doi:10.2533/chimia.2013.462 Chimia 67 (2013) 462–475 

Analytical Sciences **AS01**

## ESTASI

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In this lecture, we shall recall some electrochemical concepts underlying ionization methods for mass spectrometry such as MALDI and ESI.
Then, we shall present a novel contactless electrostatic spray method recently developed that can be used to spray from sample droplets deposited on an inert polymer substrate or from samples in a glass capillary. The gist of the method is to charge electrostatically the surface of the liquid until the electrostatic pressure overcomes the interfacial tension to emit charged droplets.

With this method, we shall show how to spray proteins or peptides being separated in a pH gradient gel by isoelectric focusing. This approach provides a direct mass spectrometry readout of IEF gels.

Then, we shall show how it is possible to spray from spy-holes in microfluidic chips where aqueous solutions are being processed. This concept is further applied to droplet-fluidic reactors.

[1] Liang Qiao, Romain Sartor, Natalia Gasilova, Yu Lu, Elena Tobolkina, Baohong Liu, and Hubert H Girault, *Anal. Chem,.* **2012**, *84*, 7422.
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Analytical Sciences **AS02**

## Evaluation of Standards for Quantitative Tissue Imaging by LA-ICP-MS

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Over the last few years, imaging of soft tissues to determine the spatially resolved concentrations or isotope ratios has emerged since the first successful laser ablation of soft tissues. [1] Recently a second direction, apart from the determination of the element concentration has called attention: quantitative immunoassay using element labeled antibodies. [2] With this technique multiple antigens can be marked on the same tissue sample. By combining the high spatial resolution of the laser ablation set-up connected to the high sensitivity of an ICP-MS this allows efficient multiplexing to sample the current status of cells within a tissue section.

Quantitative LA-ICP-MS relies on suitable internal and external standards; internal standards to correct for drift of the instrument, sensitivity differences and differences of the samples and external standards for assessing the concentration. This work investigates suitable internal standards for tissue imaging to correct for ablated mass, transport and ionisation in the ICP. Additionally a comparative approach for the quantification using external standards will be shown.

[1] P. Ek, presented in part at 1998 Winter Conference on Plasma Spectrochemistry, Scottsdale, AZ, USA, 05 - 10 January, **1998**.
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Analytical Sciences **AS03**

## Mass Spectrometry Based Disulfide Assignment in Venom Proteins

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Disulfides play a crucial role in protein structure and function. Their characterization in therapeutic protein production is necessary to ensure active pharmaceutical ingredient (API) safety and efficacy. Mass spectrometry (MS) can be used to characterize disulfide bridges in proteins to determine both the number and the connectivity of disulfides.[1] Venoms contain many disulfide-rich proteins that are potential drug candidates.[2] However, MS based disulfide assignment is challenging for venom proteins due to their knotted disulfide structure. Here we demonstrate determination of disulfide number and connectivity in Ribonuclease A and Insulin using techniques including chemical cleavage, partial reduction, pepsin digestion and collision induced dissociation. Furthermore, we show the application of techniques in MS to disulfide assignment in the three-finger toxin, Mambin.

[1] Jeffrey J. Gorman, Tristan P. Wallis, James J. Pitt, *Mass Spec. Revs.* **2002**, *21*, 183.
[2] R. Manjunatha Kini, *Toxicon* **2010**, *56*, 855.

Analytical Sciences **AS04**

## Development of an online solid phase extraction system using polyetheretherketone tubing coupled to ion trap tandem mass spectrometry

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Effective absorption of hydroxylated polyaromatic hydrocarbons to polyetheretherketone (PEEK) tubing has been recently reported, and based on this, an online solid phase extraction (SPE) system using PEEK tubing for pretreating urinary 1-hydroxypyrene (1-OHPyr) was developed (Fig. 1).

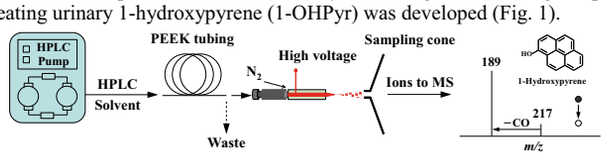


Fig. 1 Schematic diagram of an online SPE system using PEEK tubing coupled to ion trap tandem mass spectrometry (MS/MS).

Effects of HPLC flow rate and PEEK tubing i.d. were studied (Fig. 2) together with compound concentration, injection volume and system stability, demonstrating good analytical performance of the online PEEK-SPE system.

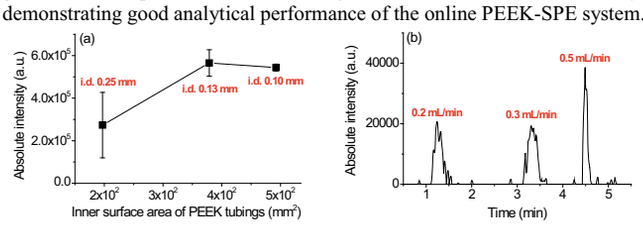


Fig. 2 Effects of PEEK tubing i.d. and HPLC solvent flow rate on absorption and desorption of 1-OHPyr to PEEK tubing.

[1] X. Li, R. Zenobi, *Anal. Chem.* **2013**, *85*, 3526.

Analytical Sciences **AS05**

**Microwave Plasma – Atomic Emission Spectroscopy: a novel technology for the analysis of impurities in gold**

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With the steep increase in the prize of gold in recent years a decrease in the quality of raw materials has been observed in the gold refining industry. Specifically, high contents of toxic elements (*e.g.* As, Cd and Se) are a serious health and environment concern. This increases the need for constant development of analytical methods.

Microwave Plasma – Atomic Emission Spectroscopy (MP-AES) is a novel technology for elemental analysis. The nitrogen plasma is an environmentally friendly alternative to currently used techniques (Inductively Coupled Plasma – Optical Emissions Spectroscopy and Atomic Absorption Spectroscopy), eliminating the need for expensive and flammable gasses. Furthermore, nitrogen can be generated locally from atmospheric air, avoiding logistical problems of supplying gas cylinders to mines in remote locations.

We have used MP-AES to analyse the contents of 15 elements in samples from all over the world. The results were compared with values previously determined with ICP-OES and found to be in excellent agreement. Moreover, the linear range of the signal over several orders of magnitude reduces the need for repeated analyses of solutions with different dilutions.

Analytical Sciences **AS06**

**Structural analysis of monoclonal antibodies by top-down and middle-down mass spectrometry**

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Comprehensive structure analysis of antibodies, and specifically immunoglobulins G (IgGs), is indispensable due to their leading role as biotherapeutics. We recently presented encouraging results obtained by high-resolution Orbitrap Fourier transform (FT) mass spectrometry (MS) in characterizing IgGs in a top-down fashion, i.e. with gas-phase fragmentation of the intact ~150 kDa proteins by electron transfer dissociation (ETD). With this technique ~32% sequence coverage was reached, but with incomplete sequencing of complementarity determining regions (CDRs), responsible for the IgG affinity and specificity. We thus applied a middle-down approach, fragmenting IgGs in solution by proteolytic cleavage with IdeS (immunoglobulin G-degrading enzyme of *Streptococcus pyogenes*), and reducing disulfide bonds. The resulting three ~25 kDa cysteine-reduced fragments (Fd, Fc/2, and light chain) were separated by reverse-phase chromatography (C4 column) and subjected to ETD tandem MS (MS/MS). Contrary to conventional approaches, original transient signals from separate chromatographic runs were acquired, summed, and processed using FT or in-house implemented filter diagonalization method (FDM). Thus, the overall quality of the experimental data was substantially improved. MS/MS spectra were analyzed with commercial software and validated manually. The combination of highly specific digestion and reduction of disulfide bonds allowed the MS characterization of variable domains of both the light and heavy chains, including the CDR2 region. Developed LC-MS/MS-methods are of direct interest for quality control of monoclonal antibodies in pharmaceutical industry, as well as for further method development toward MS-based structure analysis of mixtures of antibodies and antibody-drug conjugates in drug discovery.

Analytical Chemistry **AS07**

**Advances in azide-alkyne cycloaddition (click-chemistry) based manufacturing of molecular diagnostic peptide microarrays**

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Rapid and reliable functionalizations of solid surfaces are prerequisites for the efficient manufacturing of peptide microarrays for in-vitro diagnostic (IVD) applications. When working with biological samples such as serum non-specific binding (NSB) of complex matrix components to the spotted peptide probes and/or surrounding area must be limited in order to attain good analytical performances.

Here we present results on binding kinetics of various azide derivatized peptides to aza-dibenzocyclooctyne (ADIBO) activated surfaces based on copper-free click chemistry. Furthermore specific antibody target molecule capturing efficiencies were investigated and analytical sensitivities compared to standard microtiter plate based inverse ELISA formats.

A selection of azide-tagged "blocking reagents" of protein, polysaccharide, polyethylenglycol (PEG) and aromatic compound origin were tested for their efficacy in preventing detrimental NSB when working with serum. In fact, one of the blocking reagents revealed an exceptionally strong capacity to inhibit serum components to interact with the microarray surface.

To conclude, compatibility of reagent aspects such as reactivities, sensitivities, reproducibilities and blocking efficiencies with an automated nano-liter scale microarray spotting instrument were demonstrated with c-myc and BRCA-associated ring domain protein 1 (BARD1) cancer diagnostic model assays.

Analytical Sciences **AS08**

**Fluorescence Resonance Energy Transfer of Gas-Phase Ions under Ambient Conditions**

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The use of mass spectrometry to study biomolecules in the gas phase has been greatly enhanced due to development of soft ionization methods such as MALDI and ESI. However, the link between the structures and conformation of biomolecules in solution and in the gas phase remains unknown. Previously, Fluorescence Resonance Energy Transfer (FRET) has been used to probe conformations of gaseous polyproline-based peptides inside a quadrupole ion trap mass spectrometer. This opened a promising route to probe the conformations of large gaseous ions. Nevertheless, it requires extensive instrument modifications, and the fluorescence intensity depends on the number of ions trapped inside the mass spectrometer. In the present study, we employed a laser-induced fluorescence technique directly in an ESI plume to monitor the FRET dynamics of isolated ions as the solvent molecules are removed from the system. This study opens up a route for direct correlation between the structures and conformation of biomolecular ion in the gas phase and in solution without the need of ion trapping and complex instrumentation.

Analytical Sciences **AS09**

**Utility of chemical agents for high-throughput protein characterization**

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Mass spectrometry (MS)-based proteomics, or high-throughput characterization of proteins constitutes a comprehensive analysis of proteomes of diverse organisms. Conventional MS-based proteomics relies on protein enzymatic digestion into short, 0.6-3 kDa, peptides prior to MS analysis. Recent advances in MS instrumentation provide the necessary platform for investigating increasingly longer (3-7 kDa) peptides in a high-throughput manner. Herein, we describe a directed sample preparation workflow using chemical cleavage, as opposed to the enzymatic digestion. Chemical cleavage allows targeting of rare amino-acids, for which no proteolytic enzymes exist. As a result, the average size distribution of the generated peptides could be increased. With this approach we aim to improve high-throughput proteome characterization. The average size distribution of peptides, efficiency of peptide fragmentation methods and the probability of identifying post-translational modifications were investigated using a commercial 48 protein mixture (UPS1, Sigma Aldrich). Consecutive Methionine (M) and Tryptophan (W) cleavages were performed with CNBr. BNPS-Skatole was used for selective cleavage at W both individually, and after partial CNBr cleavage of M. Hydroxylamine was used to achieve selective cleavage after Asparagine (N). Cleavage at N terminus of Cysteine (C) residues was performed with NTCB. Resulting peptides were desalted on C4 and C18 ZipTip cartridges (Millipore, Billerica, MA), and separated on a C8 nano-LC column. Eluted peptides were supercharged post-column with 0.5% m-NBA and analyzed using Orbitrap Elite ETD FTMS. Due to the long average peptide length, high mass resolution was used for both MS and MS/MS scanning (60,000 and 15,000 at *m/z* 400, respectively). Sequence coverage and protein identification scores were obtained using Sequest, Mascot, MS-Align+, OMSSA and X!Tandem database search algorithms and compared with results produced by classical MS-based proteomics approaches.

Analytical Sciences **AS10**

**A Comparison of NMR Predictors Using Binary Tree Similarity of Spectra**

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The rooted tree method for NMR spectra comparison developed recently by the authors[1] was applied to design a methodology for comparison and evaluation of NMR prediction algorithms. For each prediction algorithm, a matrix is constructed by evaluating the similarity between the experimental spectra of 1000 molecules (rows) and their corresponding predicted spectra (columns). A query of each experimental spectrum to the database of simulated spectra is performed and NMR predictors are ranked according to their Mean Reciprocal Rank (MRR). This methodology dispenses us with the need of assigning each signal with its nucleus, which makes the task of benchmarking NMR predictors less tedious and avoids any human mistakes. We used this approach to compare four popular NMR predictors (ACD labs, Modgraph, Chemdraw, Spinus) and found that ACD labs provides the best results by far (0.6 MRR for ACD, ~0.4 MRR for the other predictors). It is worth noting that the free Spinus platform's performance is comparable to that of the other commercial alternatives. These results are consistent with those obtained using the traditional methodology of comparing deviations on predicted shifts relative to the experimental ones.

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Analytical Sciences **AS11**

**Fossil fuel analysis via high resolution mass spectrometry.**

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Fossil fuels are complex mixtures of molecules with mostly $C_cH_hN_nO_oS_S$ elemental compositions. It is imperative to identify and quantify these molecules, as they affect the physical and chemical properties of the sample. For instance, napthenic acids can corrode refinery units; sulphur-containing compounds must be removed from fuels used in transport, to reduce environmental damage. Finally, metal porphyrins found in medium and heavy crude oils degrade catalysts used in cracking and can form corrosive species. As there are many molecules with very small differences in masses, oftentimes less than 1.1 mDa, high resolution and high mass accuracy mass spectrometers are required. We have thus developed an analytical platform, which for the first time successfully employs the Orbitrap Elite Fourier transform mass spectrometer, to analyze complex fractions of fossil fuels. The platform provides advantages in comparison with other approaches and introduces several new key features, including a recalibration method which provides the required mass accuracy, and a data visualization method for sample fingerprinting and comparison. It has been tested on resin and maltene fractions of crude oil. Results demonstrate that the platform currently allows for in-depth analysis of polar components of crude oil fractions of low to medium complexity.

Analytical Sciences **AS12**

**Analysis of acidic compounds by CE in negative ESI-MS with organic solvents**

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Non-Aqueous Capillary Electrophoresis (NACE) is an attractive CE mode that consists in replacing the water of the background electrolyte (BGE) by organic solvents. This substitution alters several parameters such as pKa, dielectric constant, viscosity, zeta potential and conductivity resulting in modification of CE separation performance. In addition, the use of NACE is particularly well adapted to ESI-MS due to the high volatility of solvent and low generated currents. Organic solvents also permit to reduce the number of side electrochemical reactions at the ESI tip allowing a stabilization of ESI current and a decrease of background noise. All these features make the NACE an interesting alternative to the classical CZE mode especially to improve selectivity, sensitivity, and spray stability.
The aim of this work was to evaluate the use of NACE in negative ESI-MS for the analysis of acidic compounds with two interfaces (sheath liquid and sheathless). In a first attempt, the NACE mode was compared to the classical CZE mode for the analysis of pharmaceutical acidic compounds (NSAIDs). Then the sensitivity and separation performance achieved by both interfaces were evaluated as well as the impact of BGE and sample composition. Finally, analyses on glucuronides in urine samples were performed in order to evaluate qualitatively and quantitatively the matrix effects.

Analytical Chemistry **AS13**

## $^{14}C$ analysis of carbonates at high spatial resolution by the coupling of Laser Ablation with AMS

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$^{14}C$ is an important isotope for dating carbonates such as speleothems, corals and shells [1]. Measurements of chemically processed and graphitized samples are laborious and the achievable spatial resolution is limited. By using Laser Ablation (LA) as a sampling method rapid $^{14}C/^{12}C$ analysis can be performed at high spatial resolution. Here, $CO_2$ is generated by material decomposition upon exposure of focused high intensity laser pulses and can directly be introduced into the gas ion source of an Accelerator Mass Spectrometer (AMS) [2]. For the direct coupling of LA with AMS a LA unit was developed consisting of an ablation cell (effective volume of approximately 0.6 mL) that combines a relatively short washout time with minimal particle deposition on the cell window and walls. This specific design leads to short measurement times and also reduces cross-contamination. Furthermore, large samples (150 x 25 x 15 $mm^3$) can be hosted by the cell and moved by a positioning system at high spatial resolution relative to the laser beam. An ArF-Excimer Laser ($\lambda$ = 193 nm) is guided to the sample surface, allowing for ablation of e.g. carbonates at a scale of less than 100 µm. Simultaneous observation of the sample and the ablation process is possible. First results on the $CO_2$ production rate on carbonates during LA and first $^{14}C$ measurements will be shown.

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Analytical Sciences **AS14**

## Fluorescence Sensing of Spermine

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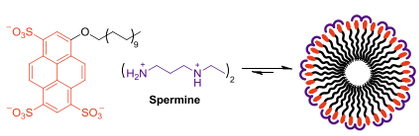
Spermine is a natural polyamine that is found in eukaryotic cells and body fluids. Monitoring spermine concentrations in urine was proposed as a tool for early cancer diagnosis.[1] Optical methods based on fluorescence are interesting alternatives because measurements are fast and easy to perform. Here, we describe a conceptually new chemosensor for spermine.[2] The sensor is based on a charge-frustrated amphiphile with a highly fluorescent head group. Analyte-induced aggregation results in pronounced fluorescence quenching. The sensitivity and selectivity of our sensor is significantly better than what has been described previously for optical spermine chemosensors. We are able to detect of spermine down to the low nanomolar concentration range. Furthermore, our system displays a very good selectivity over spermidine and other biological relevant amines. Preliminary tests with artificial urine are evidence that our sensor can be used in a complex matrix.

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This work was supported by the Swiss National Science Foundation and by the EPFL.

Analytical Sciences **AS15**

## Rapid and sensitive analysis of proteins by labeling with 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ), separation with CE-SDS and LIF detection

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Laser induced fluorescence (LIF) detection after analyte derivatization with a fluorescent tag provides ultimate high detection sensitivity for proteins separated by capillary electrophoresis-sodium dodecyl sulfate (CE-SDS). However, often the process is not quantitative and is time consuming, particularly as subsequent protein purification is necessary to isolate the protein from non reacted fluorescent reagent, as is the case with the well known fluorophore TAMRA. Additionally, TAMRA has an excitation maximum at 550 nm, thus not providing maximum sensitivity using the 488 nm argon ion laser commonly employed with CE-LIF [1].

We will discuss derivatization of proteins with 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ) for CE-SDS-LIF analysis. FQ reacts very quickly with primary amines of proteins to form a highly fluorescent product that is exited at 488 nm using the conventional argon ion laser. Non reacted reagent does not fluoresce and does not require removal [2]. Quantitative aspects of the reaction are investigated and reaction conditions are optimized. Detection of recombinant proteins produced at low levels in Chinese Hamster Ovary cell lines in bioreactors is discussed. Using FQ-labeling with CE-SDS-LIF at 488 nm limits of quantification (LOQ) <10 ng/ml are achieved.

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Analytical Sciences **AS16**

## Particle Number Concentration determined by ICP-MS

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Due to their unique properties nanoparticles are used in many different fields of application. In order to assess possible risks of nanoparticles on environment and human health, they need to be carefully characterized. Therefore, their most important properties (mass/size, elemental composition, morphology and particle number concentration) must be described. However, different complementary techniques have to be considered to obtain all these information.
Recent studies have shown that it is possible to obtain mass/size and elemental composition simultaneously using a microdroplet generation inductively coupled plasma mass spectrometer (MDG-ICP-MS)[1-3].
This work focuses on the evaluation of particle number concentration of silver nanoparticle suspensions with different nanoparticle sizes (20 nm, 60 nm, 80 nm, 100 nm) at different initial concentrations as well as different storage times comparing two techniques: single particle (sp)-ICP-MS[4] and MDG-ICP-MS.

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Analytical Chemistry **AS17**

**Mass Discrimination in High-Mass MALDI-MS**

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When complex mixtures of high-mass biomolecules such as DNA, peptides, proteins, or synthetic polymers are investigated, qualitative as well as quantitative information is of interest. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is one of the methods for investigating such mixtures. Determination of the ratio of the individual constituents is crucial. Previous studies showed that mass bias is partially caused by the microchannel plate (MCP) detectors used [1]. However, recently we have shown that high-mass molecules do not yield the same response over the whole *m/z* range when special high-mass detectors, such as ion conversion dynode (ICD) detectors, are used [2]. In this work the relative response for proteins with molecular weights from 35.9–129.9 kDa are determined for a wide range of molar ratios and factors influencing the response are discussed.

We found that the mass discrimination is less pronounced when using an ICD detector, compared to the use of conventional MCP detectors. The response was found to depend on the laser power used for MALDI; low-mass ions are discriminated against with higher laser power. The effect of mutual ion suppression as a function of the proteins used and their molar ratio is also shown: mixtures consisting of the same proteins that only differ in mass show less discrimination than mixtures consisting of different proteins with similar masses. Furthermore, mass discrimination dramatically increases for molar ratios far from 1. Finally, we present clear guidelines to choose the experimental parameters such that the measured response matches the actual molar fraction as closely as possible.

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Analytical Sciences **AS18**

**Ion mobility Spectrometry coupled to Laser-Induced Fluorescence**

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Laser-Induced Fluorescence (LIF) is a well-known spectroscopic method which is widely applied for sensitive probing of the structure of molecules due to its high specificity to the microenvironment, and to structural details. In order to enable analysis by optical spectroscopy, non-fluorescent biomolecules can be conjugated to fluorescent tags, which are small molecules with specified optical properties. Both LIF spectroscopy and Differential mobility analyzers (DMAs) have been extensively used for characterization of different compounds, but usually separately.

We report on an improved design of DMA coupled with LIF, to simultaneously retrieve two-dimensional information on the electrical mobility and fluorescence spectroscopy of gas-phase ions. This enhanced design includes an ion funnel interface at the input orifice of the DMA and a nozzle beam stage at the output of the DMA. These improvements allow detecting fluorescence not only from pure dyes and their clusters, as was demonstrated recently, but also from fluorophor-tagged biomolecules. Complex mixtures of fluorescent compounds can be separated by the DMA and studied by LIF. A FRET experiment with a BODIPY-cRh6G complex demonstrates the new capabilities of the instrument. This unique instrument combination also provides a powerful platform for probing fluorescent proteins in the gas phase. Green fluorescent protein (GFP) was tested on a new setup. In contrast to the high vacuum, where no GFP fluorescence has been detected, the presence of a LIF signal at the output of DMA could explain some specific fluorescent properties of GFP in the gas phase. Given that both conformation and fluorescence are key properties of biological molecules in the gas phase, we expect that our enhanced design will answer the question whether or not gas-phase proteins retain their liquid-phase native structure.

Analytical Sciences **AS19**

**In-silico screening for metabolite interferences in quantitative LC-SRM/MS**

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The validation of LC-SRM/MS methods in quantitative bioanalysis requires a screening for potential interferences caused by coeluting comedications or their metabolites. The limitations of current approaches have been addressed by a previously published screening strategy for comedications based on predicted LC retention times and MS precursor interferences [1].

The screening strategy has been extended from only comedications to include their most likely metabolites. The elution order of hydroxilated verapamil metabolite isomers could be predicted correctly based on their log D values if the new consensus algorithm of ACD Labs 2012 was applied.

Retention times were predicted based on a multiple linear regression between measured retention times for a set of 25 standards, the log D and additional molecular descriptors. Good accuracy has been achieved. The set of standards will be extended to several hundred compounds and its accuracy investigated in detail with the leave one out (LOO) approach.

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Analytical Sciences **AS20**

**Combining Demultiplexing and Label-free Quantification for High-resolution Data-independent Acquisition LC-MS/MS Analyses**

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There are several advantages of data-independent acquisition (DIA) over data-dependent acquisition (DDA) schemes for proteomic analyses of complex peptide mixtures (e.g. no bias toward high abundant peptides and reproducibility). One of these DIA strategies is SWATH, where a resolving Q1 window (e.g. 20-30u) is stepped repeatedly across a mass range and high-resolution MS/MS spectra are generated for all precursor ions within the Q1 window (multiplexed spectra).

While there are available tools to quantify SWATH data in a targeted manner [1], they are limited tools for simplifying MS/MS spectra (demultiplexing) including the corresponding precursor ions with high mass accuracy that can be effectively used for peptide sequence identification as well as simultaneous label-free quantification, which states the objective of this work.

The current efforts are concentrated to develop a model for precursor ion assignment based in the identification and correlation between the residual precursor ion in the MS/MS spectra and the precursor ion in the MS spectra added at the beginning of each acquisition cycle.

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Analytical Sciences **AS21**

**Selectivity and sensitivity evaluation in LC-SRM-based peptide quantification by using MRM3 or differential ion mobility spectrometry approaches**

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Selected reaction monitoring (SRM) in conjunction with stable isotope dilution has been employed as a standard workflow for peptides quantification in mass spectrometry. Generally to assess the accuracy of the measurement several transitions are recorded which increases the complexity and decrease the sensitivity of the assay. This is particularly critical for the quantification of low abundant proteins in complex biological samples.

This study attempts to evaluate the pros and cons regarding selectivity and sensitivity of alternative approaches. In particular, multiple reactions monitoring cubed (MRM³) and differential ion mobility mass spectrometry (DMS) were evaluated for quantitative peptides LC-MS analysis in Buffy coats proteins extracts. The selectivity and/or sensitivity were assessed based on different aspects such as peptide signal, matrix background level, ion transmission loss, and resolving power separation.

Analytical Sciences **AS22**

**MALDI Microarrays for rapid absolute quantification and single-cell analysis**

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We present a new sample application method for matrix-assisted laser desorption/ionization (MALDI). It is comprised of a microarray chip made of either glass or metal that contains hydrophilic spots with 100 or 200 $\mu$m diameter in an hydrophobic coating created by laser machining of a polysilazane coating. Nanoliter volumes can be reproducibly aliquoted by moving a liquid sample across the array. Single cells can be isolated by dragging a cell suspension across the array with a statistical number of single cells staying behind hydrophilic spots. After application of a MALDI matrix, the sample can be analyzed.

We have successfully applied this system for the detection of primary metabolites (e.g. phosphoenolpyruvate, adenosine triphosphate and uridine diphosphate glucose) from various unicellular organisms such as S. Cerevesiae (Baker's yeast) and different algal species. When using transparent targets we were able to measure the same single algal cells of H. Pluvialis cells both with Raman microscopy and MALDI mass spectrometry.

Recently, we have also started to apply the self-aliquoting targets for quantitative MALDI analysis. Two distinct advantages exist with our target. First, inhomogeneous matrix/analyte crystallization is not of consequence since the small spot size allows for the ablation of the whole reservoir. Second, a high number of repeats (10-20 per spot) that are possible with each sample deposition within seconds increase the statistical relevance and reliability. Calibration curves for a peptide mix showed coefficients of variation of 0.98 or better without internal standard. This is remarkable since MALDI is usually not used for quantitation due to high spatial signal fluctuation.

Analytical Sciences **AS23**

**Increasing peptide identification by mass spectrometry-friendly sample preparation and enhanced LC-MS/MS acquisition**

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In proteomics, LC-MS/MS analysis of peptides is generally performed using a data-dependent acquisition (DDA) approach to determine structural information based on the peptide precursor ion selected for fragmentation. However, the nature of this MS/MS spectra acquisition creates a sampling bias against those relatively less abundant peptides in complex matrices.

In order to yield a high proteome coverage and sample throughput for analyzing human primary monocyte derived dendritic cells (MDDCs) protein extracts, we optimized and evaluated a MS-friendly sample preparation procedure which resulted in a more than 10% increase of identified proteins. The performance of three DDA MS/MS acquisition approaches, namely: i) general technical replicates acquisition; ii) enhanced acquisition with exclusion/inclusion lists; and iii) technical replicates acquisition with extended LC separation, were also compared with the common aim to improve the number of peptide identification in MS analysis.

Analytical Sciences **AS24**

Determination of binding constants of aptamer-ligand complexes using electrospray ionization mass spectrometry

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The study of the interaction of different ligands with nucleic acids is an exciting area in modern molecular biology. A new class of molecular affinity probes called “aptamers” has gained great importance recently. Aptamers are single-stranded oligonucleotides, which can bind to other molecules with very high affinity and specificity. Despite the great promise of aptamers, very few studies exists on aptamer-ligand interactions. Most of them were carried out by optical spectroscopy techniques. Development of label-free MS methods for studying aptamer-ligand interactions will therefore be of great value. In this study we use ESI-MS and MALDI-MS to investigate aptamer-small molecule ligand and aptamer protein interactions. ESI-MS experiments were conducted with an adenosine aptamer. ESI mass spectra of only aptamer solutions resulted in ions at $m/z$ 1413 and 1696, corresponding to the −6 and −5 charge states. Upon addition of varying concentrations of adenosine, 1: 1 and 1: 2 aptamer/ligand complexes at $m/z$ 1750 and 1803 were observed. We have found that optimal instrumental conditions to observe adenosine aptamer and and adenosine complexes were: cone voltage, 0.8 kV; sampling cone voltage 30 kV, source offset 30 kV. The concentration of ammonium acetate buffer was found to be very critical to maintain aptamer-ligand interactions in nondenaturing conditions by ESI with 50 mM $NH_4Ac$ being the optimum value. Upon increasing the $NH_4Ac$ concentration from 50 mM to 100 mM, we have seen a decreasing trend in complex formation where no complex is seen at 100 mM. Addition of organic solvents helped to improve signal intensities of aptamer/ligand complexes. The dissociation constants ($K_d$) measured via nanoESI-MS were compared with solution phase ($K_d$) values.

Analytical Sciences **AS25**

**Differentiating Breast Cancer Cells vs. Normal Mammary Cells based on their volatile signature by Secondary Electrospray Ionization Mass Spectrometry**

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Recently, there has been increasing evidence for a relationship between volatile compounds released by human the body and cancers. Differentiating malignancy through its volatile metabolic signature along with identification of such volatile metabolic compounds may provide potential biomarkers for early and noninvasive diagnosis of malignant diseases [1].
We analyzed the volatile metabolic signature of three types of human breast cancer cell lines (T47D, SKBR-3, and MDA-MB-231) versus normal human mammary cells (HMLE). Volatile compounds in the headspace of conditioned culture medium were directly fingerprinted by secondary electrospray ionization-mass spectrometry. The mass spectra were subsequently treated statistically to identify discriminating features between normal vs. cancerous cell types.
Different samples were classified by using feature selection, followed by principal component analysis (PCA). In addition, high-resolution mass spectrometry and fragmentation of the most discriminant molecules can give some clue to their chemical structure.
Our study supports the hypothesis that cancerous cells release a characteristic odorous signature that may be used as disease markers.

[1] Catherine Oakman *et al., Int J Biochem Cell Biol,* **2011**, 43,1010– 1020.

Analytical Sciences **AS26**

**Binding of cancer drugs to higher-order nucleic acids**

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Higher-order nucleic acid structures like DNA quadruplexes have lately attracted increasing attention due to their proposed biological functions. DNA quadruplexes are involved in various processes of cell cycle control, including the regulation of telomere length and gene expression. These regulatory properties render DNA quadruplexes a promising target for organic and organometallic anti-tumor drugs.

Cisplatin is the cornerstone of modern cancer therapy. Its cytotoxicity is mostly attributed to the binding of adjacent guanine nucleobases, which results in 1,2-intrastand crosslinks in cellular DNA. Cisplatin was shown to influence the gas-phase dissociation of single- and double-stranded DNA as well as quadruplex DNA. The precise binding sites of cisplatin were determined by the means of high-resolution tandem mass spectrometry [1,2].

However, due to severe side effects of cisplatin, alternative organometallic compounds, including phenanthroline derivatives, ruthium-arene-complexes and multinuclear platinum compounds, are tested for future therapeutic application. The goal of this project is to elucidate the interaction of these drugs with higher-order DNA structures, to reveal the corresponding binding patterns, and to localize specific binding sites. Latest results on the DNA binding properties of (1,10-phenanthroline)-platinum(II) are presented.

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Analytical Sciences **AS27**

**Lipidomic Profiling of Algal Populations with Single Cell Resolution**

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Studying microbial populations at the single cell level is essential for understanding heterogeneity in biological systems. Their investigation can reveal a whole new level of information and improve our understanding of how microbial communities work [1]. We are currently developing a new approach for lipidomic profiling of microbial populations using a high-density microarray for matrix-assisted laser-desorption ionization – mass spectrometry (MALDI-MS). *Chlamydomonas reinhardtii* cells are dispersed on a chip consisting of a self-aliquoting microarray. Confocal fluorescence scanning locates the autofluorescent cells in the array. Thereby the fluorescence of each individual cell can be quantified. The MALDI-matrix is applied to the array using a non-contact spotting device. Using DHB in positive ion mode, high-resolution MALDI-FT-ICR measurements make it possible to detect most of the lipid classes contained in the algal membranes. Furthermore the photosynthetic pigments that are detected both by fluorescence microscopy and mass spectrometry can be used to cross-validate the two methods. The system developed shows the potential of MALDI-MS for high-throughput screening of populations.

[1] Stanislav S. Rubakhin, Eric J. Lanni, Jonathan V. Sweedler, Progress toward single cell metabolomics, *Curr. Op. Biotech.*, **2013**,24,1,95-104

Analytical Sciences **AS28**

**Analysis of nucleotide ratios from cellular metabolism using negative mode MALDI-MS**

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Single cell analysis (SCA) is becoming a technology more and more used in medical and industrial biotechnology. [1] Important areas such as diagnostics or cancer therapies increasingly call for SCA tools. Our mass spectrometric approach for SCA, which was first demonstrated in 2008, gives insights on the metabolomics level. [2]
We are interested in characterizing the energy charge in single cells and furthermore want to provide a direct and simple access to individual cellular responses. The use of matrix-assisted laser desorption ionization (MALDI) as a ionization method on our custom designed microarray allows high sensitive detection of the nucleotides in single mammalian cells. We investigate the use of 9-aminoacridine (9-AA) as a matrix in MALDI MS to characterize the ADP/ATP ratio in a biological system. 9-AA has few matrix peaks in the low mass region and is very sensitive to nucleotides (attomolar concentrations). However 9-AA tends to initiate the in-source decay of nucleotides to fragmentation products. The in-source fragmentation yield is influenced by general instrument settings (e.g. laser fluence, delayed extraction time).

**References**

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Analytical Sciences **AS29**

**Thin-film materials as reference samples for Tip-Enhanced Raman Spectroscopy**

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Tip-enhanced Raman Spectroscopy (TERS) is a very promising technique combining the electromagnetic enhancement of the Raman signal by a full metal or a metal-coated tip and the high spatial resolution of Scanning Probe Microscopy. It has the potential to become an irreplaceable tool for nanoscale spectroscopic analysis. However, TERS still has limitations: one of them is the the lack of reproducibility of the enhancement and of the spatial resolution of tips.
To overcome these limitations, there is a strong need for a reference sample allowing for a facile testing and comparison of tips. The ideal reference sample has to be a good Raman scatterer, chemically stable and mechanically resistant. We present here our first results on inorganic thin films such as diamond membranes fabricated by CVD and thin films of zinc oxide deposited by ALD, which are promising reference samples.

Analytical Sciences **AS30**

**Rapid and efficient large scale isolation strategy on MPLC-ELSD-UV using gradient transfer and enhanced pressure and temperature control**

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In natural product (NP) research, the isolation of biomarkers or bioactive compounds from complex natural extract is an essential step for *de novo* identification and bioactivity assessment. When pure NPs have to be obtained in milligram quantities, the chromatographic steps are generally laborious and time consuming. An efficient way consists in the transfer of optimised reversed phase gradient profiling conditions from analytical to semi-preparative HPLC [1]. In order to increase the sample loading, Medium Pressure LC (MPLC) represents an interesting alternative since grams of crude extract can be separated in only one step. The MPLC separations are however limited by the flow rate and pressure allowed on the glass column and typical separation are carried out over several days.
To overcome this problem, we have developed models for accurate gradient transfer from the HPLC to the MPLC scale and precise prediction of the separation of the extracts at the gram scale. A dry loading strategy adapted to most plant extracts that minimise pressure drop has been optimised. The speed of separation has been enhanced by maximising the flow rate at room temperature using a new way of controlling pressure at the column inlet. Furthermore, the pressure drop was minimised by designing a dedicated column oven for preparative separation and perform separations at up to 60°C.
The improvements of separation obtained are illustrated with the separation of three crude plant extracts, each containing different classes of molecules. In these different cases, a majority of pure NPs could be obtained in mg amounts in about one to two days.

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Analytical Chemistry **AS31**

**Laser induced breakdown detection ( LIBD) used as a sensitive nanoparticle detector to derive number based size distribution after Flow Field Flow Fractionation (AF4)**

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Nanoparticles are omnipresent in our day-to-day life. However, our knowledge about their impact and behavior is still insufficient and analytical techniques able to monitor that are of need. Only in 2011, the European Commission issued an official recommendation on a definition of nanoparticles [1]. As a consequence of the issued definition, techniques that are able to detect the number size distribution will become of great interest.
In our work we used a combination of LIBD and AF4 to determine the number based size distribution of polystyrene nanoparticles in liquid suspensions. As a first step, the experiments on the separation of mixture of nanoparticles were performed in a batch mode. This allowed us to test for the absence of aggregated nanoparticles in the AF4 channel by determining with LIBD sizes of nanoparticles. Afterwards the monodisperse and polydisperse suspensions were analyzed online. The LIBD system was calibrated based on the energy curves (or s-curves - the dependence of the breakdown probability on the laser pulse energy) recorded for different sizes and concentrations of polystyrene nanoparticles. Then as the results of the LIBD and AF4 coupling fractograms were obtained. Based on the information from LIBD and AF4 measurements we developed an algorithm converting the time resolved breakdown probabilities into particle concentrations. With the so developed AF4- LIBD system, size and number particle concentration of polystyrene nanoparticles could be determined, that meet the requirements of the definition of nanoparticles proposed by EU. Further investigation will focus on the other types of nanoparticles.

[1] Recommendation [2011/696/EU]

Analytical Sciences **AS32**

**Laser Ablation Mass Spectrometry with Ion Funnel for Trace Element Analysis in Solids.**

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Laser ablation mass spectrometry (LAMS) for elemental analysis of solids has been introduced several decades ago. The problems caused by matrix dependent ion yields however put a severe limitation before the broader acceptance of this technique. Another limitation of LAMS is the huge energy spread of the ions generated by laser ablation in vacuum.
In order to minimize the ion spread, Wang et al. used Helium at moderate pressure as buffer gas to reduce the kinetic energy of ions to improve the sensitivity especially for lighter ions when using electrostatic ion optics [1].
Another approach to thermalize ions from laser ablation is investigated in this study. An ion funnel operated at a pressure between 0.1 and 10 mbar is inserted between the ablation site and the electrostatic ion optics of a time of flight mass spectrometer (TOFMS). In contrast to previous configurations [2], this ion funnel does not require axial acceleration of the ions due to the specific gas flow dynamics inside the funnel itself. After the funnel ions exhibit a low kinetic energy spread and are more efficiently focused to the entrance aperture of the TOFMS. This simplifies the construction and operation of the funnel significantly.
First results investigating the dependence of ion transmission on operating parameters of the ion funnel as Rf-frequency and amplitude as well as buffer gas pressure will be discussed in this presentation.

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Analytical Sciences **AS33**

**Middle-Down Proteomic Analysis of Embryonic Proteins Secreted During the IVF Procedure**

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Mass spectrometry is a powerful technique capable of identifying thousands of proteins from complex biological samples. In bottom-up proteomics the proteins are digested in short, 6-20 residue long peptides, which are easier to separate and analyze. However, the technique is not ideal because of the very high number of peptides generated that often have uninformative sequences. As opposed to bottom-up, by producing mid-range (30-70 residues) peptides, the sample complexity is decreased and analysis of a single peptide can offer extensive sequence information and reliable protein identification. Although the need for this peptide size range has been recognized, the reproducible and facile production of these peptides is not trivial. We have comprehensively characterized the aspartic protease Sap9, and found that it is active in a broad pH range and maintains its activity at temperatures 15-45 °C. Peptides were separated on a nano-LC C8 column and analyzed with a state-of-the-art Orbitrap Elite ETD FTMS. 43 out of the 48 proteins in UPS1 commercial standard were identified. The same experimental conditions were applied to the analysis of secreted embryonic proteins from the culture medium. Although these proteins are expected to carry multiple phosphorylation sites, typical bottom up proteomics identified only a handful of proteins with low sequence coverage. Sap9 digestion resulted in enhanced sequence coverage of the embryonic proteins and a higher number of total proteins identified. In addition, several phosphorylation sites were reliably assigned. Therefore, we have developed a proteolytic method for protein characterization targeting mid-size peptides, and successfully applied it to solve a clinical research question.

Analytical Sciences **AS34**

**Detection of Aptamer-Protein Complexes using MALDI-MS**

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Aptamers are single stranded DNA or RNA oligonucleotides, which fold into unique three-dimensional shapes and form a binding pocket or allosteric site to accommodate their target molecules with high affinity and specificity. Although aptamers have been applied to achieve fast and sensitive detection of proteins, understanding the non-covalent interactions in protein-aptamer complexes by mass spectrometry is still a largely untouched field. Here, we presented the first report on protein-aptamer interactions by matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) . We investigated the effect of MALDI matrix, sample preparation method, laser energy and concentration of aptamer and proteins on the specific non-covalent complex detection. After a series of optimizations, 6-aza-2-thiothymine (ATT) and a modified sandwich sample preparation method provided optimum detection efficiency. By using optimized conditions, we were able to directly observe the complexes of thrombin and thrombin binding aptamer (TBA). We also confirmed that the complex observation is due to specific non-covalent interactions, rather than non-specific clusters formed in the MALDI plume. We further confirmed the capability of MALDI-MS to study protein-aptamer interactions by using lysozyme and its corresponding aptamer. The complex stability in MALDI strongly depends on the dissociation constant of the complexes in solution phase: the stronger thrombin-TBA29 complexes was observed to yield a larger fraction of signal at the mass of the intact complex than the weaker thrombin-TBA15 complex. This indicates that the non-covalent interaction strength in solution is reflected in the MALDI mass spectra.

Analytical Sciences **AS35**

**Low-volume sampling directly coupled to ESI-MS analysis using a Capillary Gap Sampler**

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Chip-based microfluidic devices coupled to ESI-MS have been commercially available for several years, but still have limitations with direct introduction of sample volumes < 1 µL as well as site specificity in sample pickup, which is both of high interest for biomedical applications. We present a "Capillary Gap Sampler" as a new, non-chip based microfluidic platform, which allows for robust and reproducible sampling of injection volumes of few nanoliters combined with direct ESI-MS analysis.
The core of the sampler consists of a liquid bridge of several nL formed within a µm-sized gap of two capillaries. The capillaries are enclosed within an air-tight chamber, which is held under a regulated overpressure of several millibars. One of the capillaries consists of stainless steel and acts directly as the ESI-MS interface. A solid pin is used for sample uptake and delivery. In combination with the liquid bridge as an "open" introduction system, this design minimizes sample adsorption/sticking on surfaces during infusion. The system has an internal volume of about 120 nL, which is appropriate for sample volumes of 10-12 nL, limiting sample dilution to a minimum. Further system characteristics are minimal peak widths of about 6 s, subfemtomole detection limits, and total injection cycle times < 12 s including pin washing and drying. Applications on peptide characterization after solid phase synthesis using single beads and bioassay monitoring demonstrate the potential of this new sampling tool for a fast and sequential analysis of samples which contain multiple analytes and are of limited sample amount.

Analytical Sciences **AS36**

**Influence of wet gas atmosphere during ablation of zircon on U/Pb ratios and resulting ages using LA-ICP-MS**

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U/Pb-based geochronology is one of the most frequently applied dating methods in the research field of geology for samples older than about 100 million years up to the oldest samples of more than 4 billion years. Zircon is the preferred mineral for this dating method, because U is incorporated in the crystal lattice during crystallization, whereas Pb is mainly rejected. This leads ideally to the absence of initial lead and allows dating single grains. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a fast and sensitive method for solid sample analysis and pairs well with the need for analysis of large sets of zircons in geological sediment studies [1].

However, the LA of zircons causes fractionation of U and Pb resulting in an unstable U/Pb ratio over the time of ablation [2]. This process is not fully understood yet, but a heat-induced phase separation with enrichment of U in the wall of the ablation pit [3] seems to be a major cause.

To reduce this effect, cooling and faster transfer of heat from the ablation pit can be achieved by applying wet ablation, i.e. under water ablation or ablation with wet carrier gas. Therefore, the LA process was studied with respect to different degrees of humidity in the carrier gas and the influence on the crater morphology and the resulting U/Pb ratios measured by ICP-MS will be presented.

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Analytical Sciences **AS37**

**Analysis of the mechanism of $NCO^-$ loss and the consecutive release of a $PO_3^-$ group from highly charged oligonucleotides**

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Mass spectrometry is the method of choice to analyze modified oligonucleotides. The elucidation of gas-phase dissociation mechanisms of modified and unmodified nucleic acids is of utmost importance to facilitate rapid and potentially automated sequence analysis of artificial DNA and RNA. In this study the fragmentation behavior of highly charged oligodeoxynucleotides (ODN) was investigated. It was found that the fragmentation of ODNs exhibiting a high charge level (ratio of the actual to the total possible charge) differs significantly from those with a low or medium charge level by following so far unreported, highly selective dissociation channels. Presence of a terminal thymine (3' or 5') results in a new fragmentation pathway involving abstraction of a cyanate anion ($NCO^-$). This process frequently coincides with the loss of water from the 3'-end or of $CH_2O$ from the 5'-end. Additionally, a consecutive release of a $PO_3^-$- ion was observed for most ODNs. Introduction of a phosphorothioate group allowed to pinpoint the $PO_3^-$ loss to the terminal phosphate group. Furthermore, experiments with methylphosphonate-modified ODNs were performed to block the phosphate-oxygen for participating in the dissociation mechanism. To elucidate the impact of the ribose sugar moiety on the $NCO^-$ loss, the gas-phase dissociation of highly charged *homo*-DNA was investigated. Since all *homo*-DNA sequences exhibited terminal cyanate loss, the abstraction of $NCO^-$ is likely to be sugar-independent. However release of water from the 4'-end or of $CH_2O$ from the 6'-end was not observed and furthermore no proof for subsequent abstraction of the $PO_3^-$ -ion was obtained.

Analytical Sciences **AS38**

**Capillary zone electrophoresis applied to the analysis of micro-heterogeneity in therapeutic monoclonal antibodies**

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The development of monoclonal antibody (mAb) pharmaceuticals has been constantly growing since the introduction of the first therapeutic mAb in 1986. However, as they exhibit a high molecular complexity, small changes in production conditions, storage or exposure to light or chemicals are sources of micro-heterogeneity, possibly affecting antibody charge, size or glycosylation pattern. As these modifications may alter the therapeutic activity and induce side effects, analytical methods capable of evaluating micro-heterogeneity are essential [1].
Capillary electrophoresis is particularly well suited for mAb analysis thanks to its high resolution and low sample consumption. Capillary zone electrophoresis (CZE) provides separation selectivity based on charge and size, and might complement capillary gel electrophoresis (size) and capillary isoelectric focusing (charge) that are routinely used in industry. A major issue in the analysis of mAbs by CZE concerns adsorption of these biomolecules to the capillary walls, leading to poor resolution and recovery.
In this work, various static capillary coatings and background electrolytes were investigated to find the optimal conditions associated with good resolution between isoforms, minimized adsorption, and acceptable repeatability for two mAbs. To monitor the adsorption of commercial intact mAbs, four criteria, namely peak efficiency, migration time repeatability, EOF change, and protein recovery, were evaluated. Finally, the repeatability and intermediate precision obtained with the selected operating conditions were assessed from the mAb patterns and applied to other commercial mAbs.

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Analytical Sciences **AS39**

**Analysis of protein therapeutics by capillary zone electrophoresis coupled to mass spectrometry**

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The development of protein therapeutics is expanding rapidly since the advent of biotechnology. However, these molecules are particularly fragile and their production and storage must be strictly controlled. Despite all precautions, these proteins can be degraded and it is essential to check the quality of the product to avoid any side effects to the patient [1]. Therefore, a wide range of analytical methods are necessary (i) to assess the overall quality of the products by comparison between batches, (ii) to identify a product if counterfeit is suspected, or (iii) to evaluate the properties of a potential biosimilar.
Capillary electrophoresis (CE) is a separation technique that is well suited to the analysis of therapeutic proteins thanks to its high efficiency and low sample consumption. High separation performance is reached if issues such as adsorption of these biomolecules to the capillary walls, leading to poor resolution and recovery, are addressed [2]. The coupling of CE with mass spectrometry (MS) enables the identification of degraded products. For this purpose, MS-compatible conditions have to be implemented.
The objective of this work was to develop an analytical method based on CE coupled to MS detection for filgrastim, a biotherapeutical drug used in cancer therapy. Experimental conditions were optimized to obtain minimized adsorption, separation of degradation products from the original protein, compatibility with MS detection, together with acceptable repeatability.

[1] Manning M.C. et al. *Pharmaceutical Research* **2010**, *27 (4)*, 544.
[2] Haselberg R. et al. *Electrophoresis* **2011**, *32*, 66.

Analytical Sciences **AS40**

**Trace element quantification in ancient copper artifacts by portable laser ablation sampling and subsequent LA-ICP-MS analysis**

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a very versatile and powerful technique for elemental and isotopic analysis of solid samples in a quasi-nondestructive manner. It allows for quantification of major, minor and trace element concentrations without any sample preparation. In order to make immovable objects (e.g. archeological artifacts in museums) accessible to LA-ICP-MS, it is desirable to uncouple the sampling process from the laboratory based ICP-MS analysis. This work aims at establishing an analytical method for the determination of trace element concentrations in ancient copper artifacts which are not accessible via conventional LA-ICP-MS setup.
The sampling step is performed on site using a portable nanosecond laser device ($\lambda$=532 nm) and fiber optics to ablate the solid sample. The generated aerosol is collected on polycarbonate membrane filters and subsequently analyzed in a laboratory based ns-LA-ICP-MS ($\lambda$=213 nm) setup. The use of an external standard allows for trace element quantification.
This analytical approach was used to quantify trace element concentrations in various copper reference materials. Good performances (±30%) were obtained for almost all trace elements. Although more accurate results and lower limits of detection can be reached when an online-LA-ICP-MS setup is used, the attempt at offline laser ablation sampling and subsequent LA-ICP-MS analysis still remains very promising for trace element quantification in samples that are not accessible to a conventional LA-ICP-MS setup.
Following the application of the offline-LA LA-ICP-MS approach to quantify trace element concentrations in copper reference materials, this method was applied to archeological copper artifacts.

Analytical Sciences **AS41**

NMR Analysis of the Components of the Reaction Mixture during the Formation of the Glucose Oxime through Spectral Aliasing.

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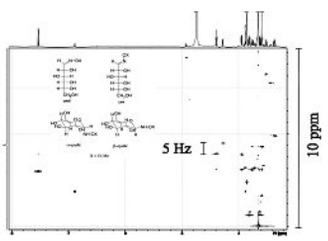
Carbohydrates often exist in different conformations making them difficult to characterize. The chemical reactions of carbohydrates give rise to complex mixtures of derivatives with relevance such as oximes and hydrazones which have found widespread applications, *e.g.* biomolecules labelling, analyzing protein–protein interactions and in vivo cell imaging.

Spectral aliasing is an NMR technique that improves the resolution in the indirect dimension (F1) and can be accessed with a reduction of the spectral window to the desired value




The aliased, $^{13}$C-HSQC recorded with 10 ppm in the $^{13}$C dimension allowed a complete assignment of $^{1}$H and $^{13}$C of all the components of the reaction mixture without any further purification.

Analytical Sciences **AS42**

**Isolation of natural products: from analytical HPLC to Mass Spectrometry guided purification by semi-preparative HPLC-MS**

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In natural product research the isolation of compounds from crude extract is a key element. In this respect, the increase of purification processes efficiency, by improvement of instrumental and methodological approaches, is a crucial point. To tackle this issue, a two-steps chromatographic strategy was developed. First, the separation of target molecules from crude extract was optimized by linear gradient at the analytical scale. Second, the gradient was geometrically transferred to semi-preparative HPLC by a gradient transfer method based on the calibration of the chromatographic systems (measurement of dwell volume and extra column volume) [1]. UV as well as MS monitoring were performed for a comprehensive detection of the various compounds present within the mixture. MS detection proved to be an important tool, not only for the purification of compounds that cannot be detected by UV or ELSD, but also for an efficient mass spectrometry guided purification of specific compounds in crude extracts. In particular, a single quadrupole mass spectrometer coupled with a semi-preparative chromatographic system (PuriFlash® - MS) was found a promising tool to increase the efficiency of constituents of interest isolation. This mass guided separation of compounds in complex mixture represent a powerful strategy, not only for the isolation of molecules present in bioactive fractions, but also for the rapid purification of biomarkers identified by UltraHPLC-MS metabolomics and dereplication process.

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

**AS43**

**Zooming in on the glucose metabolism of *S. Cerevisiae*.**

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Even in normalized cell culture, cell populations show substantial cell-to-cell differences in a number of parameters including gene expression and physiological parameters such as stress resistance and growth rate. This cellular behavior can drastically influence the response of a cell population to perturbations such as calorie restriction.

Important processes associated with cell growth and calorie restriction involve transcriptional regulation. We wish to extend this knowledge by using our single-cell metabolomic study platform called Microarrays for mass spectrometry (MAMS) to identify metabolic changes at the single cell level.

Here, we present the our preliminary results from the comparison between metabolites from individual yeast cells treated with (i) glucose-restricted, and (ii) normal cell culture medium; as well as cells grown in normal culture medium that present different growth rates. We also present here how selected MS signals from the central metabolism can be used to better understand stress resistance and differences in growth rate.

Analytical Sciences **AS44**

**OMA and OPA - Software Tool for Mass Spectra Interpretation of Natural and Modified Nucleic Acids**

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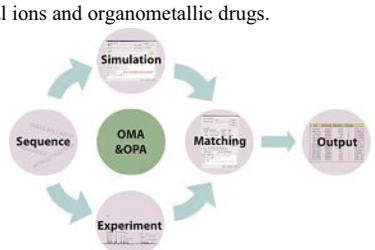
Synthetic oligonucleotides comprising unnatural building blocks have gained attention for human therapeutic applications. Due to the presence of modified nucleotides, the oligonucleotides exhibit increased biostability and bioavailability. On the other hand, cellular DNA and RNA serve as targets for various chemotherapeutic drugs, which interfere with transcriptional and translational processes (e.g. antisense, RNAi). Tandem mass spectrometry is the method of choice for sequencing of unnatural oligonucleotides and elucidation of structural alterations. Such experiments generate complex data sets, whose interpretation are laborious and requires profound knowledge of the gas-phase dissociation of nucleic acids.

The software tool OMA & OPA has been developed for interpretation of product ion spectra of natural and modified oligonucleotides and their adducts with metal ions and organometallic drugs.




Nyakas et al., *J. Am. Soc Mass Spectrom.* 2012: p. 1-8.

Analytical Sciences **AS45**

## Microarrays for mass spectrometry: Self aliquoting nanoliter reactor plates for nano-LC-MALDI-MS applications

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In this work, self aliqouting microarray plates [1] are used as a sample target for nanoliter fractions collected by a high-frequency droplet spotter [2], which is connected to a nano liquid chromatography system.
Fractions of the nano-LC (with flow rates of 250-500 nl/min) are collected in 1 Hz rates and deposited into the micro-cavities. The hydrophobic coating of the plates confines the droplets to the predefined position. The cavities further allow nanoliter volume reactions directly on the microarray plate itself. Up to 2700 spots can be collected on a plate in the dimension of a standard microscope slide (this corresponds to 45 minutes collecting time, if working at 1 Hz rates).
Peaks from a nano LC reversed phase column (3µm, 75µm x 10cm) are usually spread over at least 10 spots, and the repetitive information can be further used for an integrated enzymatic or chemical digest of the target analytes, either before or after mass spectrometric analysis. This was demonstrated by collecting the eluate from a reversed phase separation of a tryptic protein digest, in order to develop a reliable way for identification of post translational modifications. The self-aliquoting properties of the microarray plates allows further a manual pre-aliquoting of matrix, internal standards or even enzyme solutions - by just using a simple mechanical sliding device.

[1] P. L. Urban et. al., Lab. Chip., 2010
[2] S. K. Küster et.al., Anal. Chem., 2013
*both authors contributed equally to this work*

Analytical Sciences **AS46**

## Multiplexing Imaging Laser Ablation Mass Cytometry at Sub-cellular Spatial Resolution

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# Equal contribution

We describe in this work an advanced multiplexing imaging setup based on Laser Ablation (LA) coupled to a commercial mass cytometer (CyTOF®). The current measurements successfully demonstrate sub-cellular (~1 µm) spatial resolution imaging of biological tissue thin sections. One of the key components of the setup is a novel LA cell, providing short washout time and enhancing the signal to noise ratio of the LA transient signal.[1] This enables complete separation of ion signals from individual pulses generated by 20 Hz laser ablation and furthermore acquisition of analytes in the sample aerosol produced by 1 µm laser single shot. As shown in a case study, multiple biomarkers, metal-tagged antibodies were imaged simultaneously in thin sections of breast cancer tissue, using this experimental setup. The resulting high resolution biomarker images help biologists to investigate various breast cancer sub-types, and analyze cell-cell interactions.[2] The presented imaging setup will open new research opportunities for pathologists and pharmacologists and some of the potential applications will be discussed.

1 Wang, H.A.O., et al. Fast Chemical Imaging at High Spatial Resolution by Laser Ablation Inductively Coupled Plasma Mass Spectrometry. *Submitted to Analytical Chemistry* (2013).

2 Giesen, C.,[#] Wang, H.A.O.,[#] Hattendorf, B., Grolimund, D., Günther, D., Bodenmiller B. et al. *Submitted* (2013).

Analytical Sciences **AS47**

## Monitoring of fluorescent labeling reactions

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Fluorescence detection or microscopy of biomolecules often requires prior labeling with efficient fluorophores. For protein detection in particular, isothiocyanate or succinimidyl ester derivatives of fluorophores such as fluorescein are widely used to covalently react with the ε-amino group of lysines on proteins. Despite their broad use in bioanalytics, few reports have investigated the kinetics of these reactions under varying conditions, although this would allow optimization of labeling protocols.

Here we show that fluorescent labeling reactions can be monitored using fluorescence-polarization detection. The attachment of the small fluorophore to a large protein results in an increase in fluorescence anisotropy, which can be followed over time. The kinetics of several labeling reactions was determined as a function of pH, concentration and mole ratio of reagents. Furthermore, the impact of side reactions such as hydrolysis and fluorophore dimerization was assessed.

Analytical Sciences **AS48**

## Incremental Model Identification of Gas-Liquid Reaction Systems with Unsteady-State Diffusion

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Identification of kinetic models and estimation of reaction and mass-transfer parameters can be performed using the extent-based identification method, whereby each chemical/physical process is handled separately [1-3]. This method is used here to analyze gas-liquid systems under unsteady-state mass transfer. Such a situation is common in the case of diffusion-controlled reactions and is modeled by the film theory, that is, transferring species accumulate in a liquid film. In both the gas and liquid bulks, mass-balance relations describe the species dynamics as ordinary differential equations (ODE) and serve as boundary conditions for the film. On the other hand, the dynamic accumulation in the film is described by Fick's second law. The resulting partial differential equation (PDE) system is solved by discretization and rearrangement in ODEs.

The estimation of diffusion coefficients follows a two-steps procedure. First, the extents of mass transfer are computed from measurements in the two bulks. Diffusion coefficients are then estimated individually by fitting each extent of mass transfer to the extent obtained by solving the corresponding PDE. Comparison of the estimated diffusion coefficients with their literature values serves to validate the models identified in the two bulks.

The estimation of both kinetic parameters and diffusion coefficients is investigated for gas-liquid reaction systems with unsteady-state diffusion. The approach is illustrated with simulated examples.

[1] Bhatt et al, Ind. & Eng. Chem. Res. 50, 12960-12974, **2011**
[2] Srinivasan et al, Chem. Eng. J. 208, 785-793, **2012**
[3] Billeter et al, Anal. Chim. Acta 767, 21-34, **2013**

Analytical Sciences **AS49**

**Incremental Model Identification using the Concept of Extents**

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Kinetic models contribute greatly to cost reduction during the process development phase and are also helpful for process monitoring and control purposes. Kinetic models describe the underlying reactions, mass transport and operating conditions of the reactor. In the typical one-step *simultaneous method* of identification, one postulates a dynamic model encompassing the effects of all phenomena at stake, and model parameters are estimated by comparing measured data with model predictions. Simultaneous identification can be computationally costly and exhibit convergence issues in case of poor initial guesses. Furthermore, this method is characterized by high correlation between parameters, which can lead to structural mismatch.

In contrast, the *extent-based incremental method* of identification is a two-step approach, in which measured data are first transformed into extents, each one representing the effect of a particular phenomenon [1-3]. Then, for each phenomenon individually, a model is postulated and the corresponding parameters estimated by comparing the simulated and measured extents. Since each extent, and thus each effect, is handled individually, the correlation between model parameters is considerably reduced.

This presentation will give an overview of the extent-based incremental identification and will describe the procedure to analyze homogeneous and gas-liquid systems. The performance of simultaneous and incremental methods of identification will be compared via simulated examples.

[1] Bhatt et al, Ind. & Eng. Chem. Res. 50, 12960-12974, **2011**
[2] Srinivasan et al, Chem. Eng. J. 208, 785-793, **2012**
[3] Billeter et al, Anal. Chim. Acta 767, 21-34, **2013**

Analytical Sciences **AS50**

**Evaluation of supercharging molecules with the sheath liquid interface in CE-ESI-MS**

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Capillary electrophoresis (CE) coupled with electrospray ionization (ESI) mass spectrometry (MS) is a suitable technique for analysis of intact proteins. The main configuration to realize this coupling is the sheath liquid interface which is constituted of two concentric stainless steel tubes. The first one surrounds the free end of the CE capillary where a sheath liquid provides electric contact, appropriate flow and solvent composition for ionization and evaporation. The second one permits to introduce a nebulizing gas to assist the spray formation. Generally, the ionization of proteins and especially their charge state distribution (CSD) is mainly influenced by their conformation as well as the nature and composition of the ESI effluent. Thus, the sheath liquid has a direct impact on ionization and a modification of its composition can change the protein CSD. Because this interface is highly versatile, it can be used for modifying ionization without affecting CE selectivity as well as a post-capillary infusion device.
In this work, the effect of the addition of supercharging molecules [1] to the sheath liquid on the CSD of proteins was evaluated. Several supercharging agents such as nitro benzoyl alcohols (NBAs) were tested on model proteins (i.e. insulin, growth hormone, hemoglobin) presenting different properties in term of ionization, conformation and flexibility. The influence of these supercharging additives on sensitivity (i.e. protein ionization efficiency) was also estimated.

[1] S.S. Miladinovic, L. Fornelli, Y. Lu, K. M. Piech, H. H. Girault, Y. O. Tsybin, *Anal Chem.* **2012**, *84*, 4647.

Analytical Sciences **AS51**

**Sol-based optical Ammonia Gas Sensor**

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State of the art gas sensors usually are hand-held devices. The development of a gas sensor system based on flexible polymer optical fibers, which finally can be integrated into textiles, would increase sensor wearability. As a model system, sols made out of organic modified silicates doped with different mixtures of fluorescent coumarin and fluorescein were solvent casted and dried on poly(methyl methacrylate) (PMMA) plates, which were exposed to gaseous ammonia. The combination of the two dyes showed a much higher sensitivity towards ammonia than either single dye. This indicates that the Förster energy transfer between these dyes is essential for the sensing mechanism.
Significant differences in fluorescence intensity changes at 430 nm were observed for different dye mixtures, xerogel thicknesses and gas concentrations (Figure).

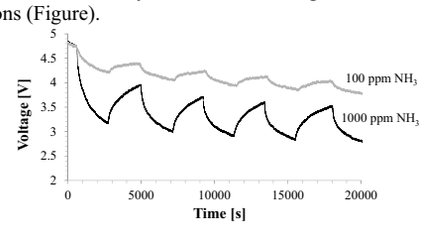


**Figure:** Dye doped 27 µm thick xerogel periodically exposed to 100 or 1000 ppm $NH_3$(g) (lowering in voltage) and $N_2$(g) (increase in voltage).

Analytical Sciences **AS52**

**Study of the behavior of individual multi-element droplets in an inductively coupled plasma by high temporal resolution mass spectrometry**

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Despite the widespread acceptance and use of inductively coupled plasma mass spectrometry (ICPMS) all the processes that an analyte undergoes in the ICP are still poorly understood. A better understanding of these processes would help to improve instrumental performance and analysis accuracy. Combination of a single droplet introduction system [1] with a prototype high temporal resolution ICP time-of-flight MS allows investigating analyte-plasma interactions on the basis of a single droplet signal. A temporal separation of the ion signal occurrences of different elements from a single droplet, which had been observed in the previous work [2], was further investigated in this study. Mixtures of different salts and particles were analysed. It was shown that the order of occurrence of the elements' signal maxima correlates with the melting/boiling temperatures of the corresponding oxides [3] and can be changed by the central gas velocity. The obtained results indicate that this signal separation is due to the spatial separation of elements, taking place during the transit of a droplet in the ICP. Moreover, it was demonstrated that particle composition, plasma parameters and the axial/radial location of analyte vaporization onset have a direct influence on the spatial separation of elements. A possible explanation for this effect and its implications on the final intensity and stability of the signal will be discussed.

[1] Gschwind *et.al.„JAAS,* **2011**, *26*, *6*, 1166.
[2] Borovinskaya *et.al.„JAAS,* **2013**, *28*, *2*, 226-233.
[3] Flamigni *et.al.„JAAS,* **2012**, *27*, *4*, 619-625.

Analytical Sciences **AS53**

**Advanced Analysis of Post-Translational Protein Modifications Using a nano-LC-MALDI-MS Interface Based on Droplet Microfluidics**

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Post-translational modifications (PTMs) are highly relevant for regulating the properties and functions of proteins. However, with conventional analytical methods, it is difficult to locate and identify these modifications. To address this challenge, we have developed a novel droplet-based microfluidic interface that combines separation by nano-liquid chromatography (LC) with detection by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The system facilitates not only enhanced aliquotation of eluted peaks at yet unmatched temporal resolution but also introduces an additional enzymatical dimension integrated into the standard LC-MS analysis workflow. This allows reliable detection of protein modifications and also the identification of the nature of the detected modification.

Droplet microfluidics is used to compartmentalize the eluate from the nano-LC into a stream of nanoliter droplets at high frequency. The droplets are spotted on a microarray with 2780 hydrophilic spots of 300 $\mu$m diameter on a hydrophobized microscope slide as described previously [1]. Due to the high sampling frequency of our spotter, typically 1 Hz, each eluted peak is aliquoted into 10 or more spots. Hence, each of these 10 or more spots contains the same analyte. By depositing a second nanoliter droplet to every other spot we then perform nanoscale reactions. The added droplet contains an enzyme which selectively removes the PTMs from the analyte. Using MALDI-MS, we are then able to reliably detect peptide modifications such as glycosylation or phosphorylation just by performing a pairwise comparison of mass spectra obtained from neighboring spots.

[1] S.K. Küster, S.R. Fagerer, P.E. Verboket, K. Eyer, K. Jefimovs, R. Zenobi, P.S. Dittrich, *Anal. Chem.* **2013**, *85*, 1285

Analytical Sciences **AS54**

**Signal processing and instrumentation for Fourier transform mass spectrometry with improved analytical performance**

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Fourier transform mass spectrometry (FTMS)-based applications necessitate improved analytical performance. The current limitations in resolution, mass accuracy, measurement precision, and other analytical characteristics of FTMS are imposed by the conventional signal processing and instrumentation. Herein, we evaluate the filter diagonalization method (FDM), which provides increased resolution compared to that of the conventional signal processing. Specifically, the resolution, mass accuracy, and dynamic range of FDM-based FTMS are characterized. Further, we implement the least-squares fitting (LSF) method, which reveals the instrumentation-limited levels of achievable analytical characteristics. Specifically, the LSF allows studying the effect of length and form of the transient signal, thermal noise of the detector, sampling rate of the digitizer, synchronization precision of the instrument electronics, electrodes geometry of the mass analyzer, and spatial spread of the ion package. Optimization of these parameters is considered in order to increase the achievable levels of FTMS analytical characteristics. Finally, we implement the high-performance data acquisition system, which allows high-definition acquisition of experimental transient signals from ions in the mass analyzer. Experiments were performed on 10 T LTQ FT-ICR MS and LTQ Orbitrap Elite FTMS (Thermo Scientific). The obtained results advance the MS-based high-resolution molecular structure analysis of complex mixtures via increased resolution, mass measurement accuracy, and signal-to-noise ratio. Particularly, we consider applications of developed methods and techniques in peptide and protein structure analysis, structural biology, and analysis of petroleum-type samples.