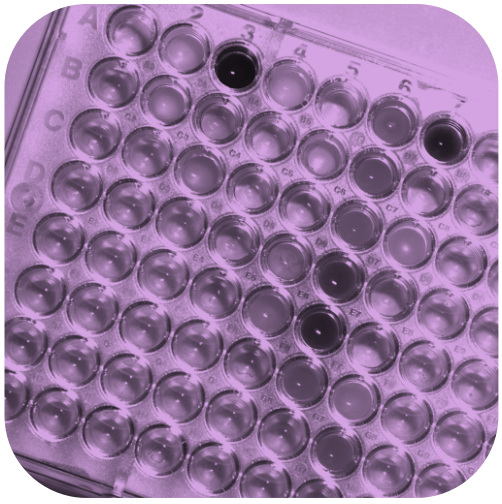
# *CH*analysis

Meeting of Swiss Analytical Scientists

November 29–30, 2013
Dorint Hotel Beatenberg

Organized by the Division Analytical Sciences
of the Swiss Chemical Society



SCS
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Division of
Analytical Sciences

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# General Information

## Organizing Committee:

Detlef Günther, ETH Zürich
Ernö Pretsch, ETH Zürich
Ralph Schlapbach, Universität Zürich, ETH Zürich
Marc Suter, Eawag

## Conference Office

Verena Schmid
c/o Eawag
Überlandstrasse 133
CH-8600 Dübendorf
Phone: 058 765 52 00
Fax: 058 765 58 01
E-mail: verena.schmid@eawag.ch

## Participation fee

CHF 250.– (includes meals and accommodation)

# Scientific program

**Friday, November 29, 2013**

12.00 Lunch

14.00 Opening

14.00 **Jürgen Schram**, Hochschule Niederrhein
*The book in chemistry*

14.45 **Sesssion 1: Imaging**
**Petra Dittrich**, ETH Zurich,
*Moderator*

14.45 **Daniel Grolimund**, PSI
*Chemical imaging goes 4D*

15.15 **X. Xie, E. Bakker**, University of Geneva
*Ion-selective and photo-switchable ion-exchanging nanospheres containing neutral ionophores*

15.30 **T.J. Stewart, J. Szlachetko, L. Sigg, R. Behra, M. Nachtegaal**, Eawag, ETH Zürich, and PSI
*Tracking intracellular Pb speciation dynamics in the green alga* C. reinhardtii

15.45 **F. Kurth, R. E. Wilson, A. J. Trüssel, D. J. Webster, R. Müller, P. S. Dittrich**, ETH Zürich
*A world-to-chip strategy for the analysis of* in vivo *stimulated cells*

16.00 Discussion

16.30 Coffee break

17.00 Poster session

19.00 Dinner

20.00 **Evening discussion:**
*Analytical sciences: Demands and opportunities*
**Ruedi Aebersold**, ETH Zürich
**Mattias Fricker**, Amt für Verbraucherschutz, Steinhausen
**Winfried Redeker**, Swiss BioAnalytics, Birsfelden
**René Schwarzenbach**, ETH Zürich
**Detlef Günther**, ETH Zürich, Moderator

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## Saturday, November 30, 2013

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9.00 **Session 2: Characterization of Nanomaterials**

9.00 **Vera Slaveykova**, Université de Gèneve
*Moderator*

9.00 **Ralf Kägi**, Eawag
*Detection and Quantification of Engineered Nanoparticles in Complex Matrices*

9.30 **O. Borovinskaya, S. Gschwind, B. Hattendorf, M. Tanner, D. Günther**, ETH Zürich
*Determination of elemental composition and mass quantification of individual metallic nanoparticles in a mixture by M-DG-coupled to a prototype ICPTOFMS*

9.45 **N. von Moos, V. Slaveykova**, University of Geneva
*Flow cytometry – an analytical tool for the investigation of nanomaterials and their interactions with cells*

10.00 **I.A.M. Worms, V. Slaveykova**, Universtiy of Geneva
*AFlFFF-ICP-MS to quantify interactions of metals in of ternary mixtures containing nanoparticles and humic substances: The case of QDs and humic fractions of Suwanee river HS*

10.15 Discussion

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10.45 Coffee Break

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11.15 **Session 3: Bioanalytics**
**Bertran Gerrits**, Novartis Pharma AG
*Moderator*

11.15 **Ralph Schlapbach**, UZH/ETH FGCZ
*Molecular analysis of biological systems: from single molecules to omics*

11.45 **K. O. Zhurov, A. N. Kozhinov, L. Fornelli, Y. O. Tsybin**, EPFL
*Analytical methods development for analysis of complex molecular mixtures with Orbitrap FTMS*

12.00 **N. Bohni, O. Schumpp, F. Mehl, S. Bertrand, M.Monod, K. Gindro, J.-L. Wolfender**, University of Lausanne, University of Geneva, Agroscope Changins ACW, Nyon, CHUV, Lausanne
*Targeted isolation of biomarkers highlighted by MS-based metabolomics in fungal co-cultures*

12.15 **H.A.O. Wang, C. Giesen, D. Grolimund, B. Hattendorf, B. Bodenmiller, D. Günther**, ETH Zürich, University of Zürich, PSI
*Multiplexed Chemical Imaging using Laser Ablation ICPMS at Sub-cellular Spatial Resolution*

12.30 Discussion

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13.00 Lunch

14.00 End of the meeting

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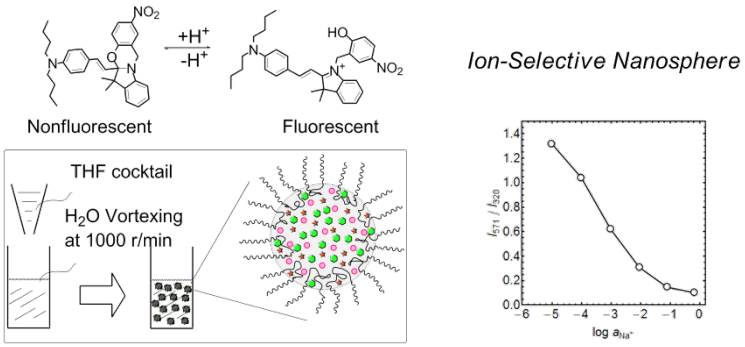
## *Ion-selective and photo-switchable ion-exchanging nanospheres containing neutral ionophores*

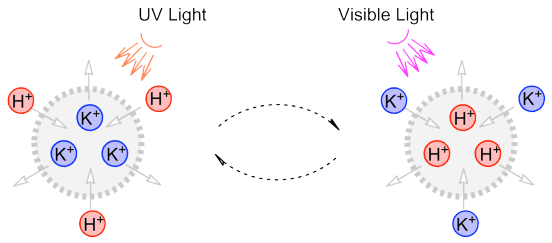
**Xiaojiang Xie and Eric Bakker**

Department of Inorganic and Analytical Chemistry, University of Geneva, Quai Ernest-Ansermet 30, CH-1211 Geneva, Switzerland.

Ion-selective indicators, whose light emission reflects the local concentration of the ion, have been used for chemical imaging purposes. Examples include indo-1, rhod-2 and fluo-3 for $Ca^{2+}$, lucigenin and SPQ (M-440) for $Cl^-$ , Sodium Green for $Na^+$ and so on. Although these indicators are widely used, they suffer from some drawbacks such as cytotoxicity, transition metal interference (i.e., selectivity), dye leakage and sequestration. Moreover, their synthesis is difficult, making these compounds expensive. The palette of detectable ions can be expanded with ion-selective optodes, which work on the principle of partitioning of ions between the sample and a sensing phase. Recently, bulk optodes have been miniaturized in order to develop micrometer or even nanometer sized ion sensors. We present here a convenient precipitation procedure to fabricate ultrasmall fluorescent ion-selective nanosensors that operate on the basis of bulk ion-exchange sensing principles.




The nanosphere matrix is composed of bis(2-ethylhexyl) sebacate (DOS) and a tri-block copolymer, Pluronic® F-127, which also functions as a surfactant to stabilize the nanoparticle. The particles can be prepared easily in large quantities without resorting to further complicated purification. Dynamic light scattering shows that these particles exhibit a monodisperse size distribution with an average diameter of 40 nm, suggesting that the nanoparticles are among the smallest ionophore-based ion-selective nanosensors reported to date. $Na^+$ and $H^+$ selective nanospheres were characterized by absorbance and fluorescence spectroscopy.




Based on this platform, we also created ion-selective nanospheres that can be triggered by light to bring about a localized ion-concentration perturbation. A photoswitchable compound, spiropyran, was incorporated into the nanospheres. Using $K^+$ as a model ion, we demonstrate that the nanospheres are able to release $K^+$ into the aqueous surroundings when irradiated by UV (365 nm) light. By illumination of visible light, they are able to uptake the $K^+$ back in and reduce the $K^+$ concentration in the surrounding solution. We aim to apply such photoswitchable nanosensors for drug delivery applications in living systems.

## *Tracking intracellular Pb speciation dynamics in the green alga* C. reinhardtii

T.J. Stewart,[1,2] J. Szlachetko,[3] L. Sigg, [1,2] R. Behra,[1] and M. Nachtegaal[3]

[1]Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH-8600 Dübendorf, Switzerland;

[2]ETH, Institute of Biogeochemistry and Pollutant Dynamics IBP, CH-8092 Zurich, Switzerland,

[3]Paul Scherrer Institute, General Energy Research Department, Bioenergy and Catalysis Laboratory, Villigen, Switzerland

Metal contamination of aquatic systems from human activities has disrupted natural biogeochemical cycles, placing pressure on organisms to cope with variable inputs of metals. Phytoplankton have developed metal regulatory mechanisms in response to changes in the bioavailability of potentially toxic metals, but a missing link in understanding these mechanisms is the ability to accurately measure intracellular metal speciation. To better understand Pb defense responses in a model green alga Chlamydomonas reinhardtii, changes in intracellular Pb speciation as a function of Pb bioavailability and exposure time were measured using Resonant X-Ray Emission Spectroscopy (RXES). Linear combination fittings (LCFs) using fingerprints of Pb standards generated from 2D-RXES maps provided high spectral detail and identified PbO, $Pb_3(PO_4)_2$, PbATP, PbS, and $Pb(GSH)_3$ as predominate intracellular species in *C. reinhardtii*. The exposure time and amount of intracellular Pb both influenced intracellular speciation, whereby initial Pb oxide and inorganic phosphate precipitation was observed. Complex formation with non-PC thiols and organic phosphate occurred at later exposure times and, under conditions of increased Pb bioavailability, formation of PbS precipitates was observed.

## *A world-to-chip strategy for the analysis of in vivo stimulated cells*

F. Kurth,[1] R.E. Wilson,[2] A.J. Trüssel,[2] D.J. Webster,[2] R. Müller,[2] P.S. Dittrich[1]

[1]Department of Chemistry and Applied Biosciences, ETH Zürich, Switzerland

[2]Institute for Biomechanics, ETH Zürich, Switzerland

In recent years, numerous novel cell analytical microfluidic platforms have been introduced to unravel cellular processes on the single-cell level and in cell cultures. However, many complex biological processes cannot be studied in these "artificial" systems, but require the natural environment of the cell. In this contribution we present a novel strategy to close the gap between in vivo models and on-chip cellular analysis.

Cell analytical platforms in microfluidics commonly rely on the use of cell suspensions and cell manipulation in artificial environments.[1–3] Although latest technological advances are able to provide more natural cellular environments *in vitro*, current culture systems cannot equivalently replicate the complex interactions among numerous cellular cues, such as biochemical, mechanical, or electrical signals. Particularly, in trabecular bone adaptation processes, dynamic mechanical loading directs bone formation and resorption responses locally towards an optimal balance between strength and minimal weight. Analyses of bone adaptation in in vivo models offer valuable insight to the interplay between the external forces and intracellular biochemical responses.[5,6] This way, alterations in protein and gene expression levels can be linked to dynamic changes in mechanical load.

As microfluidics offer great potential in biochemical analyses in terms of sensitivity, specificity, sample multiplexing and automation that outmatch standard bench top approaches, we developed a strategy to combine the benefits of *in vivo* models and microfluidic technology. The strategy combines microdissection of tissue samples by laser capture microdissection (LCM) and a microfluidic chip for cell capturing and analysis. A bone sample from a mouse caudal vertebra is harvested from a mechanical stimulated *in vivo* model. Based on strain energy density maps of the bone generated by micro finite element analysis calculated from micro-computed tomography data, single osteocytes of interest are identified. The vertebra is cryosectioned, and individual osteocytes are dissected by a focused laser beam and catapulted into open microchambers of our device, positioned above the sample. After cell dissection, the microchambers are closed by a glass slide using a clamp. The microchambers are connected to microfluidic channels allowing the subsequent supply of reagents. Thus, the cell transfer process can be characterized by nucleic acid staining, providing information on the transfer efficiency and cell integrity. As the cells are analyzed separately from each other, all retrieved data can be exactly linked to the previously defined extracellular bone environment.

Our results illustrate that our approach successfully combined LCM and microfluidic technologies to bridge the gap between in vivo models and cell analytical microfluidic platforms. Currently, the technique is optimized for the analysis of bone samples but the method can be employed for other tissues, cell cultures and biofilms, respectively.

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2. Y. Wang, J. Balowski, C. Phillips, R. Phillips, C. E. Sims, and N. L. Allbritton, Lab. Chip, **2011**, *11*, 3089–3097.
3. P. C. Gach, Y. Wang, C. Phillips, C. E. Sims, and N. L. Allbritton, Biomicrofluidics, **2011**, *5*, 32002–32012.
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## *Determination of elemental composition and mass quantification of individual metallic nanoparticles in a mixture by M-DG coupled to a prototype ICPTOFMS*

**O. Borovinskaya, S. Gschwind, B. Hattendorf, M. Tanner and D. Günther**

Department of Chemistry and Applied Biosciences, ETH Zürich, Switzerland

With the current rate of growth of nanotechology the global market of nanomaterials is expanding rapidly. Comprehensive characterization of these materials is essential for quality control and assessment of potential risks associated with their applications. Since the first attempt to use inductively coupled plasma mass spectrometry (ICPMS) for the analysis of individual nanoparticles,[1] this approach has received a lot of attention. Mass/size quantification and determination of particle number concentration can be realized using ICPMS. Moreover, employing a micro droplet introduction system, calibration without NP standard materials can be conducted.[2] However, to assess the properties of nanoparticles using commercially available ICPMS, which are based on sequential mass filters, the elemental composition of particles has to be known in advance. In contrast, utilization of an instrument which can rapidly measure all the masses of interest simultaneously for characterization of single nanoparticles would not require this prerequisite anymore.

Therefore, a prototype ICP time-of-flight MS (ICPTOFMS) which can read out the data at the rate of the extraction frequency (every 30 μs) was designed and constructed in our group in collaboration with TOFWERK AG (Thun, Switzerland).[3] The current characteristics of this instrument allow detecting multiple isotopes in the mass range of 7-238 Da in a time-resolved mode even from a single nanoparticle. The capabilities of this instrument for determination of elemental composition and quantification of individual particles will be presented and the results of mass/size quantification of Ag, Au and AuAg nanoparticles in the mixture will be demonstrated.

**References:**

1. C. Degueldre and P. Y. Favarger, Colloids and Surfaces A: Physicochemical and Engineering Aspects, **2003**, *217*, 137-142.
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## *Flow Cytometry – an analytical tool for the investigation of nanomaterials and their interactions with cells*

**N. von Moos and V. Slaveykova**

Environmental Biogeochemistry and Ecotoxicology, Institute F.-A. Forel, Earth and Environmental Science, University of Geneva, Versoix, Switzerland

Flow Cytometry (FCM) is a laser-based instrument for the multiparametric analysis of heterogeneous particles in suspension that measures particle number, size, granularity and fluorescence. Traditionally, it has mainly been used for basic research and medical purposes (blood diagnostics etc.) but in recent years its application has also been extended to environmental hazard assessment, especially in combination with biomarker detection by means of fluorescent probes. In the context of nanotoxicology, FCM can be a powerful tool for the investigation of interactions between nanoparticles (NPs) and living cells as well as their toxic effects in complex media.

There are two ways in which to distinguish NPs from cells. The first and simplest distinction can be based on the pattern obtained in the size (forward scatter) vs. granularity (side scatter) plot. Generally, NPs are smaller in size and exhibit less granularity and structural complexity compared to living cells. Thus, the NPs and cells generally form two distinct populations in the scatter plot. However, this approach is less straightforward when nanoparticles engage in direct interaction with cells and adhere to their surface and/or when nanoparticles form aggregates in the same size range as the cells of interest. In this case, the second possible distinction based on fluorescence is more accurate. In this way, photosynthetically active cells, such as microalgal cells, can easily be distinguished from nanoparticles by their natural autofluorescence, given the nanoparticles do not have overlapping emission.

This approach additionally offers ample opportunities to be exploited by intelligent experimental design based on cells and nanoparticles (e.g. quantum dots) with emission wavelengths lying wide apart. In combination, these two approaches enable the accurate distinction of single and aggregated nanoparticles from cells as well as cells in direct contact with nanoparticles. However, the full power of FCM lays in the additional use of fluorescent probes for cellular biomarkers of stress, such as for example Propidium Iodide for cell integrity, CellRox Green for intracellular reactive oxygen species, CM-H2DCF for intracellular esterase activity or C11-BODIPY581/591 for lipid peroxidation, to name just a few. We demonstrate how $TiO_2$ can be discerned from Chlamydomonas reinhardtii cells and how fluorescent probes can provide insight into the mechanisms of NM toxicity.

## *AFlFFF-ICP-MS to quantify interactions of metals in of ternary mixtures containing nanoparticles and humic substances: The case of QDs and humic fractions of Suwanee river HS*

**I.A.M. Worms and V.I. Slaveykova**

University of Geneva, Faculty of Sciences, Institute F. A. Forel, 10, route de Suisse, CH-1290 Versoix, Switzerland

The asymmetrical flow field-flow fractionation (AFlFFF) coupled on-line to UV- detection and inductively coupled plasma-mass spectrometry was used in the present study to quantify the size and the elemental distributions in a system containing carboxyl-terminal group polymer coated CdSe/ZnS quantum dots (QDs) as model engineered nanoparticles, Suwannee River fulvic (SRFA) or humic (SRHA) acids and trace metals (Cu or Pb). Obtained results showed a narrow QD size distribution with an average hydrodynamic diameter of 12.8 ± 0.5 nm. Addition of both 20 mg $L^{-1}$ SRFA, or SRHA, and 100 µg $L^{-1}$ or Cu and Pb did not significantly influence the QDs hydrodynamic size distributions. The AFlFFF-UV-ICP-MS set up also offers considerable potential for characterization of the distribution of trace metals such as Cu and Pb within natural and engineered nano-sized particles fraction. In the ternary system containing QDs, humic acids and metals, the amount of Cu or Pb bound to the QDs was 12 and 5 times higher than that associated to the SRFA or SRHA, respectively. Environmental implications of the results are discussed with respect to the role of nanoparticles as potential trace metal vectors and consequences on metal bioavailability was exemplified by measuring the bioaccumulation of Cu and Pb in tree green microalgae differing in their cell-wall composition.

## *Analytical methods development for analysis of complex molecular mixtures with Orbitrap FTMS.*

**Konstantin O. Zhurov, Anton N. Kozhinov, Luca Fornelli, Yury O. Tsybin**

Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

Recent hardware-based advances in high-resolution mass spectrometry enable analysis of complex mixtures of organic origin (e.g., proteomic, metabolomic, petroleomic, etc). However, to insure that efficiency of any such analysis of a complex mixture is maximized, corresponding software-based techniques also needed to be developed. We present two data processing and one data analysis methods that aim to augment the depth of sample characterization and facilitate analysis of ensuing data on thousands of compounds present therein. The methods were field tested on data obtained with an Orbitrap Elite FT mass spectrometer, with resolution of 480,000 at 400 m/z, with eFT option ON.

The first method relates to the ubiquitous problem of identifying analyte vs. noise components in any given mass spectrum that ultimately determines the degree of confidence in assignation of low level analytes. Effectively, a plot of component density vs. log intensity is used to separate the noise vs. analyte distributions, and, based on that, a noise thresholding value is proposed that optimizes for the number of false positives vs. false negatives. Petroleomic and proteomic samples are used to illustrate method performance.[1]

The second method involves post-acquisition recalibration of the obtained mass spectra. As complex mixtures may contain up to 200 peaks per Dalton, a very high resolution is required to separate them. In-built calibration procedures generally provide mass errors on the order of one to several ppms. However, in order to successfully assign the analyte peaks, very high mass accuracy is required, at sub ppm levels. An iterative empirical method is proposed that uses some of the analytes as internal calibrants, resulting in RMS ~350 ppb mass errors for highly ordered analytes, such as petroleomics.[2]

The third method allows for fast and facile overview of the entire complex mixture, based on grouping of all the assigned compounds by common heteroatom content. In essence, a 2D hexagonal projection of the 3D axis, labelled with N, O and S atoms, is used to map the entire complex mixture. Total peak intensity of a given heteroatom class is converted into a colour abundance scale, thus additionally providing relative abundance % for all identified heteroatoms. The hexagonal class representation may be used to compare multiple samples on a single projection, based on differences in geochemical origin, storage, sample processing, ionization method, etc.[3] The method can be readily applied to characterize crude oil samples, bio-oils, coal, dissolved organic nitrogen, environmental samples, etc.

The developed platform, encompassing the presented methods, is thus applicable to analysis of moderately complex crude oil samples, MS/MS spectra of large biomolecules such as antibodies, and can be extended to analysis of highly complex molecular mixtures of organic origin. Notably, the degree of sample complexity feasible for analysis with this platform is primarily limited by the maximum resolution obtainable on the instrument. This issue is currently being addressed by application of advanced signal processing methods[4] and attainment of longer transient signals.

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4. Kozhinov, A. N.; Tsybin, Y. O. Analytical Chemistry **2012**, *84*, 2850-2856

## *Targeted isolation of biomarkers highlighted by MS-based metabolomics in fungal co-cultures*

**Nadine Bohni,[1] Olivier Schumpp,[2] Florence Mehl,[1] Samuel Bertrand,[1] Michel Monod,[3] Katia Gindro,[2] Jean-Luc Wolfender[1]**

[1]School of Pharmaceutical Sciences, University of Lausanne, University of Geneva, Geneva, Switzerland

[2]Mycology group, Agroscope Changins ACW, Nyon, Switzerland

[3]Departement of Dermatology and Venereology, Laboratory of Mycology, CHUV, Lausanne, Switzerland

The prevalence of Fusarium spp. as causative agent of onychomycoses is rising and Fusarium spp. as well as other non-dermatophyte fungi appear to be insensitive to systemic standard treatment.[1] Hence, new antifungal agents active against Fusarium spp. are needed. In a large screening,[2] different plant and human pathogenic fungi were co-cultured with Fusarium spp. in Petri dishes. Few fungi were able to keep Fusarium at bay, among them the Basidiomycete Hohenbuehelia reniformis. Furthermore, co-culture of both fungi on the same Petri dish strongly induced the release of red pigments in the growth medium. A metabolomics study using UHPLC-TOFMS analyses proved to be efficient to detect up-regulation of several metabolites. On the other hand, the up-regulation of pigments could only be confirmed using a specific UHPLC-UV analysis. In fact, these compounds were of low abundance in the fungal extract and due to their strong chromophore, their quantity was overestimated in UV-detected analyses. This explains their rather low ion intensity in the MS-detected analyses.

Still, isolation of biomarkers (significant loadings highlighted by statistical analysis (OPLS-DA) of the UHPLC-TOFMS datasets) revealed to be difficult. In many cases, very minor constituents with good ionizability in MS were highlighted in the MS-based metabolomics study and were thus difficult or impossible to isolate. In addition, extracts of the mycelium of fungi grown on agar contain mainly saccharides as confirmed by NMR of the total extract. To get around this, prefractionation with the resin HP20SS was successfully applied to separate secondary metabolites from the saccharides that made up for ~70% of extract mass. Thus, ELSD – evaporative light scattering detection, a universal and quantitative detector for LC – was chosen to devise an isolation strategy to purify the biomarkers that are potential antifungal compounds involved in the capacity of H. reniformis to repel Fusarium spp.

This work shows that ELSD is especially well adapted for isolation of fungal metabolites given the large chemical diversity of molecules produced by fungi. The analytical strategy for the isolation of biomarkers and Fusarium pigments from fungal co-cultures grown on solid medium is presented.

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## *Multiplexed chemical imaging using laser ablation ICPMS at sub-cellular spatial resolution*

**H.A.O. Wang,[1] C. Giesen,[2] D. Grolimund,[3] B. Hattendorf,[1] B. Bodenmiller,[2] D. Günther[1]**

[1]Trace Element and Micro Analysis Group, ETH Zurich, 8093 Zurich
[2]Institute of Molecular Life Sciences, University of Zürich, 8057 Zürich
[3]microXAS Beamline Project, Swiss Light Source, PSI, 5232 Villigen PSI

High spatial resolution multiplexed chemical imaging on biological samples is an emerging field of application of LA-ICPMS, since the understanding of cellular phenotypes as well as cell-cell interactions requires sub-cellular feature recognition and simultaneous multi-protein detection. In this study, an imaging setup using a 193nm Laser Ablation system coupled to a high temporal resolution ICP-TOFMS (CyTOF®) is demonstrated. A fast washout LA cell was used to enhance the signal to noise ratio of the LA transient signal, enabled complete separation of ion signals from individual pulses generated by 20 Hz LA.[1] As a result, sub-cellular (~1 µm) spatial resolution imaging of biological tissue thin sections could be achieved with the presented system. In a case study, up to 32 metal-tagged antibodies were imaged simultaneously in thin sections of breast cancer tissue. The resulting high resolution images help biologists to differentiate various breast cancer sub-types, and analyze the effect of the cell microenvironment.[2] The presented imaging setup will open new research opportunities for cell biologists, pathologists and pharmacologists, to study cancer metastasis, to better categorize cancer patients, or to develop personalized medicines.

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

## *Direct arsenic sensing by a renewable gold nanoparticle plated Ir-based microelectrode*

**Romain Touilloux, Mary-Lou Tercier-Waeber, and Eric Bakker**

Dept. of Inorganic and Analytical Chemistry, University of Geneva, Sciences II, 30 Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland

Arsenic, originates from both natural and anthropogenic sources, is ubiquitous in all natural waters. We aim to determine arsenic (III) in water at nanomolar range and at natural pH. For this purpose we have developed a microelectrode capable of quantifying As(III). This microsensor consists of a gold nanoparticle plated Ir-based microelectrode (AuNP-IrM). One of the key advantages of this device is the possibility to renew the AuNP layer by electrochemical control. Moreover, it is possible to tune the gold layer thickness, which can be approximated by the reduction charge of Au(III) to Au(0). The characterization of this microsensor for the detection of As(III) in synthetic media at natural pH (pH 8) using Square Wave Anodic Stripping Voltammetry is reported. The data reveal that reproducible linear calibration curves are obtained for arsenic (III) concentrations in a range of 10 to 50 nM using a 3 minute preconcentration time. The Cu interference is negligible for Cu:As concentration ratio d 10:1. The measurements are reproducible in time with different gold layers for a given arsenic solution. The gold nanoparticle layer has a lifetime of at least one day.

## *Enhancing the experimental optimization of SRM transitions sensitivity using an automated approach*

**Bandar Alghanem,[1] Aivett Bilbao,[1,3] Ying Zhang,[1] Dario Bottinelli,[2] Frédéric Nikitin,[3] Markus Mueller,[3] Frédérique Lisacek,[3] Jeremy Luban,[2] Caterina Strambio De Castillia,[2] Emmanuel Varesio,[1] Gérard Hopfgartner[1]**

[1]Life Sciences Mass Spectrometry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland

[2]University of Massachusetts, Medical School, Program in Molecular Medicine, Worcester, MA, USA

[3]Proteome Informatics Group, Swiss Institute of Bioinformatics, Geneva, Switzerland

Quantitative proteomics is essential to system biology to obtain information at the molecular level. Selected reaction monitoring (SRM) also known as multiple reaction mentoring (MRM) in conjunction with stable isotope dilution is employed as a standard workflow for peptides quantification in proteomics. Crucial steps in the development of SRM assays are: the identification of representative peptides, the best selection of SRM transitions pairs (precursor/fragment ion), and the optimization of the instrument parameters, in particular collision energy (CE). In general there are three ways to select transitions in SRM assays; to perform shotgun proteomics experiments, to consult proteomics databases or to generate synthetic peptides libraries. While with shotgun experiments and SRM database the building of SRM assay is straightforward, the optimization of SRM methods with synthetic standards can be time intensive. In the present work the selection of the best SRM transitions (selectivity and sensitivity) for synthetic peptides and the tuning of instrument parameters were performed using a fully automated approach considering the acquisition parameters (charge state, CE, declustering potential) resulting in the selection of most favorable transitions.

Hundred and six isotopically labeled (`heavy`) peptides were investigated on a QTRAP 5500 individually by flow injection analysis (FIA). MS2 spectra were acquired by ramping the CE voltage (10 steps) for different precursor ions charge-state using a batch builder script on analyst 1.6 (AB SCIEX). Predominant charge state of each peptide, fragment ion assignments, and CE profiles generation were obtained using in-house developed java-based software (SRMoptimizer). The results of the approach were compared with regards to sensitivity to those obtained from SRMAtlas or Skyline.

## *All solid state chronopotentiometric ion-selective electrodes based on ferrocene-functionalized PVC*

**Zdeňka Jarolímová, Gastón A. Crespo, and Eric Bakker**

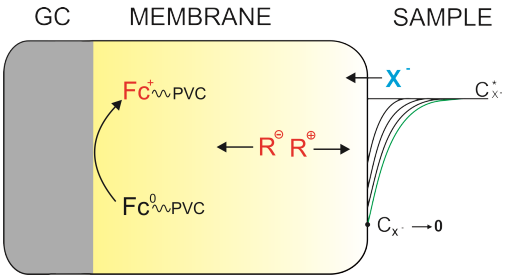
Department of Inorganic and Analytical Chemistry, University of Geneva, Quai Ernest-Ansermet 30, CH-1211 Geneva, Switzerland

Since a great variety of ion-to-electron transducers are available nowadays, many electrodes configuration that operated with liquid-solid transducers have become in all-solid-state electrodes.[1–2] The most prominent example is the ion-selective electrode operated with potentiometry.[3] Very clear advantages have been reported when a solid material (nanostructure materials, conducting polymers) replaces the inner liquid solution.[2,4–7] Miniaturization (chip and single use device), easy-construction and diverse positioning sensing (not only vertical) are some of the strength features.[8]

In that context, we present an all solid contact ion-selective electrode based on poly(vinyl chloride) covalently modified with ferrocene (Fc) moieties that allows one to operate the membrane in a chronopotentiometric sensing mode. The membrane is considered as initially non-perm-selective towards anions, and an applied anodic current provokes a defined anion flux in direction of the membrane. As shown in Scheme 1, Fc has the role of the ion-to-electron transducer at the glassy carbon/membrane interface. Furthermore, the oxidation of Fc to $Fc^+$ "turns on" the membrane sensing mechanism at the sample interface for anions in this case. With this protocol, a variety of anions (chloride, nitrate, thiocyanate and perchlorate) can be depleted at the membrane surface. In absence of an ionophore, their order of preference follows the expected Hofmeister selectivity sequence. The all solid-state configuration tolerates an imposed current density of 1.7 μA $mm^{-2}$, which translates into an upper detection limit of ca. 1.4 mM. Higher current densities of up to 31.2 μA $mm^{-2}$ are possible with addition of freely dissolved alkyl ferrocene derivative for an expected upper detection limit of 17.0 mM. In view of developing an analytical sensor, different anions are tested. A linear range of two orders of magnitude from 0.01 to 1 mM is found. The membranes are evaluated over several days, displaying practically the same slopes and intercepts, with a RSD of less than 2%. Electrochemical limitations of free Fc and bound Fc are critical evaluated.[9]

On the other hand, if the membrane contains an ionophore (selective to carbonate) the Hofmeister selectivity sequence is modified, in analogy, to potentiometric experiments. Therefore, carbonate ion can be also measured using chronopotentiometry mode.[10] A titration example will be shown with the aim to mimic the determination of total alkalinity "in-situ" conditions.

Finally, this approach should allow one to develop a new family of solid-state chronopotentiometric ion sensors that require relatively high current densities. In fact, the development of an all-solid-state electrode for detecting polyions in undiluted human blood is currently performed in our lab.




Scheme 1. Schematic illustration of the all solid state membrane electrode mechanism.

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## *Parallel ultra high pressure liquid chromatography - mass spectrometry for the quantification of HIV protease inhibitors using dried spot sample collection format*

**Kyoko Watanabe,[1,2] Emmanuel Varesio,[1] Gérard Hopfgartner[1]**

Life Science Mass Spectrometry, School of Pharmaceutical Sciences, University of Lausanne, University of Geneva, 24 Quai Ernest Ansermet, CH-1211 Geneva 4, Switzerland

Global Application Development Center, Shimadzu Corporation, Kyoto, Japan

For the quantification of pharmaceuticals and their metabolites in biological matrices, mass spectrometric detection using selected reaction monitoring (SRM) mode has become essential for developing sensitive and selective assays. Ultra high pressure liquid chromatographic (UHPLC) separation allows reducing the analysis time while maintaining very good separation efficiency. However, for multiple analytes SRM mode acquisition with short dwell time (≤ 10 msec) is mandatory to maintain good peak shapes and accurate and precise quantitative values.

To allow high sample throughput while maintaining good chromatographic performance we developed a parallel UHPLC system with two low pressure gradient pumps combined to a triple quadrupole mass spectrometry for the quantitative analysis of eight HIV protease inhibitors using tube based dried spot sample collection format. This sample collection format has several advantages over card format : 1) sample collection and sample preparation is performed with the same device, 2) larger sample volumes can be collected. Under ambient conditions the sample drying time is about 2 hours. Using microwave heating this time could be shortened to only 5 minutes without any analyte degradation. The assay was validated for human plasma and blood spiked with HIV protease inhibitors in the range 25-20,000 ng/mL. The LLOQ was found to be 25 ng/mL and 50 ng/mL for all compounds in plasma and blood respectively. Analysis of clinical samples was also demonstrated. The parallel UHPLC system allowed reducing the analysis time from 6 to 4 minutes and therefore sample throughput was improved significantly without any compromising analytical performance. The assay was applied to analyze clinical plasma samples using a QUAL/QUAN approach with SRM mode for quantification and product ion scan mode with fast scan speed (15000 u/sec) for confirmatory analysis.

## *Quantification of the hormone peptides ghrelin and des-acyl ghrelin in human plasma with high selectivity by cubic selected reaction monitoring (LC-SRM3/MS)*

**Jonathan Sidibé, Emmanuel Varesio, Gérard Hopfgartner**

Life Sciences Mass Spectrometry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest Ansermet 30, 1211 Geneva 4, Switzerland

Ghrelin is a bioactive peptide initially identified in rat stomach which stimulates appetite and growth. Two forms of the peptide were observed, the acyl-ghrelin with an octanoyl modification on the third serine residue and the des-acyl ghrelin lacking this modification. The current method for the quantification of ghrelin in biological samples is ELISA. However, ELISA may over-estimates ghrelin concentration because the immunoassay may not be selective enough to differentiate ghrelin from desacyl-ghrelin or from a common precursor the prepro-ghrelin. LC-MS analysis is particularly adapted to overcome this selectivity issue especially when using the selected reaction monitoring (SRM) mode for quantitative analysis. Peptides such as ghrelin, are generally ionized, in electrospray, in several charge states ($4^+$ to $8^+$) depending on the mobile phase conditions. Beside the loss in sensitivity the charge state distribution may changes over the concentrations range and sample background which affects the limit of quantification, the accuracy, the precision and the dynamic range of the assay. To overcome these limitations a method based on the sum of multiple charged states and their corresponding fragments was developed for the quantitative analysis of ghrelin and des-acyl ghrelin in human plasma. By summing, SRM transitions of the $6^+$; $5^+$ and $4^+$ charge states of the ghrelin peptides, quantification performances were improved in term of sensitivity, accuracy and reproducibility.

However, limited selectivity of the SRM transitions has been observed in human plasma samples at low concentrations (100 pg/mL). Thus, a LC-MS/MS/MS method based on a triple quadrupole linear ion trap was developed, where second generation product ions from multiple charge states peptides are selected and summed (LC-SRM3/MS). Compared to LC-SRM/MS with LC-SRM3/MS the selectivity of the assay was significantly improved. The quantification method has been validated and the limit of quantification (LOQ) was 50 pg/mL and 75pg/mL for des-acyl ghrelin and ghrelin respectively. Quantification of both peptides in human plasma samples has been achieved, and concentrations ranging from 92.1 to 179.7 pg/mL for des-acyl ghrelin and from 80.5 to 225.3 pg/mL for the ghrelin were measured.

## *Unexpected occurrence of caffeine in sleep inducing beverages*

**C. Mathon[1,2] S. Bieri,[1] P. Christen[2]**

[1]Official Food Control Authority of Geneva, Quai Ernest-Ansermet 22, 1211 Geneva 4, Switzerland

[2]School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland

Herbal food supplements (HFS) are widely marketed with various health claims, easily available and, have generally not been through a rigorous testing process. The Official Food Control Authority of Geneva controls the composition of HFS, and during a survey of more than 100 herbal food supplements by high performance liquid chromatography coupled to hybrid mass spectrometry (HPLC-MS/MS),[1] caffeine was often detected in slimming food supplements but also in sleep inducing foodstuff.

Caffeine acts as a weak psychostimulant and is known to cause trouble sleeping.[2] Thereby, the presence of caffeine in sleep-aid supplements was surprising, and further investigations were conducted to exclude any possible misidentifications. The botanicals of the sedative mixtures were analysed individually by Ultra Performance LC coupled with a time of flight (TOF) MS, and caffeine was detected in linden (Tilia spp.) extracts. The presence of caffeine was confirmed by the direct analysis of powdered linden by thermal desorption coupled with a GC×GC-TOFMS.

Once caffeine was unequivocally identified it was quantified in 11 samples containing linden, with a validated Ultra Performance LC-MS/MS method. Caffeine concentrations were between traces (< LOQ) and 110 mg kg-1 while those in prepared sedative beverages ranged from traces (< LOQ) to 237 µg per cup of infusion.

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## *In-line desalination and potentiometric detection of anions for seawater analysis*

**Bastien Néel and Eric Bakker**

Department of inorganic and analytical chemistry. University of Geneva, Sciences II, 30, Quai Ernest-Ansermet, CH-1211 Geneva, Switzerland

A better understanding of our environment begins by a better monitoring of relevant chemical and biological parameters. Potentiometry, by measuring the activity of specific ions, is a widely used technique because of its cost-effectiveness and its easy implementation.

While limits of detection for lipophilic anions can reach nanomolar levels – as for iodide or perchlorate – potentiometric devices selective for more hydrophilic ions do not share the same performances either for the detection limit or for selectivity, for example for nitrate, or phosphate.

Moreover, measuring anions in seawater meets the challenge of chloride interference, where it is several orders of magnitude more abundant than nitrate or phosphate.

We report here on the sodium chloride removal of a small amount of seawater coupled to online potentiometric detection for in situ analysis in oceanic environment.

## *AC dielectrophoresis driven algal arrays on-chip for biosensing of freshwaters*

**Coralie Suscillon,[1] Orlin D.Velev,[2] Vera I. Slaveykova[1]**

[1]Aquatic Biogeochemistry and Ecotoxicology, Institute F.-A. Forel, Earth and Environmental Science, Faculty of Sciences, University of Geneva, 10 route de Suisse, CH-1290 Versoix, Switzerland

[2]Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, USA

Microalgae are largely used in standard ecotoxicity bioassays and are common components in biosensors and microarrays.[1] Nonetheless, the reproducible immobilization of the living cells for biosensing purposes while maintaining their viability is still a major challenge.[2] Recent developments in the area of electrokinetic cell manipulation e.g. by dielectrophoresis, opened the possibility for rapid on-chip separation, concentration and immobilization.[3] The present work explores the capability of the alternating current electrophoresis for trapping and chaining of a green alga Chlamydomonas reinhardtii on a chip in freshwaters of different origin (e.g. lake, pond and river) and bioassay media. The efficiency of algal chaining was characterized in terms of the percentage of cells in chain and the length of chains. The results demonstrated that AC-field frequency and intensity, duration of the applied field as well as the composition of the exposure media are critical for obtaining efficient and reproducible cell trapping and chaining in one-dimensional array. The highest percentage of cells in chain was obtained at 20 V $mm^{-1}$ and 1 kHz field applied for 600 s. High concentrations of nitrate, dissolved organic matter and the overall high conductivity in surface water decreased the cell chaining efficiency, while high concentrations of phosphate and citrate favor the chaining process.[4] Finally AC-field had no measurable influence on algal physiology, characterized in term of auto-fluorescence, oxidative stress and oxidative damages, showing the promise of AC dielectrophoresis for rapid algal immobilization in the development of live cell based sensors.

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

**Ying Zhang,[1] Dario Bottinelli,[1] Aivett Bilbao,[2] Bandar Alghanem,[1] Frédéric Nikitin,[2] Markus Müller,[2] Frédérique Lisacek,[2] Jeremy Luban,[4] Caterina S. De Castillia,[4] Emmanuel Varesio,[1] Gérard Hopfgartner[1]**

[1]University of Geneva, Life Sciences Mass Spectrometry, School of Pharmaceutical Sciences, Geneva, Switzerland;

[2]Swiss Institute of Bioinformatics, Proteome Informatics Group, Geneva, Switzerland

[3]University of Massachusetts, Medical School, Program in Molecular Medicine, Worcester, MA, USA

Sample preparation and MS acquisition workfloware critical stepsin MS-based proteomics. In order to yield a high proteome coverage and sample throughput for analyzing human primary monocyte derived dendritic cells (MDDCs) protein extracts, we evaluated thesample preparation procedureincluding the protein precipitation and protein reconstitutionstepsand MS data acquisition applying exclusion/inclusionlists. For sample preparation acetone and TCA/acetone protein precipitation methodswere investigated fortheMDDCs cell lysate, which containing high amount of MS incompatible surfactant. The performance of sodium deoxycholate, anacid cleavable detergent, was evaluated as an additive to increase the protein solubilization during the reconstitution step. On the other hand, to overcome the sampling bias against less abundant peptides in the complex mixturein MS/MS acquisition inclusion and exclusion lists were generatedbased on the confidence of peptide sequence identification and retention time obtained from prior technical replicates. These alternative approaches were comparedwith the original DDA approach based on the dataset obtained formultiple technical replicates.

Acetone precipitation gave the best results with overall 34% more protein identifications than the TCA/acetone method. In addition, the addition of sodium deoxycholate in the protein reconstitution solution further increased the proteome coverage of MDDCs, as indicated by the increased number of identified proteins especially the hydrophobic proteins (14% and 28%, respectively). Furthermore,with the help of the incorporated exclusion/inclusion lists into selected DDA approach, we were able to enhance the MS data acquisition efficiency in regards to distinct peptide and protein identifications by an increase of 13% and 23%, respectively, compared to the results from the same number of technical replicates with regular DDA. In the meanwhile, the application of exclusion/inclusion lists allowsmore peptides of low-abundance to be identified during MS/MS acquisition.

## *Effects of gas atmosphere compositions during ablation of zircon on Pb/U ratios using LA-ICPMS*

**Steffen Allner, Daniel Tabersky, Detlef Günther**

Laboratory of Inorganic Chemistry, ETH Zurich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland

The two radioactive decay systems from $^{238}U$ –> $^{206}Pb$ and $^{235}U$ –> $^{207}Pb$ are widely used in geochronology to date geological samples in the range of 100 million up to 4.4 billion years before present. The accessory mineral zircon ($ZiSiO_4$) is preferred as sample, because Pb is selectively rejected during crystallisation and U is incorporated only. Therefore, the common lead fraction that is interfering with age determinationis virtually zero.Thus, single grains of zirconcan be dated directly.

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) has been used to determine the age of a wide range of zircons. No time consuming and error prone digestion, matrix separation, and preconcentrations steps are required and, therefore, it allows the analysis of large sample sets as demonstrated in sediment studies.[1] Nevertheless, LA of zircons results in an increase of the Pb/U ratio over the ablation process.[2] In current investigations, a phase transition through melting at the rim of the ablation pit is observed when nanosecond lasers are used.[3] As the ablation process is influenced by the properties of the gas atmosphere, first experiments with oxygen addition the carrier gas showed a reduction of this fractionation effect.[4]

We will present results for a wider range of gas mixtures and their impact on of Pb/U spot ablation fractionation and resulting Pb/U ratios.

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## *2D imaging at different wavelengths: elucidating the role of the plasma in elemental fractionation during LA-ICP-MS analyses*

**Luca Flamigni, Joachim Koch, Detlef Günther**

Swiss Federal Institute of Technology, Laboratory of Inorganic Chemistry,
ETH Zürich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland

Accurate quantification by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) requires the use of matrix-matched standard materials for calibration. This is due to the occurrence of so-called elemental fractionation, which may happen when sample and standard are of different composition. Elemental fractionation has been shown to originate at different steps of the analysis, in particular during the laser ablation process[1] and inside the plasma, where vaporization, atomization and ionization take place. Developments in laser systems towards lower wavelengths and shorter pulse duration have helped improving the accuracy of LA-ICP-MS analyses, but not much has been done to understand the role of the ICP. Further, a few studies have shown that increased humidity of the laser-produced aerosols can improve the quantification accuracy and lower the limits of detection.[2–4] To obtain experimental evidence of the role of the plasma in the elemental fractionation and to better understand why the addition of water can be beneficial for LA-ICP-MS analyses, a Czerny-Turner optical spectrometer operated in 2D imaging mode was used to capture side-on emission of the plasma. Different wetting approaches were employed, allowing for a direct control over the wetness of the aerosol. Our results revealed a merging of the axial emission maxima of atomic emission clouds of different elements when adding water to the laser-produced aerosols. These findings, together with diagnostic MS measurements, and LA-ICP-MS quantification at different degrees of humidity indicate that a shortening of the temperature "jump" region inside the plasma when adding water can be the reason for the improved quantification capabilities of the system. By comparing wet and dry quantification and figures of merit reported in the literature,[2–4] the contribution of the ICP to the elemental fractionation in LA-ICP-MS was estimated to be of about 15-20 %. This finding implies that advances on the ICP side could allow for significantly more accurate analyses by LA-ICP-MS when matrix-matched calibration is not possible.

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## *Improved flexibility in LA-ICPMS using a gas exchange device*

**Ladina Dorta,[1] Marcel Burger,[1] Reto Glaus,[1] Joachim Koch,[1] Robert Kovacs,[1] Kohei Nishiguchi,[2] Daniel Tabersky,[1] Keisuke Utani,[2] Detlef Günther[1]**

[1]ETH Zurich, Department of Chemistry and Applied Biosciences, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland

[2]J-SCIENCE LAB Co., Ltd, 3-1, Hiuchigata-cho, Kamitoba, Minami-ku, Kyoto 601-8144, Japan

Applications of laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) are increasingly growing in many research fields for the direct analysis of solid samples. Its quasi non-destructive sampling and the high spatial resolution make it a powerful technique. However, samples have to be confined for the laser ablation process into a cell with a finite volume. Large samples need to be cut into fragments, which is sometimes not desirable, either because of their commercial or idealistic values (archaeological artefacts), or because of loss of information (speleothems) in this kind of destructive sample preparation. To overcome this limitation, larger cells[1] or open cells[2] were developed. The development of a gas exchange device (GED)[3] enabled LA without the use of an ablation cell. The aerosol is aspirated form the ablation site in to the GED, where air is exchanged to Ar, providing stable ICP operation.

The use of atmospheric aerosol sampling with LA-ICPMS using a GED was investigated in 2010 by Kovacs et al.[4] In the following, the setup was tested for various applications. The coupling of the GED to a multi collector (MC)-ICPMS enables the isotope ratio determinations of the larger samples.[5] The development of non-contact entrainment device and the development of large capacity gas exchanged membranes improved the aerosol entrainment and washout and thus enabled the study of stalagmites in high spatial resolution.[6] Furthermore, the coupling of the system to a fibre-coupled laser device permitted the ablation of arbitrary sized objects with uneven surfaces.[7]

The used of the GED system therefore enhances the flexibility of the LA-ICPMS, as the samples do not have to fit into an ablation cell. The setups, figures of merit and selected applications will be discussed.

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## *Quantification of nanoparticle mass and number concentration*

**Sabrina Gschwind, Lourdes Aja Montes, Daniel A. Frick, Harald Hagendorfer, Detlef Günther**

ETH Zurich, Wolfgang-Pauli Str. 10, 8093 Zurich, Switzerland

The comprehensive characterization of nanoparticles (NPs) which are used in many different application fields required up to now the consideration of various analytical techniques to gain information about their most important properties such as mass, particle number concentration and composition.

In this work, a technique based on the introduction of diluted NP suspensions via monodisperse single droplets into an inductively coupled plasma mass spectrometer (ICP-MS) is presented.

Therefore, two different strategies to couple the microdroplet dispenser (MDG) to any ICP-MS will be shown[1,2] and compared for different application. Further, quantification results of various NP suspensions in respect to mass and particle number concentration will be given and pros and cons in respect to commonly applied techniques (e.g. Transmission Electron Microscopy (TEM), Field Flow Fractionation (FFF)3 and single particle (SP)-ICP-MS4) discussed.

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## *$^{14}$C Analysis of carbonates at high spatial resolution by coupling laser ablation with accelerator mass spectrometry*

**C. Münsterer,[1,2] L. Wacker,[2] B. Hattendorf,[1] M. Christl,[2] J. Koch,[1] R. Dietiker,[1] H.-A. Synal,[2] D. Günther[1]**

[1]Laboratory of Inorganic Chemistry, D-CHAB, ETHZ, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

[2]Laboratory of Ion Beam Physics, ETHZ, Schafmattstr. 20, HPK, 8093 Zurich, Switzerland

Carbonate records such as speleothems and corals are of great interest to paleoclimate research and related fields. High resolution $^{14}$C-data of speleothems can amongst other applications provide new dating techniques.[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 relatively short washout times with minimal particle deposition on the cell window and walls. This specific design leads to short measurement times while reducing 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 used for the ablation at a scale of less than 100 µm. First $^{14}$C measurements on standard material and on real stalagmite samples will be presented.

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## *Synthesis of oriented nano-wires on a microfluidic platform*

**Mario Lenz,[1] Benjamin Z. Cvetković,[1] Josep Puigmartí-Luis,[2] and Petra S. Dittrich[1]**

[1]Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland

[2]Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Campus Universitari de Bellaterra, Barcelona, Spain

Oriented nanowires offer novel building blocks for next-generation electronic devices. Localized formation of well-defined nanowires in bulk is challenging due to limited control. Control of reaction and also localization is provided by microfluidic systems with volumes of a few hundreds picoliter. Controlled and reproducible orientation of formed nanowires however remains challenging today but is essential for further applications.

Here, we analyze the controllability and reproducibility of position and orientation on the bottom-up formation of metalorganic nanowires.

Here, in a microfluidic channel system of polydimethylsiloxane polymer with pneumatic valves, silver is electroless deposited after reduction of a Ag(I) salt and reacts with tetracyanoquinodimethane (TCNQ). Consequently, AgTCNQ wires are formed by diffusion-controlled self-assembly which are influenced by narrow microchannel or additional confined geometries in the reaction vessel.

Controlling the position and orientation of newly formed structures provides enhanced possibilities of application of AgTCNQ but also other metal-organic nano-wires for synthesis and in-situ application for microelectronic circuits that could be used as gas-sensing elements.[1]

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## *Integrating microarrays into the workflow of nLC-MALDI-MS: Advanced identification and characterization of protein N-glycosylation*

**Martin Pabst,* Simon Küster,* Petra Dittrich, Renato Zenobi**

Department of Chemistry and Applied Biosciences, ETH Zürich,
Wolfgang-Pauli-Strasse 10, 8093 Zürich, Switzerland

Protein N-glycosylation is one of the most abundant post translational modifications and appears to be highly variable. Dozens of glycan structures on distinct glycosites are usually summing up to a plethora of combinations.

The complex nature of glycosylation makes both, their finding and characterization, a demanding task. We demonstrate here an advanced method for a reliable mining of glycopeptides from complex proteolytic digests as well as the subsequent characterization of the site specific glycosylation heterogeneity, by integrating a microarray platform into the workflow of standard nLC-MALDI-MS. A nLC separation of tryptic fragments of glycoproteins is fractionated onto a microarray using a microfluidic interface, where one chromatographic peak is spread over at least 10 individual microarray-spots. The microarray enables further a specific treatment of each second spot with PNGaseF. The final MALDI-MS analysis generates two traces: I) one LC-trace with PNGAaseF treated spots and II) a native trace, as directly fractionated from the nLC. Mining for distinct glycosites is now done by searching for the deglycosylated peptides in the treated trace. An identified peptide leads then directly to the position of the intact glycopeptide clusters in the neighboring spots (before and after). Unknown glycosites can be identified by a sequential comparison of neighbored peak lists (between treated and untreated spots). Furthermore, the deglycosylated peptide can be sequenced in a simple CID MS/MS experiment and standard tools like mascot become accessible. We applied the microarray approach to the site specific glycosylation analysis of IgG and IgM from blood plasma.

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* both authors contributed equally

## *Microarray-MALDI-TOF-MS for quantitative applications: Analysis of saquinavir from lysates of PBMCs and a validation using inverse confidence limits*

**Martin Pabst,* Stephan Fagerer,* Klaus Eyer, Robert Steinhoff, Jasmin Krismer, Renato Zenobi**

Department of Chemistry and Applied Biosciences, ETH Zürich, Wolfgang-Pauli-Strasse 10, 8093 Zürich, Switzerland

We demonstrate the usefulness of our recently introduced microarray plates for quantitative MALDI-MS applications for the analysis of the HIV protease inhibitor saquinavir from lysates of peripheral blood mononuclear cells (PBMCs) using standard MALDI-TOF-MS. The microarray plates in the size of a standard microscope slide contain parallel lanes of hydrophilic spots, where samples are not spotted by pipetting, but deposited by dragging sample droplets with a metal slider along these lanes. With a few microliters of sample, we rapidly generate 10 replicates (each ~10 nanoliters) for every data point. The small aliquots dry immediately after deposition (more homogeneous crystals) and are further fully consumed within seconds during MALDI-TOF-MS analysis. As a consequence of the small and accurate spot volumes, combined with a high number of replicates the microarray-MALDI-MS method allows a quantitative analysis with highest confidence. To validate our quantitative results obtained for saquinavir, we determined average standard deviations and the 95% confidence belt of the calibration curve as well as the standard error and the 95% inverse confidence limits of the estimated concentrations of the quality control samples.

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* both authors contributed equally

## *Structure determination of a novel covalent monolayer on the nanometer length scale using tip-enhanced Raman spectroscopy*

**Lothar Opilik,[1] Payam Payamyar,[2] Andri Schütz,[2] Carolin Blum,[1] Junji Sakamoto,[2] A. Dieter Schlüter,[2] Renato Zenobi[1]**

[1]Department of Chemistry and Applied Biosciences, ETH Zürich, Wolfgang-Pauli-Strasse 10, 8093 Zürich

[2]Department of Materials, ETH Zürich, Wolfgang-Pauli-Strasse 10, 8093 Zürich

Laterally extended two-dimensional polymers are of particular interest in materials science because of their special properties that differ from any three-dimensional material. Methods to obtain layered inorganic material are harsh and lack the control of an organic synthesis approach. Controlled bottom-up synthesis of large polymeric films with a defined repetition unit would open new and exciting possibilities. These two-dimensional materials could be designed for specific applications, e.g., new materials with extraordinary thermal or mechanical stability, molecular sieves or 2D scaffolds. The first experimental demonstration was recently accomplished using a photoinduced cycloaddition reaction.[1] In a similar approach, it was found that a polymerization based on anthracene dimerization of ordered amphiphilic monomer units at an air-water interface can lead to an even more extended covalent monolayers.

Raman spectroscopy has previously been used to probe the structural changes after the polymerization event.[1] Because of the intrinsic weakness of spontaneous Raman scattering, this has so far not been possible on single/few layers of the 2D polymer. The present study focuses on the use of tip-enhanced Raman spectroscopy (TERS) for an in-depth investigation of the internal structure of single/few layers of the two-dimensional film after transferring it to a solid support. TERS is a surface analytical method, which combines the high lateral resolution of scanning probe microscopy (SPM) with a greatly enhanced Raman scattering intensity. The high spatial resolution (< 50 nm) and the significantly improved sensitivity (down to the detection of a few monomer units) provide new insights into the structure of this new and exciting material. Besides single point measurements, spectroscopic images of extended areas of the polymer layers are presented.

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## *Linking of toxicity and adaptive response pathways across the transcriptome, proteome and phenotype of* Chlamydomonas reinhardtii *exposed to silver*

Smitha Pillai,[1,*] Renata Behra,[1,2] Holger Nestler,[1] Marc J-F Suter,[1,2]
Laura Sigg,[1,2] Kristin Schirmer[1,2,3]

[1]Eawag, Swiss Federal Institute of Aquatic Science and Technology, Duebendorf, Switzerland
[2]Department of Environmental Systems Science, ETH Zürich, Switzerland; [3]School of Architecture, Civil and Environmental Engineering, EPF Lausanne

Understanding mechanistic and cellular events underlying a toxicological outcome allows the prediction of impact of environmental stressors to organisms living in different habitats. A systems based approach aids in characterizing molecular events, and thereby the cellular pathways, that have been perturbed. However, mapping just adverse outcomes of a toxicant falls short of describing the stress or adaptive response that is mounted to maintain homeostasis on perturbations and may confer resistance to the toxic insult. Silver is a potential threat to aquatic organisms due to the increasing use of silver-based nanomaterials, which release free silver ions. The effects of silver were investigated at the transcriptome, proteome and cellular level of *Chlamydomonas reinhardtii*. The cells instigate a fast transcriptome and proteome response, including perturbations in copper transport system and detoxification mechanisms. Silver causes an initial toxic insult, which leads to a plummeting of ATP and photosynthesis and damage due to oxidative stress. In response, the cells mount a defense response to combat oxidative stress and to eliminate silver via efflux transporters. From the analysis of the perturbations of the cell's functions, we derived a detailed mechanistic understanding of temporal dynamics of toxicity and adaptive response pathways for *C. reinhardtii* exposed to silver.

## *Analysis of phosphorylated nucleosides by MALDI MS*

**Steinhoff, R.F., Krismer, J., Fagerer, S., Pabst, M., Zenobi R.**

ETH Zürich, Wolfgang-Pauli-Strasse 10, 8049 Zürich, Switzerland

A current problem in the life sciences is the fast detection of nucleotides from biological cell populations. Nucleotides are key players in the central energy metabolism of cells and due to their regulatory functions in cells of high interest in the life sciences. Here we present a fast method to analyse nucleotides from cell lysates by direct negative ion MALDI mass spectrometry using 9-aminoacridine as matrix. Nucleotides tend to fragment in-source by dephosphorylation to smaller counterparts. We investigated the behavior of adenosine-5'mono-, di-, and triphosphate (AMP, ADP, ATP), as well as the cofactors coenzyme A and acetyl-coenzyme A (CoA, AcCoA), and nicotinamide adenine dinucleotides ($NAD^+$, NADH) in detail. So far we identified the applied laser power and the extraction pulse delay to have a strong influence on the degree of in-source decay. At standard instrument settings, the 9-aminoacridine matrix resulted in a much higher in-source decay compared to 2,4,6- trihydroxyacetophenone. We were able to determine the degree of in-source decay during an experiment by adding $^{13}C$-labelled ATP to a cell lysate. Analysing a cell extract of the monocytic cell line THP-1 with $^{13}C$-ATP as internal standard, we were able to obtain values for the energy charge that were similar to those determined by a reference LC-ESI-MS method.