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Targeted on-line breath analysis discriminates COPD patients vs. healthy controls and subjects suffering from asthma

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Introduction: Recently we identified markers in exhaled breath discriminating patients with chronic obstructive pulmonary disease (COPD) from healthy controls using real-time mass spectrometry. The aim of this study was to validate the previously found disease specific metabolic profile of COPD in an independent cohort of patients suffering from chronic obstructive lung disease, i.e. COPD, asthma, or healthy controls using real-time mass spectrometry (validation study).

Method**:** On-line breath analysis was performed in patients suffering from COPD or asthma as well as in healthy subjects, using secondary electrospray ionization-high resolution mass spectrometry (SESI-HRMS). The previously reported COPD specific mass spectrometric markers were then analysed in this new cohort with respect to their statistical significance and classification performance.

Results: Breath analysis was performed in a total of 133 subjects (COPD n=49, asthma n=31, healthy controls n=53). Using this bigger, independent, more heterogeneous cohort, we were able to reproduce and validate our previous findings. Many of the COPD specific markers that were reported in the previous study were also significantly altered in this new dataset (figure 1) and allowed for discrimination between COPD patients and asthma/healthy controls. We thereby could obtain additional information about which biomarkers are the most robust in this heterogeneous group of subjects.

Conclusions: These preliminary results confirm our recent finding that breath analysis by real-time mass spectrometry allows the identification of mass-spectral features discriminating COPD patients from an independent cohort of asthmatics and healthy controls with good accuracy. Untargeted real-time mass spectrometry is a powerful and easily applicable method for the diagnosis of COPD.

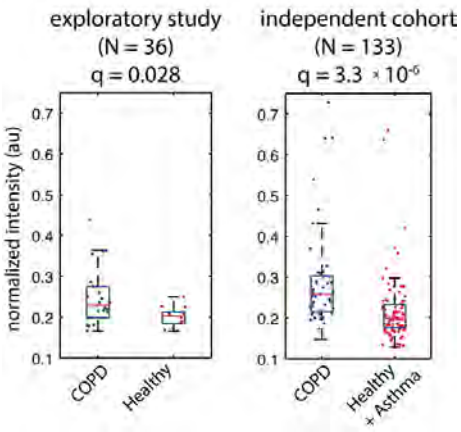

Figure 1: Boxplots of the average intensities of the mass spectral feature of exhaled breath at 201.0702 Da in positive mode, for the old (left) and new (right) cohort. Besides others, this compound was recently reported as a putative biomarker for COPD. In the old cohort (22 COPD vs. 14 controls) this compound was significantly increased in COPD patients, which was also true for the new independent cohort of 49 COPD patients, 31 asthma patients and 53 healthy controls.

Laser Ablation Time of Flight Mass Spectrometry using Ion Funnel for Trace Element Analysis in Solids

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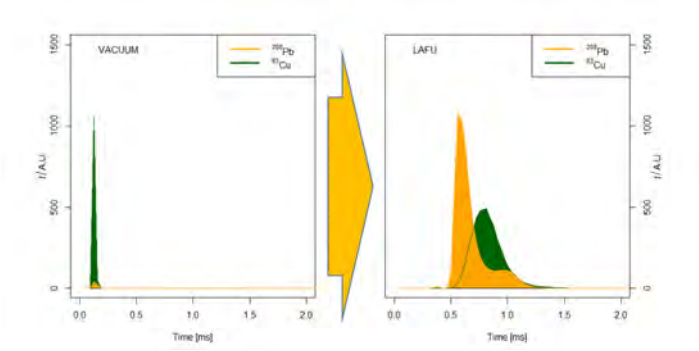
The generation of ions during high energy laser ablation has already in the early 1960s been utilized in fundamental[1] and analytical purposes[2]. The advent of new laser technology, femtosecond lasers in particular, makes this approach attractive again for analytical purposes. This technology, however, leads to ions with a broad initial angular and energy distribution, which makes transmission to the mass analyzer a challenge. In this work we investigate a novel laser ablation ion funnel ("LAFU") ion source, in order to exploit laser generated ions for spatially resolved elemental and isotope analysis of solids. The ion funnel was first described by Shaffer et al[3] to improve ion transmission in electrospray ionization mass spectrometry and is based on the theoretical work of Gerlich[4]. Briefly, it consists of stacked ring electrodes with decreasing inner diameter towards the mass spectrometer orifice. Radiofrequency (RF) power is applied to the ring electrodes at alternating phase (180° shift) between adjacent electrodes. Classically a DC gradient is superimposed on the ion funnel to drive ions across the funnel; in the here developed setup the DC gradient is avoided and the transmission relies solely on the gas dynamic effects produced by a convergent-divergent supersonic (CD) nozzle placed between the sample and the ion funnel. In addition to spatial focusing, the kinetic energy of the ions in the funnel is dissipated through collisions with a buffer gas (e.g. He) during their transit through the ion funnel.



Initial results with the "LAFU" ions source show for the first time that low m/Q elemental ions can be successfully focused in an ion funnel. Overall the transmission efficiency could be increased up to 200 times in comparison with ablation in high vacuum. Still the ion transmission through the ion funnel showed a pronounced dependence on m/Q, as a result of the pseudostationary fields created near the ion funnel electrodes. A parametric studies was performed using Brass standards to assess the influence of the operating condition used. Low m/Q species have better transmission with lower RF amplitude and higher Frequency, conversely behavior is found for high m/Q species.

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Towards quantitative depth profiling of Sn/Cu solder bumps

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Progress in flip-chip technology, a vital element of semiconductor industry, is emerging and constantly bringing along novelties on the material science side. One important innovation for solder bumped flip-chips is the use of Sn/Cu double layer bumps, which serve to make direct contact between devices and circuit boards. The quality of the resulting interconnects strongly depends on the degree of organic contamination in the Sn/Cu bump. To shed light on the amount of incorporated impurities, depth profiling of the two layers before being annealed is required. This is a challenging task, as there is no quantitative method that would allow analysis of the laterally highly confined bump arrays. Furthermore, the very distinct material characteristics of Sn and Cu (e.g. color, hardness, electrical and thermal conductivity) are demanding for the analysis method.

In this report, a miniature reflectron time-of-flight mass spectrometer (LMS) combined to a fs laser ablation/ionization source[1] is applied to approach the complex problem via investigation of a simplified model system: electrodeposited Sn/Cu bilayers. These bilayers are much thinner and laterally more extended than the target Sn/Cu bumps. This makes their analysis straightforward and allows to focus on the main challenges faced when trying to obtain a depth profile of a multi-component entity with rather different physical characteristics as well as the unknown influence of the interface separating the two layers. To our knowledge, this is the first dedicated report on fundamental studies on these aspects for Sn/Cu bilayers.

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Micro-device integrated platforms for Point-Of-Care Therapeutic Drug Monitoring

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Therapeutic Drug Monitoring (TDM) allows personalized treatment for diseases where continuous control of drug dosage is required in order to avoid adverse effects. Currently, this process is demanding for the patient, costly and time consuming. For this reason, single-step whole blood tests are desirable as they eliminate analytical errors arising from the complex process of sample preparation (i.e. transportation of the sample, centrifugation, dilution, extraction).To address this issue, we introduce the ability of paper-like membranes to run quantitatively clinical chemistry using Fluorescence Polarization Immunoassay (FPIA) as tool for direct quantification of small molecules in whole blood. Even though paper is extensively used in diagnostic tests[1], the direct quantification of small drugs combined with sample preparation is still limited. Moreover, optical techniques, especially Fluorescence Polarization (FP) have not been associated with paper mainly due to the intrinsic fluorescent background. In our case, the feasibility of such measurement, directly within paper is given by using a near infrared-fluorescent (NIR) labeling, which has low interference with whole blood sample and with the paper itself while the competition was investigated within the reservoirs created by the paper-based microstructures. Our approach showed good analytical performance for Tobramycin a small molecule antibiotic, with a limit of detection and limit of quantification of 0.2 and 0.6 µg/mL, respectively. To further test the assay with reduced and simplified sample pre-processing for Tobramycin, a miniaturized FP analytical demonstrator was designed. The measurements were performed within glass capillaries with a 300 µm diameter while preserving the analytical performance (Fig. 1).

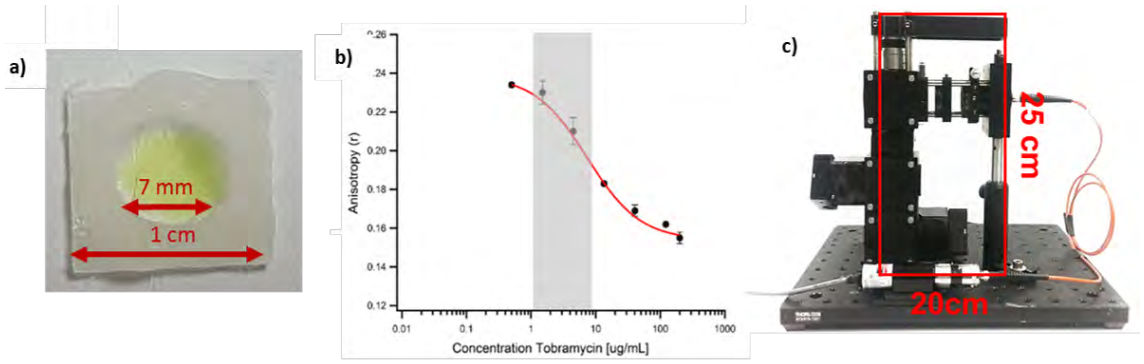

Fig. 1: Whole blood filtration and quantification of Tobramycin through FPIA: a) the design of paper-based microchamber for drug analysis; b) FPIA dose-response curves within paper were performed during three consecutive days: Tobramycin quantification in whole blood showed good stability and reproducibility between measurements with a mean Coefficient of Variation (CV) of 30%; c) a miniaturized optical analytical device for drug quantification.

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High precision spectroscopic measurement of N_2O clumped isotopic species

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Nitrous oxide (N_2O) is a major greenhouse gas and the most important ozone destruction species. Understanding formation mechanisms and clarifying its disperse and highly variable sources and sinks is important for mitigating the emissions. Measuring the doubly substituted "clumped" isotopocules of N_2O can add new and unique opportunities to fingerprint and constrain the N_2O biogeochemical cycle. A similar strategy has recently been applied for other atmospheric constituents such as CO_2, CH_4, and O_2.[1-4]

Within this project, we are developing an analytical technique for the selective and precise analysis of the most abundant clumped N2O isotopic species: $^{15}N^{14}N^{18}O$, $^{14}N^{15}N^{18}O$, and $^{15}N^{15}N^{16}O$. The measurement setup is based on a dual quantum cascade laser absorption spectrometer (QCLAS) with an astigmatic Herriott multi-pass absorption cell.

We will present the measurement concept, in particular the selection of wavelength regions for maximum sensitivity for the clumped species as well as for simultaneous analysis of singly substituted isotopologues, required for referencing the measurements. The absorption lines of singly substituted and clumped N_2O isotopocules are verified in terms of their frequency and line-strengths by standard addition experiments. Clumped N_2O gases for this verification were prepared by thermal decomposition of chemically synthesized double labeled ammonium nitrate. Their isotopic purity was determined by quadrupole MS. Finally, measurement conditions such as pressure and concentration of N_2O as well as instrumental parameters including tuning of the laser source and settings of the spectroscopic software were optimized with respect to overall analytical precision and drift.

We demonstrate that this novel analytical technique is a very promising alternative to the currently emerging high-resolution mass spectrometric approaches [5] in terms of ease-of-use, field deployability, sample throughput, precision, and most importantly, its inherent selectivity for the clumped isotopomers $^{15}N^{14}N^{18}O$ and $^{14}N^{15}N^{18}O$. The performance of the novel QCLAS technique with respect to clumped N_2O isotopes can offer a broad range of prospective applications.

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Novel Chemiluminescence-based Method for the Quantification of the Total N-nitrosamines Concentration in Water

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N-nitrosamines (NOAs) are potent carcinogenic and mutagenic compounds that can be present in water, biological fluids, foodstuffs and cigarette smoke. For instance, NOAs can be formed during oxidative treatment of wastewater and drinking water. Several studies have shown that the formation of NOAs in water can arise from the reactions of amine precursors with chemical oxidants such as chloramines, chlorine or ozone. So far most of the studies published on NOAs in water have focused on only nine specific NOAs selected by the US-Environmental Protection Agency (EPA). However several recent studies have shown that the NOAs selected by the US-EPA might account only for ~5-15% of the total NOAs concentration (TONO) in water.[1] Therefore, quantifying the TONO is necessary to assess the overall risk associated with the presence of NOAs in water. To determine whether *N*-nitrosodimethylamine and other specific NOAs of current interest are dominant or minor components of the total NOAs pool, several chemiluminescence-based methods using chemical denitrosation agents such as HI_3, HBr or CuCl have been developed.[2,3] However, denitrosation agents used in chemiluminescence based-methods are very unstable and may form potent nitrosating agents such nitrosyliodide (NOI) that might (re)form NOAs during the analytical process.[4] Alternatively, NOAs can be photolysed by UV light yielding $NO^{\bullet}$ that can be detected by a nitric oxide chemiluminescence analyser. Photochemical reactions are usually easier to control since no chemical reagents are involved and the reaction is controlled by the UV dose. Based on the issues related to chemical denitrosation of NOAs and the promising features of UV photolysis, we have developed and evaluated a photolysis chemiluminescence-based system for the analysis of TONO concentrations in aqueous samples.[5] The analyses of pre-concentrated water samples have shown that the TONO concentrations in two wastewater effluent samples were 49.8 ± 5.1 nM and 50.2 ± 4.9 nM, whereas in greywater the TONO concentration ranged between 9.3 and 18.3 nM. These TONO concentrations are in the same range as previously reported in the literature and suggest that *N*-nitrosodimethylamine likely constitutes only of small fraction of the total NOAs pool. By utilizing a capillary microphotochemical reactor to reduce the sample cross-section and volume, irradiation and resulting NOAs photo-decomposition to $NO^{\bullet}$ were optimal to reach a sensitivity level comparable to chemical denitrosation (LOD=0.1 mM; LOQ=0.3 mM). Moreover, the reproducibility was enhanced compared to other methods. Because UV-photolysis is more convenient and more reliable to work with, this method simplifies the determination of total NOAs concentration in water.

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High-resolution depth profile analyses of Al/Cu and Ni/Cr superlattices with periodicities ≤ 100 nm by ns/fs-LA-ICPMS

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To fulfill ever-growing requirements in materials science the utilization of superlattices, *i.e.*, modulated metal/semiconductor heterojunctions and oxide thin films, has become of major interest for engineering and fabrication processes of future thermo-electrical and electro-photonic devices [1]. Worth mentioning, individual layers of such periodic composite materials are of thicknesses in the sub-100 nm range which makes them ideal test targets for researchers to (dis-)prove concepts of quantum confinement, a collective term commonly used in the framework of the discretization of an object´s physical properties (*e.g.*, optical, thermal, or electrical) when shrinking its dimensions to the nanoscale. To adapt the properties of superlattices to a specific set of applications material structure (crystalline/amorphous) and thickness, and composition (major, minor, and/or traces) of layers are adjusted and hence need to be analyzed.

In this paper, the capabilities of nanosecond and femtosecond laser ablation inductively-coupled plasma mass spectrometry (ns/fs-LA-ICPMS) for the sub-100 nm depth profile analysis of (semi-)conducting materials composition were explored [2]. Two state-of-the-art ArF ns- and Ti:Sapphire fs-LA systems operated at wavelengths and pulse widths of 193 nm/25 ns and 400 nm/150 fs, respectively, both equipped with laser radiation homogenization assemblies were applied to the analysis of well-characterized Cr/Ni and Al/Cu metal superlattices (substrate material: SiO_2, periodicity: 60-100 nm, total thickness: 0.6-1 mm) and Cr/brass plain coating (thickness: 5 mm). Our data suggests fs-LA to permit the analysis of individual metal layers by ICPMS with depth resolutions ranging from approximately 10 nm for Cr/Ni to < 100 nm for Al/Cu; no such depth resolution could be achieved through ns-LA due to the strong heat diffusion, which gave rise to instantaneous melting of material [3]. By comparison, depth resolutions achievable for single Cr/brass transitions using either of the LA systems were found to be in a range of approximately 500 nm. Still, progressive changes of Cu/Zn responses acquired by ns-LA-ICPMS indicated the occurrence of heat diffusion and, thus, re-distribution of substrate material in the course of analysis.

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Advances in Stray Light Testing and Automated Performance Verification for UV/VIS Spectrophotometers

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In UV/VIS spectroscopy regular performance verification is essential to ensure accurate instrument performance. Widely accepted guidelines are described in the US Pharmacopeia (USP). Here, we assess the methods for measuring stray light according to the current and previous version of the USP and present efficient automatic measurement of this and other optical performance parameters.

http://www.mt.com/uvvis

Microscale biopatterning on surfaces with hydrodynamically confined liquids on a scanning probe

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Patterning and immobilization of biomolecules on surfaces are central to surface biological assays. **We present a novel method which allows contact-free sequential delivery of reagents to a reaction site on a surface, with a characteristic switching time of 560 ms over a transport distance of 60 cm.** We use a standard off-chip selection valve to switch between reagents, which are immediately encapsulated as droplets in an immiscible liquid. The droplets confine the dispersion between reagent interfaces, and are delivered to a microfluidic probe head. This confined flow can be brought into contact with any surface, including cell tissues, to drive a reaction. In close proximity to the apex, we implemented a passive phase-separation microstructure that allows complete removal of the oil phase, such that only the aqueous phase exits the chip and reaches the reaction surface. The separator consists of an array of capillaries, which are subjected to a negative pressure and, owing to hydrophobicity differences between phases, allow removal of the continuous phase, such that only the aqueous phase reaches the reaction surface. With this **we demonstrate two key applications of the method for measurements of receptor-ligand reaction kinetics and protein response to chemical stimuli.** We believe the method will be useful for studying the dynamic response of cells and proteins to various stimuli, as well as for highly automated multi-step assays.

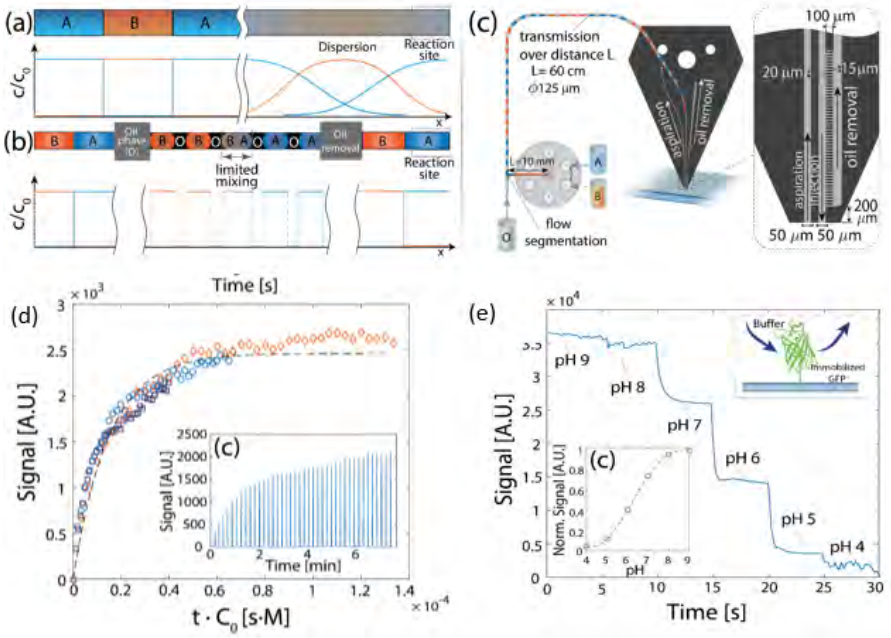

Figure 1: Schematic illustration of the operation principle and applications which allows sharp interfaces to be maintained between individual plugs over long distances and applied on a surface using a MFP.

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Capillary DBDI and APPI as efficient ionization sources for direct interfacing between SPME and MS

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Direct interfaces of solid-phase microextraction (SPME) with mass spectrometry (MS) are rapidly spreading in the MS community thanks to ambient ionization sources developed in recent years such as DART, DESI and DBDI. One of the limitations that needs to be addressed when dealing with complex sample matrices is the matrix effect, which can severely affect reproducibility and sensitivity. Two of the most promising approaches to directly ionize compounds extracted from SPME, i.e., DBDI-MS and APPI-MS, were compared in terms of tolerance towards different kinds of sample matrices. This approach enabled results to be available within minutes, rather than hours required when chromatography is used, saving precious time when fast and accurate results are needed.

Both sources were built in-house, in a capillary format that can be directly connected to the MS using a leak-tight connection. The compounds were thermally desorbed from the SPME device and the resulting gas phase ionized. Quantitation was performed by high-resolution mass spectrometry using a Thermo LTQ Orbitrap. Commercial SPME fibers, including matrix compatible PDMS/DVB/PDMS fibers, were employed for quantification from complex sample matrices. Compounds of different polarity and chemical classes were employed in this study. Quantitation of several classes of molecules in complex samples was achieved. Several complex matrices were spiked with target compounds. Among these, fruit juices, milk, body fluids (urine and blood plasma) and soil were chosen. Illicit drugs, pesticides and other environmental pollutants were considered.

The matrix tolerance observed with both sources allowed the analysis of complex samples while avoiding the need for sample pre-treatment, derivatization and, notably, permits one to skip conventional and time-consuming chromatographic separation. A complete (>99%) thermal desorption of the compounds from the SPME devices usually required less than 1 minute, enough time to perform quantitation, with no need for any cryofocusing system. Matrix component could be retained on the fiber for longer times. For example, sugars or phenolic compounds from food matrices were highly persistent on the SPME fiber. Our results show that chromatography can be circumvented in many cases where the complexity of the sample matrix is not extremely high, especially when adequate pre/post-extraction procedures are employed. A thorough comparison of the efficiency and selectivity (compounds of different polarity) of the DBDI and APPI ionization mechanisms in the direct ionization of complex samples is being carried out and will be used to extend this approach to more complex samples matrices.

Probing Site-Specific Slow Motions of Side Chains in Proteins

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The activity of proteins is intimately linked to molecular dynamics. Several different motions are simultaneously present at ambient temperatures, in different parts of the protein and occuring on different time scales (from picosseconds to seconds). Untangling these motions and assigning their functional roles is a major challenge. Solid state NMR is emerging as a unique powerful tool to simultaneously observe different protein motions either in a site-specific or group specific manner.

Here, we introduce an approach to measure slow motions, occurring on the ms-ms time scale, site-specifically in protein sidechains. We use methyl group nuclear spin relaxation as the motional probe, especially as they play an important role in the formation of hydrophobic cores (which are of critical importance for protein formation and function).[1-2] We present measurements on a sample of microcrystalline GB1, synthesized using specifically labelled precursors that lead to selective labelling of $^{13}CHD_2$ in isoleucine, leucine and valine side chains. With this approach, all other carbon and proton sites in the protein are ^{12}C and ^{2}H.

We measure both longitudinal and transverse relaxation parameters of methyl ^{13}C and ^{1}H in a site-specific manner. In order to remove potential interference from the other interactions we perform experiments at MAS spin rates of 105 kHz on deuterated protein. ^{1}H-^{13}C distances are extracted by using the VCCP method. Moreover, we also measure backbone ^{15}N relaxation parameters. We analyse the data with model free (SMF), extended model free (EMF) and Gaussian axial fluctuation models (GAF), and discuss the resulting dynamical picture for the sidechains.

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Stabillity of extracellular enzymes in surface waters: Comprehensive structural and functional analysis by proteomics techniques and enzymology

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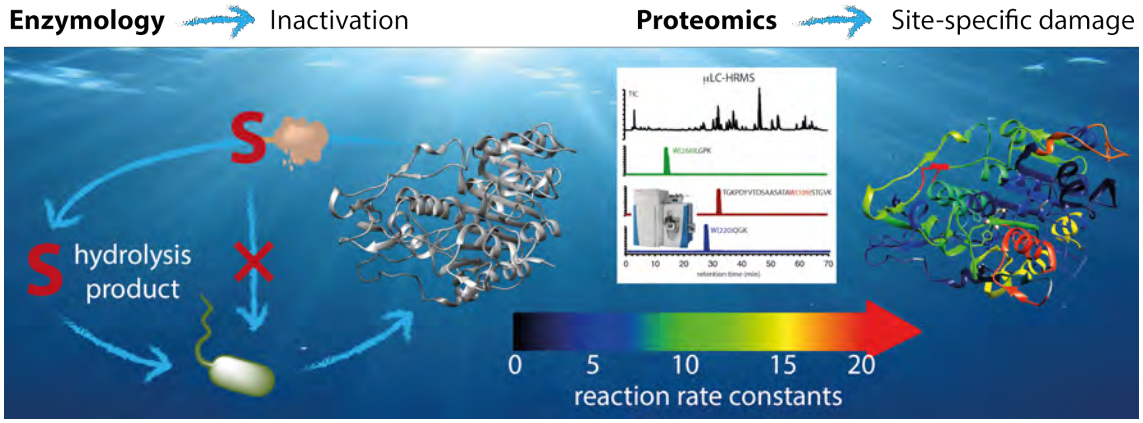
Various bacteria and algae excrete extracellular enzymes that play central roles in the biogeochemical cycling of nutrients and carbon by breaking down macromolecular organic matter. Activities of extracellular enzymes are ubiquitous in surface waters and the ability of these enzymes to influence aquatic systems critically depends on their stability. Once released by the cells, extracellular enzymes are susceptible to various transformation processes that can lead to inactivation, including biodegradation, sorption, and light-induced reactions, which we focus on in the presented work.

By employing enzyme specific proteomics techniques and enzymology we study site-specific damage in the macromolecular structure of enzymes and inactivation rate constants. Our approach to study intermolecular processes within the higher order structure of enzymes can also be applied to various processes besides photochemistry.



To study the effects of photochemical reactions, we expose enzyme solutions to enhanced UV light in absence or presence of natural antioxidants. We monitor activity by enzymology and degradation in the enzyme structure by proteomics techniques during the exposure time. Refined proteomics protocols allow us to monitor peptide fragments with microLC-HRMS accounting for 80-100% sequence coverage of each protein. Hence, we can produce nearly complete pictures of the site-specific damage within the enzyme structure.

We demonstrate that tryptophan (Trp) oxidation within the protein structures tracks the inactivation kinetics during exposure to light. Furthermore, inactivation as well as Trp-peptide degradation are both slowed down in the presence of natural antioxidants. Here, photochemically produced radical cation intermediates of Trp react with antioxidants by electron transfer which forms parent Trp and decelerates photodegradation. These phenomena can be observed across the three model enzymes. In addition to oxidation of Trp containing enzyme fragments, secondary reactions can be observed. For example, oxidation of tyrosine-containing moieties via a (proton coupled) electron transfer or disulphide reduction in proximity to photoexcited tryptophan.