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Inorganic and Coordination Chemistry **1**

Metal Ions Guide Nucleic Acid Structure and Function

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RNAs and DNAs are inevitably associated with cations: We investigate how metal ions guide the folding of complex RNA structures, stabilize local structures, participate in catalysis, and how they build-up linear arrays within nucleic acid helices.[1,2] RNAs in our research focus are ribozymes, i.e. catalytic RNAs, as well as riboswitches that regulate gene expression by specifically binding to small metabolites.
The yeast mitochondrial group II intron ribozyme Sc.ai5γ is highly dependent on Mg^{2+} and very sensitive to the presence of, e.g., Ca^{2+}.[3] By single molecule FRET, NMR and other techniques [3-5] we are characterizing the effect of Mg^{2+} and Ca^{2+} on catalysis, local structures and folding: Unexpectedly, the active state is reached only transiently, and Mg^{2+} enhances the dynamics of domain docking.[5]
In the case of the *btuB* riboswitch from *E. coli* we showed that the corrin side chains of the coenzyme B_{12} moiety are the determinants for the correct structural change of the riboswitch, whereas the axial ligands of B_{12} regulate the affinity towards the RNA.[6]

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2

Analytical Chemistry in the Bernese Oberland: Detection and Identification of Chemical Warfare Agents at the SPIEZ LABORATORY

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The SPIEZ LABORATORY is the Swiss centre of expertise in protection against nuclear, biological and chemical (NBC) threats and hazards.
It supports national partners and cooperates with international organisations such as the Organisation for the Prohibition of Chemical Weapons (OPCW) which is located in The Hague. The OPCW is the implementing body of the Chemical Weapons Convention (CWC) which entered into force in 1997. Since 1998 the Analytical Chemistry Group of the SPIEZ LABORATORY is one of the so-called designated laboratories of the OPCW. The group is specialized in the off-site analysis of the chemicals covered by the CWC, their precursors and degradation products. The detection and identification of these compounds in environmental samples imposes a formidable challenge: hundreds of thousands of compounds are possible. The samples can be solid, liquid or gaseous and complex matrices have to be expected. The concentrations are in most cases at trace level. Thus, sophisticated sample preparation methods, general unknown screening procedures together with selective and sensitive separation and detection techniques are needed. In addition, a specialized synthesis laboratory is necessary to provide the respective reference chemicals. The main analytical tools used at the SPIEZ LABORATORY analysis are GC/MS and gas chromatography in combination with different detectors, liquid chromatography/mass spectrometry (LC/MS), and nuclear magnetic resonance (NMR) together with sample preparation procedures which allow the extraction and derivatization of the relevant chemicals prior to the analysis.
This presentation gives an overview about the Analytical Chemistry at the SPIEZ LABORATORY, the various steps in the verification analysis of unknown samples with special focus on the screening concepts using LC/MS.

3

Investigating ruminant digestive physiology using element markers and ICP-MS

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Ruminants retain food in the form of fluids, small and large particles over different periods within their rumen. Although small particles are excreted faster than large ones, it has been unclear whether differences in large particle size have an effect on retention.
We investigated these questions in cattle, applying a complex marker system to their rumens. Markers consisted of Cobalt-EDTA dissolved in water, while fibres of different length were mordanted with Chromium (2mm), Cerium (10mm) and Lanthanum (20mm). Faeces were collected in regular intervals after marker application and analysed by ICP-MS for marker concentrations after microwave digestion.
The resulting excretion curves confirm known differences between fluids, small and large particles. Unexpectedly, however, the two large particle size classes (10 and 20mm) did not differ in their excretion pattern, suggesting a size-threshold rather than a continuous function of particle size for retention. While producing new insights into ruminant digestive physiology, our study demonstrates that ICPMS analysis of element markers allows a rapid and sensitive investigation of the movement of complex substrates consisting of several distinct phases (like fluids, different-sized particles). Although the organic samples (faeces) typically are comparatively heterogenous, the resulting concentration curves allowed a clear distinction of excretion patterns.

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HPLC-MS for the investigation of bile acids as potential new biomarkers

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Bile acids are the major degradation products of cholesterol and they undergo considerable structural modification through hepatic and intestinal metabolism. They are biologically important as mediators of dietary lipid absorption as well as ligands of the nuclear receptor farnesoid X receptor (FXR) and hence regulators of lipid and carbohydrate metabolism. Since bile acids are reabsorbed through an efficient enterohepatic circulation, quantification of their precursor 7alpha-hydroxy-4-cholesten-3-one (C4) is used to provide information about the direct input of *de novo* biosynthesis out of cholesterol in contrast to the input coming from reabsorption of bile acids from the intestine. Our interest lies in determining the diagnostic or prognostic significance of bile acid quantification in patients suffering from various diseases including metabolic syndrome.
Quantification of bile acids requires a highly sensitive method since these compounds are present in the micromolar range in serum. Methods based on reverse-phase chromatography coupled to mass spectrometry were developed for the differentiated quantification in serum of the 15 major human bile acids as well as for their precursor C4.
Preliminary data show considerable intraindividual variation in concentrations of these compounds throughout the day, which is caused by food intake and circadian rhythm. This consequently renders the preanalytical phase conditions more difficult to establish.
Two sensitive methods were developed and validated for the quantification of bile acids and their precursor in human serum. These methods will be further applied to healthy volunteers and patients in order to better characterize the significance of bile acid metabolites in various diseases.

5

New insights in intact protein analysis with CE-TOF/MS

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The on-line combination of capillary electrophoresis (CE) with mass spectrometry (MS) is an attractive option for intact protein analysis (*i.e.*, no digestion, no derivatization). On the one hand, CE presents features such as great efficiency due to low diffusion coefficients. Moreover, CE allows working under aqueous conditions and without stationary phase. On the other hand, MS provides selectivity and ability to identification. TOF (time-of-flight) analyzer is particularly well suited to intact protein analysis, due to high mass range capability and mass accuracy. This lecture will illustrate the interest of CE-ESI-TOF/MS for this particular purpose with two developments.
In a first application, an efficient, rapid, and simple CE-TOF/MS method was developed to analyze natural human growth hormone (hGH) and recombinant growth hormones (rhGH) without sample preparation. The method presented original analysis conditions that allowed distinguishing hGH from rhGH. It was successfully applied to seized samples in a forensic case. In a second application, a complete analytical strategy based on CE-TOF/MS was developed to detect intact hemoglobin-based oxygen carriers (HBOC) in plasma samples collected for doping control. HBOC (such as Oxyglobin®) are purified proteins obtained from polymerized bovine hemoglobin that are misused as performance enhancers. A sample preparation based on immunodepletion was mandatory to remove most abundant proteins that interfered with CE separation and altered ESI.

6

Proposed Redefinition of the Unit Mole

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In parallel to the evolution of science and technology our units of measurement improve continuously. The early man made units have been replaced in the course of time one by one by more stable units preferentially based on constants given by nature. The last unit of the International System of Units (SI) that is still based on a human artifact is the unit of mass, the kilogram.
In the wake of the redefinition of the kilogram there have been launched discussions on the necessity of the redefinition of other units, amongst other on the redefinition of the unit mole. The current definition of the mole is defined as the amount of substance of a system which contains as many elementary entities as there are atoms in 0.012 kilogram of carbon 12, its unit is "mol". When the mole is used, the elementary entities must be specified and may be atoms, molecules, ions, electrons, other particles, or specified groups of such particles. It follows that the molar mass of carbon 12 is exactly 12 grams per mole, M(12C) = 12 g/mol [1]. In the proposed new definition the mole is defined as the amount of substance of a system that contains exactly 6.022 141 79 x 10^{23} specified elementary entities, which may be atoms, molecules, ions, electrons, other particles, or specified groups of such particles [2].
This contribution critically examines the submitted arguments to justify the proposed redefinition of the unit mole by 2011 for their persuasive power to change a scientific and cultural good such as a unit of measurement. It aims at increased awareness of the chemical communities of the proposed changes and seeks their views.

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7

Isobaric tagging-based selective quantitation of CSF proteins using reporter calibration curves

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High throughput quantitation is essential for the validation of potential biomarkers in biological fluids. In the past few years, mass spectrometry (MS) has emerged as a practical tool for the multiplexed measurements of these markers by isotope dilution. Isobaric tandem mass tags (TMTs [1]) were used in this study for the monitoring and quantitation of potential protein biomarkers in cerebrospinal fluid (CSF) by MS.
TMT sixplex reagents were used to label CSF and 4 identical mixtures synthetic proteotypic peptides (representative of the targeted proteins), which were prepared at 4 chosen concentrations. Labeled samples were mixed and the pooled sample was subjected to OFFGEL electrophoresis as first dimension separation. The fractions were analyzed by LC-MS/MS on both ESI Orbitrap and MALDI TOF/TOF instruments.
After collision-induced dissociation, each TMT label releases a specific reporter ion. When reporting TMT reporter peak areas along chromatograms, it clearly appears that simplified patterns are observed for the reporters at m/z = 128, 129, 130 or 131 (related to the amount of the synthetic peptides) with respect to reporter at m/z = 126 which reflects the complexity of the CSF sample. The reporter ions generated by the TMT-labeled reference synthetic peptides were used therefore as a constrained tool to selectively identify these peptides in CSF and to quantify them using the internal calibration curve generated this way. This approach shows that protein quantitation using isobaric tags is a selective, fast, efficient and straightforward method.

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8

Nanoscale Imaging and Chemical Analysis Using Combinations of Atomic Force Microscopy and Raman Microspectroscopy

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Macroscale properties of materials are often influenced by their structure and chemical composition at the nanometer scale. The efficiency of thin-film solar cells, for example, is affected by concentration gradients at the nanometer scale, nanoparticles consisting of segregates or contaminants, and the presence of secondary phases. Analytical tools with high spatial resolution and chemical contrast are needed for the optimization of production processes and for quality control of such materials. High resolution chemical imaging is also expected to yield important insights into highly complex biological systems, such as bacterial biofilms and lipid membranes.
Combinations of atomic force microscopy (AFM) and Raman microspectroscopy allow imaging and chemical analysis with sub-μm resolution. Tip-enhanced Raman spectroscopy (TERS) with metal-coated AFM tips provides chemical analysis of surfaces with a resolution of 20–50 nm. We will present technical improvements of TERS, such as highly enhancing TERS tips and a proof-of-principle for TERS in liquids, as well as applications of AFM, Raman microspectroscopy and TERS in biology and materials science. In bacterial biofilms, for example, nanostructures involved in the adhesion of bacteria to surfaces, such as pili and extracellular polymeric substances (EPS) have been detected as well as inorganic nanoparticles, such as dolomite nanoglobules in *H. meridiana* biofilms, a species involved in biomineralization. The investigation of solar cell materials, such as $CuInS_2$ (CIS), has shown that Raman microspectroscopy can provide images with approx. 400 nm resolution and chemical contrast that allows the identification of chemical species, segregates, contaminants and even different crystal structures of the same compound.

9

Water in chromatographic interfaces – computational studies

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We present results from MD simulations for a model of a reverse phase liquid chromatography (RPLC) system. Our ultimate goal is to devise a methodology for determining retention times of various pharmaceutically relevant compounds in the process of their separation with the RPLC technique. In the present work we have studied an eluent in order to establish how the solvent structure is influenced by various functionalizations of the RPLC silica-alkyl chains interface. We have studied three models consisting of silica layers with alkyl chains attached so as to represent the RPLC setup. The studied systems differed between each other in functionalization of the alkyl chains. The eluent in all the models was represented by a mixture of water (TIP3P model) and acetonitrile in even proportions.
Our results from the computational studies of the model with non–functionalized alkyl chains confirm existence of microheterogenity in the mixture of water and acetonitrile. This phenomenon was observed experimentally [1] and in computational simulations [2]. We also found stable water filaments connecting bulk solvent with the silica layers. Additionally, we have observed different kinds of water molecules that can be differentiated by their mobility. This kind of taxonomy of water molecules on the silica surfaces was observed experimentally almost 30 years ago by Scott [3].

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10

Electrothermal treatment of laser generated aerosols: a route to online separation of elements for LA-ICPMS

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Electrothermal vaporization as a sample introduction system for inductively coupled plasma mass spectrometry (ICPMS) can achieve matrix separation before vaporization of analytes, thus reducing polyatomic interferences [1]. Recently its ability to resolve isobaric interferences has been demonstrated [2]. As far as laser ablation is concerned, matrix or element separation prior to the introduction into the ICPMS has not been realized up to now. Based on an initial study of Vaculovic et al. [3] this work explored the use of thermal treatment of laser aerosols in order to achieve selective, online element separation. For this purpose, laser generated aerosols from the standard reference material NIST 610 were heated within a Perkin Elmer HGA-600MS graphite furnace before the introduction into an ICPMS system. The behavior of ion signals of 30 trace elements was investigated depending on furnace temperature, showing element specific signal suppression. The temperature of initial signal suppression was found to correlate with the corresponding melting point of the elements oxides and the suppression reached 99% for volatile elements. At ~1000 °C signal suppression for all elements measured was detected. The size of aerosol particles was characterized after thermal treatment by means of laser light scattering spectroscopy and scanning electron microscopy. At temperatures between 900 and 1500 °C the formation of particles larger than 200 nm was observed which may explain the observed signal loss because such large particles can not be completely vaporized in the ICP.

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11

Sandwich mixer-reactor: Influence of the diffusion coefficient anf flow rate ratios

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Chemical modifications of peptides and proteins is now commonly used to complement mass spectrometry (MS) analysis, in order to isolate specific subclasses of proteins by affinity baits, or for quantitation purposes. Recently, an electrospray microchip for MS including a passive mixing unit was designed for on-line derivatization of cysteinyl peptides [1].

An alternative microsystem, so-called "sandwich mixer-reactor" has been investigated [2]. A sandwich mixer consists of mixing two solutions in a channel, one central laminar flow being sandwiched between two outer flow solutions. The present numerical study considers the convection-diffusion of two reacting species A and B, provided respectively by the two incoming solutions. The simulations show how the diffusion coefficient and flow rates ratios influence, via the transversal diffusion length and reaction kinetics, the reaction extent at the end of the sandwich mixer. First, this extent can be enhanced up to 60% if the species with the lowest coefficient is located in the outer solutions where the flow velocity is small compared to that of the central part (higher residence time). Secondly, decreasing the outer flow rates (to confine the reaction close to the walls) and increasing the local concentration to keep the same flux ratio improve the extent by 300%. These conclusions are also demonstrated for consecutive reactions, showing an amplification of the effects described above.

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12

Spectral Validation of Rank Deficient Kinetic Models

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Kinetic hard-modelling is often the method of choice in order to determine kinetic parameters of reactions by non-linear optimisation and fitting of measured data. In spectroscopy, kinetic hard-modelling allows decomposing time and wavelength resolved data into concentrations, obtained by integration of the postulated mechanism (rate law), and absorptivity spectra, which are linearly deduced by a calibration-free approach [1]. The validity of the kinetic model can then be assessed by comparing the estimated absorptivity spectra with independently measured ones.

The calibration-free approach inherently leads to a mathematical ambiguity referred to as rank deficiency if concentrations are linearly dependent, since absorptivity spectra cannot be uniquely estimated. Strategies have been developed to circumvent this rank deficiency, but, until recently, the validation of the kinetic model was frequently hampered, as estimated absorptivity spectra could be unknown linear combinations of the real spectra.

We have recently introduced a systematic method for the experimental and data analytical design of spectroscopic kinetic data that allows identifying the suitable strategy to circumvent rank deficiency [2]. Also, linear combinations observed in the estimated absorptivity spectra can be elucidated, allowing spectral validation of any postulated kinetic model, even with rank deficient concentrations [3]. In this contribution, we will present the impact of our systematic method on various simulated and experimental kinetic models commonly encountered in mid-IR and UV-vis spectroscopy.

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13

A Single-use Film as LDI Target Plate

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Single-use aluminum foil-based laser desorption/ionization (LDI) target plates have been developed for MS analysis, and provide detection results comparable to those of commercial stainless steel plates whilst offering a convenient way to avoid the time-consuming surface cleaning process. Additionally, arrays of TiO_2 nanoparticle spots are coated on the foil either by screen-printing or rotogravure-printing [1] followed by sintering to form mesoporous spots to act as anchor for sample deposition (Figure 1). These TiO_2 spots provide optional functions to the Al foil, such as matrix-free LDI or specific affinity for *in-situ* enrichment of phosphopeptides [2]. These single-use foils are cheap to produce, easy to use, and well suited for high-throughput proteomics research, and can also be of interest for protein post-translational modifications study.

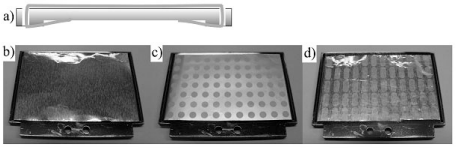

Figure 1. a) Schematic illustration of assembling foils on a modified commercial target plate. Pictures of modified target plate assembled with b) Al-foil, c) screen-printed TiO_2-Al-foil and d) rotogravure-printed TiO_2-Al-foil.

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A Kinetics Model for Space-confined Enzyme Catalyzed Reaction

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A kinetics model is proposed to illustrate the proteolysis catalyzed by the nanoporous-matrix immoblized enzyme. The entrapped enzyme could speed up traditional time consuming in-solution protein digestion.[1] A modified one-by-one proteolytic mechanism[2] was employed to simulate the kinetics of the reaction. Digestion of myoglobin was used to show the high efficiency of the novel proteolysis method, while angiotensin 1 and ACTH(1-14) was selected as models to validate the simulation. The investigations are in good accordance with the theoretical simulation that confinement effect is a major factor for the faster digestion, where the primarily digested peptides can be restricted in the matrix pores and then further proteolysis can be highly accelerated (Figure 1b). Meanwhile, the diffusion limitation is also an unnegligible factor (Figure 1a). The present work can help understanding many enzyme catalyzed reactions for high throughput protein profiling in proteomics research, and can promote the study of enzyme action occurred in the cell.

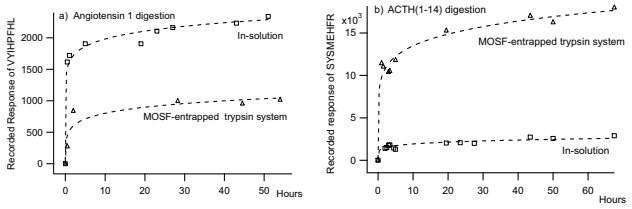

Figure 1. The produced digest plotted as a function of digestion time.

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Mapping magnetic forces produced by rectangular permanent magnets in static microsystems

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Magnetic beads (MBs) have become a powerful and widespread tool for both *in vivo* and *in vitro* applications, due to a nowadays well-developed surface chemistry and a tailor-made functionalisation. They have been used for many purposes in microfluidics, such as analyte labelling, sorting and separations of cells, mixing, transport of MBs using magnetic forces instead of a liquid flow and finally immunoassay. As the literature often focuses mainly on the biological application itself, relatively little information on the magnetic aspects has been published. Indeed, experimental parameters as the kind of magnets, their number, their strength, their size and/or configuration are often overlooked.

Finite element numerical simulations were carried out in 2D geometries to map the magnetic field and force distribution produced by rectangular permanent magnets as a function of their size and position with respect to a microchannel. A single magnet, two magnets placed in attraction and in repulsion have been considered. The goal of this work is to show where magnetic beads are preferentially captured in a microchannel. These simulations were qualitatively corroborated by microscopic visualizations of magnetic beads plug formation in a capillary. The results show that the number of plugs is configuration dependent with: in attraction, one plug in the middle of the magnets; in repulsion, two plugs near the edges of the magnets; and with a single magnet, a plug close to the center of the magnet.

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16

Rapid detection of small organic molecules and proteins in biological fluids using ultrasound-assisted Extractive Electrospray ionization mass spectrometry (EESI-MS)

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A novel method based on ultrasound-assisted EESI-MS has been developed and applied to rapidly detect the presence of small organic molecules in complex matrices, such as melamine in raw milk. Moreover, the fact that high mass proteins can be measured simultaneously opens up possibilities of this technique for analysis of biological samples in the liquid phase without sample pre-treatments. This technique required no sample preparation, and is ideal for high throughput measurements with low sample consumption.

To show the performance of this setup, a 3 μL aliquot of untreated milk spiked with 100 ppm melamine was analyzed and the corresponding mass spectrum, after background subtraction, is observed. Many different peaks appear. Protonated melamine was observed at m/z 127. It took only ca. 2 min to run four measurements. Afterwards, raw milk spiked with melamine at different concentrations (500, 100, 10 and 1 ppm) were measured in series, giving LOD ~ 500 ppm. [1] Furthermore, we simultaneously detect a broad ion distribution over a mass range of 900 to 1600 in raw milk. In the deconvoluted mass spectrum, the peak at m/z 24092 could be identified as β-casein in cows' milk. Other minor peaks could be assigned to other molecules of the casein family. Also, other biological matrixes have been measured. For instance, we found that the direct analysis of one drop of whole blood using ultrasound-assisted EESI resulted in the observation of multiply protonated haemoglobin subunits.

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Multiplexed Immunohistochemical Assays Using Time-Resolved Lanthanide Luminescence Imaging in a Lab-on-a-Chip Device

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The development of miniaturized bio-analytical systems is an ever-growing field of research[1] for the characterization of predictive biomarkers in malignant tumors and the follow-up of the disease during treatment.[2] We present here the design and implementation of a new PDMS-based microfluidic device that uses lanthanide luminescent probes for the simultaneous detection of multiplex tumor markers on cancerous tissues. This approach involves the development of a panel of Ln-labeled tumor associated antibodies which allow time-resolved imaging and trace the localization of tumor markers on the cancerous tissue where the immune complexes have bound.

The red-emitting Eu-W8044 and green-emitting Tb-W14016 chelates (PerkinElmer) were used in this assay, as they features adequate photophysical properties and possess an active group for easy protein coupling. Duplex assay results for the simultaneous detection of estrogen receptors (ER) and the human epidermal growth factor receptors 2 (Her2/*neu*) on breast cancer tissue sections are presented and discussed with respect to the conventional procedure.

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Metabolite profiling of plant extracts by ultra-high pressure liquid chromatography at elevated temperature coupled to time-of-flight mass spectrometry

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Detailed metabolite profiling of crude plant extracts, mandatory for both quality control and metabolomics purposes, requires high-resolution separation with a reasonable sample throughput. In this respect, the use of ultra-high pressure liquid chromatography working at high temperature and coupled to time-of-flight mass spectrometry (HT-UHPLC-TOF-MS) was evaluated in terms of achievable peak capacity for a given analysis time.
In a first step, it was shown that the longest column does not compulsory provide the maximal peak capacity for a given analysis time in UHPLC. From a theoretical point of view, a 150 mm column should be preferentially selected for gradient lengths up to 60 min at 30°C, while longer columns are attractive only for higher analysis times. Compared to 30°C, peak capacities were increased by about 20-30% for a constant gradient length at 90°C and gradient time decreased by 2-fold for an identical peak capacity.
In a second step, profiling of crude plant extracts was evaluated. Extracts from the model plant *Arabidopsis thaliana* and from a *Ginkgo biloba* phytopreparation were analyzed. For metabolites spread over a large polarity range (e.g., *Arabidopsis thaliana*) the use of high temperature (HT) was found beneficial. However, for the analysis of extracts containing more polar analytes (e.g., *Ginkgo biloba*), HT was found detrimental and causes a decrease in retention and thus resolving power.
Stability under HT conditions was evaluated and no apparent degradation was evidenced for both standard mixture and crude extract analyses. HT represents thus an additional parameter that can be considered for improving high-resolution profiling of extracts with relatively apolar metabolites.

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Comparison between the performance of columns packed with sub-2μm and fused-core particles for peptides analysis.

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Compared to the determination of small molecules, the analysis of peptides is more complex and necessitates specific chromatographic conditions. These last years, there has been a strong demand for fast and efficient peptide analysis, particularly for the quality control of peptides produced by biotechnological procedures or in the field of proteomics (peptide mapping).

The main objective of this study was to test two types of stationary phases for peptides masses ranging from 1000 to 5000g.mol^{-1}. In this context, columns packed with sub-2μm particles were tested to increase throughput while maintaining excellent performance. On the other hand, to limit the backpressure generated by small particles, columns packed with semi porous particles of 2.7μm that consist in a 1.7μm solid core covered with a 0.5 thick shell of porous silica were alternatively studied. The latter should provide faster mass transfer, particularly beneficial for large biomolecules. In this work, the mobile phase temperature was also increased up to 90°C to further improve chromatographic performance. Finally, the best conditions were selected for analyzing a tryptic digest and results obtained compared with regular HPLC analysis.

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CE-ESI-TOF/MS for human growth hormone analysis

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Endogenous human growth hormone (hGH) is secreted by anterior pituitary gland under several isoforms, the 22 kDa being the most abundant one. Other forms, including the 20 kDa isoform, are commonly called non-22-kDa. hGH is pharmaceutically prescribed to principally treat growth failure and hGH deficiency. Since the end of 1980s, recombinant hGH (rhGH) were developed through genetic engineering. rhGH have an identical sequence to the naturally occurring 22 kDa hormone, but some modifications of pharmaceutical rhGH, such as deamidation, oxidation or cleavage, may arise during purification process, as well as protein storage.
An analytical strategy based on capillary electrophoresis (CE) was developed to characterize hGH and rhGH. First experiments with basic pH enabled the identification of a deamidated hGH form, but bad repeatability was revealed. Acidic BGE conditions were tested in combination with organic modifiers to achieve stable and fast separations. Experimental design procedures were used to optimize CE coupling to ESI-TOF/MS. The developed method permitted the discrimination between natural hGH and rhGH according to their respective mobility. TOF/MS detection provided exact mass measurements, allowing for a supplementary distinction between hGH and rhGH by presence, respectively absence, of the 20 kDa isoform. The methodology was finally applied to the successful identification of rhGH in several seized samples.

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Blood doping with hemoglobin-based oxygen carriers (HBOC): analysis by CE-UV and CE-ESI-TOF/MS

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Blood doping is defined by WADA (World Anti-Doping Agency) as the use of products that enhance the uptake, transport, or delivery of oxygen to the blood. One approach uses artificial oxygen carriers, known as hemoglobin-based oxygen carriers (HBOC). These products are made of bovine or human hemoglobin (Hb), which is intra- or intermolecularly cross-linked, polymerized, or conjugated. Although several HBOC are under development or in clinical trials, only a few have been approved by authorities. Among them, Oxyglobin® (Biopure, Hb of bovine origin) was the only one approved for veterinary purpose by the FDA.
A complete analytical strategy based on capillary electrophoresis (CE) was developed to detect intact Oxyglobin® in plasma samples collected for doping control. The main issue consisted of ensuring sufficient electrophoretic resolution between Oxyglobin® and Hb that could be released from mechanical haemolysis. On-capillary detection was performed with UV at 415 nm, offering selectivity for hemoproteins (such as Hb and Oxyglobin®). Online MS detection with TOF analyzer was also used to provide accurate mass on CE peaks and unambiguous determination of Oxyglobin® uptake. A sample preparation based on immunodepletion was mandatory to remove most abundant proteins that interfered with CE separation and altered electrospray ionization (ESI). The developped methodology allowed reaching LOD of 0.3 g·dL-1 in plasma.

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Evaluation of a sheathless interface based on a porous tip for CE-MS coupling

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The hyphenation of Capillary electrophoresis (CE) with ESI Mass Spectrometry (MS) is a powerful method to provide high efficiency, sensitivity and selectivity. The successful operation of CE-ESI-MS system requires closed electrical circuits for both the CE separation and electrospray process. A wide range of interfaces has been proposed to satisfy this requirement. One of these is the new sheathless nanospray interface based on a porous tip developed by Moini [1]. With the latter, the electrical connection is achieved by inserting the capillary outlet consisting of a porous terminal section of fused silica into an ESI needle filled with a conductive liquid. The needle is independently grounded to close both CE and ESI circuits.
This interface was evaluated for hyphenating a CE instrument (ProteomeLab™ PA 800 Protein Characterization System, Beckman Coulter) with a single quadrupole MS instrument (1100 MSD, Agilent). Investigations aimed at reaching the best performance in terms of sensitivity (*S/N* ratio), resolution and stability. Numerous parameters were studied, taking into account each stage of the analytical process (*i.e.*, CE, interface, and ESI-MS). Various pharmaceutical compounds were tested (*e.g.,* amphetamines, β-blockers) and performance was compared to that obtained with a commercially available coaxial sheath liquid interface.

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23

Sphingo- and Phospholipidomic Approach in the Search for Biomarkers for Atherosclerosis

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Sphingolipids and phospholipids are structurally very heterogenous and encompass more than 1000 different molecular species. They play prominent roles in many critical metabolic and biochemical processes such as the formation and functioning of cellular membranes and plasma lipoproteins as well as signal transduction. Despite their great structural and functional diversity, phospho- or sphingolipids themselves have been seldom investigated for their pathogenetic contribution to disease and seldom exploited for diagnostic purposes. Studying and quantifying the complexity of lipids requires an advanced analytical technique, such as mass spectrometry. We developed methods utilizing LC-MS/MS for the analysis of ceramides (CER), sphingosine-1-phosphate (S1P), sphingomyelins (SM), phosphatidylethanolamines (PE), phosphatidylinositols (PI), phosphatidylcholines (PC), phosphatidylglycerols (PG), phosphatidic acids (PA) and their lysolipids in plasma.

The developed LC-MS/MS methods combined the neutral-loss and precursor-ion scanning ability of the triple quadrapole mass analyzer, together with HPLC separation. This approach allows the analysis of both the total endogenous amount of lipids within the same class (i.e. having the same head groups), as well as the concentration of each molecular lipid species (i.e. having a distinct fatty acid chain length) within a given class. It has been shown that the method allows reproducible semi-quantitative determination of the different lipid classes.

Preliminary results from the differential profiling of plasma samples from patients and healthy individuals showed the potential of the method to be applied in biomarker evaluation studies.

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Novel Analytical Approaches in Anticancer Metallodrug Research

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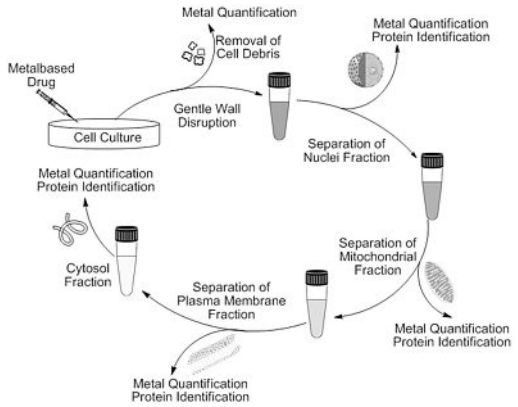
By the hyphenation of powerful separation methods such as multidimensional liquid chromatography and electrophoresis to ultra-high resolution mass spectrometers such as Fourier transform – ion cyclotron resonance (FT-ICR) and inductively coupled plasma (ICP) MS, new insights into the mode of action of already established but also novel metal-based anticancer drugs can be gained. The techniques allow highly sensitive investigations on the stability of the complexes under physiological conditions (important for pharmaceutical preparations), their interactions with DNA as well as human serum and cellular proteins *in vitro* and *in vivo*, requiring only minute sample amounts. It is expected that implementation of the proposed techniques at an early stage of the drug development process can greatly improve efficiency.



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Development of a Standard Sample for Tip-Enhanced Raman Spectroscopy

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Chemical information about structures on the nanometer scale from miniaturized manufacturing processes and the nanometer scale constitution of biological samples are currently under intense research. High resolution techniques, such as SEM, STM and AFM mainly yield topographic information, yet do not allow chemical assignment. Typical methods delivering chemical information such as Raman, IR or NMR lack the necessary high spatial resolution. Tip enhanced Raman spectroscopy (TERS) offers a combination of topographic imaging using atomic force microscopy with spatial resolutions of around 20 nm and Raman spectroscopy to obtain chemical information from the same small area.

An investigation of a potential standard sample based on inorganic nanoparticles, allowing the comparison of TERS instruments will be presented. The development of such a sample for the evaluation of the enhancement factor delivered by TERS tips in different labs using various approaches and setups is of great importance. Only an independent standard will allow real comparison of the data published by different groups in literature an put them into perspective.
Different materials and preparation methods have been evaluated for the special needs of such a standard sample. To calculate the enhancement factors this sample should allow measurements of enhanced Raman bands on (ideally) nanometer sized structures. Confocal measurements with (ideally) the same amount of Raman active material and background measurements without the Raman scatterer or with a different Raman scatterer are necessary to calculate the enhancement and check tip cleanliness, respectively. Natural diamonds are proposed and evaluated as a promising candidate to be used as such a sample. Different preparation methods and measurements from as prepared samples will be shown.

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Extended Domains of Organized Nanorings of Silver Nanoparticles as SERS Sensors for Molecular Detection

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Surface-enhanced Raman spectroscopy (SERS) is a powerful technique for identification of molecules due to the unique set of vibrations modes of any molecular species combined with tremendous signal enhancements observed on the surface of noble metals. However, SERS can be envisaged as an analytical tool only if substrates with reproducible enhancement can be produced. Substrates made of electrochemically roughened gold and silver surfaces show good enhancement factors but are often problematic regarding reproducibility and stability. In order to solve this problem, new substrates with well-defined nanostructures have recently been tailored in accordance with a detailed comprehension of the SERS mechanism. The huge electromagnetic field enhancement occuring near metallic surface irregularities in the SERS effect is due to the excitation of localized surface plasmons by light. Here we present the synthesis and SERS study of large ordered areas of silver nanoparticles on a Si(111) surface fabricated using electroless metal deposition and the nanospheres lithography method. We show for the first time the possibility to organize these nanoparticles in nanorings using a very simple method. Massive and reproducible SERS enhancements have been recorded. The possibility to control the size, the interdistance and the crystallinity of these nanoparticles allowed us to systematically investigate their influence on SERS. Combined with a rapid and easy procedure to clean these substrates in order to re-use them, promising applications as large-size, regenerable and low-cost chemical/biochemical sensors can be envisaged.

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Analysis of Plasma Degraded Biomacromolecules in a Plasma Air Purifier

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Infectious diseases take an enormous toll on people and on the economy in the whole world. Airborne pathogens cause many of such diseases and the biggest majority is uniquely adapted to spread in indoor environments. One method to avoid these contaminations in the air is by using a plasma air purifier. This plasma air filter is capable to degrade biological compounds without any absorbing material.
The aim of this project is to discover the mechanisms behind the degradation of biomacromolecules by using a plasma air filter (Askokoro, Zürich, Switzerland). It is known that a non-thermal plasma has a high potential in air cleaning technology,[1] but a possible disadvantage could be that in some cases unwanted degradation products are formed which could be more harmful than the original molecule. First, a system was designed which allowed nebulizing a compound, passing it through the plasma filter, and collecting the resulting fragments. For the performance check of the set-up, bovine serum albumin (BSA) was used for the nebulisation. BSA is a relatively high mass protein (MW=66.43 kDa) and available in large quantities. For the quantification of the sampled BSA an external calibration was made using high performance liquid chromatography (HPLC). The generated calibration curve of BSA has a coefficient of determination (r^2) of 0.9974 and the limit of detection (LoD) is 0.10 μmol L^{-1}.The highest sampling efficiency of the system for BSA was found for an air velocity of 0.25 m/s and the efficiency of the switched on plasma filter (6.1 kV) was 66.5% to 82.1%. Particularly fragments below 1000 m/z were noticed.

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Rapid Characterization and Monitoring of Food Quality by Extractive Electrospray Ionization Mass Spectrometry

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Mass spectrometry (MS) is the first choice for molecular characterization of complex samples, due to its unparalleled ability to acquire qualitative and quantitative data with high information content, as well as its exceptional sensitivity and specificity.

Herein, we report a simple yet universal method, termed extractive electrospray ionization (EESI) mass spectrometry to characterize and monitor quality of olive oil and honey samples. With this technique, olive oils and honey samples were characterized without any sample pretreatment. Using the EESI mass spectral fingerprints, 4 extra virgin olive oil samples (1 pure, 3 adulterated with 5% of edible oils), which are difficult to differentiate by smell, were successfully separated with high confidence in PCA score plots. Moreover, the rancid olive oil could also be differentiated from fresh olive oil samples. Besides, 2 honey samples (substandard and grade A honey) were also successfully characterized by EESI. The levels of hydroxymethylfurfural (HMF), a molecular marker to determine the quality of honey were identified by performing MS/MS. EESI-MS is an attractive tool to study the fundamental chemistry of complex viscous liquids without involving sample pretreatment steps.

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Coupling of parallel capillary electrophoresis and matrix-assisted laser desorption/ionization mass spectrometry for metabolite analysis

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Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) is a widespread technique mainly in proteomics, but recently, several applications to small molecule detection are showing a new potential for this ionization method. MALDI-MS coupled with a separation step, such as capillary electrophoresis (CE), can be a powerful analytical platform able to cope with some of the numerous challenges that metabolomics poses.
We present a simple method and apparatus to detect analytes separated in parallel capillaries via MALDI. The coupling is obtained by deposition onto custom-designed MALDI target plates.
The plates are coated with an omniphobic layer and patterned with parallel grooves that act as recipients of the effluent from the capillary. The matrix is loaded into the grooves prior to deposition of the analyte and the plate acts as ground electrode during the CE step. The deposition is achieved by dispensing the capillary effluent along the grooves onto the matrix layer previously deposited. This operation is facilitated by using an XY-plotter. The omniphobic polysilazane coating facilitates the deposition of a homogenous layer of matrix into the grooves and aids reliable deposition of the capillary effluent. The CE and deposition setup incorporate a UV detector as well.
We show MALDI-MS analysis in positive and in negative ion modes, using two different matrices, of a sample containing different classes of analytes such as peptides and nucleotides. We also demonstrate application of this method to the analysis of a crude cell extract spiked with a standard solution of phosphorylated metabolites.

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Design of a laser ablation cell for quantitative analysis of large samples with high spatial resolution

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Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) is a powerful technique for quantitative trace element analysis. Yet design and characteristics of the ablation cell is one of the crucial prerequisites for successful quantitative analysis.
Common ablation cells for LA-ICP-MS have a small volume in order to reduce the washout time of the laser generated aerosol, yielding a fast transport to the ICP. However, small volume cells can only hold small samples, that is a disadvantage when fast analysis is required or preservation of the sample is inevitable. We have developed a large ablation cell with fast washout for high resolution trace element analysis of a stalagmite slab, as a case study. Laser ablation scanning along the axis of growth of this stalagmite, rather than single hole drilling, gives highly resolved variations of trace element distribution in the stalagmite's annual layers. Fast washout of the aerosol is essential for this application, because mixing of the aerosols from different locations on the sample must be minimized in order to maintain the high resolution from ablation. Gas flows within the cell were modeled with Computational Fluid Dynamics (CFD) for further understanding of washout characteristics. The washout of the aerosol was measured to be 70% faster (for ^{23}Na) in the new cell compared to our standard ablation cell, which is in agreement with the simulations. One large sample with maximum dimensions of 230x29x9 mm (LxWxD) or a series of small samples can be mounted in the cell.

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Quantitative analysis of caffeine in beverages and food by HPLC-UV and qNMR

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Numerous food products and beverages contain caffeine, and caffeine is among the most widely consumed natural products. The quality control of caffeinated products usually relies on HPLC-UV and, therefore, the method is well established. Quantitative ^{1}H-NMR (qNMR), on the other hand, is increasingly used for quantification purposes because of its speed, simple sample handling and versatility [1]. There are currently few validated qNMR methods for quality control. Here, we developed a qNMR assay for the quantification of caffeine in beverages (energy drinks, tea and coffee) and solid food products (guarana powder, chocolate). The method uses an internal standard (1,3,5-trimethoxybenzene) and was validated (selectivity, precision, accuracy and robustness). HPLC-UV and qNMR assays were compared with respect to precision (0.7% and 1.2%, respectively) and overall error (3% and 2%, respectively). The comparison shows that qNMR can be an interesting option for quantitative assays of natural products lacking a chromophore and, hence, difficult to analyze by HPLC-UV.

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Copper-β-amyloid peptide complexes studied by electrospray ionization mass spectrometry

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Beta-amyloid peptides (Aβ) play a key role in the pathogenesis of Alzheimer's Disease (AD). Transition metal ions such as Zn, Cu, and Fe, concentrated in the amyloid deposits are presented to promote the abnormal Aβ aggregation[1], which is one of the hallmark of AD. The interaction of redox-active copper ions and Aβ is suggested to contribute to the toxicity of the peptides mediated by the generation of reactive oxygen species (ROS) through the oxidation of Cu^{+}[2]. However, the reactions involved and their role in the mechanism of oxidation remain unclear.

Electrospray ionization mass spectrometry (ESI-MS) is a powerful tool for studying biological molecules and can provide a great deal of information regarding the molecular weight and structure of the organometallic complexes. Moreover, sacrificial copper electrodes can electrogenerate copper ions for probing both Cu^{+}- and Cu^{2+}-peptide interactions in ESI-MS[3]. Since a few of direct studies of Cu^{+} binding or reactivity with Aβ have been reported, the complexes between copper and Aβ42 were investigated at first. Cu^{2+}-Aβ42 complexes were observed and the binding regions of Cu^{2+} were identified in the hydrophilic N-terminus by MS/MS. Then, interactions between copper ions and Aβ16 were investigated more in details either by mixing the solution with a copper(II) electrolyte solution or by using a sacrificial copper anode. Therefore, Cu^{+} complexes are observed and the comparison with Cu^{2+} complexes will be shown.

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Alternative in-source fragmentation pathways of benzylpyridinium ions produced in ESI

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The survival yield method is widely used to calibrate the internal energy of ions produced in mass spectrometer ion sources, in particular in electrospray ionization (ESI) sources. In this method, the so-called "thermometer ions" are used to probe internal energy the analyte obtain in the ion source. [1] Benzylpyridinium salts exhibit a simple fragmentation pattern and a broad dissociation energy range, and are therefore extensively used as thermometer ions. [2, 3]
In the present work, we critically address the reliability of the survival yield method and the use of benzylpyridinium salts as thermometer ions. The fragmentation patterns of several benzylpyridinium ions were studied by collision-induced dissociation (CID) mass spectrometry. Although benzylpyridinium ions have long been considered to have only a single dissociation channel, our findings clearly demonstrate that both the CID and the in-source fragmentation patterns are actually more complex. Quantum chemistry calculations (*ab initio* and DFT) were used to evaluate the uncertainty in energy calibration introduced by the extra channels found. Overall, our results suggest a more complex behavior of benzylpyridinium ions during the ESI process than generally believed.

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Rapid visual detection of endogenous biological cyanide

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Cyanide is one of the most toxic anions to humans and almost all other forms of life [1]. Nevertheless it is produced in nature by cyanogenic microorganisms or integrated and stored as cyanogenic glycosides in more than 2000 plants like sorghum, flax, giant taro, bamboo and cassava. The latter one is a staple food of about 500 million people in Africa and South America. To date the incomplete removal of cyanide during food processing leads to significant public health problems [2].
Although many different methods for the detection of cyanide are known, the simple and selective detection of cyanide in water is still a great challenge [3].
We demonstrated the use of vitamin B12 and cobalt corrinoids as nontoxic optical sensors [4]. Recently we reported the influence of the side chains of cobalt corrinoids on the sensitivity and selectivity in the sensing of cyanide [5]. We will present the colorimetric detection of endogenous biological cyanide in different biological samples as well as directly on its surface. The selective coordination of the sensor with cyanide was proven by UV-vis and NMR spectroscopy [6].

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Competition experiments followed by aliased 2D-NMR spectra. Affinity study of a small library of carbohydrates for a borohydrate.

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In recent years, we have developed NMR methodologies [1] allowing chemists to follow complex mixtures undergoing chemical reactions or dynamic processes [2] directly in the NMR tube. In order to avoid the overlap problems in 1D spectra, we use 2D HSQC to spread signals in a carbon dimension. Spectral aliasing of the carbon dimension makes it quick and easy to obtain the high resolution necessary to study similar molecules. The poster illustrate the power of NMR to study boronate esters and obtain structural as well as thermodynamic information about the molecules involved.

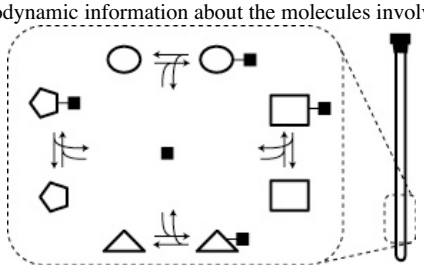

Figure 1: NMR study of dynamic libraries by NMR. The open shapes represent different carbohydrates and the filled square a borohydrate.

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Nanoscale Analysis of Biological Samples Using AFM and Raman Microscopy

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Biological samples are known to be highly heterogeneous on the nanometer scale. For example, biofilms consist of aggregates of microorganisms and extracellular polymeric substances (EPS), such as polysaccharides, proteins and humic substances. Essential characteristics like biofilm adhesion and biofilm formation, for instance, are controlled by EPS. Although biofilms play a significant role in technical processes like wastewater treatment, and have undesirable effects such as biofouling in industrial plants, the detailed structure and composition is not known. However, deeper insight might be used to develop more effective biocides as well as for process optimization in wastewater treatment. To obtain a better understanding of the structure within biofilms, spatially resolved chemical information is needed.
Biofilms of different origins, e.g. grown in hot springs or tunnel systems, were examined by atomic force microscopy (AFM) and Raman microspectroscopy. The obtained Raman map based on the intensities of specific marker bands was in good agreement with the different topological features of the AFM image, thus combining topographical and chemical information. Additionally, morphological and morphometric data of sperm cells were obtained by AFM, which were used to explain the differences in the reproduction properties of either the same or different fish species. Furthermore, the potential of Raman microscopy in the analysis of sperm cells has been demonstrated. Raman spectra were collected that reveal different chemical compositions of different parts of the cell. Raman bands collected on the heads of the sperm cells, for example, could be assigned to DNA.

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Machine learning applied to UPLC-TOF/MS metabolic fingerprinting for the discovery of wound biomarkers in Arabidopsis thaliana

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The metabolic response to wounding in plants involves a complex network of regulators. A high throughput metabolic fingerprinting approach based on ultra performance liquid chromatography (UPLC) and high resolution time-of-flight (TOF) mass spectrometry (MS) was previously developed for the detection of wound biomarkers in the model plant *Arabidopsis thaliana* [1]. Complex data of high dimensionality, hardly exploitable with standard data analysis tools, were generated.
In this study, machine learning methods are applied to identify the metabolites involved in the wound response and standalone classifiers or combinations of classification and feature selection algorithms are concurrently assessed. Cross validation resampling procedures are used to avoid overfitting. Remarkable performances are achieved with various methodologies, while models' stability shows the robustness and the interpretability potential of the methods. These models allow a notable insight of the wound phenomenon by highlighting numerous relevant biomarkers depicting its spatio-temporal development. The study demonstrates that machine learning methods represent valuable tools for the analysis of UPLC-TOF/MS metabolomic data in the context of stress signaling.

Keywords: machine learning, feature selection, metabolomics, UPLC-MS.

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38

Simultaneous selective detection of surface and liquid phase species by PM-IRRAS at solid-liquid interfaces: First design and performance

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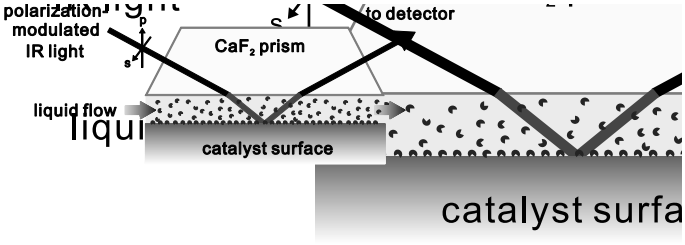
Probing chemical processes occurring at solid-liquid interfaces is a big challenge due to the complexity given by the presence of solvent and spectator species in concentrated forms. We present a novel vibrational spectroscopic method which allows simultaneous but separate monitoring of dynamic surface and liquid phase chemical processes occurring at solid-liquid interfaces. The designed PM-IRRAS cell allows stimulation of the surface processes by deliberate concentration and temperature modulation, thus exciting surface chemical processes. The technique offers great opportunities for mechanistic investigations of dynamic surface and solid-liquid interface processes by the time-resolved detection of surface and liquid phase species and the correlation of their dynamic behavior. Time-resolved studies on dynamic adsorption of acid molecules on metal oxides and CO oxidation over Pt are presented.



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Charge Separation in Inductively Coupled Plasma Mass Spectrometry (ICPMS).

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Inductively coupled plasma mass spectrometry (ICPMS) is a power technique for elemental analysis. Despite the high analyte sensitivity offered by current instrumentation, the detection efficiency is still only in range of 10^{-4} at best [1]. This is mainly due to the vacuum interface required to transform atmospheric pressure plasma into an ion beam for MS analysis.
It was shown that the ICP can be considered electrically neutral when entering the first stage of vacuum interface of ICPMS [2]. In current instrumentation the ion beam is formed after a skimmer where the plasma density is getting sufficiently small to achieve charge separation be the electrostatic field of the ion optics [3].
To improve ion transmission a new approach based on a configuration without skimmer is under investigation. It uses an electrodynamic ion funnel instead of common ion optic to guide ions from the sampler cone to MS detector. The ion funnel can focus ions by an RF field, created by the stack of ring electrodes of continuously decreasing inner diameter. The achievable fields at the funnel however were too small to allow for charge separation and formation of an ion beam. To avoid electrical contact of the plasma with the electrodes a new configuration is currently under investigation, which should allow the formation the ion beam upstream the funnel. The set-up consists of an arrangement of electrodes, placed within the expanding plasma, which serve to establish a strong field for more efficient charge separation.

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Establishment of an activity scale for clinical applications using ion-selective electrodes in a flow system.

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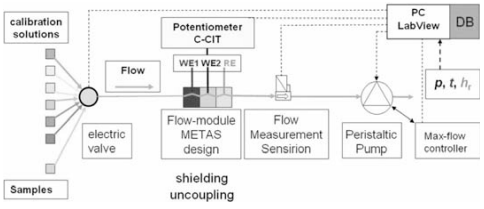
In clinical chemistry, the relevant quantity is often the free ion activity of the analyte and not the free content or concentration of substance as traditionally specified. Ion selective electrodes (ISE) are widely used to directly measure the activity of ions. ISE measurement is a standard method in point of care testing units (POCT) for continuous monitoring for e.g. haemodialysis, cardiac surgery and in intensive care units. The problem that the quality control faced today is that the IFCC guidelines recommends measuring and reporting ionized metal content as a substance concentration relative to the concentration in primary aqueous calibrators.



The EMRP Joint Research Project T2.J10 Traceable measurement of ion biospecies and ion activity in clinical chemistry "*TRACEBIOACTIVITY*" covers the improved reliability of ISE measurement results for monovalent and divalent ionic species. The main aim is to build up a scale for chemical activity of clinical relevant ions that is consistent with the already existing and accepted activity scale for pH.

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Microfluidic bio-assays based on electrostatically micro-patterned beads

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Protein functionalized microparticles (beads) are widely being used in combination with microfluidics for realizing on-chip bio-assays [1]. For performing bead-based on-chip bio-assays it is a pre-requisite to pattern and immobilize the beads inside the microfluidic channel. We have demonstrated a simple method for micro-patterning of streptavidin-coated beads on a glass substrate through electrostatic force-mediated self-assembly [2]. We use positively-charged aminosilane micropatterns, present on the microchannel surface, for electrostatically attaching the negatively-charged streptavidin-coated beads (Fig. 1). Our recent study demonstrates the possibility of performing high sensitivity (few hundred pg/ml of IgG) immunoassays and multiple antigen detection on a microfluidic chip using the electrostatically patterned streptavidin-coated beads as the assay substrate.

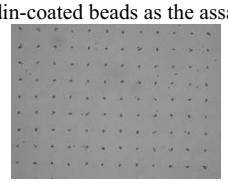

Fig. 1: Optical micrograph showing 1 μm diameter sterptavidin-coated beads electrostatically patterned on a glass substrate using aminosilane micropatterns.

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MEMS based compact FT-IR spectrometer

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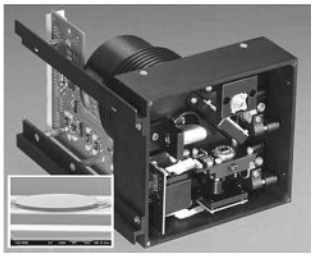
With the advent of small, compact micro-electro-mechanical systems (MEMS) it became possible to build pocket sized spectrometers for various spectral ranges, including the near-IR or mid-IR. Recently, we succeeded to construct a fully portable Fourier Transform spectrometer, based on a translatory MEMS micro-mirror component [1] (see inset). Using a nearly inertia-less MEMS devices to modulate the IR light allowed building a highly compact spectrometer with sub-millisecond scan times, which is immune to shock and vibrations. The Figure below shows the spectrometer prototype, including the optics and all on board electronics.



With a spectral range of 2-6 μm and a resolution of 10 cm^{-1} and its inherent multi-analyte detection capabilities, this spectroscopic sensor will open new application fields for smart, fast sensors.
Here we will present the device and show results from first measurement in order to characterize its performance in much detail.

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MEMS Scanning Grating IR Spectrometer Measures CO_2 in Beverages

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One of the main parameters for the quality control of carbonized beverages and beer is the amount of dissolved carbon dioxide. At present dissolved CO_2 in beverages is determined mainly by expanding the volume above the sample and measuring the partial gas pressure. The spectrometric approach offers an alternative for the measurement of CO_{2aq} with the advantage of not being interfered by other gases. A recently developed compact spectrometer[1] based on a micro-electro-mechanical reflective scanning grating[2] has been further developed for mid-IR operation, especially to observe the absorption band of CO_{2aq} which is located at 2343cm^{-1}. For the measurement of CO_{2aq} with a 25μm flow cell in a transmission setup, 3000 single scans were averaged to generate an adequate SNR yielding a repetition time of about 20 seconds. A calibration curve was obtained by measuring different CO_{2aq} absorptions which result from different concentrations of $NaHCO_3$ in a citric acid puffer at pH3. The resulting calibration curve is linear in a very good approximation. However, with this setup the detection limit was determined at 0.5g/l CO_{2aq} which shows the need for improvements. MEMS is an enabling technology to realize compact and reliable NIR and mid-IR analyzers with good spectral properties and fast response times. Furthermore the excellent mechanical stability of MEMS has the potential for being used as in-line sensor flanged directly to the main process pipeline.

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Major element analysis of various glass reference materials by IR-fs and UV-fs Laser Ablation-Inductively Coupled Plasma Mass Spectrometry

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a well-known technique for trace element analysis. Quantification is commonly carried out by external calibration using internal standardization. The internal standard is determined using complementary techniques, such as XRF or EPMA. Since few years a 100% normalization approach has been successfully applied, which is based on measurements of all major element oxides for internal standardization.
This study was focused on the determination of major elements (Na, Mg, Al, Si, K, Ca, Ti, Mn, Fe) in e.g. BCR-2G, BHVO-2G, BIR-1G and the MPI-DING series using IR- and UV-fs laser ablation. The ablation parameter were similar to those typically applied for trace element determinations (185 μJ – 2 mJ, 10 Hz, 45 to 170 μm crater size, 60 s ablation). NIST 610 was used as external calibration material and Si was used as internal standard.
The results indicate that fs-LA-ICP-MS is not significantly improving precision and accuracy for major element analysis. The influence of fluence or wavelength on the quantitative results is negligible. Precision for major elements was always in the range of 0.3 to 2.1 % and independent of crater size. Surprisingly, Na was consistently determined lower than the reference values (10-22%). However, using one of the above mentioned reference materials for calibration the accuracy improved to 3-5 %. Significant deviations were also observed for K All other major element concentrations agreed with the reference values within 1 to 10 % and indicate that matrix effects are most dominant for easy ionizable elements.

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Electrochemical Biosensor for Monitoring the Impact of Nanoparticles on Aquatic Microorganisms

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During the last few years a series of manufactured and engineered nanomaterials -such as nanoparticles, nanotubes or nanowires- have been increasingly used by nanotechnology industries due to their enhanced optical, electrical, catalytic or mechanical properties. Consequently, their potential release in the environment and subsequent ecotoxicological impacts is becoming a main concern that needs to be addressed. In this context, the present work describes the development of a biochip platform which is able to carry out multiplexed on-line analysis of the substances excreted throughout cells in their suspension medium. This original non-invasive cell monitoring approach relies on the implementation of ultramicroelectrode arrays (UMEAs) known for their outstanding analytical performances –such as high sensitivity, high signal-to-noise ratio, short response time- due to radial diffusion regime [1]. Each UMEA is biofunctionalized with a specific recognition enzyme via potential-control patterning procedures such as electrodeposition or electropolymerization. Selected targets are reactive oxygen species (ROS) such as superoxide and hydrogen peroxide, and malondialdehyde (MDA) that is typically produced by cells during lipid peroxidation. This amperometric biosensing platform is currently being used as a complementary tool to assess the effect of functionalized quantum dots and gold nanoparticles on green microalgae such as *Chlamidomonas reinhardtii* and *Chlorella kersseli* that constitute the base of the aquatic food chain [2].

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Comparison between nanoESI-MS and MALDI-MS combined with cross-linking for the analysis of non-covalent complexes

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Nondenaturing electrospray ionization mass spectrometry (ESI-MS) is an established technique to study non-covalent interactions in quaternary protein structures. However, matrix assisted laser desorption/ionization MS (MALDI)-MS combined with chemical cross-linking is a complementary approach to analyze protein complexes. Four complexes, creatine kinase (CK, a homodimer), glutathione S-transferase (GST, a homodimer), ribonuclease S (RNase S, a heterodimer) and leucine zipper (LZ, a homodimer) were studied by both nanoESI and MALDI. In all cases, cross-linking followed by MALDI-MS clearly shows the protein complex. CK and GST dimers were also easily detected using nanoESI. However, in the case of RNase S and LZ, only a small amount of RNase S complex was observed, while no LZ dimer was detected. The dissociation constants for all these systems are similar, in the μM to nM range. A potential explanation of the difference in the observation of complexes by nanoESI may thus be the nature of the interactions involved. In CK and GST dimers, hydrogen bonds and polar interactions, which should be maintained during the ionization process, prevail. In contrast, LZ and RNase S complexes are based on hydrophobic interactions, which are known to be difficult to survive the ionization. Chemical cross-linking combined with MALDI-MS can thus be used for all different types of protein complexes, whereas we showed that nanoESI is not suitable for non-covalent complexes based on hydrophobic interactions.

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Mechanistic Chemistry Studies: From Instrumental Design and Insitu Detection to Suitable Methods for Reaction Modelling.

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ETH Zurich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland

Detailed mechanistic studies of complex chemical reactions are challenging. They require state of the art insitu instrumentation, a well-defined reaction environment, powerful chemometric methods to elucidate the kinetics and thermodynamics, and chemical intuition. Kinetic and thermodynamic analysis are well-established under quasi-ideal conditions, e.g. for dilute homogeneous systems [1]. However, in contemporary synthetic chemistry, highly concentrated (solvent-free) conditions are often encountered, or heterogeneity can be present for example as in the formation of nano particles (sol-gel). Then, kinetic/mechanistic analysis generally becomes very complex.

We have developed a new high performance reactor offering a well characterised, versatile and highly controlled reaction environment suitable to study chemical systems of such complexity [2]. In this contribution, we present our studies on a four step consecutive reaction including preceding reactant dissolution. Deterministic model development based on mass transfer and time evolution of the dissolving solid particles including their breakage are discussed. Novel methods to elucidate the evolution of the particle size distribution from insitu laser reflectance and ATR-IR measurements during the course of the reaction will be presented.

Financial support by the Swiss National Science Foundation (grant no 200021-113473) is gratefully acknowledged.

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Evaluation of HILIC materials for the fast separation of polar compounds. Application to the analysis of substrates/metabolites and saccharides.

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Hydrophilic interaction chromatography (HILIC) or "aqueous normal phase" is a term introduced by Alpert [1] to describe a technique for separation and analysis of hydrophilic and polar compounds. It is characterized by a hydrophilic stationary phase and a mobile phase that contains an organic solvent percentage higher than 70%. These last years, numerous works have been reported on HILIC columns with conventional geometry, but there is an increased demand for fast separations with an improved resolution. Such separations can only be obtained with the new generation of columns and instrumentation. Today, a few manufacturers offer HILIC stationary phases packed with sub-2μm particles (UHPLC technology) or packed with semi porous particles of 2.7μm that consist of a 1.7μm solid core covered with a 0.5μm thick shell of porous silica (fused-core technology).
The aim of this study was to compare the performance and the chromatographic behavior of several HILIC columns packed with 1.5μm, 1.7μm and 2.7μm fused-core particles. It has been demonstrated that fused-core column generates efficiency 30% lower and a backpressure two to three times lower than that of UHPLC columns. However, as the backpressure always remains low in HILIC mode (low mobile phase viscosity), the columns packed with sub-2μm particle were far more interesting in terms of performance. Finally, the applicability of these columns was tested for two representative separations: i) a mixture of substrates and their metabolites, ii) various native saccharides. Retention of these polar analytes was acceptable on the different investigated columns and analysis time cuts to less than five minutes.

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Use of LC-PDA-MS and off-line microprobe NMR for the profiling of *Iris germanica* extracts

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The roots of German iris (*Iris germanica* L., Iridaceae) have been traditionally used for various topical applications including the treatment of sores and freckles [1]. Characteristic constituents of the root are isoflavones which reportedly show anti-inflammatory and anti-oxidative properties. For these reasons iris root extracts are used as cosmetic ingredients.
Lipophilic and polar extracts of iris root were submitted to a phytochemical profiling by semi-preparative HPLC and off-line NMR measurements in a 1 mm TXI microprobe (active volume 5 µL) [2]. A total of 18 compounds were purified in sub-milligram to milligram amounts via two successive chromatographic steps on a SunFire column (10 x 150 mm; 5 µm, Waters) with a gradient of acetonitrile in water containing 0.1% HCOOH. The compounds were identified as isoflavones, isoflavone glycosides and acetovanilllone by analysis of on-line MS and PDA, and off-line NMR data including HSQC and HMBC spectra. The activity of the isolated compounds on the proliferation of endothelial cells is currently being investigated. The example demonstrates the applicability of the off-line HPLC microprobe NMR approach as a robust means for a rapid chemical and biological characterization of the constituents of plant extracts.

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UPLC profiling of phenolics in Single Malt Scotch Whiskies

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[2] Waters AG, Täfernstrasse 4, CH-5405 Baden-Dättwil, Switzerland

Distilled spirits contain numerous phenolic constituents which are extracted, in part, from the wood cask during maturation. Such compounds may provide information on authenticity and quality of the products. Analyses have so far focused on the determination of the volatile constituents by GC. Few HPLC methods for whisky analysis have been published. However, they are time-consuming [1] or afford only limited resolution [2]. This prompted us to explore the potential of Ultra High Performance Liquid Chromatography (UPLC) for the analysis of whiskies. Using a HSS T3 column (2.1 x 100 mm; 1.8 µm, Waters) and a gradient of methanol in water containing 0.1% TFA over 60 min, more than 15 compounds, including phenolic acids, aldehydes, phenols and furans could be identified from their chromatographic and UV properties, and by comparison with authentic samples. In addition, several lignans were identified after semipreparative isolation and ^{1}H NMR measurement using a 1mm TXI microprobe (Bruker).
The UPLC assay was subsequently used for the quantification of the constituents in a selection of Single Malt Scotch Whiskies that have been classified into ten different clusters on the basis of their organoleptic properties collected from hundreds of tasting notes [3].

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Non-Radioactive Analysis of all Phosphoinositides by LC/MS

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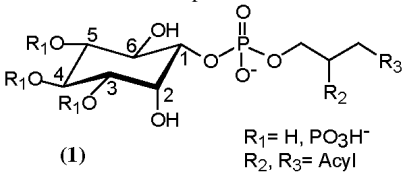
Phosphoinositides (PIs; (**1**)) play fundamental roles as signalling molecules in numerous cellular processes.



(**1**)

Direct analysis of PIs is typically accomplished by metabolic labelling with ^{3}H-inositol or inorganic ^{32}P followed by deacylation, ion-exchange chromatography, and flow scintillation detection. This analysis is laborious, time consuming, and involves massive amounts of radioactivity. To overcome these limitations we established a non-radioactive LC-ESI/MS assay for separation and selective detection of deacylated PIs. Separation of PIs was achieved by ion-pair chromatography on a polar endcapped RP-18 column. For the first time all PtdInsP and PtdInsP$_2$ isomers could be separated. We applied the method to various cell types to study changes in PI levels upon specific stimulations.

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High Performance Reaction Calorimetry with In-situ ATR-IR Spectroscopy for Tracking Lithiation and Alkylation/Acylation Reactions

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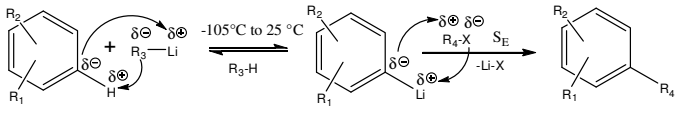
In fine chemical industry, the use of organolithium compounds is a modern and powerful technique for selective C-C bond formation. Lithiation reactions are generally performed at low temperature; they can range from as low as -105°C up to +25°C [1].



It is of great importance to optimally schedule the dosing of the alkylation/acylation electrophile (Step 2 in the Figure) to avoid recombination with the nucleophilic lithiation agent (e.g. n-BuLi) possibly remaining from Step 1. For a relevant case study, we show how our novel low-temperature (-70°C) dedicated reaction calorimeter with in-situ ATR-IR detection can be used to follow the course of the reaction [2], and to tackle this task to optimize reaction conditions.

We gratefully acknowledge financial support from Lonza AG, Visp.

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Towards single molecule enzymology of encapsulated enzymes

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Single molecular fluorescence spectroscopic studies of enzymes have paved the way for a better understanding of how enzymes work at the molecular scale. Previously we have shown that it is possible to measure molecular turnover of single enzymes (horse radish peroxidase) at glass surfaces previously functionalized with a biotin-derivatized polylysin-*g*-polyethyleneglycol polymer by using various fluorogenic substrates in combination with total internal fluorescence fluorescence correlation spectroscopy (TIR-FCS)[1, 2].

Polymeric nanocontainers with reconstituted OmpF have been reproducibly tethered to glass surfaces previously patterned by microcontact printing using BSA/BSA-biotin. The enzyme acid phosphatase was encapsulated in the nanoreactors and the fluorogenic substrate ELF-97 was used as a fluorescent probe in order to determine enzyme kinetic parameters using both fluorimetry and confocal fluorescence microscopy[3]. The kinetic parameters obtained from the free, the encapsulated, and the surface-tethered encapsulated enzyme are compared. Our results show that it will be possible in a near future to study enzyme kinetics at the single molecule level inside nanoreactors. This technique could potentially be used to develop new biosensors for screening enzymatic activity at an unprecedented sensitivity.

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Towards nanoscale molecular analysis and chemical imaging at atmospheric pressure by near-field laser ablation mass spectrometry

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For chemical analysis with high spatial resolution, methods that work at atmospheric pressure are especially suitable for the chemical characterization of biological samples such as tissue or even live cells.

Laser ablation mass spectrometry routinely reaches a spatial resolution of 50 - 100 μm and normally requires the sample to be in vacuum. In previous work [1], our group was the frist to demonstrate that near-field laser ablation at atmospheric pressure can in principle be coupled to mass spectrometry (SNOM-MS), ultimately allowing a spatial resolution below the optical diffraction limit (λ/2).

In the past years, we have developed an improved setup that combines near-field laser ablation at atmospheric pressure with an ion-trap/time-of-flight mass spectrometer.[2] With this instrument, spatially resolved molecular analysis of organic substances at atmospheric pressure could be shown with a lateral resolution as low as 5 μm.[3] The spatial resolution for our instrument is currently limited by the sensitivity of the MS. Different studies have been performed to gauge which factors restrict the sensitivity, and to help us develop approaches which could overcome these limitations, ultimately allowing analysis at the nanoscale.

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An in-Solution Molecular Approach to Solve the Disulfide Bridge Pattern of Recombinant Human Growth and Differentiation Factor 5.

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Native homodimeric growth and differentiation factor 5 (GDF5) belongs to the transforming growth factor β (TGF-β) superfamily which is involved in many developmental processes. Each monomer contains a cystine knot formed by three intrachain disulfide bridges and the monomers are connected via an interchain disulfide bridge [1].
Even tough the disulfide bridge pattern of recombinant homodimeric rGDF5 was recently elucidated by X-ray diffraction [2] no approach for *in-solution* determination of this pattern was available.
By applying a combination of proteolytic degradation and subsequent analyses of the disulfide-linked peptides by electrospray- and MALDI-TOF mass spectrometry, amino acid analysis, and Edman degradation we were able to unambiguously identify of the disulfide bridge pattern of the monomeric mutant rGDF5(C84A) and of the homodimeric rGDF5 in solution [3].

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