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Chromatographic resolution of racemic compounds on optically active polymers as chiral stationary phases (KGF-SCS Senior Industrial Science Award Lecture 2016)

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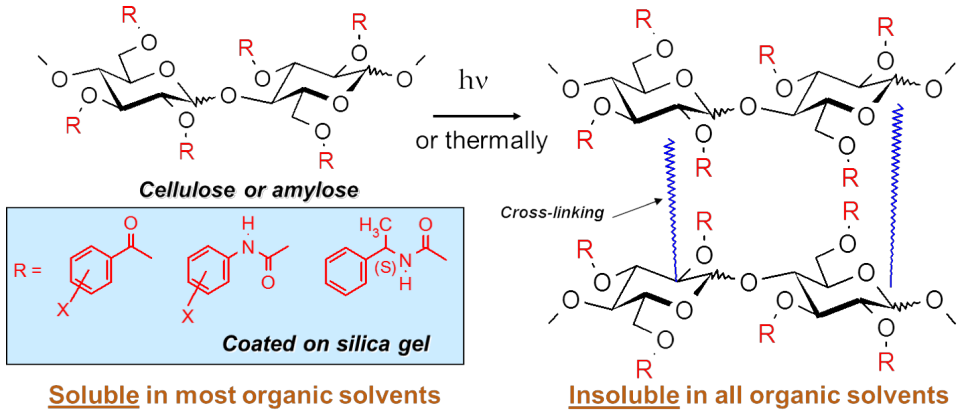
The awareness of the importance of chirality on the biological activity of chiral drugs, fungicides, insecticides, hormones, or fragrances is now well established. Over the last 30 years it has led to a rapidly increasing demand for tools addressing this fundamental question and for methodologies dealing with the chemistry challenges associated to this molecular feature.

Among all developed tools, enantioselective chromatography using chiral stationary phases has become a key technology for the analysis and preparative separation of the enantiomers of racemic compounds. It is now the standard technique for determining the optical purity of chiral substances, replacing almost completely the classical methods such as optical rotation.

In this context, polysaccharide derivatives have emerged as remarkably powerful chiral materials for the purpose of separating stereoisomers by chromatography, showing an exceptional chiral recognition capability not only for analytical determination of optical purity but also for the preparative utilization of this technology to produce optically pure compounds from mg to ton scale. The approach has now become the standard everyday process to access the single pure stereoisomers of potential new chiral drugs in drug discovery. Moreover, with the invention and introduction of immobilized polysaccharide-based chiral stationary phases, we have been able to considerably improve and extend the applicability of enantioselective chromatography to a broad variety of chiral molecules [1-3]. The process is based on a photochemical reaction which leads to a cross-linking of the polysaccharide chains.



The new generation (immobilized) of polysaccharide-based stationary phases has been gradually introduced on the market since 2004 and these phases are now used worldwide as the state-of-the art materials in almost all research laboratories dealing with chiral molecules both in academia and industry, as well as in many development and production units.

The advance of enantioselective chromatography has undeniably permitted to develop technological opportunities which were not conceivable some years ago in the field of large scale separation of stereoisomers of chiral drugs. Furthermore, this development has also favored the advent of the multi-column and continuous separation technology such as simulated moving bed (SMB) chromatography in the pharmaceutical environment, and the resurgence of the packed supercritical fluid chromatography (SFC) technique in general.

[1] E. Francotte, D. Huynh, *J. Pharm. Biomed. Anal*. **2002**, *27*, 421.
[2] E. Francotte, T. Zhang, Patent, PCT Int. Appl. (1997), WO 9704011 .
[3] E. Francotte, *Chimia.* **2009**, 63, 867.

Towards a better understanding of spectral similarity between structurally related compounds

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High-resolution tandem mass spectrometry (HR-MS/MS) is a vital tool in compound identification in environmental samples, *e.g.*, detecting unknown transformation products (TPs) that are produced when emerging contaminants are subjected to natural or anthropogenic processes. Fragmentation of a compound is induced during measurement and it has in general been assumed that structurally similar compounds will have similar spectra since they are likely to produce similar fragments. Furthermore, this tenet has been proposed as a way to improve unknown identification. This hypothesis was tested here using a set of 199 related pairs (parent compounds and their structurally related transformation products (TPs)) which were measured with HR-MS/MS using higher-energy collision-induced dissociation (HCD) fragmentation.

Using purchased reference standards, each compound was measured with liquid chromatography coupled to HR-MS/MS over a range of HCD energies. Spectra were cleaned and recalibrated with the R package “RMassBank”. TPs were paired with their respective parent compounds and included different modification reactions, such as N- or O-dealkylation, hydroxylation, or conjugation. The spectral similarity of a pair was calculated as the dot product of aligned intensity vectors. The influence of collision energy on the similarity of the spectra was investigated, as well as the use of merged spectra from different HCD energies. Additionally, it was hypothesized that shifting the MS/MS fragments of the TP by the mass difference of the transformation would lead to increased similarity between the spectra of each pair.

The highest spectral similarity scores were achieved at high collision energies, indicating that small fragments produced at these energies, or the combination of many small fragments, retained structure-specific information. Also critical was the removal of the precursor peak during comparison to reduce false positive matches. Merged spectra which included both the measured fragments and fragments which were adjusted for the mass of the transformation performed the best of all scenarios tested. Under these conditions, at an optimum similarity score of 0.12, 80% of related pairs had a spectral similarity above this value, while 90% of unrelated pairs were below this threshold. Still, structural similarity of pairs as estimated by the Tanimoto coefficient was not strongly correlated to the similarity of the spectra, indicating that even small changes in a molecule may influence fragmentation. The mechanisms governing this phenomenon need to be further investigated so that spectral similarity between known and unknown spectra can be successfully used for the purposes of prioritization of unknown for nontarget identification.

[1] Stravs, M. A., Schymanski, E. L., Singer, H. P., Hollender, J. (2013). Automatic recalibration and processing of tandem mass spectra using formula annotation. *Journal of Mass Spectrometry, 48*(1), 89-99. doi:10.1002/jms.3131

Understanding the cellular distribution and protein targets of a ruthenium (II) anti-cancer compound, RAPTA-T via mass spectrometry

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Newer generation anti-cancer ruthenium (II) complexes such as RAPTA-T are promising clinical candidates which possess interesting anti-metastatic properties. However, the mechanism of action of RAPTA-T is poorly understood. Here we apply imaging mass spectrometry to elucidate the differences in distribution of RAPTA-T in highly metastatic breast cancer cells, MDA-MB-231 in contrast to MCF-7 cells which are lowly metastatic. We see clear nuclear accumulation of RAPTA-T in the metastatic cell line. We also find that unlike previously speculated, the activation of RAPTA-T involves not just aquation but possibly detachment of the arene. We then apply a novel proteomic profiling method to probe for interesting protein targets in these cells. We find two interesting targets phospholipase D3 and methionine adenosyltransferase 2A, the former being linked to metastasis and the latter being involved in cancer progression.

[1] R. F. S. Lee, S. Escrig, M. Croisier, S. Clerc-Rosset, G. W. Knott, A. Meibom, C. A. Davey, K. Johnsson and P. J. Dyson, *Chem. Commun.*, 2015, **51**, 16486–16489.
[2] A. Chernobrovkin, C. Marin-Vicente, N. Visa and R. A. Zubarev, *Scientific Reports*, 2015, **5**, 11176.

Combined GC- and UHPLC-HR-MS based metabolomics to analyze durable anti-fungal resistance processes in cereals

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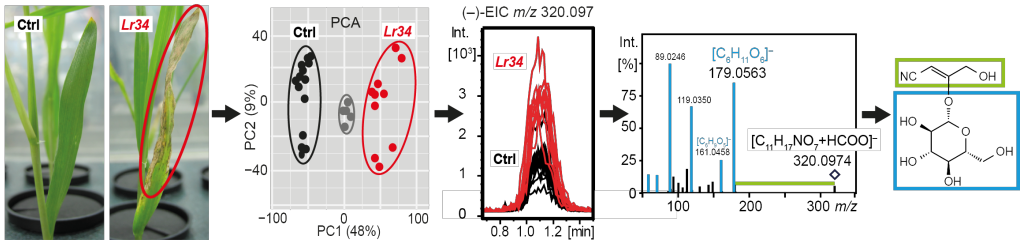
Introduction of durable resistance genes in crops is an important strategy to prevent yield loss caused by fungal pathogens and to maintain food security. The resistance gene *Lr34* of wheat (*Triticum aestivum*) durably confers resistance to four major fungal pathogens leaf rust, stripe rust, stem rust and powdery mildew. *Lr34* is functionally transferable to barley (*Hordeum vulgare*) [1] and rice (*Oryza sativa*). The molecular resistance mechanism of *Lr34*, encoding for an ATP-binding cassette transporter [2], is not known yet. The overall aim of this multi-disciplinary project was to increase the understanding of the molecular function and defense response of durable disease resistance in cereals.

To characterize *Lr34* functionality at metabolite level, a metabolomics approach based on combined UHPLC-HR-MS and GC-MS technology was applied. Comprehensive metabolic profiles of *Lr34* barley, rice and wheat grown under different conditions were investigated and a broad range of structurally diverse primary metabolites (e.g. amino- and organic acids, sugars), lipids and secondary metabolites (e.g. flavonoids) were identified. UHPLC-HR-MS/MS allowed the annotation of a variety of defensive secondary metabolites [1] contributing to the understanding of the durable, multi-pathogen resistance *Lr34* in different crop species.



[1] Harsh Chauhan, *et al.*, *Plant J.*, **2015**, *84*, 1, 202-215.
[2] Simon G. Krattinger, *et al.*, *Science,* **2009**, *323*, 1360-1363.

Compound-specific isotope analysis of environmental organic micropollutants: challenges and possibilities

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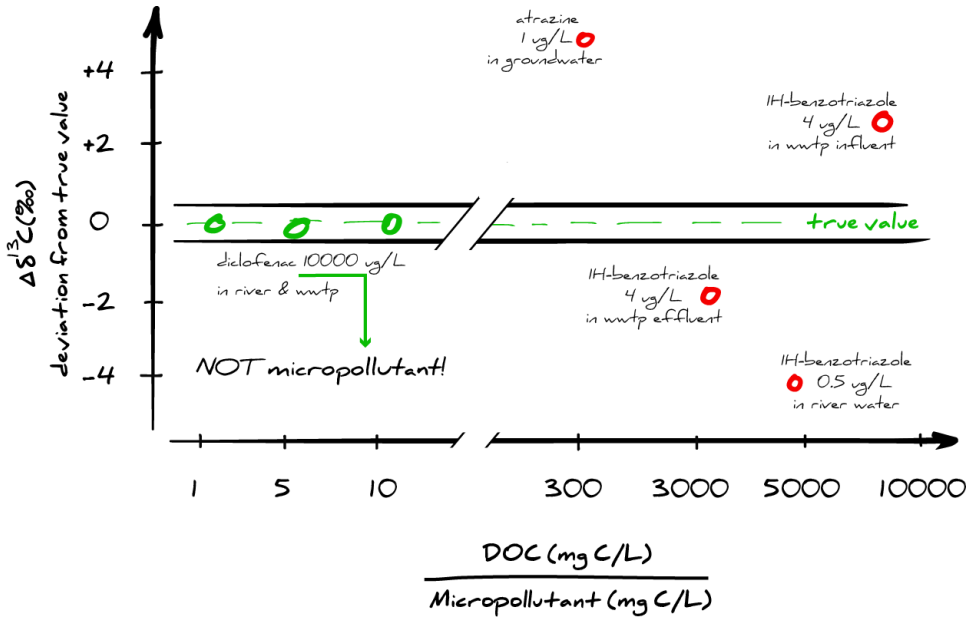
Occurrence of organic micropollutants in water raises major concern to both human health and the environment. Hence, it's necessary to assess the fate of these chemicals in the environment where compound-specific isotope analysis (CSIA) offers invaluable information that cannot be simply obtained by concentration dynamics. However, CSIA for environmental organic micropollutants currently encounters major challenges that impedes its use, especially for the polar micropollutants. These challenges can simply be summarized by two facts, among others: 1) organic micropollutants occur in natural systems at very low concentrations (i.e. ng/L - µg/L) which fall below the typical limits of gas chromatographs coupled to isotope-ratio mass spectrometer (i.e. mg/L) by factors easily exceeding 50000. 2) The latter fact dictates that very large volumes of water (e.g. ≥ 10 L) must be enriched by the corresponding factor. These two facts inevitably lead to partial co-extraction of interfering substances which consequently deteriorates the quality of the acquired isotopic data (see graph). Since there is no substitute for good chromatography in CSIA, there is a great interest in introducing selectivity in the sample preparation to get rid of the interfering substances.

In this scenario, we explore the use of molecularly-imprinted polymers (MIP), tailor-made synthetic materials specific for certain class of compounds, as a strategy for specific enrichment of typical anthropogenic micropollutants. Chloro-s-triazines and benzotriazoles were chosen as model compounds representative for pesticides in agricultural catchments and consumer chemicals in waste water, respectively. Characterization of the developed MIP-CSIA procedures demonstrates the viability of such an approach in eliminating the interfering matrices. Whereas, the studied model compounds are specifically retained on the corresponding MIP and recovered without interferences. Furthermore, comparison of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratio measurements before and after the specific retention shows no consequences on the integrity of the isotopic ratios using the optimized methodologies. The developed strategy was tested in typical aquatic environments, such as leachates from soil lysimeters for triazines and domestic waste water from wastewater treatment plants for benzotriazoles.



Electrochemical Proton Transfer Based Polyaniline Films for Thin Layer Titrations

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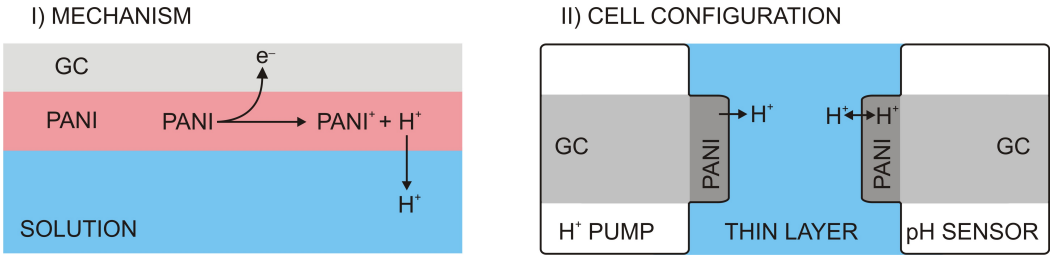
Polyaniline (PANI) is univocally one of the most relevant electronic/conducting polymers, which has been applied for broad range of applications from batteries to sensors [1-3]. PANI is proposed here for the first time as a promising material for analytical applications where a targeted release of proton is required. Therefore, this research was mainly focused on the proton release properties of PANI layer. The PANI layer was synthesized by electropolymerization of aniline on the surface of glassy carbon (GC) by cyclic voltammetry [4, 5]. The coated PANI layer was then applied as a proton pump to release protons in thin layer coulometric mode (Scheme I).

The electrochemical arrangement consisted of one sensor and one pump placed opposite each in order to define a thin layer gap. The pump was used to release protons while the sensor was utilized as potentiometric readout to determine the local pH. A linear relationship between the duration of the applied pulse and the released charge (correlated with the potentiometric readout) allowed one to titrate the sample by adding a charge package, thereby mimicking a classical titration [6, 7]. Here, one PANI layer was used as a proton selective sensor and another PANI layer was applied as a proton pump (Scheme II).

We introduce here for the first time that a solid contact material such as PANI may provide an alternative technology with more pronounced mechanical and chemical robustness (compared to perm-selective membranes or other approaches based on water electrolysis) for both releasing and detecting protons in solution. Finally, the presented approach is applied for the titration of real samples including sea, river and lake waters for the determination of total alkalinity.



[1] Chetna Dhand, Maumita Das, Monika Datta, *Biosensors and Bioelectronics*, **2011**, *26,* 2811-2821.
[2] Alan G MacDiarmid, *Angewandte Chemie*, **2001**, *40,* 2581-2590.
[3] James Y Shimano, Alan G MacDiarmid, *Synthetic Metals*, **2001**, *123,* 251-262.
[4] M Radoicic, Z Saponjic, G Ciric-Marjanovic, *Applied Catalysis B: Environmental*, **2013**, *136,* 133-139.
[5] Shaolin Mu, *Synthetic Metals*, **2004**, *143,* 259-268.
[6] Majid Ghahraman Afshar, Gaston A Crespo, Eric Bakker, *Angewandte Chemie*, **2015**, *127,* 8228-8231.
[7] Majid Ghahraman Afshar, Gaston A Crespo, Eric Bakker, *Analytical Chemistry*, **2015**, *87,* 10125-10130.

Persistent organic pollutants in white-blooded Antarctic fish Champsocephalus gunnari and Chaenocephalus aceratus

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Persistent organic pollutants (POPs) are ubiquitous environmental chemicals and can be found even at very remote areas such as the Antarctic Ocean. Via long-range atmospheric transport (LRAT), global distillation processes and cold condensation POPs reach the Antarctic ecosystem and bioaccumulate in aquatic biota. Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and former widely used pesticides such as g-hexachlorocyclohexane (g-HCH), hexachlorobenzene (HCB), and p,p'-DDT are lipophilic organic chemicals with high potential to bioaccumulate in Antarctic species. Antarctic fish, which hold high trophic positions, appear to possess low endogenous elimination rates for POPs and are expected to show increasing levels of these chemicals with rising anthropogenic pollution. Therefore two fish species of Antarctic icefish, originating from the Southern Ocean around Elephant Island, were caught and analyzed for their levels of PCBs, PBDEs, HCB, HCHs, and DDTs. The two species included the planktivorous *Champsocephalus gunnari* and the piscivorous *Chaenocephalus aceratus*. The species of white-blooded icefish were caught during a cruise with the research vessel 'Polarstern' during March 13 to April 9, 2012, around Elephant Island and the South Shetland Islands. POPs were analyzed in muscle and ovary tissue of mature, female fish. Lyophilized tissue of muscle or gonads was extracted using a speed-extractor (E-914, Büchi, Switzerland) with a mixture of n-hexane/dichloromethane (1:1). Extracted lipids were spiked with $^{13}C_{12}$ labeled internal standards and cleaned-up by treatment with concentrated sulfuric acid and liquid chromatography on multilayer silica gel. Quantitative determination of the target analytes was carried out by gas chromatography/high resolution mass spectrometry (GC/HRMS) at a mass resolution of 8'000.

Our results revealed higher contaminat levels in ovary than in muscle tissues of both species. Most analyte concentrations and the toxicity equivalents (TEQs), as well as the bioanalytical equivalents (BEQs) were lower as in temperate species. Comparison with data from the literature points to higher PCB and DDT concentrations than those measured in icefish in the 90's. For the other contaminants no temporal trend could be identified. Higher bioaccumulation was found for HCB and DDTs in C. aceratus compared to C. gunnari. However there was no general species-specific accumulation pattern of the different classes of POPs between the two icefish. Thus, the expected link between contaminant burdens of *C. aceratus and C. gunnari* and their ecological traits was only weakly supported for these species [1].

[1] Anneli Strobel, Peter Schmid, Helmut Segner, Patricia Burckhardt-Holm, Markus Zennegg, Chemosphere 2016, 1 - 8 (article in press)

Field-scale in-situ analysis of ambient N_2O isotopic composition to trace source processes in an intensively managed grassland

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Nitrous oxide (N_2O), a strong greenhouse gas and an important ozone-depleting substance, is primarily emitted from pristine and fertilized soils. A multitude of biogenic and abiotic N_2O producing processes have been identified, but their relative contribution to total N_2O emission and relevance for different ecosystems is currently not known, as related processes are highly dispersed and variable. For the development of effective N_2O mitigation strategies, however, detailed knowledge of processes and their temporal and spatial variations is essential. Analysis of site-specific N_2O isotopic composition has proven potential to disentangle source processes based on their characteristic isotopic signatures (Wunderlin et al. 2013).

In recent years we developed an analytical technique for real-time high-precision analysis of N_2O site-specific isotopic composition, consisting of a quantum cascade laser absorption spectrometer (QCLAS) coupled to an automated preconcentration device, called TRace gas EXtractor (TREX) (Wächter et al. 2008, Eyer et al. 2016). In a pilot field study N_2O isotopic signatures could be interpreted in relation to management events and meteorological conditions (Wolf et al. 2015). Since then the field-applicability of the setup was significantly improved by advanced temperature control for both QCLAS and TREX, and installation of the instrumentation in a 19” rack. In addition, the novel device offers the possibility for simultanous analysis of $\delta^{17}O$-N_2O.

Here we present first results from a field study which will be carried out at an intensively managed grassland site in southern Bavaria, Germany (Fendt, 600 m.a.s.l.)between June and July 2016, as a sub-module of the ScaleX 2016 campaign organised by IMK-IFU. We will focus on the discussion of ambient N_2O isotopic composition measurements above the grassland site that are expected to shed light into different N_2O source processes based on their isotopic source signature. These results will be combined with atmospheric transport simulations and footprint analysis to interpret spatial variability. It is foreseen to evaluate N_2O isotopic information in conjunction with $\delta^{15}N$ values of nitrogen precursors (NH_4^+, NO_3^-), management events and additional supporting soil and metereological parameters. Results from the field study will be discussed in relation to complementary approaches: A biogeochemical soil model (L-DNDC) with an isotope sub-modul currently developed at IMK-IFU, and a ^{15}N tracer approach applied by Thünen Institute.

Wunderlin P., Lehmann M.F., Siegrist H., Tuzson B., Joss A., Emmenegger L., Mohn J. (2013): Environmental Science and Technology 47(3). 1339-1348.
Waechter H., Mohn J., Tuzson B., Emmenegger L., Sigrist M.W. (2008): Optics Express 16 (12). 9239-9244.
Eyer S., Tuzson B., Popa, M. E., van der Veen C., Röckmann T., Rothe M., Brand W. A., Fisher R., Lowry D., Nisbet E. G., Brennwald M. S., Harris E., Zellweger C., Emmenegger L., Fischer H., Mohn J. (2016): Atmospheric Measurement Technique 9, 263-280.
Wolf B., Merbold L., Decock C., Tuzson B., Harris E., Six J., Emmenegger L., Mohn J. (2015): Biogeosciences 12(8): 2517-2531.

At the interface between climate research and metrology: Gas adsorption and desorption on high pressure standard cylinders

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Long term atmospheric monitoring of trace gases require great attention to precision and accuracy. For a globally integrated and well established greenhouse gas observation network, World Meteorological Organization (WMO) has set recommended compatibility goals within the framework of its Global Atmosphere Watch (GAW) Programme. To achieve these challenging limits, the stability of the primary and secondary gas standards are of great importance.

For high pressure standard gas mixtures used in atmospheric trace gas analysis, there exists only a limited amount of data and few attempts to quantify the surface processes. Here, we focus on instabilities in gas composition due to surface processes, in particular, adsorption and desorption and it's temperature and pressure dependency. Specifically, we investigate adsorption/desorption phenomena on steel and aluminum cylinders for the species CO, CO_2 and CH_4 by using a cavity ring down spectroscopy analyzer. In the present study a set of experiments are designed to test the temperature dependency in the range of -10 °C to +50 °C and pressure dependency from over 100 bars to atmospheric pressure. Moreover, measured concentrations are fitted to a simple adsorption model in order to quantify the parameters of adsorption. For CH_4 no distinct difference between aluminum and steel cylinders are observed. CO_2 showed clear temperature dependency for steel and only minimal for aluminum cylinders, whereas CO needs further investigation.

Enyzme-Substrate Complexes Studied by Native Electrospray Mass Spectrometry: First Steps Towards Gas-Phase Enzymology

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The question of whether, and under what conditions, electrosprayed biological molecules and biomolecular complexes retain their "native" forms in the vacuum environment of a mass spectrometer remains an intense area of research [1-4]. To date, most experiments seeking to tackle this question have used a structural approach, relying on measures of ion size, reactivity, and dissociation properties. In this work, we attempt to address this question using a new, functional approach, with trypsin as a model enzyme. The aim was to isolate gaseous non-covalent trypsin-substrate complexes in the gas phase, and to see whether the acylenzyme (with the C-terminus of the substrate having dissociated after specific enzymatic cleavage) can be produced upon activation of these complexes. The successful formation of the gaseous acylenzyme would act as strong evidence for a correctly organized enzyme active site in the gas phase.

Electrospray ionization (ESI) mass spectra were acquired using gold-coated glass capillaries with a Waters Synapt G2 mass spectrometer. ESI solutions contained trypsin and/or model substrate (N-benzoyl-capped synthetic hexapeptides of type Bz-XXRGGG) at equimolar (10 micromolar) concentrations. Ammonium acetate (500 mM) was used for "native" ESI. Measurements at low pH were done in 1% aqueous acetic acid.

Native ESI mass spectra of trypsin-substrate mixtures indicate that, for many peptides, the hydrolysis reaction goes to completion in the nano-ESI emitters prior to MS analysis. However, trypsin-bound N-terminal fragments are clearly visible in the high mass regime, in accordance with trypsin's N-terminal recognition properties. Hydrolysis rates can be slowed by lowering pH, though this appears to hinder substrate binding affinity. Nonetheless, mass spectra of electrosprayed trypsin-substrate mixtures at low pH show peaks corresponding to the bound substrate, though these exist in low abundance. Upon isolation and collisional activation of these complexes, peaks corresponding to covalent detachment of the C-terminus of the substrate are apparent in tandem mass spectra. This is consistent with a gas-phase enzyme reaction having occurred in the gas phase. As a control experiment, trypsin complexes with peptides containing D amino acids (which react slowly in solution) were shown also to survive the electrospray process; however, in this case, no evidence for a gas-phase enzyme reaction is observed upon collisional activation. Building on these preliminary results, we are currently extending these studies to a larger library of peptides, to assess whether the specificity of the enzyme reaction depends on amino acid sequence in a manner similar to that in solution. Furthermore, to enable studies of the short-lived complexes of trypsin with good substrates under native solution conditions, we are currently working towards implementing a rapid-mixing device prior to MS analysis. In these experiments, the mass spectrometer acts as a useful readout to monitor potential proteolytic activity in the gas phase, as the trypsin-substrate complex and acylenzyme have distinct masses which are easily distinguishable in a mass spectrum. It is easily envisioned that this methodology could be implemented to study other enzyme reactions in the gas phase.

[1] K. Breuker, F.W. McLafferty, Proc. Natl. Acad. Sci. USA, **2008**, 105, 18145.
[2] E. Jurneczko, P.E. Barran, Analyst, **2011**, 136, 20.
[3] Z. Hall, C.V. Robinson, J. Am. Soc. Mass Spectrom., **2012**, 23, 1161.
[4] J. J. Brady, E. J. Judge and R.J. Levis, Proc. Natl. Acad. Sci. USA, **2012**, 109, E207.

Studies on discrete samples using a microdroplet generator combined with ICP-Time-of-Flight Mass Spectrometry

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Conventional liquid introduction for inductively coupled plasma optical emission (ICP-OES) and mass spectrometry (ICP-MS) is typically realized by means of pneumatic nebulizer combined with a spray chamber. However, drawbacks of this approach include sample consumption and incomplete sample transport, which can limit its application in various fields, namely forensic, toxicological, biological, and clinical studies, where only microliters of sample are available [1]. In this study, a microdroplet generator (MDG) was used as an alternative sample introduction system for ICP-MS. With this setup, monodisperse droplets (20-40 µm in diameter) are generated with a user defined frequency (1 Hz to 2000 Hz) and result in time-separated and discrete ICP-MS signals of about 300 µs in duration. Droplets are produced on-axis to the ICP and introduced into the plasma via a stream of helium and argon gas to focus their trajectories and partially dry them [2]. This system configuration routinely enables 100% transport efficiency. In combination with a recently developed ICP-Time-of-Flight Mass Spectrometer (ICP-TOFMS), we record complete elemental spectra from each generated droplet and can examine the composition of ICP-TOFMS signals from droplets at a time resolution of 30 µs. Thanks to the narrow size distribution of the droplets, the time required to undergo complete desolvation, atomization, excitation and ionization is highly reproducible from one droplet to another, making this discrete sample introduction system an ideal candidate tool for investigating fundamental ICP processes [3]. Here, we report studies on the effects of analyte mass and water load for multi-elemental detection from individual droplets. Importantly, because droplets serve as proxy for other mass-limited discrete samples, such as single cells or nanoparticles, these studies indicate the potential of multi-element ICP-TOFMS analysis for these species. Despite its numerous advantages, poor limits of detection (LODs) in terms of concentration (above 1 ng/g) for single-droplet analysis remain a limitation of MDG sample introduction. While absolute LODs are excellent (10s of attograms), the lowest concentration detection limit achievable was in the ng/g range for ^{238}U, which compares poorly to conventional solution-based introduction systems. To overcome this limitation, signal averaging may be employed. With this approach the concentration LOD for ^{238}U is improved to 2 pg/g with 500 droplets averaged. These results make MDG-ICP-TOFMS competitive with conventional solution-based sample introduction for ICP-MS. Finally, in this presentation, we will discuss how the characteristics of discrete microdroplets affect the performance of the ICP and how the fate of these individual entities directly correlates to nanoparticles behavior within the plasma.

[1] J. L. Todolí and J. M. Mermet, Sample introduction systems for the analysis of liquid microsamples by ICP-AES and ICP-MS, *Spectrochimica Acta Part B: Atomic Spectroscopy*, **61** (2006), 239-283.
[2] (a) Gschwind, S.; Hagendorfer, H.; Frick, D. A.; Gunther, D., Mass Quantification of Nanoparticles by Single Droplet Calibration Using Inductively Coupled Plasma Mass Spectrometry. *Anal Chem* **2013,** *85* (12), 5875-5883; (b) Borovinskaya, O.; Gschwind, S.; Hattendorf, B.; Tanner, M.; Gunther, D., Simultaneous Mass Quantification of Nanoparticles of Different Composition in a Mixture by Microdroplet Generator-ICPTOFMS. *Anal Chem* **2014,** *86* (16), 8142-8148.
[3] (a) Olesik, J. W., Investigating the fate of individual sample droplets in inductively coupled plasmas. *Appl Spectrosc* **1997,** *51* (5), A158-A175; (b) Borovinskaya, O.; Aghaei, M.; Flamigni, L.; Hattendorf, B.; Tanner, M.; Bogaerts, A.; Gunther, D., Diffusion- and velocity-driven spatial separation of analytes from single droplets entering an ICP off-axis. *J Anal Atom Spectrom* **2014,** *29* (2), 262-271.