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Relevance of solid forms and its implication on the performance of pharmaceutical drug products

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Appropriate selection of the crystalline form of an Active Pharmaceutical Ingredient (API) is a key decision in developing patient-centered drug products as this influences the safety, efficacy and performance of the pharmaceutical product. On top of these considerations, it is aimed also to facilitate development of robust drug manufacturing processes while guarantying appropriate key quality criterion during the shelf life of the drug product[1]. Advanced characterization tools are needed to analyze the crystalline drug substance in formulated drug products, especially when multicomponent systems and low concentrations are involved. Laboratory and synchrotron-X-ray powder diffraction are seen as complementary techniques among the tools that involve different vibrational spectroscopic techniques and imaging technologies. This presentation will exhibit case studies aiming to illustrate applications of recent development of these methods and continuous innovations that are deployed to assess and mitigate risks when unpredictable event occurs.

[1] P.H. Stahl, B. Sutter, A. Grandeury, M. Mutz, ‘Alternative salt forms: Salts’ in Polymorphism in the Pharmaceutical Industry: Solid Form and Drug Development, **2019**, Wiley-VCH , R. Hilfiker, M. von Raumer (Eds.)

Aptapaper – An aptamer-based glass fiber paper platform for rapid upconcentration and detection of small molecules

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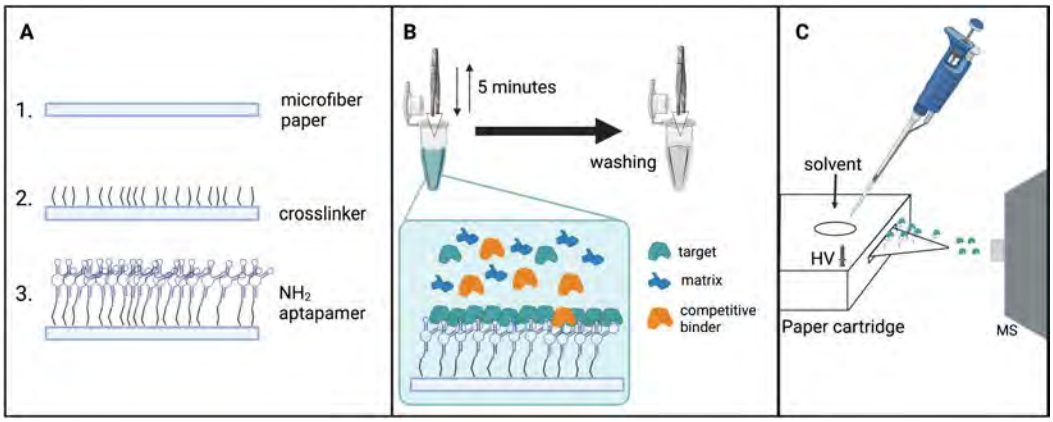
We characterized and tested a paper-based platform ("Aptapaper") for the upconcentration and analysis of small molecules from complex matrixes. As a proof of concept, we used two well-characterized aptamers, quinine and serotonin binding aptamers (QBA and SBA). We used specific aptamer-friendly conditions to ensure the correct folding and binding of both aptamers. We then characterized the QBA aptapaper system using fluorescence microscopy. We used paper spray ionization coupled with high-resolution mass spectrometry (PS-MS) to detect the target molecules. A washing step after the molecule's binding was incorporated to lower the limits of detection (LOD) and reduce matrix effects.



LODs were determined to be 81 pg/mLfor quinine 1.8 ng/m for serotonin, respectively, from different matrices. Random absorption was analyte-specific and observed for quinine but not serotonin. Based on the results obtained, we are now adapting the system to other aptamers of relevance in different areas that require the analysis of compounds present in low concentrations, such as environmental monitoring or food safety. The use of glass microfiber paper renders this approach low cost, and the sampling conditions allow non-experienced persons to collect samples from remote areas. In principle, selective binding and paper spray ionization can later be applied to more portable techniques such as miniature mass spectrometers. The shelf life of bound molecules on the aptapaper was investigated for 4 days with no sample loss for quinine, proving that the samples can be stored and sent to a facility for MS readout [1]. However, each aptamer must be tested separately to optimize the specific conditions necessary for the aptamer to fold properly.

[1] Sandra Martínez-Jarquín, Alina Begley, Yin-Hung Lai, Giovanni Luca Bartolomeo, Adam Prusŕa, Christian Rotach, and Renato Zenobi, Analytical Chemistry, 2022, 94, 5651−5657

Temperature-Controlled Electrospray: A Window into Solution Thermochemistry of Non-Canonical Nucleic Acid Complexes

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Repeated DNA sequences account for more than 50 % of our genome. These sequences can fold into non-canonical DNA structures playing essential roles in cellular function. Guanine-rich direct repeats can form G-quadruplexes (GQs) and partially complementary strands can form branch-like structures, such as three-way junctions (TWJs). Misfolding or persistence of these structures can lead to DNA genomic instability causing severe cellular damage, apoptosis, or abnormal cell growth. To develop ligands that specifically target these structures, detailed information about binding sites, affinity, thermodynamics, and structure aid in developing targeted genetic disease therapeutics. The challenges using conventional methods arise from the structural complexity and variability of these structural assemblies. This can be overcome by temperature-controlled nanoelectrospray ionization (TC-nESI), a method to simultaneously analyze individual forms of DNA or DNA-ligand complexes and characterize their thermodynamics.[1] Here we introduce a custom-built TC-nESI source, which is designed such as the nESI emitter is placed between two copper blocks containing a Peltier element, which guarantees uniformly distributed heat and allows for temperature gradients between 13 – 90 °C.

MS thermal denaturation experiments were designed to acquire mass spectra with increasing source temperature and to observe unfolding steps of individual intermediates of multi-stranded oligonucleotide constructs. We observed changes in melting temperatures (T_m) of individual DNA domains depending on the type of domain, the number of domains, their position, the presence of ligand, and more. Following a detailed van't Hoff analysis, the changes can also be used to calculate thermodynamic parameters.

A DNA TWJ is a complex comprising three double helices, half-complementary to each other, which converge toward a central branchpoint (the potential ligand binding site).[2] TCnESI analysis of TWJ confirmed an unfolding pathway with three different duplex intermediates. Van't Hoff analyses of MS thermal denaturation experiments showed a stabilizing effect of additional base pairing in overhangs of intermediates. We propose that the competition between the formation of the duplex and the TWJ (represented by ΔG°) depends on the formation of additional base pairs between overhanging strands. Effects of the TWJ-binding ligands on the unfolding pathway and thermodynamic stability were also investigated. These ligands bind specifically to the cavity in the center of the branchpoint and greatly affect the TWJ unfolding thermodynamics.

In conclusion, our TC-nESI methodology is a powerful tool for identifying binding events, characterizing their thermodynamic aspects, and quantifying unfolding intermediates in the wide temperature range. Therefore, TC-nESI is a promising method to fill up the gaps in the current therapeutic drug development and be a powerful alternative to current methods.

[1] A. Pruška, A. Marchand, R. Zenobi, *Angew. Chemie - Int. Ed.,* **2021**, 60, 15390–15398.
[2] L. Guyon, M. Pirrotta, K. Duskova, A. Granzhan, M. P. Teulade-Fichou, D. Monchaud, *Nucleic Acids Res.,* **2018**, 46, e16.

Advancing Cyclic Ion Mobility Mass Spectrometry Methods for Studying Biomolecules: Towards the Analysis of Mega Dalton Protein Aggregates

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Mass spectrometry (MS) has developed into a powerful tool for investigating the structure of proteins. The advent of electrospray ionization MS (ESI-MS) made it possible to transmit large, non-covalent complexes without significantly disrupting their structure. When ESI-MS is coupled with ion mobility spectrometry (IMS) it becomes possible to investigate the conformational dynamics of a biomolecule. Furthermore, a temperature-controlled ESI-MS (TC-ESI-MS) source can be used to allow for the continuous monitoring of temperature-induced changes in a biomolecule, providing insight into its thermodynamics and aggregation properties. For larger complexes, this information cannot be readily obtained using conventional methods, highlighting the usefulness of this approach. However, this analysis requires careful instrument tuning to ensure that a biomolecule is transmitted without perturbing its gas-phase structure. The goal of this work was to provide a framework for tuning a cyclic IMS mass spectrometer for the analysis of biomolecules and demonstrate how this instrument can be coupled with TC-ESI-MS to elucidate the structure of temperature-induced mDa protein aggregates.

The cyclic IM mass spectrometer used in this work has three phases during standard IMS experiments: an ‘*inject*’ phase, where ions are moved on to the cyclic array located in the cyclic IMS cell, a ‘*separate*’ phase, where the ions a pushed off the array for IMS separation, then finally, the ‘*eject and acquire*’ phase in which ions pass around the cyclic IMS cell, and are guided out of the system by the cyclic array. The two settings which had the greatest effect on ion transmission were the array offset and the so-called "racetrack bias" during the ‘*separate*’ phase. We found that similar voltages are required for effective ion transmission, otherwise, ions get caught in the array, and will not move around the cyclic IMS cell, which is evident in the IMS chromatogram as a peak with a drift time starting just after the ‘*separate*’ phase. The degree of these effects was dependent on the mass of the protein being analyzed. Optimized IMS settings were then used in the TC-ESI-MS analysis of Jack bean urease. This analysis was performed to investigate temperature-induced effects on the structure of this protein. Prior to heating, the two highest oligomeric states of this protein were the hexamer (~ 550 kDa, native) and a dodecamer (1.1 MDa, nonspecific). An increase in temperature led to urease forming octadecamers. Three distinct gas-phase conformations were observed for the dodecamers, the prevalence for two of which were affected by temperature.

Quantification of endotoxins in bacterial bioreactor samples and correlation of the endotoxin content to optical density and dry cell weight

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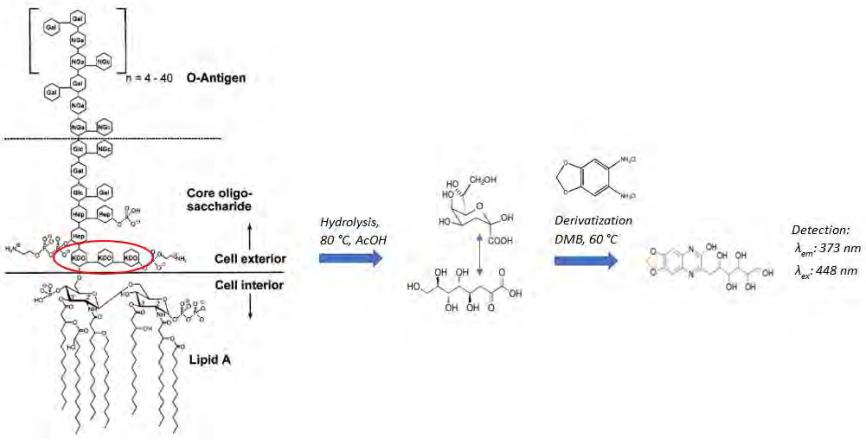
The novel chemical Kdo-DMB-LC endotoxin (ET) assay [1] allows the accurate and economic determination of the ET content in supernatants of Gram-negative bacteria bioreactor samples.

During mild acidic hydrolysis, the ET specific sugar acid 3-deoxy-D-manno-oct-2-ulsonic acid (Kdo) is quantitatively released. Kdo is reacted with 1,2-diamino-4,5-methylenedioxybenzene-2 HCl (DMB) to obtain the highly fluorescent derivate Kdo-DMB. It is separated from the reaction mixture by RP-HPLC and detected by fluorescence. The Kdo content is converted to the ET content of the sample.

The evolution of the ET content in dependence on the cultivation time is shown for two batch cultivations of *Escherichia coli* K12 and a fed-batch cultivation of *Pseudomonas putida* KT2440. A linear correlation between the ET content and the easy-to-access bioreactor parameters optical density (OD_{600}) and dry cell weight (dcw) is presented. It follows that OD_{600} and dcw may serve as leading indicators for an economic real-time estimation of the ET content at different cultivation time points and cultivation conditions, e.g., in production bioreactors. The OD_{600} can further be used to establish simple sample dilution schemes for ET quantification in samples of unknown ET content, e.g., for the Limulus Amoebocyte Lysate (LAL) or the Kdo-DMB-LC assay. The ET content measured with the Kdo-DMB-LC assay [ng / mL] and the ET activity obtained by the compendial LAL assay [endotoxin units (EU) / mL] showed a high correlation for both bacteria bioreactor samples.



Working scheme of the Kdo-DMB-LC assay, endotoxin molecule adapted from [2]

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Combining Activity Profiling with Advanced Annotation to Accelerate the Discovery of Natural Products Targeting Oncogenic Signaling in Melanoma

E. Garo[1], T. Hell[1], A. Rutz[2,4], L. Dürr[1], M. Dobrzyński[3], J. K. Reinhardt[1], O. Pertz[3], M. Hamburger[1], J. Wolfender[2,4]

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HPLC-based activity profiling is routinely used in our lab to track active compounds in plant extracts and was applied here to a project aiming to identify natural products (NPs) inhibiting oncogenic signaling in melanoma [1]. An in-house library of 2’576 plant extracts was screened in an image-based high-content assay. A total of 140 hits were found, and 44 of them were selected for activity profiling. The informed selection of extracts prior to a preparative isolation of active compounds is key to success in NP-based drug discovery, given the large number of samples to process. Linking high-quality metabolite annotations in crude extracts with activity would significantly facilitate this selection step.

We therefore combined advanced UHPLC-HRMS/MS metabolite profiling with HPLC-based activity profiling to leverage the information obtained from both approaches. This strategy was successfully applied to a subset of the library, at the level of sub-milligram amounts of crude extract. Advanced annotation and data organisation allowed the identification of compounds that were likely responsible for the activity of these extracts. The overall workflow will be presented and illustrated with selected examples of structurally diverse compounds.

[1] Lara Dürr, Tanja Hell, Maciej Dobrzyński, Alberto Matei, Anika John, Nathanja Augsburger, Gloria Bradanini, Jakob K Reinhardt, Florian Rossberg, Milos Drobnjakovic, Mahabir Gupta, Matthias Hamburger, Olivier Pertz, Eliane Garo, *J. Nat. Prod.* **2022**, 85, 1006-1017.

Does digitalization in R&D slow down innovation and take away flexibility? What is the risk of ignoring the benefits of digitalization?

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The most scientifically sound regulations for digital records are made from Pharma authorities. However, they also are the most expensive and inflexible ones.

Recent papers suggest that science has problems with reproducibility maybe caused by pressure for publications. The pressure, for instance, may lead to selected results, supporting a certain hypothesis. Records get lost and investigation is difficult. In 2020 it even hit a noble price winner, who had to recall a paper.

Selective results in Pharma regulations are called “testing into compliance”. If revealed, it will have drastic financial and reputation consequences for a pharma company.

Rules for digitalization do not have to be reinvented for science, but should leave some more flexibility, e.g. when it comes to validation documents.

Facilitation of digitalization is in the focus of instrument suppliers through technical controls that enforce compliance and enable reinvestigation of “sensational results” to confirm they were not accidental.

The downside of digitalization is the integration cost and effort. Missing standards is only one aspect of this. Our solution is commercially available platform software, maintained by the supplier. It connects a multitude of instruments and different applications in a single effort.

Influence of Chlorinating Agents on the Formation of Stable Biomarkers in Hair for the Retrospective Verification of Exposure

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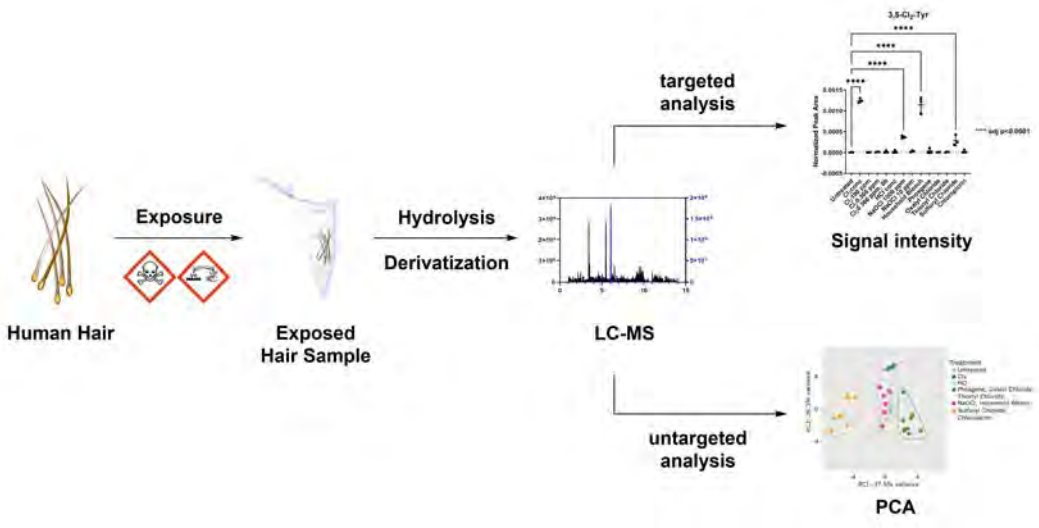
Abstract: Chlorine, as a dual-use chemical, is an essential industrial chemical which has been used as a chemical weapon in the past due to its toxicity and availability. The retrospective verification of chlorine intoxication is often especially challenging and unambiguous markers are still missing. In this study, the effects of different chlorinating and oxidizing agents on human hair were investigated. Samples were exposed to a variety of chlorinating chemicals for a short time and then completely hydrolyzed by a HBr solution to break down its keratin proteins into individual amino acids. After derivatization and targeted LC-MS analysis, 3-chlorotyrosine and 3,5-dichlorotyrosine were unambiguously identified from human hair exposed to chlorine, hypochlorite and sulfuryl chloride. Our results show long-term stability of these markers in the biological matrix, as the chlorotyrosines can still be found 10 months post-exposure at the same levels. Finally, an untargeted analysis was able to discriminate between some of the different intoxicants.



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[3] Crow, B. S.; Quinones-Gonzalez, J.; Pantazides, B. G.; Perez, J. W.; Winkeljohn, W. R.; Garton, J. W.; Thomas, J. D.; Blake, T. A.; Johnson, R. C., Simultaneous Measurement of 3-Chlorotyrosine and 3,5-Dichlorotyrosine in Whole Blood, Serum and Plasma by Isotope Dilution HPLC-MS-MS. *J Anal Toxicol*, **2016**, 40 (4), 264-71.
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Rapid Spectroscopic Detection of Volatile Organic Compounds With Widely Electrically Tuneable Quantum-Cascade Lasers

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Fast, accurate, and in-situ detection of volatile organic compounds (VOCs) is beneficial for quality control in many industrial fields, environmental monitoring, and medical diagnostics. Laser-absorption spectroscopy (LAS) has the potential to fulfil these requirements, even though VOCs exhibit broad and spectrally overlapped absorption spectra, however it requires light sources with broad spectral coverage. Extended-tuning quantum-cascade (QC-XT) lasers [1] are a promising candidate that can combine rapid, high-resolution spectral scanning with broad coverage.

In this work, we present the development and characterisation of a compact mid-IR spectrometer for the high-precision and simultaneous measurement of small VOCs. The analyser uses a QC-XT laser (Alpes Lasers) coupled to a multi-pass cell with 76 m of optical path. By means of the Vernier effect, the laser can be operated in six different spectral windows (each ~1.5 cm^{-1} wide) distributed between ~1163 cm^{-1} and ~1102 cm^{-1}. A complete spectrum is measured every 360 ms by rapid switching between and tuning within the six windows.

VOC measurements were performed in a flow-through configuration at low pressure (~50 mbar) to minimise the broadening of spectral lines. The spectra were recorded at high resolution (< 10^{-4} cm^{-1}), which is not available in the literature for most VOCs. Thus, we generated our reference database and developed a concentration-retrieval algorithm.

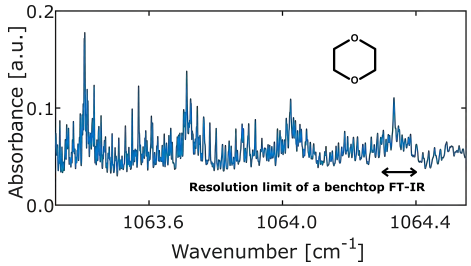

Figure 1. Absorption spectrum of 1,4-dioxane recorded in one of the spectral windows. Several VOCs, containing up to four carbon atoms (C4), reveal significant fine structure in their ro-vibrational spectrum. Such distinct narrow features were also observed for larger molecules (~C6) with rigid molecular structure or high-order symmetry.

Our instrument is well-suited for the detection of small oxygen-containing VOCs at amount fractions down to tens of ppb. We have achieved a typical precision of ~1 ppb for 25 s averaging time, demonstrated for methanol at an amount fraction of 10 ppm, and a large linearity range over 3 orders of magnitude. Excellent selectivity of the method that enables multi-compound measurements with a relative expanded uncertainty of <2% (k=2) was achieved thanks to the broad measuring range, high spectral resolution, and the unique spectral fingerprints of the investigated VOCs.

The system is currently being further developed for breath analysis in the framework of Zurich Exhalomics [2].

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[2] https://www.exhalomics.ch/ (Accessed: 14.03.2022)

Visualizing Surface Phase Separation in PS-PMMA Polymer Blends at the Nanoscale using Tip-Enhanced Raman Spectroscopy

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Polymer blend films have a wide range of applications in display devices, solar cells, high-density information storage media, catalysis, and biotechnology.[1] Control of the polymer phase separation is crucial for a successful application of polymer blends in the above-mentioned technologies. Vertical and lateral polymer phase separation can occur at the nanometer length-scale,[2] and therefore, requires analytical tools with ultrahigh sensitivity and spatial resolution for its analysis.

In the last two decades, tip-enhanced Raman spectroscopy (TERS) has emerged as a label-free and non-destructive nanoanalytical tool. In this work, using correlative topographical, molecular, and elemental information, we demonstrate that besides the lateral phase separation,[3] observed by AFM and confocal Raman spectroscopy (Figures 1a and 1b), a vertical phase separation also takes place at the top *ca.* 20 nm of the polystyrene (PS)-poly(methyl methacrylate) (PMMA) blend surface. Comparison of confocal Raman and TERS imaging (Figures 1b-1e) revealed a uniform PS layer at the blend surface. Complementary XPS measurements reveal the presence of PMMA within the top 9.2 nm of the surface implying that continuous PS layer is present at the sample sub-surface.

Whilst AFM, XPS, and confocal Raman spectroscopy have been used for characterization of polymer blends before, this is the first time that a clear vertical phase separation within 20 nm of the PS-PMMA blend surface was revealed, due to the TERS surface sensitivity. Notably, the full picture is revealed only by correlating the complementary structural, molecular, and elemental information. Given the demonstrated unique ability of TERS to probe surface phase separation, we envisage that it can become an important complementary analytical tool for nanoscale polymer characterization and contribute significantly to the advancement of polymer-based technologies.

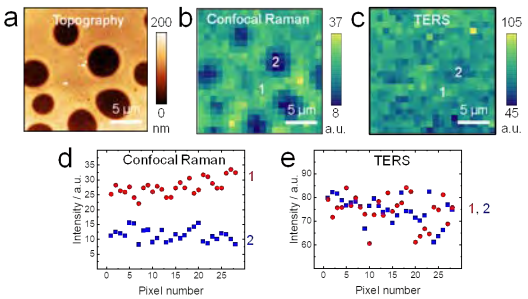

Figure 1: Correlative (a) AFM, (b) confocal Raman, and (c) TERS images of a PS-PMMA blend region. The PS signal intensity was used to construct the confocal Raman and TERS images. The PS intensities obtained by (d) confocal Raman and (e) TERS in the two blend phases marked in Panels b and c, respectively.

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Pure Isotropic Proton Solid State NMR

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In solid state NMR, the linewidths of tens of kilohertz observed in ^{1}H spectra are mostly due to the contribution of homonuclear dipolar couplings. Magic-angle spinning (MAS) greatly improves spectral resolution and at the highest rates available rates today (100-150 kHz)[1] linewidths can be reduced to hundreds of Hertz. Nevertheless, the interactions are not completely removed due to the imperfect nature of coherent averaging and the residual linewidths are still orders of magnitude larger than those encountered in solution state NMR.

Here, we propose that instead of optimizing an averaging scheme, we can parametrically map the residual terms due to the imperfect averaging of MAS[2-3] and remove them in a k-space representation.[4] More precisely, by acquiring a series of MAS spectra at increasing rates, the pure isotropic proton (PIP) signal is extracted in a parameter fitting approach.[4] This method has been demonstrated on eight different organic solids, obtaining isotropic spectra substantially narrower than the correspondent fastest MAS spectra, with linewidths down to as little as 48 Hz (Figure 1). Currently, our work is focused on the exploration of the limitations and fidelity of the approach by extending its domain of application to more complex spectra, studying the origin of artefacts in the spectra and testing new approaches of the data processing.

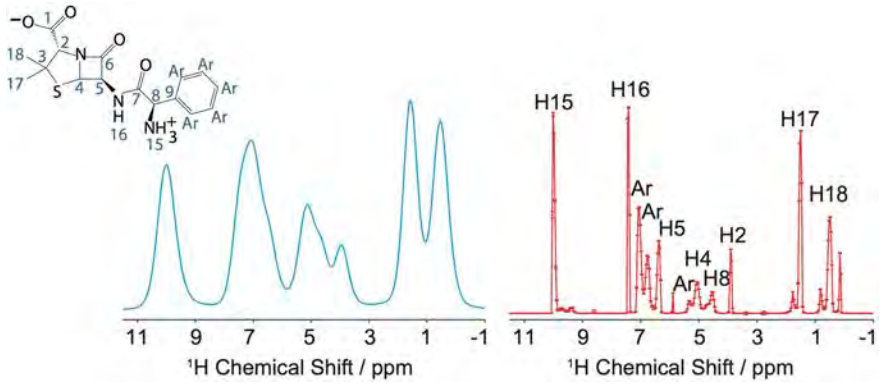

Figure 1. The 100 kHz MAS spectrum (blue) and the pure isotropic proton (PIP) spectrum (red) of ampicillin.

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[3] Bruno Simões de Almeida, Pinelopi Moutzouri, Gabriele Stevanato, Lyndon Emsley, *J Chem Phys*, **2021,** 155, 8, 084201.
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Compressed Sensing for Rapid Signal Retrieval in X-ray Absorption Spectroscopy

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X-ray Absorption Fine-Structure Spectroscopy (XAFS) which is a powerful analytical technique, that works with almost any sample matrix, for obtaining elemental and chemical information in many fields such as biosciences, material sciences, catalysis and physical chemistry. XAFS utilizes a large bandwidth radiation that is scanned sequentially to capture the entire spectrum. The entire scanning of certain samples can take relatively long times. High brightness, which is essential for enough sensitivity, can be destructive for certain samples over time. Additionally, time resolved XAFS needs complex optical setups and fast signal processing techniques resulting in a data deluge. These source features are obtained at synchrotron beamlines. Ideally, one would like to have a rapid acquisition of the entire spectrum, i.e. faster than the chemical reaction being studied. Furthermore, the source should operate at below the sample damage flux, without sacrificing information and the required resolution. Advantageously, this method should be available in each laboratory. The aim of this work is to tackle this challenge and enable XAFS on a tabletop. To achieve the target, first step is the development of a tabletop X-ray laser source and the second step is the utilization of unique data collection methods to compensate for the limitations of the source. A compact terawatt chirped pulse amplification laser has been developed for generating X-rays in the lab, based on the principle of Laser produced plasma, where a high energy laser pulse is incident on a solid target to achieve ionization in a Ne-like or Ni-like stable ground configuration; enabling the emission of bright X-rays on a scale of nanosecond to picosecond time duration. For bypassing the limit of sequential scanning, a compressive data collection method is realized. Compressed Sensing (CS) is a well-known in signal processing technique used to acquire and reconstruct under-sampled data sets without losing any important information about the signal. Taking advantage of the sparsity of the spectral signal in a fixed basis and when sampled randomly, the XAFS data acquisition can be dynamic. Aided by convex optimization solvers, faster and reliable data acquisition is possible with competent data reconstruction. A case study of a Co Foil is presented; showing proof of concept that XAFS raw data can be under sampled, reconstructed and translated well into K-space and R-space with an error of less than 1% and a compression factor of more than two. Reliable data about the edges, oxidation states, atomic distances, and structure are extracted with post-processing.

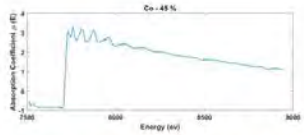

Figure 1a: Co Foil XAFS raw data with 45 % under sampling.

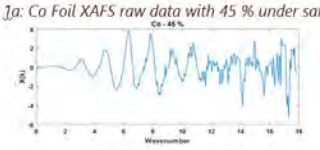

Figure 1b: Co Foil XAFS under sampled data translated in K-Space.

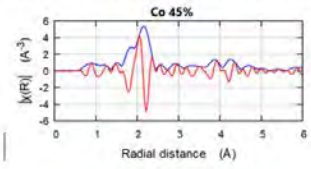

Figure 1c: Co Foil XAFS under sampled data shown in R-space.

Impact of Ablation Cell Design in LA-ICP-MS on Elemental Fractionation

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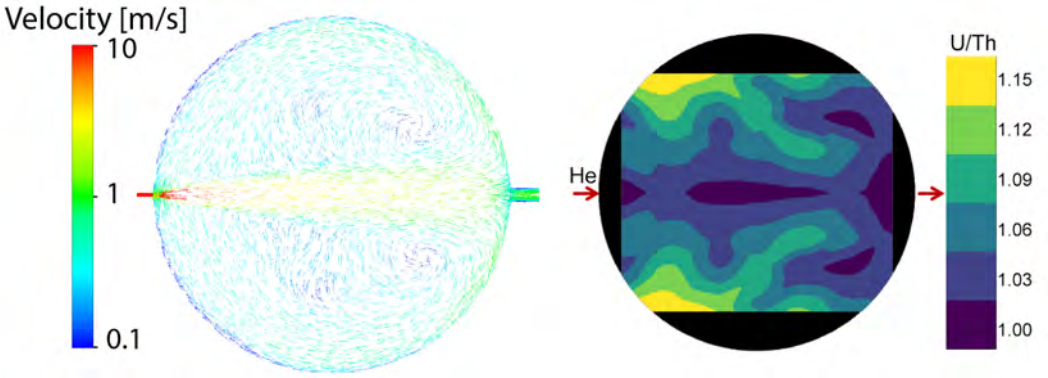
Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been used routinely in the past few decades for the analysis of samples in geology, biology, material sciences, forensics and more. Large volume ablation cells are commonly used for the purposes of isotope and bulk analysis. The laser generated aerosol is highly dispersed, resulting in stable signals optimal for sequential mass analysers. For purposes that require spatial information, such as imaging and depth profiling, low dispersion laser ablation cells have been developed to resolve individual laser pulses. However, due to their fast aerosol washout nature, these ablation cells necessitate the use of simultaneous mass analysers, such as time of flight mass spectrometers, in order to perform accurate multi-elemental analysis. The most common working principle makes use of a two volume approach, where samples are placed on a 3D-stage within a large volume, below a smaller volume with a narrow opening in-between.[1,2]

This work focuses on the differences of the aerosol transport between these two types of ablation cells. Transport phenomena of the laser generated aerosols were investigated based on their particle size distributions, elemental fractionation and gas flow simulations. The differences between low and high dispersion were investigated, as well as the effect of aerosol smoothing after low dispersion ablation. The three setups were compared in regards to reproducibility and accuracy. This work contextualizes the importance of ablation cell design to reduce multiple sources of elemental fractionation.



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Harnessing low-temperature plasma chemistry to distinguish alkylated aromatic isomers with mass spectrometry

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Low-temperature plasma sources, for example, atmospheric pressure chemical ionization (APCI), dielectric barrier discharge (DBD), or direct analysis in real time (DART), are used in mass spectrometry to ionize gaseous samples. These sources generate reactive neutrals, excited species, and ions that generally result in the intact molecular analyte ion. However, variation in the operating conditions can lead to chemically surprising products, for example, we recently reported that excited state *N* atoms react with aromatic hydrocarbons to form *N*-heterocycles at high operating voltage. Here, we use the voltage-dependent changes in plasma chemistry to differentiate alkylated aromatic hydrocarbon isomers with varying alkyl chains (at low voltage) and the number of alkyl substituents (at high voltage).

We infused pure alkylated aromatic hydrocarbon isomers with the formulae C_8H_{10} and C_9H_{12} through a nitrogen dielectric barrier discharge ionization source (DBDI) coupled online to an orbitrap mass spectrometer. We determined the products of the compounds at low (2.44 kV_{pp}) or high (3.44 kV_{pp}) operating voltage from their exact masses and fragments produced by collision-induced dissociation (CID). The products and mechanisms were compared to electron impact ionization database spectra.

At low voltage, the products vary depending on the branching of the alkyl substituent. At the same concentration and plasma conditions, dimethylbenzene C_8H_{10} isomers form the radical cation $[M]^{\bullet+}$ and lose a methyl group, whereas ethylbenzene is further fragmented. Likewise, all isomers of C_9H_{12} form the radical cation $[M]^{\bullet+}$, but propylbenzene also forms $[C_7H_7]^{\bullet+}$, isopropylbenzene $[C_8H_{11}]^{\bullet+}$, 1-ethyl-4-methylbenzene $[C_9H_{11}O]^{\bullet+}$, and trimethylbenzenes lose a methyl group. These reaction products are attributed to reactions with ions (N_2^+, N_3^+, N_4^+, NO^+) and atomic oxygen $O(^3P)$ from impurities in the plasma gas. At high voltage, all alkylated aromatic hydrocarbons form two ionization products: an N-addition product $[M+N]^+$ and an N-replacement product $[M\text{-}C+N]^{\bullet+}$, which are attributed to elevated concentrations of nitrogen ions and excited state N atoms $N(^2P)$, respectively. The branching ratio of the addition and replacement products depend on both the concentration and degree of substitution of the aromatic hydrocarbon. The greater the gas-phase concentration of the analyte, the higher the ratio of N-replacement to -addition product. In a preliminary experiment, when adjusted for concentration, the higher the number of alkyl substituents (toluene<xylene<mesitylene), the lower the ratio of the substitution to replacement product.

Probing the Stability of the β-Hairpin Structure of GB1P in the Gas Phase by Coupling Mass Spectrometry and Fluorescence Spectroscopy

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Mass spectrometry-related innovations: This is the first application of tmFRET in a mass spectrometer to probe whether β-sheet structures retain their solution-phase structure in the gas phase.

Introduction: Whether GB1p, a small peptide forming a β-hairpin, unfolds after desolvation was the object of previous research and is still debated. The stability has never been thoroughly investigated by approaches that combine mass spectrometry with fluorescence spectroscopy-based methodologies such as FRET, because the compact structure of β-sheets falls below the working range of FRET. However, recently, transition-metal FRET (tmFRET) in the gas phase was reported, which provides a working range suitable for β-sheet structures. Thus, this work reports on the utilization of tmFRET to probe the stability of the GB1p β-hairpin structure in the gas phase.

Methods: Wild-type GB1p was modified by attaching a cysteine residue to the C-terminus and a histidine-glycine-histidine motif to the N-terminal side. While the first provides a labeling site for a donor dye, carboxyrhodamine 6G maleimide, the latter non-covalently binds a copper ion (Cu^{2+}) that acts as a fluorescence quencher. Fluorescence lifetime measurements of the labeled peptide in the gas phase were performed for every major charge state (z = 2+,3+, 4+) in MS/MS mode. After adding copper chloride ($CuCl_2$) to the sample solution, similar lifetime measurements were carried out. Additional ion mobility spectra provided complementary experimental values and together with the fluorescence-based findings were compared to molecular dynamics simulations to obtain further structural information.

Preliminary data: Lifetimes of carboxyrhodamine 6G for every measured charge state without the addition of copper chloride did not differ significantly from each other and matched the expected value of t = 6.8 ns. When copper ions (Cu^{2+}) were bound to the di-histidine motif, a decrease of fluorescence lifetime was observed regardless of the charge state, which proves the occurrence of tmFRET. The reduction in fluorescence lifetime decreased systematically for higher charge states. This is likely a result of Coulombic-driven unfolding of the β-hairpin, resulting in higher dye-copper distances. A stronger quenching effect for lower charge states (2+, 3+) could be interpreted as a retention of the solution-phase native fold of the β-hairpin structure of GB1p after desolvation, due to the close proximity of carboxyrhodamine 6G and the copper ion. Ion mobility data supported the trend observed for spectroscopic experiments by allocating lower collisional cross sections (CCS) for lower charge states. Finally, ongoing molecular dynamics simulations provide predicted gas-phase candidate structures of GB1p that match our experimental findings, i.e. comparable CCS and dye-quencher distances.

In-Cell Quantification of Drugs by Magic-Angle Spinning Dynamic Nuclear Polarization NMR

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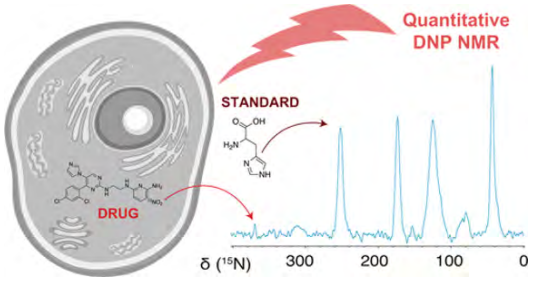
[1]EPFL, Laboratoire de Résonance Magnétique, [2]AstraZeneca, Early Chemical Development, [3] AstraZeneca, Early Chemical Development,, [4]AstraZeneca, Bioscience, Research and Early Development, [5]AstraZeneca, Oral Product Development, [6]AstraZeneca, Oncology

In pharmacology, the quantity of drug inside cells is key to understand the activity of the molecules. The quantification of intracellular drug concentrations would provide a better understanding of the drug function and efficacy. The most accurate quantification methods for drugs in-cells should be performed without modification of either the drug or the target, and with the capability to detect low amounts of the molecule of interest. Here, typically, the relevant range of detection should be in many cases in the µM to nM (pmol to fmol per million cells) range. Thus, it is currently challenging to provide direct quantitative measurements of intracellular drug concentrations that simultaneously satisfy these requirements. Here, we show that magic-angle spinning dynamic nuclear polarization (MAS DNP) can satisfy all these requirements. We apply a quantitative 15N MAS DNP approach combined with 15N labeling in order to quantify the intracellular amount of the drug [15N]CHIR-98014, an activator of the Wingless and Int-1 signaling pathway. We determine intracellular drug amounts in the range of tens to hundreds of picomoles per million cells.[1] This is, to our knowledge, the first time that MAS DNP has been used to successfully estimate intracellular drug amounts. The method should pave the way to access in-cell pharmacokinetics data.



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Visualizing Zeolite ZSM-5 Catalyst Deactivation at the Micro- and Nano-scales using Confocal and Tip-enhanced Fluorescence Microscopies

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Zeolite H-ZSM-5 is a widely used solid catalyst in the chemical industry to convert methanol into gasoline as well as base chemicals, such as ethylene and propylene, in the so-called methanol-to-hydrocarbons (MTH) process [1]. However, the deactivation of zeolite H-ZSM-5 catalysts via the formation of coke species during MTH is not yet well understood, primarily due to the lack of analytical techniques with sufficient specificity, sensitivity or spatial resolution. Herein, we demonstrate that hyperspectral confocal fluorescence imaging is an effective tool to investigate formation of coke species during the MTH process. We performed confocal fluorescence investigation of zeolite ZSM-5 crystals subjected to 10 (10-ZSM-5) and 90 (90-ZSM-5) min of reaction. These measurements revealed a preferential formation of smaller coke species in the central region of the crystal, whereas larger coke species preferentially formed at the edge and the apex regions. Additionally, the 90-ZSM-5 crystals were found to produce more graphite-like species compared to the 10-ZSM-5 crystals. This correlates very well with the previous observations [2, 3]. Furthermore, nanoscale tip-enhanced fluorescence (TEFL) imaging showed a higher amount of coke formation at certain topographic features (such as the crystal steps) on the 10-ZSM-5 and 90-ZSM-5 crystals as depicted in the representative example, shown in Figures 1d-1f. Our study demonstrates that hyperspectral fluorescence imaging could be a powerful tool to investigate deactivation of catalytic materials of industrial importance, such as zeolite crystals at the micro- and nano-scales.

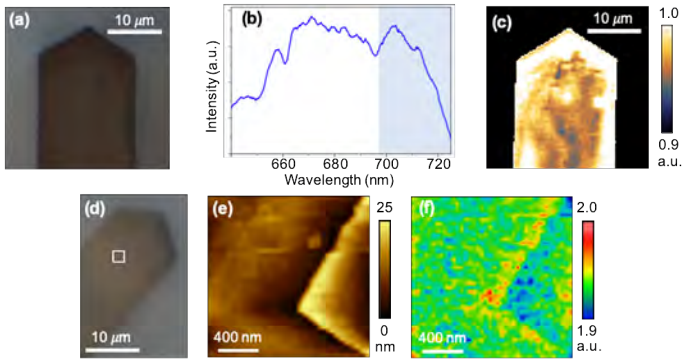

Figure 1. (a) Optical image of a zeolite 10-ZSM-5 crystal on a Si substrate. (b) Average fluorescence spectrum of a zeolite 10-ZSM-5 crystal. (c) Confocal fluorescence image of the blue marked spectral region in (b) measured from the zeolite crystal shown in (a). Step size: 600 nm. Spectrum acquisition time: 1 s. A low P1/P2 ratio is observed in the edge regions indicating a higher EFAL compared to the center. (d) Optical image of a zeolite 10-ZSM-5 crystal. (e) AFM topography image of the marked area in (d). (f) TEFL image of the area shown in €. Spectrum acquisition time: 0.25 s. Step size: 25 nm.

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Novel Analytical Strategies for the Characterization of Peptide Complexes by Temperature-Controlled Cyclic Ion Mobility Mass Spectrometry

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Over the last few decades, electrospray ionization mass spectrometry (ESI-MS) has evolved into a gold standard for the analysis of biomolecules in the process of drug discovery and drug development. Despite the availability of very high-resolution MS instruments, the analysis of isomeric/isobaric compounds such as peptides or peptide complexes, glycosylated proteins or micro-heterogeneities in antibody formulations remains challenging and time-consuming. With the recent development of high-resolution ion mobility (IM) devices, the experimental possibilities have been brought to another level. Here we combine this technology with our home-built temperature-controlled nano-ESI (TCnESI) source to develop novel analytical strategies for the above-mentioned challenges [1,2]. The combination of our TCnESI source with a cyclic IM-MS instrument (cIM, Waters) gives us unique access to thermodynamic and kinetic information of complex mixtures, such as the co-formation and stability of isomeric/isobaric peptide complexes, which is currently not possible by any other technique. In an initial approach, we used our method for the label-free analysis of isomeric collagen model peptides (CMPs). Preliminary results show that cIM allows the separation of three isomeric CMPs with the same ionization profile, differing only in the position of an aspartic acid (Fig. 1). Applying more passes in the cIM is expected to give a clear separation of these monomers and allow analyses of other functionalized isomeric/isobaric peptides at same m/z ranges. We plan to apply this method to fully characterize the specificity of heterotrimer formation in complex mixtures of three or even more label-free peptides. The expansion of this CMP toolbox and characterization by cIM-MS is expected to afford a much deeper understanding in the controlled formation of collagen triple helices.

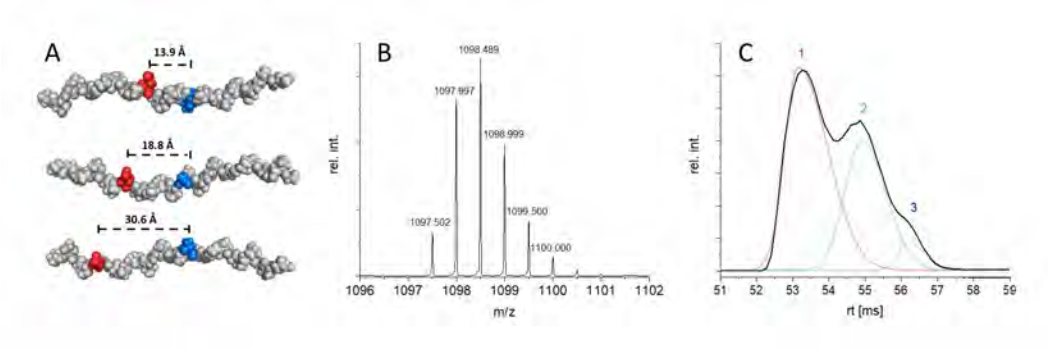

Figure 1

A) Models of three isomeric collagen model peptides ([POG]7 MW = 2198.34 Da, pdb: 3B0S) each modified with an (4S)-aminoproline (blue) and an aspartic acid (red) group in different positions. The Cα to Cα distances of both amino acids are shown above. **B)** Native ESI mass spectrum of a 1:1:1 mixture of all three peptides (50 µM in 100 mM ammonium acetate), recorded in ESI negative mode. The overlapping peak distribution of the doubly charged monomers at around 1098 m/z shows an identical pattern as each individual monomer species.

Solvent vapor removal for the downward-pointing ICP-TOFMS by using combined desolvation devices

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Previous droplets and single cell studies have shown that the configuration of a vertical downward-pointing inductively coupled plasma time-of-flight mass spectrometry (prototype ICP-TOFMS) ensures sample introduction at high dispensing rates of up to 1000 Hz with a transfer efficiency of 100%. Furthermore, the samples can reach the plasma independent of its mass, size and shape due to the gravitational force. Microdroplet generator (MDG) and autodrop pipette were used for the sample introduction [1][2]. Based on previous results, the prototype ICP-TOFMS is a suitable candidate to couple it with a high throughput (> 1000 cells/s) instrument such as the cell sorter flow cytometer for single-cell analysis.

However, the flow cytometer (FACSAria IIIu) generates larger droplets compared to the MDG's and the autodrop pipettes commonly used in our group. This is quite troublesome because the solvent vapor will drastically cool down the plasma and thus, lowering the ionization efficiency. Additionally, high oxide formation is also expected. Therefore, desolvation devices are needed to prevent this outcome. It has already been demonstrated that either a gas exchange- or a helium filled falling tube device upon heating can remove the extra solvent vapor beforehand [1][2].

Therefore, we have recently combined these desolvation devices and coupled them to the prototype ICP-TOFMS. The setup was built so that the droplets are first introduced into the the falling tube device and then to the gas exchange device. It is expected that the combined desolvation devices can completely desolvate droplets that are generated by the flow cytometer at 1000 Hz or even higher dispensing rates. Before that, the current goal is to introduce successfully the 93 µm droplets – generated by a 70 µm autodrop pipette – at 1000 Hz while keeping the oxide ratio below 3%.

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A new approach for determining RNA G-quadruplexes structures

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G-quadruplexes are non-canonical nucleic acid structures formed in guanine-rich DNA and RNA sequences. Typically, one G-quadruplex consists of several stacking planes each comprised of four guanines interacting through hydrogen bonds [1]. Since their discovery, the focus was mainly on DNA G-quadruplexes. In recent years, RNA G-quadruplexes have come into focus because of their regulatory roles in translational regulation, 3'– end processing, transcription termination, mRNA localization, and alternative splicing. G-quadruplexes can adopt different overall topologies essential for the various biological functions. While DNA G-quadruplexes exist in parallel, anti-parallel, and hybrid topologies, RNA G-quadruplexes only adopt the parallel conformation due to the C3'-endo preference of the ribose sugar [2]. Nevertheless, RNA G-quadruples can be highly dynamic due to the exchange between four consecutive guanines along the backbone or from the insertion of guanines from the loops into a G-tetrad. These dynamics can be minimized by the removal of all additional guanines outside of the stacking planes, locking the structure to one single conformation that yields higher quality NMR spectra. Elucidating the structure is of the utmost importance for determining their biological function, but until now no unimolecular RNA G-quadruplex structure has been determined.

The current method to elucidate G-quadruplexes structures by NMR relies on solid-phase oligonucleotide synthesis, allowing for site-specific labelling. However, this approach is unsuitable for RNA sequences due to high costs and low yields. Instead, uniformly labelled RNA constructs can be prepared in good yield and high purity by *in vitro* transcription. Hitherto, no linear assignment strategy is available for solving uniformly labelled G-quadruplex structures. Thus, we designed a step-by-step protocol using a set of NMR experiments that in principle can be applied to any G-quadruplex. The assignment starts from a single guanine to a whole G-tetrad up to the correct arrangement of the individual planes. In this approach, a pair of HNN-COSY spectra is the focal point, allowing the unambiguous assignment through the hydrogen bonds. Applying this new strategy, we have solved the first monomolecular RNA G-quadruplex structures.

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Development of a targeted proteomics method to monitor phosphorylation dynamics of multiple proteins within the mTOR pathway in zebrafish

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The mechanistic target of rapamycin (mTOR) is a crucial signalling pathway known for its role in the regulation of cell growth and proliferation [1]. Signal transduction within the mTOR pathway occurs through protein phosphorylation, which has been traditionally studied with antibody-based methods. Since appropriate antibodies are not always available for non-mammalian proteins, we are proposing an alternative approach using mass spectrometry-based targeted proteomics, which we developed for zebrafish (*Danio rerio*), an important model organism in human health and environmental toxicology fields. Based on a literature review we selected 69 unique peptides representing phosphorylated or non-phosphorylated protein targets along the mTOR pathway. To develop multiple reaction monitoring (MRM) assays to monitor their abundance and phosphorylation changes, we followed the workflow by Tierbach et al. [2] with the addition of an enrichment step for phosphopeptides. This step improved the detection sensitivity for low abundant phosphopeptides. Commercially produced synthetic peptides were used for MRM assay optimization, i.e. to determine specific retention times and the 2-3 strongest transitions for the target peptides. The instrument sensitivity for the peptide targets could be increased by up to 50% through collision energy optimization. To ensure reliable quantification for all targeted peptides, we further tested the developed method in regards to (1) intra- and inter-day variability, (2) effects of the sample matrix before and after enrichment, and (3) response linearity of the target peptides. Only peptides with a linear response and a precision of below 20% CV (coefficient of variation) were considered for quantification. Our approach enables a simultaneous assessment of phosphorylation changes for multiple peptide targets along the mTOR signalling pathway. In the following steps, we will use our method in zebrafish PAC2 cells sampled at different growth stages, i.e. the exponential and stationary phases, or after exposure to pharmacological modulators of the mTOR pathway activity, to investigate mTOR-mediated signalling and its role in growth regulation in fish cells.

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R-based Automatic Spectra Evaluation Routine (RASER) for the selective and rapid analysis of chlorinated paraffins and olefins

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[1]Swiss Federal Institute for Materials Science and Technology Empa, [2]Department of Chemistry, University of Zürich, [3]Zürich University of Applied Sciences ZHAW

Technical chlorinated paraffins (tCPs) are currently produced in a large scale of 1 million t/y and are used in various applications as plasticizers and flame-retardants in plastic and as metalworking fluids [1]. tCPs are complex mixtures with millions of constitutional isomers and stereo-isomers of poly-chlorinated *n*-alkanes with carbon-chain lengths of C_{10}-C_{30} (C-homologues) and chlorination degrees of Cl_3-Cl_{20} (Cl-homologues) [1]. The analysis of CPs is challenging because of mass spectrometric interferences of the numerous isomers, the absence of suitable reference materials and the presence of side-products or transformation products such as chlorinated olefins (COs) [2]. Because of their toxicity and environmental risks, the use of short-chain CPs (C_{10}-C_{13}) has been restricted since 2017 and SCCPs are regulated as persistent organic pollutants (POPs) under the Stockholm Convention [3]. This forced a shift to the production and usage of medium- (C_{14}-C_{17}), long- (C_{18}-C_{21}), and very long- chain ($C_{>21}$) CPs. Therefore, it is crucial to develop analytical procedures that allow to study CPs and their transformation products for all carbon- and chlorine-homologues.

We developed a liquid-chromatography method coupled to atmospheric pressure chemical ionisation and high resolution mass spectrometer (LC-APCI-Orbitrap-MS) to study complex mixtures of up to 1'320 homologues of CPs and olefinic transformation products. Such spectra are indeed complex, containing up to 30'000 signals. An R-based Automatic Spectra Evaluation Routine (RASER) was developed and used to read-out defined CP and CO signals of specific homologues. The potential of the method was evaluated on plastic materials we are exposed to in daily life. With RASER, we obtained C- and Cl-homologue distributions of CPs and COs from these plastic materials. RASER reduced the workload for the spectra evaluation from weeks to hours. Visual validation of measured and expected isotope clusters and the reconstruction of full-scan mass spectra showed the presence of hundreds of CP- and CO-homologues. Mass spectrometric abundances were highest for medium-chain CPs and COs. CO-homologues were detected in abundances up to 40 % with respect to the corresponding CP-homologues.

The soft-ionization technique applied favours the formation of quasi-molecular chloride-adduct ions without a formation of fragments. The high-mass resolution allowed to distinguish CPs and olefinic materials and RASER provides the possibility to evaluate complex spectra of tCP-mixtures containing up to 30'000 ions. This combined approach can now be applied to various CP-containing materials of different origin such as plastic from consumer products or environmental samples.

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Development of an Automated Total Nitrosamines (TONO) Analyzer

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Formation of *N*-nitrosamines in water and wastewater treatment is of concern due to their high carcinogenic potency, e.g., *N*-nitrosodimethylamine (NDMA) is estimated to be several hundred times more potent than the regulated trihalomethanes in drinking water. Rather than a direct release from specific sources, *N*-nitrosamines are known to be mainly formed *in situ* during disinfection processes such as chlorination, ozonation, etc. They are categorized as disinfection byproducts (DBPs). In principle, nitrosation can take place with any nitrogen-containing organic precursor present in water with a potential formation of a variety of *N*-nitrosamines. However, robust analytical tools available to date are somewhat limited to the commonly known individual *N*-nitrosamines, e.g., NDMA. In 2017, Breider and von Gunten developed an analytical platform which, based on UV photolysis and chemiluminescence, determines the total content of *N*-nitrosamines in water samples.[1] This system had many shortcomings and therefore, the TONO analyzer is currently upgraded, aiming for a complete automation of the analytical procedure from sampling to detection including data analysis without human intervention. All the hardware modules such as autosampling platform, syringe pumps, valves, photoreactor, etc. were 3D-designed, 3D-printed and assembled in-house. Open-source microcontrollers (i.e., Arduino) were used to control individual hardware modules with firmwares written in C++ and the user interface written in Python for serial communication with microcontrollers.

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Time-resolved continuous measurements of DNA disposition kinetics using charged nanoparticles anchored on the ion-selective membrane of an aptamer-based electrode

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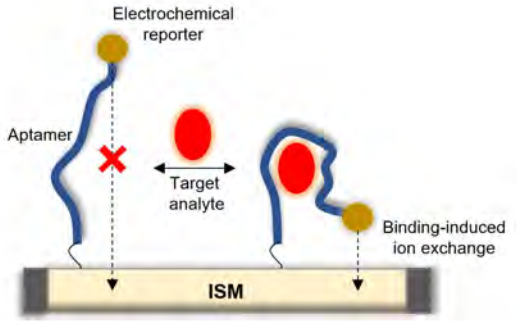
An aptasensor belongs to a class of biosensors where the recognition phase consists of a DNA or RNA aptamer. The aptamer recognizes the molecular target against which it was previously *in vitro* selected. Electrochemical aptamer-based sensors exploit an electrode-bound, redox-reporter-modified aptamer, as the biological recognition element [1]. The presence of the specific target induces a conformational change in the aptamer, which is easily measured based on the interaction of the electrochemical probe with the electrode surface, as shown in Figure 1. Following the incorporation of an electrochemical reporter, the sensing mechanism of previous works relies on the redox properties of those species, including ferrocene and methylene blue, for example [2]. In this study, a redox reporter is replaced by a charged nanoparticle, which can strongly affect the ion-exchange process on the surface of an ion-selective membrane (ISM). The sensing principle in this case, put forward here for the first time, does not depend on the redox process of electroactive probes.

This is accomplished by modifying the surface of gold nanoparticles (100 nm diameter) with cysteamine (cysteamine hydrochloride) and arginine (L-Arginine), by means of a peptide conjugation using an EDC-mediated crosslinking protocol (EDC: N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride) in phosphate buffer saline (PBS, pH 7.1). The final particles have their surface covered with positively charged groups that can be detected by a cation responsive PVC-based membrane. To attach the aptamer to the electrode surface, the PVC is first modified with sodium azide in bulk solution. Using a Cu-catalyzed azide-alkyne cycloaddition click chemistry protocol, the azide-modified PVC membrane is coupled with alkyne-PEG-NHS (Alkyne-PEG-N-hydroxysuccinimidyl ester) cross-linking reagent. The NHS linker can be used to attach the biorecognition elements, including aptamers, to the sensor surface by an efficient conjugation to primary amines at physiologic pH. The aptamer sequence should be modified with a thiol group at its 5’ end, which will bind to the electrochemical probe, while the amine at its 3’ end will be attached to PVC-PEG via NHS reaction. As shown in the scheme below, the presence of a specific target induces a conformational change in the aptamer. By affecting the ion-exchange interaction at the membrane interface, this produces a measurable electrochemical output that can be used to measure molecular concentrations in real-time.



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Capacitive Readout of pH-Sensitive Membranes using a Symmetric Flow Cell towards Environmental Applications

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Constant potential coulometry with ion-selective electrodes (ISEs) allows for an increased precision compared to direct potentiometric readout. Bobacka and coworkers introduced this novel approach using an ion-to-electron transducing material acting as a capacitive layer on solid-contact electrodes [1]. A constant potential is applied between the reference electrode and the ISE. Thus, any variation in ion activity, resulting in a potential change at the ISE, must be compensated by an opposite potential change in the capacitive layer. This process generates a transient current that can be integrated to obtain the charge, which was found to be proportional to the logarithmic ion activity [2]. Our group further improved this method by replacing the solid-contact material by an electronic capacitor to avoid unideal behavior of the capacitive layer and by adding an electronic control to automatically short circuit the capacitor. The electronics were then miniaturized and integrated in a portable potentiostat (PotentioCap) with the aim to achieve *in situ* measurements.

Even if the precision of constant potential coulometry is high, the stability of the open-circuit potential (OCP) at the membrane remains crucial to obtain a reproducible current signal. Potentiometric measurements with ISEs are known to drift with time and with temperature. Rumpf et al. described a symmetrical potentiometric cell where two Ag/AgCl electrodes were immersed into two cambers separated by an ion-selective membrane [3]. One of the solutions was kept constant and the corresponding electrode acted as a reference. The symmetry of the setup allows to compensate for the drift of potential over time or resulting from temperature change as it similarly affects both electrodes.

This work aims to combine the precision of capacitive readout with the stability of symmetric potentiometric cells to measure pH with ultra-high precision. The experimental setup is composed of a flow cell divided into two compartments connected with a pH-sensitive membrane. A commercially available Ag/AgCl wire is connected on each side of the membrane to record the potential. A Pt wire is inserted on one side to act as a counter electrode for the constant potential coulometry measurement. One compartment is filled with reference solution while the other one can be filled with a sample solution to perform a coulometry measurement or the reference solution to record the OCP. This will allow for reversible quantification of pH by constant potential coulometry and may serve as basis for a future environmental probe.

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Exploring Gas-Phase MS Methodologies for Structural Elucidation of Branched N-Glycan Isomers

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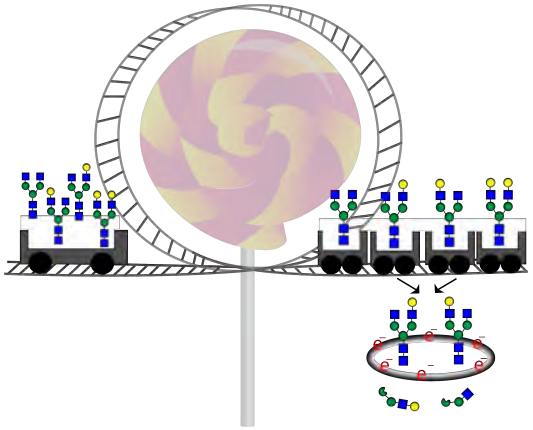
Structural isomers of N-glycans that are identical in mass and atomic composition provide a great challenge to conventional mass spectrometry. This study employs additional dimensions of structural elucidation including ion mobility (IM) spectroscopy coupled to hydrogen/ deuterium exchange (HDX), and electron capture dissociation (ECD) to characterize three main A2 N-glycans and their conformers. A series of IM-MS experiments were able to separate the low abundance N-glycans and their linkage-based isomers (α1,3 and α1,6 for A2G1). HDX-IM-MS data indicated the presence of multiple gas-phase structures for each N-glycan including the isomers of A2G1. Identification of A2G1 isomers by its collision cross section was complicated due to the preferential collapse of sugars in the gas phase, but was possible by further ECD fragmentation. The cIM-ECD approach was capable of assigning and identifying each isomer to its IM peak. Two unique cross-ring fragments were identified for each isomer: *m/z* = 624.21 for α1,6 and *m/z* = 462.16 for α1,3. Based on these key fragments, the first IM peak, indicating a more compact conformation, was assigned to α1,3 and the second IM peak, a more extended conformer, was assigned to α1,6.



Effect of inert environment on Tip-Enhanced Raman Spectroscopy of biological molecules

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Tip-Enhanced Raman Spectroscopy (TERS) has emerged as a promising technique to study two dimensional materials (such as graphene, thiol SAMs, transition metal dichalcogenides, surface bound catalysts) [1] and, biological molecules (such as peptides, proteins, DNA, cells, model cell membranes, etc).[2,3] However, most of these works are carried out in an ambient environment. Precise understanding and characterization of such materials are underpinned on the accurate interpretation and origin of Raman bands, especially in the fingerprint region of the Raman spectrum.

In this work we discuss the origin of spurious signal due to adventitious carbon species [4] and their contribution to the Raman spectrum and TERS mapping of the aforementioned systems. We show that depending on the type of the sample (packed self-assembled monolayer vs. drop-cast samples) the incidence of contamination signals increases significantly. Furthermore, we also propose a strategy of performing TERS in an inert, nitrogen rich (N_2) environment to reduce the frequency of the contamination signals in such samples. Based on the type of sample, the reduction in the contamination signals in an N_2 rich environment can vary from 33-80% compared to measurement in air. Moreover, we found enhanced tip stability in the inert environment, helping us in performing high-resolution scans without the loss of plasmonic enhancement. Lastly, we also found less variation in the amount of contamination signals for various measurements hinting towards a more control over the experimental conditions as well as sample contamination. We hope such strategies will help mitigating sample contamination and accurate interpretation of the TERS spectra of various samples.

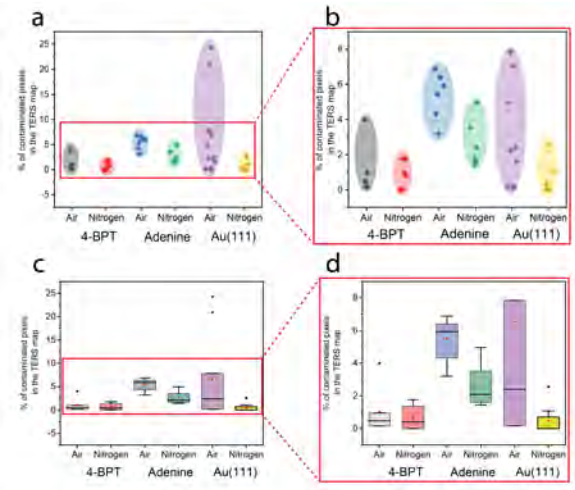

Figure 1: (a) Scatter plot (c) box plot showing the percentage of pixels with contamination signal in a TERS map for a 4-biphenylthiol (4-BPT) SAM, adenine drop-cast sample and Au (111) surface, in air and N_2 respectively. (b) Magnified view of (a), (d) magnified view of (c).

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XUV Desorption of Polymer Matrices studied with TOF-SIMS and XPS

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Desorption is well established to perform a rapid and spatially resolved microanalysis. The use of short wavelength radiation has been proposed to enhance the absorption and thus eliminate the heat-affected zone [1]. Sub-destructive sampling with extreme ultraviolet radiation (XUV, λ= 5-50 nm) has benefits in microanalysis due to its photochemical interaction within any matrix. XUV can excite the electrons and ionize the surface molecules or atoms efficiently. It is important to study the interaction between XUV photons and materials as well as the photo chemical effects on various material surfaces. Polymer matrices (PMMA and PTFE) were primarily irradiated by a pseudospark XUV source without focusing, i.e. at low irradiance (200 μJ/cm^2). Time-of-flight secondary ion mass spectroscopy (Tof-SIMS) was utilized to observe the effects on the irradiated surface. Fragmentation of the main chain and the side chain scission was observed in both polymers. Surface oxidation and fluorine depletion of PTFE was presented, which was also verified with X-ray photoelectron spectroscopy (XPS). XPS results also showed the change of the chemical composition between the pristine and the XUV-exposed samples. However, the chemical modification of PMMA is negligible. Additionally, the wettability of both polymer increased after EUV treatment, because there are more O generated and remained on the surface. Since it is problematic to modify the very stable PTFE, the surface modification of PTFE with EUV photon is extremely interesting, which can provide multiple applications for PTFE. This study presents a wide range of possibilities for the further development of the surface modification/microanalysis by XUV irradiation/ablation.

Keywords: XUV ablation, surface modification, TOF-SIMS

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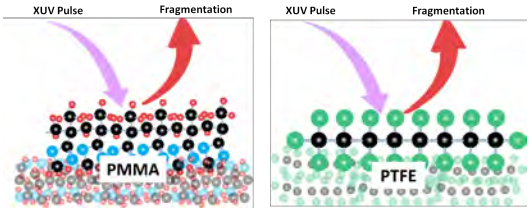

Observation of calcium carbonate prenucleation species via dissolution dynamic nuclear polarization.

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Calcium carbonate is one of the most important inorganic compounds that exist in this world. It has numerous polymorphs and is usually formed from the reaction between Ca^{2+} and CO_3^{2-}. However, the mechanism of this transformation remains elusive. Precursors that include prenucleation clusters, liquid-like precipitates and amorphous nuclei have been proposed. Because the carbon atom in these proposed precursors has different chemical environments, ^{13}C NMR would be a powerful tool to study the nucleation mechanism of $CaCO_3$. While powerful, the conventional NMR methods are limited by their intrinsically low sensitivity and would be expected to struggle with the determination of such short-lived species in small concentrations. Recently, the advances in dissolution dynamic nuclear polarization (dDNP) have made it possible to observe chemical species with low concentrations and short lifetimes.

Here we investigate the early-stage nucleation process of $CaCO_3$ via ^{13}C NMR dDNP. When Ca^{2+} ions are combined with CO_3^{2}- ions that are previously hyperpolarized at liquid-helium temperature, we observe *in situ* a previously unseen signal with distinct chemical shift distribution that decays at a different rate than the free carbonate which we observe in our spectra too. After the reaction is done, this signal is no longer seen in the thermal spectra. We ascribe this signal to the pre-nucleation clusters of calcium carbonate which is the first direct observation of calcium carbonate pre-nucleation species using NMR.

A Comprehensive Method to Elucidate Pyoverdines Produced by Fluorescent Pseudomonas ssp. by UHPLC-HR-MS/MS

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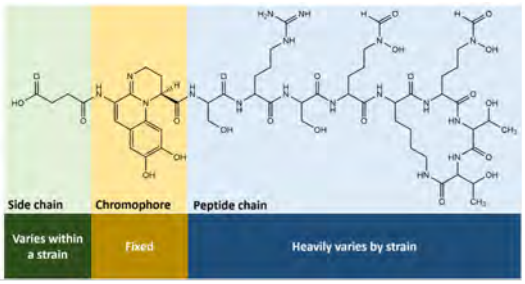
Microbial secondary metabolites represent a rich source for drug discovery, plant protective agents and biotechnologically relevant compounds. Among them are iron-chelating molecules called siderophores that play key roles in bacterial community assembly. Some siderophores such as certain pyoverdines can act as plant protective agents due to their pathogen control properties [1]. These biologically active pyoverdines are commonly not identified due to their complex chemical structure and the lack of simple and rapid analytical procedures despite great interest. They are solely produced by fluorescent *Pseudomonas* members and consist of different peptide chains specific to each bacterial species often incorporating unusual amino acids (see figure) [2].



Hence, a generalized high-throughput UHPLC-MS/MS pipeline was developed for the structural elucidation of pyoverdines using a Q Exactive MS. Liquid bacterial culture samples were purified by a small-scale solid phase extraction (SPE) and directly submitted to liquid chromatography. All ion fragmentation (AIF) generated mass spectra containing the characteristic fragments of the biological precursor of pyoverdine, ferribactin, leading to the revelation of the mass of secreted pyoverdines. Targeted MS/MS experiments at multiple collision energies accomplished the full structure elucidation of the pyoverdine peptide chain. The interpretation of MS/MS spectra was simplified using a mass calculator and a fragmentation predictor programmed in Excel. The method robustness and applicability were demonstrated by the analysis of 13 unknown pyoverdines secreted by sampled bacterial cultures. Among these, 4 novel pyoverdine peptide chains were discovered that were not previously reported in literature.

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Pressure drops set-up: an efficient way to rapidly measure the pressure drops contribution of novel catalysts.

A. Sacchetti[1]

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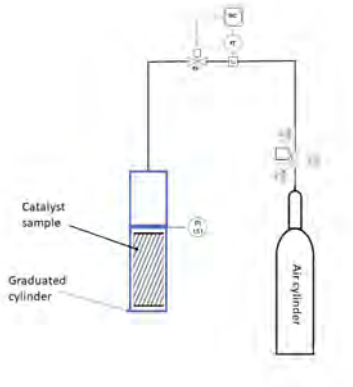
CASALE SA is a global supplier of technologies and engineering solutions for the production of Ammonia, Urea, Methanol, Nitrates, Phosphates, complex fertilizers, Syngas and Melamine. For most of these processes, especially for Ammonia, Methanol, Syngas and de-NOx, the catalyst covers a fundamental role, and the optimization of a technology passes undoubtedly through the development of an optimal catalyst. It is well known that, apart the chemical proprieties, the catalyst performances are strongly affected by the shape which have a direct impact on the catalyst's mechanical proprieties and its behaviour in terms of liquid hold up, regime (diffusional and kinetic regime) and pressure drops. These latter present a pivotal parameter for the energy consumption in a system: the higher the pressure drop, the greater the amount of energy consumed to maintain the desired process flow. In view of industrial applications of the developed catalyst, it is therefore fundamental to assess the catalyst impact on the pressure drops. Many studies are found in literature, highlighting the importance and effect of the catalyst shape for industrial processes, combining experimental and simulations approaches [1,2]. As pioneer of innovative and fast solutions, CASALE SA developed an approach that enable the development of new catalyst projecting it in an industrial system. Indeed, the common path for the selection of a winning catalyst is composed of different steps: after selecting the most suitable formulation in terms of chemical proprieties, it is fundamental the evaluation of fluid dynamic behaviour according to the shape proprieties. Besides CFD simulations, experimental evidence on the pressure drops contribution from the catalyst can be observed, using real catalyst pellets or 3D-printed shapes. This latter method allows the fast exploration of innovative and undiscovered shapes, that can be tested under industrial flow regime. The tests functioning is fully automatized thanks to LabVIEW communication, limiting manual operations. Moreover, the measure is not only very fast (about 20 minutes per measure) but is also non-destructive, to minimize catalyst wastes. Therefore, through the rapid experimental results offered by this home-made and simple set-up, it is possible to quantify the pressure drops encountered with new solid catalysts. Even more, the validation of the pellets formed with a 3D printer, open new possibilities to rapidly explore the effect of innovative shape on the process efficiency.



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Portable and efficient suitcase for water measurements in Syngas and Ammonia Plants.

A. Sacchetti[1]

[1]Casale SA

CASALE SA is a leader in developing new technologies and engineering solutions for the synthesis of Ammonia, Syngas, Urea, Methanol, Nitrates, Melamine and all the fertilizers-chain products. Apart to the development of new technologies and the revamping of pre-existing industrial plants, CASALE SA offers fast and effective solutions that allow to monitor and analyse stream coming from industrial applications. For example, in the plant for ammonia synthesis, the amount of oxygenated compounds present in the fresh make-up gas is a critical parameter to monitor. Indeed, it is well known that the most common ammonia catalyst, the iron-based, is deeply affected by the amount of oxygen present in the feed, leading to reduction of the catalyst performances with final deactivation[1,2]. This is translated in negative effect in terms of ammonia production and earlier catalyst replacement. Typical sources of oxygen are H_2O, CO and CO_2 that are inevitable residues coming from the previous operations. Within regards of iron-based catalyst, the content that should not be surpassed of Oxygen is 10 ppm, in order to avoid unwanted consequences on the catalyst. Therefore, an accurate measurement of the H_2O concentration that is fed into the reactor is fundamental to avoid unexpected loss of catalyst's performances.

Thus, CASALE SA developed and manufactured a portable instrument, allowing water measurements and quantification in situ from the streams of Ammonia plant that allows precise determination of oxygen flowing to catalytic bed. This system avoid the needs of customized gas chromatographs and expensive instruments[3]. The device is equipped with a dedicated line for the collection of the stream with following Karl-Fischer titration set-up, and can be easily moved from one part of the industrial feed to another. This solution allows the facile and rapid detection of water amount in ammonia streams, which cover a pivotal role in terms of efficiency for the ammonia catalyst.

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Urea: from the laboratory to the plant scale to sustain the world's growth.

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Urea covers a fundamental role in feeding the world: among all solid fertilizers, it contains the highest amount of solid nitrogen, making it largely exploitable as nitrogen-release fertilizer. Indeed, urea production reaches 181 million t/year[1] in 2020 and it is forecast to increase up to 302 million t/year by 2030[2]. In the soil, urea, is broken down into ammonium, which is rapidly taken by the plants and is exploitable as nutrient by these latter. Moreover, some bacteria present in the terrain are capable to oxidize the ammonium into nitrate, that are rapidly absorbed by the plants to contribute to their growth. The basic process (named Bosch-Meiser) dates back to 1922 and consist of two equilibrium reactions. Ammonia and carbon dioxide reacts at high temperature to produce ammonium carbamate via an exothermic reaction. This latter converts into urea and water, within and endothermic process. The urea synthesis plant is often found adjacent to the ammonia process: this allows not only to avoid $_{NH3}$ transportation but permit the exploitation of the CO_2 emitted as byproduct in the ammonia synthesis process due to natural gas consumption.

Urea covers a fundamental role in CASALE SA business, which is deeply engaged in finding innovative and unconventional solutions in all the nitrogen-value chain processes, starting from ammonia and ending up with urea, nitric acid, melamine and complex fertilizers. Besides developing tailored solutions for industrial plants (e.g: an Urea On-Line Raman Analyzer (AURORA[3]) for operando measures of KPIs in Urea plants), CASALE SA is engaged also in lab-scale experiments to understand new possible reactive conditions relevant to improve the design of the urea synthesis reactors.

Tests are executed in a lab-scale ALLOY59 autoclave and, by changing reaction parameters (N/C and H/C, Temperature and Inert pressure in the system), it is possible to screen rapidly many different conditions that have a direct impact on Urea selectivity. After performing the reaction for the desired time, the liquid phase is analyzed via HPLC for urea quantification, while the analysis on gas-phase present in the system allows the determination of NH_3, CO_2 and H_2O %. With this double analysis it is possible to investigate in detail the liquid-vapor equilibrium that occur during the reaction and how it is influenced by the reaction parameters.

To conclude, Urea is undoubtedly protagonist of the present and the future agricultural and fertilizers scenario. Despite its synthesis at industrial level is one century old, many improvements can be done. The solutions proposed by CASALE SA (AURORA device, scrubber units etc) are aimed to optimize the productivity and minimize the wastes in industrial plants. Moreover, aware of the importance of explorative experiments at lab scale, CASALE SA combine the development of innovative industrial-scale solutions with laboratory experiments that open new frontiers in terms of analysis that would not be possible on a plant site.

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Photoluminescence brightening of single-walled carbon nanotubes through conjugation with graphene quantum dots

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Spanning the tissue transparency window, the near-infrared (NIR) SWCNT photoluminescence (PL) single-walled carbon nanotubes (SWCNTs) can optically penetrate biological tissue for deep-tissue imaging and optical sensing. SWCNTs are often functionalized with single stranded DNA (ssDNA) to yield sensors that are biocompatible, responsive, and selective. However, the low brightness of these ssDNA-wrapped sensors restricts the depth at which such sensors can be implanted in the tissue. In this work, we demonstrate the PL enhancement of ssDNA-wrapped SWCNTs through the incorporation of biocompatible graphene quantum dots (GQDs). Two kinds of GQDs, pristine (PGQDs) and nitrogen-doped (NGQDs), were fabricated and characterized. Thermodynamically, both GQDs were shown to significantly increase the fluorescence efficiency of ssDNA-SWCNTs with the same degree of PL enhancement after 3 h. A correlation between the diameter of the SWCNTs and the PL enhancement factor was approved, the larger the SWCNT diameter, the higher the PL increase upon addition of GQDs. For example, a 30-fold enhancement was achieved for (8,6) chirality while it was only 2-fold for the (6,5) chirality. Our experiments showed that the addition of GQDs leads to an increase in the surface coverage of SWCNTs suspended by ssDNA, limiting water molecules access to the nanotube surface and thus increasing the fluorescence efficiency. Kinetically, NGQDs brightened SWCNTs much faster than PGQDs. The PL intensity reached a plateau in 2 min following addition of NGQDs while it was still increasing even after 1 h upon addition of PGQDs. We show that NGQDs can act as reducing agent to decrease the dissolved oxygen, which quench the SWCNTs PL. This advancement provides promising tools for engineering the brightness of NIR sensors for biomedical applications such as single-molecule imaging of individual SWCNTs using NIR confocal microscopy and deep tissue sensing.

Simple micropreparative gel electrophoresis technique for purification of nanoscale materials

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Many biochemical, biomedical, and material applications hinge on the ability to effectively separate and purify nanoscale materials. Though this need is largely addressed with biological macromolecules using a variety of chromatographic and electrophoretic purification techniques, such techniques are usually laborious, time-consuming, and often require complex and costly instalments that are inaccessible to most laboratories. Synthetic nanoparticles face similar purification challenges, often relying on techniques that are material-specific. In this work, we introduce a versatile micro-preparative (MP) method based on polyacrylamide gel electrophoresis (PAGE) to purify biological samples containing proteins, nucleic acids, and complex bioconjugates, as well as synthetic nanoparticles based on graphene quantum dots (GQDs). Using a conventional vertical slab PAGE, we demonstrate the extraction of purified DNA, proteins, and DNA-protein bioconjugates from their respective mixtures using MP-PAGE. We apply this system to recover DNA from a ladder mixture with yields of up to 90%, compared to the 58% yield obtained using specialized commercial devices. We also demonstrate the purification of folded enhanced yellow fluorescence protein (EYFP) from crude cell extract with 90% purity, comparable to purities achieved using a two-step size exclusion and immobilized metal-ion affinity chromatography purification procedure.Moreover, we demonstrate the successful isolation of an EYFP-DNA bioconjugate that otherwise could not be processed using the two-step chromatography procedure. Finally, the technique was further extended to demonstrate size-dependent separation of a commercial mixture of GQDs into three different fractions with distinct optical properties. MP-PAGE thus offers a rapid and versatile means of purifying biological and synthetic nanomaterials without the need for specialized equipment.

Self-referencing Pulstrode: Further Optimization and New Electrode Designs

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With an ever-increasing world population and life expectancy, public health often ranks as the second sector in terms of budgetary spending worldwide. Finding ways to reduce the costs, whether it is in the preventive care or therapeutic domain, is a crucial component of developing a sustainable health system. As a result, Point-of-Care Testings (POCTs) and wearable sensors have attracted a tremendous interest in the past decades. As opposed to traditional analysis, which are costly and time-consuming, POCTs and wearable sensors present, among others, the following advantages: they are cost-effective and allow rapid or continuous measurements, which lead to better reaction time and thus fewer costly complications [1]. Electrochemical sensors in that regards represent a good example of POCTs.

The reference electrode is an essential component of an electrochemical system, resulting in a high research activity in that domain [2]. The gold standard remains the Ag/AgCl double junction reference electrode. However, owing to its electrolyte-filled inner compartment its design is cumbersome and impractical for wearable sensors applications, which require miniaturization. From that point of view, all-solid state reference electrodes provide a promising alternative.

Gao *et al.* proposed a solid-state reference electrode which relies on an Ag/AgI element and acts as a pulstrode to self-generate a reference potential [3]. The pulstrode protocol consists of four distinct steps: 1) potentiometric measurement of the initial state of the system (OCP), 2) a cathodic current pulse, leading to the reduction of Ag^+ into Ag and the local release of a controlled amount of iodide, 3) measurement of the EMF (reference pulse) 4) application of the original OCP to regenerate the system into its initial state. The protocol has proven its reliability in terms of precision and stability over cycles on a macro-electrode. This work investigates the application of the pulstrode protocol to different electrode designs such as a fine silver wire acting as a micro-electrode or screen-printed electrodes provided by the Swiss Center for Electronics and Microtechnology. Additionally, an attempt to improve the robustness of the described system against sample convection and sample density fluctuations was made by covering the electrode surface with an agarose gel layer.

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Selective copper binding for accurate distance determinations by gas-phase transition metal FRET

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Transition metal FRET (tmFRET) employs a fluorophore donor and a transition metal cation acceptor pair for the determination of small (10-40 Å) [1] inter chromophore distances in biomolecules. This approach was investigated in both solution [1] and in the gas phase [2], utilizing a double histidine motif to non-covalently bind copper to the peptides. Although the histidine pair enables binding close to the peptide backbone, facilitating the interpretation of inter chromophore distances in their biological context while reducing laborious labelling steps, it can also present some drawbacks. The close proximity to the peptide backbone in the gas phase can lead to confirmational rearrangements of the biomolecule due to the presence of a highly charged transition metal cation in the absence of a shielding solvent. Additionally, simulation results have indicated that the copper is likely ultimately bound to a single histidine residue in the gas phase. [2] Moreover, some amino acid residues (i.e., aromatic systems) could present a similarly favorable binding site for copper in this environment, thus rendering its binding nonselective. In this study, alternative binding sites are explored employing a chelating linker (nitrilotriacetic acid) [1], which is covalently bound to the biomolecular backbone. This approach promises superior binding affinities, resulting in higher ionization efficiency and fluorescence intensities, while reducing redundant interactions. Therefore, the binding site is well defined, thus facilitating the biological interpretation of distances obtained from energy transfer efficiencies.

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Solvatochromic Co-extraction-based Optical Nanosensors for Monitoring Carbonate Speciation

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Carbonate is a highly relevant ion for environmental analysis. The chemical balance of marine carbonate systems governs the formation of biogenic calcium carbonate by various calcifying organisms and, therefore, influences living conditions of many marine species such as corals and plankton. This balance is constantly shifted by the ocean uptake of anthropogenic CO_2, which might eventually lead to undersaturation of subpolar surface waters accompanied by dramatic changes in ocean biota [1]. To understand and study these processes it is necessary to develop techniques for the *in situ* mapping of carbonate species concentration in a simple and robust manner. Optical sensors have the potential to meet all these requirements. Optical sensing of carbonate and carbon dioxide has attracted notable attention, however, most of the approaches focus on the implementation of macroscopic sensors [2], while carbonate-selective optode nanosensors have not been reported so far.

This contribution will describe our current progress in the development of carbonate-selective nanoemulsions. The roadblocks in transitioning the classical chromoionophore-based approach to the nanoscale, potentially originating from a high surface affinity of the ion-ionophore complex, were overcome with the introduction of new solvatochromic sensors with a co-extraction-based response mechanism. Carbonate extraction into the sensor phase is facilitated by the addition of a second cationic dye into the solution. This dye itself also acts as an optical reporter and allows for a ratiometric readout (Fig. 1A). The resulting sensors are reversible and demonstrate good selectivity in the presence of major interfering ions (Fig. 1B) and are confirmed to directly measure carbonate speciation. While the approach bears the inherent drawback of some sample manipulation, which is unfavorable for *in situ* applications, it lays the foundation for further development of a robust carbonate-selective nanosensor.

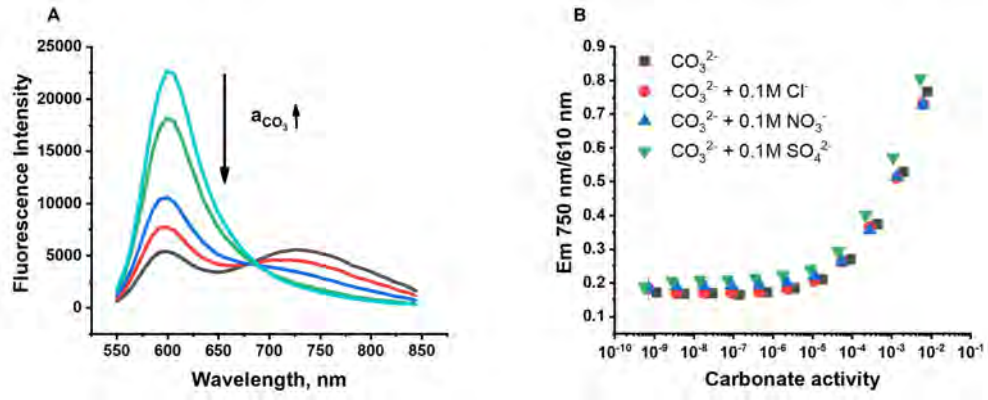

Figure 1. (A) Fluorescence emission spectra of sensing particles in presence of X3 in solution; (B) Sensor calibration curves in mixed solutions, containing fixed concentration of an interfering anion.

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New multiple nuclei and ultra-high resolution Spinsolve benchtop NMRs for 1- and 2D NMR assisted structure verifications

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The sensitivity and resolution of Benchtop NMR spectrometers has been steadily improving over the years as more and more powerful magnets and electronics are made available. With the launching of the Spinsolve 90, Magritek has taken another step towards achieving higher resolution and sensitivity on the bench. These powerful magnets are now available with automatic multinuclear probes which makes it possible to measure multiple nuclei on the same instrument (Spinsolve Multi X).

As the performance and capability of benchtop NMR spectrometers increase, so does the range of applications that the instrument can address. In this presentation, the latest benchtop NMR applications will be shown with extensive examples of NMR data (e.g. 1- and 2D NMR structure verification of Brucine) highlighting the ever-increasing potential of these systems. The Spinsolve 90 has the highest sensitivity that enables advanced experiments, such as HSQC-ME, to be acquired in just 2 minutes. Modern techniques such as NUS and NOAH can make experiments even quicker reducing the time chemists have to wait for NMR results. The versatility of the Spinsolve Multi X probes, which can automatically switch between several X-nuclei like 13C, 31P, 7Li, 29Si, (among others) enabling advanced multinuclear measurements to be made without operator intervention will be shown for on- and off-line analysis. Thanks to the large versatility of the systems, a wide range of applications e.g. on-line monitoring of chemical reactions or qNMR studies are possible [1,2].

Moreover, we will show the superior performance of the ULTRA systems in terms of their resolutions and benefits for e.g. highly effective solvent suppression techniques.

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Robustness of the pure isotropic proton solid state NMR method

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Proton-based structure determination by solid-state NMR is hindered by the poor resolution that originates mainly from residual homonuclear dipolar couplings even at the highest MAS rates achievable today. One way of enhancing the resolution of proton one-dimensional spectra up to pure isotropic spectra was recently proposed by Moutzouri et al. [1]. Spectra with ultra-high resolution were achieved for six different compounds by parametrically fitting sets of MAS spectra acquired at different MAS rates (from 30 to 100 kHz). The principle behind this approach relies on a k-space mapping of any error resulting from the imperfect coherent averaging induced by MAS in such a way that it can be removed in a multi-dimensional grid. Here, we aim to evaluate the robustness of this method.

More specifically, we investigate the effects of: (i) the range of MAS rates acquired, (ii) the static B^0 field, (iii) the digital resolution of the MAS spectra used and (iv) experimental imperfections such as phase and baseline corrections on the resulting pure isotropic proton spectra. We also investigate the fidelity of the method and exclude the possibility of overfitting by: (i) studying inhomogeneously broadened spectra, which *a priori* cannot be narrowed by magic angle spinning and (ii) applying it to fully deuterated histidine samples in which the residual dipolar coupling has been reduced to minimum, making the linewidths essentially MAS-independent.

Finally, we inspect the origin of artifacts that are currently seen in pure isotropic ^{1}H spectra, tracing them back to the fitting of the spectra in regions, imposed by the current computational limitations, and to the presence of MAS lineshapes with different characteristics than those currently predicted by the model.

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Hydrogel matrices for near-infrared monitoring of ascorbic acid release

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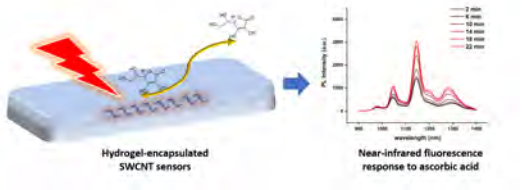
Single-walled carbon nanotubes (SWCNTs) have excellent optical properties for sensing applications. On excitation, semiconducting SWCNTs emit fluorescence that varies with the chirality and the environment of the nanotube. Because the near-infrared fluorescence emissions are minimally absorbed by biological tissue and biofluids, SWCNTs can be used for deep-tissue imaging and sensing. These applications, however, require SWCNT immobilization and encapsulation strategies that are stable and biocompatible.



Herein, we develop a hydrogel-encapsulated SWCNT sensor to study the release of ascorbic acid, a model agent for delivery applications. We examine the response of SWCNTs wrapped in $(GT)_{10}$, $(GT)_{20}$, $(GT)_{40}$, $(CCG)_4$, $(CCG)_8$ and $(AT)_{15}$ DNA sequences to ascorbic acid. The strongest response is observed for the $(GT)_{10}$ sequence, which underwent a 2.5-fold increase in intensity on the addition of 100 µM ascorbic acid. The $(GT)_{10}$-wrapped SWCNT sensors also show the greatest sensitivity to ascorbic acid concentrations over the range of 10 - 100 µM. We further compared the performance of the $(GT)_{10}$-wrapped SWCNT sensor in different hydrogels, including alginate, hyaluronic acid, and agarose matrices loaded with ascorbic acid. The agarose gels show the most promising performance, undergoing the largest intensity change on the release of 100 µM ascorbic acid. Scanning electron microscopy (SEM) images of the agarose hydrogel loaded with and without the SWCNTs confirm no significant perturbation of this matrix on SWCNT encapsulation under the tested conditions. Finally, the encapsulated sensor was applied to monitor cyclic ascorbic acid loading and release. We demonstrate a reversible fluorescence response over 3 addition-wash cycles of 100 µM or 1mM ascorbic acid. These results highlight the promising application of SWCNT hydrogels for the reversible optical monitoring of bioactive agents.

Combining ion mobility spectrometry and fluorescence spectroscopy for structural characterization of biomolecules in the gas phase

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Introduction:

Conformational studies on biomolecules in the gas phase based on Ion mobility mass spectrometry (IM-MS) and Förster resonance energy transfer (FRET) have been of increasing interest in recent years.

Methods:

In this study, we propose a powerful new structural characterization method by combining of a home-built differential ion mobility spectrometry (DMS) device, to IM-MS (i.e., TWIM and cIM), and gas-phase fluorescence spectroscopy based on a modified quadrupole ion trap (QIT) MS. The DMS can be easily moved between instruments, allowing FRET measurements, as well as determination of the differential mobility(ΔK), and collision cross sections (CCS). Molecular dynamics (MD) simulations were then conducted to provide conformational insights.

Results:

The $[M+3H]^{3+}$ ion of a singly-labelled polyanaline peptide (P1-Atto 532) showed two separated peaks in DMS with 0.3 mol % isopropanol as gas modifier in the buffer gas. Downstream IM-MS measurements confirmed 3 and 4 conformations with different ratios for each DMS separated peaks. Further, isomeric doubly-labelled polyanaline peptides (5+, 6+, and 7+ charge states) could also be separated in DMS with 0.4 mol % ratio acetonitrile as gas modifier. FRET measurements showed that the calculated donor-acceptor distances (r_{DA}) of the $[M+6H]^{6+}$ ions of P1-crh6g-QSY7 and P2-crh6g-QSY7 are 77.4 ± 1.0 Å, and 71.5 ± 0.9 Å, respectively. The structural information stored in the differential mobility (ΔK) due to ion-modifier clusters binding (Gibbs free energy, ΔG), shape or CCS, and r_{DA}, could define structural constraints for MD simulations. The combination of these three complementary techniques promises more accurate information and thus a better picture on gas-phase biomolecular conformations together with computational studies.

Self-powered potentiometric-optical transduction with capacitive electronic components

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Self-powered sensors are attractive because they are environmentally friendly and allow for sensor miniaturization. However, developing self-powered potentiometric sensors is still quite challenging because only limited energy can be harvested by this measurement principle. Thus, the signal transfer cannot rely on a continuous energy source.

Resistive components such as LEDs consume energy in to provide an optical signal and are poor candidates for self-powered transduction of potentiometric sensors. Capacitive electronic components such as liquid crystal displays (LCDs) and e-paper are considered energy storage elements, and zero energy will be consumed in principle to maintain a signal. Their absorbance may be precisely controlled by an applied voltage applied, which are attractive properties for a potential signal transducing element.

While chemical materials such as Prussian blue films have been reported earlier to visualize the signal of a potentiometric probe,[1,2] this contribution will focus on the use of a liquid crystal display (LCD) and e-paper. These materials exhibit less energy consumption, a faster response time and wider dynamic range for sensor readout.

LCDs as signal transducer are shown to be attractive because their capacitance is about 50pF per pixel, and a negligibly small charge (~0.5 nF) is needed to drive it. This allows us achieve the direct energy transfer from a pH glass electrodes to an electrochromic display for the first time.[3] Unfortunately, however, the nature of the LCD cannot tolerate a continuous DC voltage, requiring the intermittent regeneration of the pixel by an electronic switch. To improve the approach for practical use, e-paper is explored here. Compared to an LCD, e-paper exhibits no threshold voltage and may be driven by an DC voltage to allow a continuous optical observation of the potentiometric signal.

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Calibration Gas Generator for Secondary Electrospray Ionization High-Resolution Mass Spectrometry

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As one ionization method for gaseous analytes, secondary electrospray ionization (SESI) offers high sensitivity down to parts per trillion, soft ionization conditions, and the capability to ionize compounds of various polarities in both the positive and the negative ionization mode.[1] These advantages led to the use of this ionization method for online breath analysis in the context of clinical research, through which candidate biomarkers were found for several diseases using SESI coupled to high-resolution mass spectrometry (HRMS). For clinical use of these biomarkers, quantification is needed. Whereas other gas-phase ionization methods enable quantification of the detected metabolites through reaction rates, this is not possible for SESI. Rather, a precise delivery of gas standards of potential biomarkers at low concentration is needed for quantification. For this purpose, a vapor generation system was adapted to be interfaced with SESIS-HRMS.[2] This system is based on the controlled evaporation of a liquid analyte and its diffusion in a carrier gas stream. It is capable of generating gas standards at various concentrations down to low parts-per-trillion concentration levels with varying degrees of relative humidity.

To test the capabilities of this calibration gas generator coupled to SESI-HRMS, several questions had to be answered and calibration curves of selected compounds had to be obtained. The first was the determination of the limits of detection and the limits of quantification for target analytes. Additionally, the repeatability and robustness were determined through repeated measurements and measurements on different days. The first class of compounds from which gaseous standards were generated was short-chain fatty acids. SCFA play a critical role in the interplay between gut microbiota and diet in metabolic health[3]; their appearance in breath is also linked to gut microbial activity.[4] The lowest concentration quantified with these acids was in the low part-per-trillion region. The influence of humidity on the detection of these acids was also investigated.

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Laser ablation mass spectrometry for imaging with improved sensitivity and throughput

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Laser ablation inductively coupled plasma mass spectrometry (LAICPMS) has been widely used in element analysis and biological tissue imaging due to its high sensitivity and ease of operation[1]. However substantial use of argon gas introduces additional cost and high argon-based interference peaks[2, 3] which hinders analysis for some elements. Transport of aerosol from ablation chamber to ICP torch lowers analysis speed which further limits the throughput of imaging. To overcome these and meet the growing need for complex biological tissue imaging, a laser ablation funnel focused time of flight system was developed and further modified to gain better performance[4].

The system consists of a positioning system, a femtosecond laser, a custom made convergent-divergent nozzle and RF-only funnel for collision cooling and focusing, ion optics for transmission of ions to the time-of- flight where analysis is achieved. The femtosecond laser allows for directly desorbing and ionizing sample while minimizing heat-affected zone compared with conventional-used nanosecond laser[4], thus preserving the structure of adjacent area.

A frequency doubled femtosecond laser with τ=250 fs, λ = 400 nm is used for direct sampling of analyte which is placed on a 3D translating stage. Ions are formed under elevated pressure condition with reduced kinetic spread by collisional cooling and further focused in ion funnel and transported to ion optics and time of flight for analyzing.

Initial experiments with the new setup were carried out to determine suitable operating conditions with respect to gas flow rates, pressure in the sample chamber. Preliminary investigation and characterization of corona discharge coupled time of flight system were also achieved to show compatibility of this configuration.

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