, *63*, 527-570

Analytical Chemistry **AC 37**

The Role of Nebulizer Gas Pressure in Electrosonic Spray Ionization

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Electrosonic spray ionization (ESSI) source, first developed by Takáts et.al. in 2004, is an important variant of electrospray ionization (ESI).[1] The main difference between ESSI and ESI is the pressure of the nebulizer gas: in ESSI, the gas pressure is high (≥20 bar) while in ESI it is normally much lower (≤ 5 bar). Assisted by high pressure, some unique advantages have been demonstrated in ESSI, for bimolecule analysis. However, to date, very little work has been done on the ESSI mechanism, especially, on how the high pressure gas helps to keep proteins in a near-native conformation. In this work, we investigated the ESSI mechanism using both ESSI-mass spectrometry (MS) and numerical simulations. Two globular proteins, cytochrome c and myoglobin, were systematically analyzed under different experimental conditions. With a small sample flow rate (1-5 μL/min) and a neutral buffer solution, neither of these two systems showed observable changes in charge state when the gas pressure was varied from 5 to 45 bar. However, at a high sample flow rate (≥200μL/min), the intensity ratio of peaks representative of the denatured form to those assigned to native form decreases obviously when the gas pressure increases. When the sample flow is large, much larger droplets will be formed and these droplets can then easily be torn apart by the high gas flow, shortening the solvent evaporation process and consequently preserving the conformation of proteins in the droplets. This idea is also supported by our numerical simulations. This work not only provides systematical details of the mechanism for ESSI but also helps to develop new application for ESSI or other gas-assisted ambient ionization sources in the future.

[1] Z. Takáts, J.M. Wiseman, B. Gologan, R.G. Cooks, *Anal. Chem.* **2004**, 76, 4050.

Analytical Chemistry **AC 38**

Single Cell Analysis of Microbial Metabolites by Synchrotron FTIR Spectromicroscopy

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Microorganisms play a significant role in the processing of organic and inorganic compounds in the environment. These transformations range from the decay of biological matter to the global cycling of such elements as sulfur and phosphorus. Understanding the detailed biochemical processes that mediate microbial transformation of the surrounding medium is a key issue in microbiology, environmental studies and biotechnology. Many of the processes develop in a heterogeneous environment and need to be resolved in space, on the scale of a single cell or small cell clusters, and in time, on the scale of minutes to hours.
We discuss the use of synchrotron FTIR spectromicroscopy to investigate microbial metabolism. The technique provides information on composition, concentration and time evolution of chemical processes. Most importantly, it allows the study of living samples. We present specific applications to the characterization and investigation of metabolism in bacteria belonging to the genera *Thiomargarita* and *Beggiatoa*. We show the possibility of identifying the composition of inorganic and organic inclusions within single cells, and of following in real time the redox changes of inorganic oxianions and organic macromolecules.

Analytical Chemistry **AC 39**

Studying of the optical properties of fluorescent biological tags in the gas phase.

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One of the most powerful methods for analyzing large biological molecules is labeling them by fluorescent markers. This allows one to visualize, track, and quantify molecules and events in living cells as well as to study biological molecules themselves. In this work we performed studies of the optical properties of different fluorescent labels in the gas phase, in solution, and inside an electrospray plume. The data obtained can be used for studies of biological samples in the gas phase, and as a benchmark for theoretical fluorescence spectra calculation and prediction.
We performed a series of experiments for studying the fluorescence and absorption of different compounds in the gas phase as well as of the same substances dissolved in different solvents. We present results obtained for the following compounds: Nile red, Nile blue, Rhodamine 6G, Rhodamine B, and Dansyl chloride.
In the present work we studied fluorescence properties of these substances in the ESI plume as well as in high vacuum (at pressures less than 2×10^{-8} Torr) inside the ICR cell of the mass spectrometer. We explored optical properties of positively and negatively charged molecules. The fluorescence spectra in the gas phase differ from the spectra of the dissolved compounds. We show that the fluorescence maxima in the gas phase in some cases are red-shifted by 50 nm in comparison with the dissolved compounds. Absorption coefficients of the particular substances were also measured with action spectroscopy. We believe that fluorescence spectra in the gas phase can be used for studying of the properties of different biological molecules as well as reference spectra for theoretical studies of the particular compounds.

Analytical Chemistry **AC 40**

PTV Back-Flush Injection GC with NCI-MS/MS: A Robust, Sensitive and Selective Method for the Determination of Ethylglucuronide in Hair

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Introduction: Ethylglucuronide (EtG) is a direct metabolite of ethanol and is used as a retrospective long-term marker for alcohol consumption. A robust, sensitive and selective method for the determination of EtG in hair was developed and validated.

Results:

LOD, S/N ≥ 3:1	LOQ, S/N ≥ 10:1	Lin. Range (n = 10)	R^2
1.5 pg/mg	3.5 pg/mg	5 – 500 pg/mg	0.9931

Intraday Precision(level 35 pg/mg)	Interday Precision (level 35 pg/mg)
4.8 %	3.2 %

Table 1. Selected validation data

Conclusion: The use of the PTV back-flush injection system clearly demonstrates the advantages of saving time and costs. The GC-NCI-MS/MS method shows good linearity, accuracy and reliability for a wide concentration range.

Analytical Chemistry **AC 41**

Supercharging of large proteins in electrospray ionization mass spectrometry

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Electrospray ionization (ESI) is a technique used in mass spectrometry to produce intact multiply charged ions from the analyte solution. The multiple charging reduces mass-to-charge (*m/z*) ratios of the ions. It makes possible to observe large molecules with a narrow *m/z* range mass analyzer and also increases the tandem mass spectrometry (MS/MS) efficiency. The addition of the supercharging reagent to electrospray solution increases the multiple charging of the analyte [1]. The effect of two supercharging reagents, *m*-nytrobenzyl alcohol (*m*-NBA) and glycerol, was investigated on large (MW>12,000) proteins such as cytochrome C, carbonic anhydrase, bovine serum albumin (BSA), and immunoglobulin G. The proteins were obtained from Sigma-Aldrich. The experiments were performed on a Thermo linear ion trap (LTQ) FT-ICR mass spectrometer. Addition of a small amount of *m*-NBA (0.7%) to the 10 μM solution of the cytochrome C (47% Water/50% Methanol/3% Acetic acid) increased the maximum charge state of the most abundand ion from 16+ to 18+. On the other hand, the addition of glycerol (50%) to the 10 μM solution of the cytochrome C (97% Water/3% Acetic acid) produced the bimodal ion signal distribution where the most abundant signals had charges of 11+ and 19+. The decreased extent of the charge-enhancement was observed when supercharging agents were added to the solutions of larger proteins. Addition of 0.5% of *m*-NBA to 5 μM BSA solution (49% Water/50% Acetonitrile/1% Formic acid) increased the charge state of the most abundant ion from 54+ to 55+, whereas the addition of glycerol (up to 55%) produced polymodal ion distribution with the most abundant signals with 60+ and 31+ charges. Thus, the known supercharging agents are not efficient for supercharging large proteins and we continue the quest for an appropriate agent and tailoring the experimental conditions.

[1] A.T. Iavarone, E.R. Williams, *Int. J. Mass Spectrom.* **2002**, *219*, 63.

Analytical Chemistry **AC 42**

The fate of burned CeO_2 nanoparticles: ICPMS analysis of nanoparticles in different compartments of a municipal solid waste incinerator

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CeO_2 nanoparticles are produced in high quantities because of their catalytic properties, which are enhanced by their increased surface to volume ratio compared to the bulk material. Due to the accompanied environmental impact and health issues, their transfer capabilities into lung cells and maize plants as well as their behavior in waste water treatment plant have been investigated [1-3]. To improve the understanding of the environmental impact of such nanoparticles, their fate into municipal solid waste incinerator was experimentally studied by feeding CeO_2 nanoparticles into the waste and by adding them to the NH_3 solution used for selective non catalytic reduction of NO_x. This experiment was designed to determine the CeO_2 nanoparticle pathway through the incineration facility. To follow the CeO_2 particles, samples from 5 locations of the plant were taken and measured by ICPMS. The structure of the nanoparticles after incineration was investigated by SEM and TEM. For quality control and validation of digestion procedure and ICPMS measurements, fly ash reference material was analyzed as well as flame synthesized CeO_2 nanoparticles. Digestion procedure and figures of merit of the developed analytical method will be discussed in detail.

[1] Limbach L.K., *Environ. Sci. Technol.,* **2005***,* 39*,* 9370.
[2] Birbaum K., *Environ. Sci. Technol.*, **2010**, 44, 8718.
[3] Limbach L.K., *Environ. Sci. Technol.*, **2008**, 42, 5828.

Analytical Chemistry **AC 43**

Applications of TERS in Nanoscale Chemical Analysis of Biological and Materials Samples

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Macroscale properties of materials are greatly influenced by their structure and composition at the nanometer scale. For example, the efficiency of solar cells is affected by concentration gradients at the micro- and nanometer scale, and the arrangement of biomolecules in cell membranes influences essential processes. For studying such samples, analytical tools are needed that provide imaging and chemical analysis with nanoscale resolution.

In our lab, two instruments for tip-enhanced Raman spectroscopy (TERS) are used for this task: An AFM-Raman combination based on an inverted microscope allowing the investigation of transparent samples, and an upright microscope equipped with modules for AFM and STM that enables measurements of opaque samples. In both cases, Ag or Ag-coated tips placed into a laser focus strongly confine and enhance the electromagnetic field leading to enhanced Raman spectra from a sample area with a diameter of 10–50 nm. This enables chemical analysis at the nanometer scale.

TERS experiments with molecular monolayers on gold substrates, biopolymers, and supported lipid layers have shown the possibility to map the distribution of various compounds with nanometer-scale resolution. A proof-of-principle study has demonstrated that TERS can even be operated in liquids, which is an important aspect when studying cell membranes in their native environment. The combination of AFM with high-resolution Raman microscopy also gave new insight into novel thin-film solar cell materials. Concentration gradients of Ga in the photovoltaic absorber material $Cu(In,Ga)Se_2$ have been studied at the micrometer scale, and the formation of $MoSe_2$ close to the interface with the Mo substrate has been visualized with Raman microscopy. TERS in a new upright configuration will allow studying such heterogeneities with nanometer resolution.

Analytical Chemistry **AC 44**

Multiparameter Analysis of Single Algal Cells by Integrating Raman and Fluorescence Microscopy with Mass Spectrometry

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A vast number of bioanalytical protocols use samples containing a large number of cells. However, populations of cells, even those that have similar genetic constitution, can display substantial phenotypic variability. Single-cell analysis is essential to gain an unbiased insight into biochemical processes that occur in heterogeneous populations of cells. A thorough understanding of single-cell systems requires multiparameter measurements by applying various orthogonal analytical techniques, in which different components of the cellular phenotype are assayed.

This contribution describes a facile label-free approach for performing multidimensional chemical analysis on individual single-cell organisms by combining optical, auto-fluorescence and Raman microspectroscopy with laser desorption/ionization mass spectrometry (LDI-MS). Single unicellular algae are seeded on a bare stainless steel plate and analyzed microspectroscopically. This provides information on the content and distribution of photoactive species, such as β-carotene (based on its Raman signature), as well as chlorophyll and other components of the photosynthetic apparatus (based on their fluorescence emission). Exactly the same cells are then analyzed by MS. Phospholipid species are readily ionized by LDI of intact cells, without the need for an auxiliary matrix. This not only facilitates sample preparation, but also preserves high spatial resolution and high sensitivity. Using this method, we were able to study the content and arrangment of proplastids and photosystem components, as well as the amounts of various phospholipid species in individual algal cells. The methodology can be used in fundamental biological studies on unicellular organisms, which require information on internal structure and chemical composition of single cells.

Analytical Chemistry **AC 45**

Detection of primary metabolites in single HeLa cells by MALDI-MS

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Any population of living organisms shows heterogeneity due to various underlying reasons, e.g. stochastic gene expression.[1] It is known that this variability is decisive for the population's fitness and therefore of importance, for example, for the heat resistance of salmonella or development of potent antibiotics.
In the past, we have developed various methods for targeted metabolomics of single cells by matrix-assisted time-of-flight mass spectrometry (MALDI-TOF-MS).[2-4] Currently, we are employing the MicroArrays for Mass Spectrometry (MAMS) technology as a platform.[6] These transparent chips host a dense array of recipient sites whose size allow for deposition from single to hundreds of cells.
In the present study we cultured HeLa cells on a MAMS slide, where they grew preferably in the hydrophilic reservoirs. We were able to reproducibly detect various primary metabolites (e.g. ATP) from single HeLa cells.

[1] Aertsen, A.; Michiels, C. W. *Critical Reviews in Microbiology* **2004**, *30*, 263-273.
[2] Amantonico, A.; Urban, P. L.; Fagerer, S. R.; Balabin, R. M.; Zenobi, R. Analytical Chemistry 2010, 82, 7394-7400.
[3] Urban, P. L.; Jefimovs, K.; Amantonico, A.; Fagerer, S. R.; Schmid, T.; Madler, S.; Puigmarti-Luis, J.; Goedecke, N.; Zenobi, R. Lab Chip 2010, 10, 3206-3209.
[4] Amantonico, A.; Oh, J. Y.; Sobek, J.; Heinemann, M.; Zenobi, R. Angewandte Chemie-International Edition 2008, 47, 5382-5385.

Analytical Chemistry **AC 46**

Structural analysis of immunoglobulins by top-down mass spectrometry

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Immunoglobulins G (IgGs) are tetramers composed by two light and two heavy chains, with an overall molecular weight of 150 kDa. As one of the most important classes of therapeutic and bioengineered proteins, IgGs are commonly analyzed by mass spectrometry (MS), in order to confirm their sequence and obtain a glycosylation profile. For this purposes, bottom-up MS strategies, consisting in the proteolitic digestion of the protein into peptides prior to MS measurements, are generally applied. However, these approaches lack the possibility of obtaining information about the three-dimensional structure of the protein, are not selective to the glycoforms and may introduce experimental errors upon proteolysis. Top-down, consisting in the fragmentation of the intact IgGs, could overcome the mentioned limitations. Nevertheless, due to the complexity of the fragment population this technique is normally limited to proteins of reduced size.

Recently, we successfully applied high-resolution mass spectrometry to the detection of the product ions obtained from IgGs by electron capture dissociation (ECD) and electron transfer dissociation (ETD) tandem MS. These radical-driven fragmentation techniques not only allowed us to extensively sequence both heavy and light chains of the IgGs, including the important variable domains (responsible for the antigen recognition), but also to elucidate some three-dimensional features of the intact protein, which showed not to undergo dissociation in specific disulfide-protected regions, and conversely to be highly fragmented at different, less structured positions. Moreover, the analysis of the fragmentation pattern could distinguish between IgGs belonging to different isotypes or deriving from different source (e.g., human and mouse).

Analytical Chemistry **AC 47**

NMR: from similarity to structure elucidation

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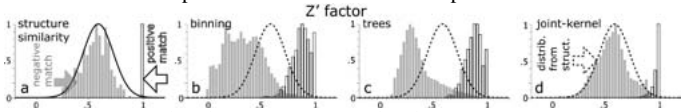
Nuclear spins are exquisitely sensitive to subtle modifications of their environments that reflect in the position of their NMR signals. Standard distance measurements fail to recognize those shifts and thus underestimate the real similarity. Thus, Pretsch and coworkers [1] introduced a comparison based on subsequent division of the spectrum into bins (Fig. b). We present a solution based on binary trees (Fig. c) that are more suitable for the analysis of 2D/3D data, since they focus only on the regions of the spectra that contain signals, thus describing the data in a very compact form. In addition, we show a method that permits to mix structural and spectral information.



To do this, trees of 296 representative molecules were paired with their corresponding vector of structural descriptors and a joint-kernel was calculated. The results, displayed as Z' factors, (Fig. d) were found closer to those obtained with structures (Fig. a), used as a reference. Moreover, this joint-kernel allows predicting the descriptors corresponding to any unknown tree. The predictions were found promising and represent a step toward structural elucidation.

[1] Bodis, L., Ross, A and Pretsch, E., *Chemometr. Intell. Lab.* **2007**, 85, 1-8.

Analytical Chemistry **AC 48**

Ionic Liquids as Matrices in High Mass MALDI-MS

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Matrix-assisted laser desorption/ionization (MALDI)-MS usually generates low charge states. Thus the m/z-ratio can in some cases become rather large and even exceed the detection range of the commonly used detectors. This led to the development of special high-mass detectors based either on superconducting tunnel junctions or on ion-to-ion-conversion. Solid matrices usually leads to heterogeneous sample spots. As an alternative ionic liquids have been used. Some of the advantages of ionic liquids are their low melting point and their good vacuum stability [1]. Up to now, the largest ion measured using an ionic liquid matrix is the trimer of urease at m/z 270'000 Da [2].

The use of ionic liquids as MALDI matrix allows also the coupling of microfluidic devices to a spotter, which deposits the sample on the sample plate. This leads to a higher throughput and a higher reproducibility. For this reason several ionic liquids consisting of protonated alkyl amines in combination with sinapinic acid anion were synthesized and tested for their suitability for high-mass MALDI with respect to resolution and signal intensity.
We were able to measure biomolecules with masses exceeding 500 kDa and therefore it was possible to extend the range of MALDI accessible by ionic liquids.

[1] S. Carda-Broch, A. Berthod, D. W. Armstrong, *Rapid Commun. Mass Spectrom.*, **2003**, *17*, 553.
[2] J. A. Crank, D. W. Armstrong, *J. Am. Soc. Mass Spectrom.*, **2009**, *20*, 1790.

Analytical Chemistry **AC 49**

Comparison of different approaches and tools for studying small amount of natural products by NMR

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The bio-guided isolation of natural products often affords mass-limited samples. Their structural elucidation by NMR still remains a challenge in terms of analysis speed, quality of spectra and sensitivity [1]. The improvement of these aspects will enable NMR to be used as a tool for trace chemical analysis [2]. To increase sensitivity, three main approaches are considered: upgrade of the magnetic field strength, thermal reduction of noise during detection (cryogenic probes, cryoprobes™), decrease of the coil diameter and increase of the filling factor of the receiver coil (capillary probes, CapNMR™).
In this study, the quality of 1D and 2D spectra recorded on a Varian Unity Inova 500 MHz equipped with a CapNMR probe (manual injection with a syringe or injection by an automatic liquid handler with OMNMR system) and on a Bruker Avance III 500 MHz spectrometer equipped with a DHC 5mm Cryoprobe, were compared. Quinine (100 µg) was used as reference compound. Moreover, different systems available on the market (shigemi tubes, micro tubes with capillaries and MATCH™ system) for the minimization of sample volume were tested and the results were further discussed. Practical aspects related to the isolation of natural products at the microgram in terms of purification strategy and sample handlings were also detailed.

[1] Lambert M., Wolfender J.-L. *et al*, *Anal. Chem*. **2007**, *79*, 727.
[2] Schroeder F.C. and Gronquist M., *Angew. Chem. Int.* Ed. **2006**, *45*, 7122.

Analytical Chemistry **AC 50**

The Role of Isotopic Fine Structures in Protein Identification by High Resolution Mass Spectrometry

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Mass spectrometry is a key method for protein identification in proteomics [1]. The common protein identification procedure is based on tandem mass spectrometry (MS/MS) of proteolytic peptides derived from the protein enzymatically. However, modern MS/MS analysis alone is insufficient to identify peptides unambiguously [2]. Therefore, additional complementary techniques are being developed to improve identification consistency. These techniques are based on accurate mass, retention time, or isotopic abundance measurements. Nowadays, state-of-the-art high resolution mass spectrometry allows measurements of relative abundances of "isotopic fine structures" of peptides, i.e. species with the same elemental composition, different isotopic composition, but the same number of neutrons and therefore the same mass number. Here, we investigate the potential benefits of having peptide isotopic fine structure information for improved peptide and protein structural analysis. The experiments were performed on a hybrid high-performance ESI LTQ FT-ICR MS instrument (Thermo Scientific, Bremen, Germany) equipped with a 10 T super-conducting magnet. Peptides were obtained from Sigma-Aldrich and Bachem AG or synthesized in-house and used without further purification. The Python-based software was developed to perform computational analysis and deal with experimental data. Potential of the technique was computationally estimated. Its performance was computationally and experimentally characterized. For the technique to be effective required abundance measurement accuracy was obtained.

[1] R. Aebersold., M. Mann, *Nature*, **2003**, *422*, 198.
[2] R.A. Zubarev *et al.*, *J. Am. Soc. Mass Spectrom.*, **2008**, *19*, 753.

Analytical Chemistry **AC 51**

Quantification of long-chain fatty acids with GC-MS

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Fatty acids are ubiquitous molecules which are present in all organisms in high concentrations. They are mostly present in esterified form and are part of different lipids. Due to their chemical structure they undergo different chemical reactions.
Fatty acids are mainly analysed by gas chromatography coupled to mass spectrometry (GC-MS) because of its high resolution power and good sensitivity. In order to be able to analyse the different fatty acids in different samples materials (blood, animal tissues) a GC-MS method has been developed for the simultaneous quantification of 11 long-chain fatty acids and has been validated in EDTA-plasma.
50 µl of EDTA plasma have first been subjected to saponification, then the total fatty acids were derivatized to methyl esters and extracted with n-hexane:t-butyl methyl ether (1:1, v/v). The total fatty acids have been analysed with GC-MS in full scan mode from *m/z* 40 to 600 with a scan time of 0.56s on a non-polar fused silica column. The compounds were identified by comparison of the respective retention times and the mass spectra with a commercially available standards and electronic library (NIST).
The method validation resulted in good precision an accuracy data over the whole analytical range. The stability experiments demonstrated that the addition of butylated hydroxy toluene (BHT) already in the sample tube was necessary to prevent the samples from oxidative degradation
In conclusion, the GC-MS method is sensitive, precise and accurate to detect 11 different fatty acids in EDTA plasma and can be applied as well in other sample materials.

Analytical Chemistry **AC 52**

Online patient monitoring for opiates by secondary electrospray ionization mass spectrometry

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Patient monitoring by breath analysis for opiates such as morphine is a challenge for modern analytics. The concentrations of the analytes are very low and the sampling is delicate. We introduce a setup to monitor breath of patients during surgery by mass spectrometry. The method, based on secondary electrospray ionization [1,2], even allows to quantify small amounts of opiates such as morphine (about 0.3 ng/L). The setup we published recently [3] was developed further to work in the situation of an artificially ventilated patient. A first animal experiment carried out last year (not published yet) showed that the blood concentration of morphine drops significantly after it passed the lungs. We used our mobile, online configuration as well as classical offline technics (such as Tedlar bags) in this experiment. However, the concentration measured was lower than 0.1ng/L (in breath). Therefore it was below the detection limit of our online system. We have further optimized the sample introduction and transport to get lower limits of detection and well defined sample volumes and flows. Experiments with a breathing machine (artificial breath) show how to sample breath more quantitatively and reproducibly. Especially for morphine, the sampling and ionization showed to be the most critical steps. Animal experiments with the new setup will also be shown.

[1] Ding, J. H., S. P. Yang, et al. (2009). Analyst 134 (10) 2040-2050.
[2] Dillon, L. A., V. N. Stone, et al. (2010) Analyst 135(2): 306-314.
[3] Berchtold, C., L. Meier, et al. (2011). IJMS 299 (2-3) 145-150

Analytical Chemistry **AC 53**

Molecular Processes in an Atmospheric Pressure Non-Thermal Plasma Driven Air Purifier

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Plasma air purifying systems that are becoming commercially available are an interesting alternative for cleaning air compared to the commonly used high efficiency particulate air filters. Such a plasma air purifier should decompose all contaminants (even viruses) to harmless degradation products.

In the present study, a non-thermal plasma based air purifying system with very low power consumption was tested. A new interface was designed to directly connect the exhaust of the air purifying system with a mass spectrometer. The ionization of the compounds studied was either accomplished using the plasma air purifier itself or with a miniaturized atmospheric-pressure afterglow ion source. The plasma was generated with an high voltage AC power supply, which can be manually adjusted up to a voltage of 9.5 kV. A commercial mass spectrometer (LCQ Deca, Thermo Finnigan, San Jose, USA) equipped with a self-made elongated inlet cone was used for the analysis.

Hexyl-, octyl- dipenthyl- and dibutylamine were used as model compounds to characterize the air purifying system. Surprisingly, no decomposition fragments of the studied substances could be seen. Instead of degradation to smaller molecules, larger molecules were observed in the exhaust of the air purifying system. The influence of the high voltage applied by the air purifying system on the four different compounds was studied. The highest voltage used produced polymer-like mass spectra for the primary amines as well as for the secondary amine. Several side chain oxidation products were observed for the amines, most likely due to OH radical reactions.

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A simple measure to monitor the progress of chemical cross-linking of protein-protein noncovalent interactions

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Chemical cross-linking in combination with Matrix-Assisted Laser Desorption/Ionization mass spectrometry (MALDI-MS) has emerged as a powerful tool to study noncovalent protein complexes. [1] Due to their high reactivity towards primary amines, N-hydroxysuccinimide esters are the most commonly applied reactive groups. Simultaneously, the cross-linker is hydrolyzed by water. Although the underlying amidation has a fairly high reaction rate, careful optimization of reaction times has to be undertaken to guarantee maximum yields of stabilized complexes, avoid degradation of delicate sample materials and minimize peak broadening due to excessive "decoration" of the protein surface. Consequently, a tool monitoring the extent of the cross-linking reaction could avoid time-consuming optimization procedures. This study focused on the pH value of the reaction mixture as a monitoring tool. First, pH changes occurring during hydrolysis of the cross-linker at different pH values were investigated and compared with mass spectrometric hydrolysis data. Subsequently, the progress of the cross-linking reaction of several protein complexes was monitored by MALDI-MS. Bis(sulfosuccinimidyl) suberate (BS^3), was selected as a cross-linker due to its water solubility and thus simplified handling for MS sample preparation. A low buffer concentration of 10 mM, and thus a low buffer capacity, was chosen in order to be able to monitor changes of the solution composition by measuring the pH value. Both pH measurements and mass spectrometric detection indicated a complete hydrolysis and cross-linking reaction after equivalent reaction times. Thus, we recommend using the pH to monitor the extent of chemical cross-linking reactions.

[1] A. Sinz, *Mass Spectrom. Rev.* **2006**, 25, 663.

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Laser Ablation Using Diode Pumped Solid State Lasers

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) allows for trace element quantification of solid samples with limits of detection in the sub-ppm range. In order to enable LA-ICPMS for samples outside the lab, e.g. for archeological samples or museum objects, an offline laser ablation sampling strategy was developed. Diode pumped solid state (DPSS) lasers promise to be suitable for preliminary removal of solid materials as they are compact, air-cooled and provide sufficient pulse energies of up to a few mJ per pulse. Additionally, DPSS lasers allow for selecting repetition rates from Hz up to the MHz range.

The ablation of opaque glass and metallic samples was performed using a DPSS laser at a wavelength of 532 nm with ablation frequencies ranging from 10 Hz up to 1 kHz. The produced aerosols were collected on membrane filters and were later quantified by reablation using LA-ICPMS. The sampling efficiency and elemental fractionation effects were shown to be widely independent of the ablation frequency. Using an ablation rate of 1 kHz allows sampling sufficient amounts of material within seconds for further offline quantification of major and trace elements with the performance similar to that of conventional LA-ICPMS. The experimental design and figures of merit of the sampling approach will be explained in detail.

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Analysis of small gas samples in sealed recipients with mass spectrometry using static gas dilution

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A sample preparation method is described enabling to analyse small amounts of gases in sealed recipients using vacuum extraction and static dilution with ultra pure nitrogen. Combining trace gas analysis with ion-molecule reaction mass spectrometry (IMR-MS), high precision vapour pressure measurements and gas chromatography allowed determining the chemical composition of the impurities and gas quantities corresponding to partial pressures below 1 mbar. The equations for the dilution are established relating the measured amount of substance fractions in the sample mixture to their partial pressures in the sealed recipient and in the case of oxygen to the air pressure. The method was applied for the quality control of liquefied pressurized gas (LPG) used in implantable medical drug pumps. The precise dosage of the drug requires that its pressure is independent of the filling volume of the reservoir. This is realised with a metal bellows wall surrounded by a sealed recipient with the LPG in its gas-liquid equilibrium state. At a given temperature its pressure is independent of the volume and is only determined by the chemical composition and the purity.
The developed preparation method, the dilution with ultra-pure nitrogen and the analytical methods can generally be used to measure most gaseous analytes down to trace levels even for ubiquitous substances like oxygen [1].

[1] www.euromet.ch, project 937, www.euramet.org/index.php?id=tc-mc-prjects&no_cache=1&ctcp_projects[cmd]=details&ctcp_projects[uid]=814.