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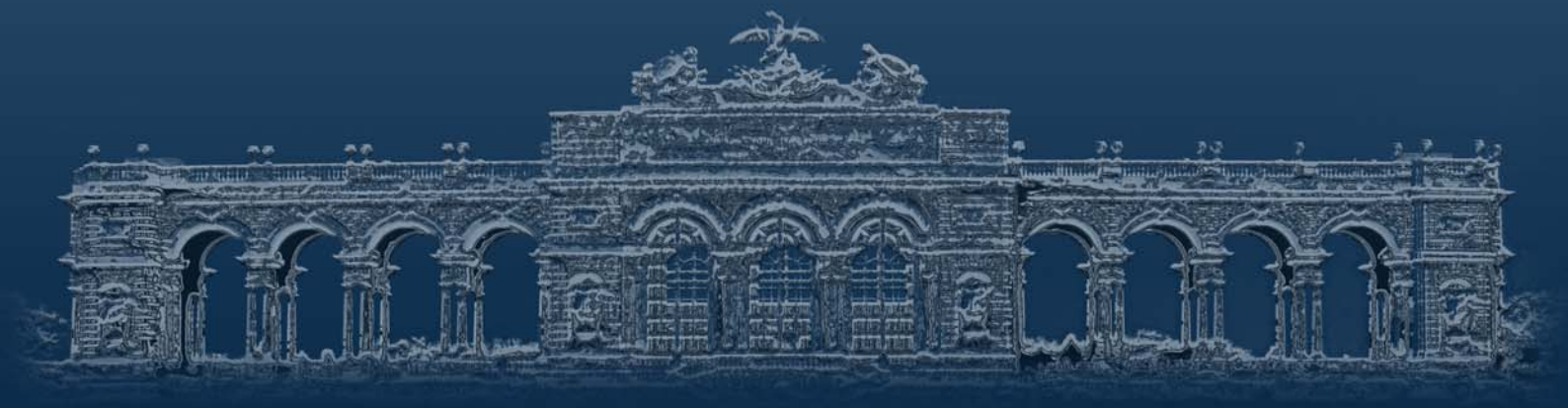
Book of Abstracts



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IL1

A journey through the world of iron

Crichton, Robert R.

In the beginning was **IRON**. The principal element in the molten mass that gradually cooled and coalesced to form the planet that we now inhabit was iron, which constitutes the most important constituent of the Earth's core. And until photosymthetic blue green began to gradually transform the predominantly reducing atmosphere into the oxygenated environment we know today, iron as Fe^{2+} was readily available. This changed dramatically the bioavailability of iron, now mostly present as Fe^{3+}, which hydrolysed, polymerised and precipitated to form the characteristic red deposits of iron oxides which characterise the Pre-Cambrian.

As human evolution and civilisation evolved, the Stone Age was superseded by the Bronze Age and finally by the Iron Age. And as living organisms evolved, so the requirement of iron for cell growth and division meant that systems to acquire this quasi-essential metal became a fixed parameter in the evolution and survival of species, from bacteria, through fungi, plants, animals and finally man. The need for iron characterises the virulence of microbial pathogens, the lack of iron limits the cultivation of plants for animal and human requirements, the capacity of cancer cells to replicate rapidly depends on their ability to acquire iron , while iron deficiency affects one third of the world's population and genetic disorders characterised by toxicity associated with excess iron accumulation affect many of our fellow human beings.

So, it was into this biological world of iron that I began my journey with the small electron transport protein cytochrome c in 1963, and have continued through the oxygen transporting haemoproteins of insect larvae to the proteins of iron storage and transport, ferritin and transferrrin, and ultimately to the homeostatic mechanisms which regulate iron balance in man, and to the disorders which characterise imbalances in this homeostasis. I have been fortunate to make this journey through the iron world as it developed from an almost Stone Age simplicity to reach what has been the Golden Age of Iron Metabolism of the last 20-30 years, and the lecture will illustrate some of the key steps along the way.

IL2

From innate immunity to hormone and extracellular matrix biosynthesis - how posttranslational modifications of the heme cofactor modulate catalysis of human peroxidases

Obinger, Christian

The peroxidase-cyclooxygenase superfamily is comprised of seven families with the chordata peroxidases (family 1) forming the latest evolutionary descendants including human thyroid peroxidase (TPO), lactoperoxidase (LPO), eosinophil peroxidase (EPO) and myeloperoxidase (MPO). Based on an updated phylogenetic tree, the available X-ray structures (LPO, MPO) and biophysical and kinetic investigations, we analyse the evolution of structure and function of these heme oxidoreductases as well as their relation to human peroxidasin (PXD) (family 2). Among other aspects the proximal and distal H-bonding network(s) and the impact of posttranslational modifications of the prosthetic group and the formation of heme to protein linkages on catalysis are presented. It is demonstrated how these modifications modulate the electronic features and redox properties of these iron proteins and influence the kinetics and thermodynamics of the two-electron oxidation of the preferred substrate molecules, i.e. the (pseudo-)halides chloride, bromide, iodide and thiocyanate. It will be discussed how the reaction products hypohalous acids and hypothiocyanite, are utilized as antimicrobial agents (LPO, EPO, MPO) or in specific reactions like biosynthesis of the thyroid hormons triiodothyronine (T_3) and thyroxine (T_4) (TPO) or in formation of specifc sulfilimine links in collagen IV protomers, an event essential to basement integrity (PXD).

IL3

Analytical chemistry tools for metallomics: revisited and emerging

Lobinski, Ryszard

Metallomics refers to the entirety of research activities aimed at the understanding of the molecular mechanisms of metal-dependent life processes. The status of a mineral element in a biological sample can be defined by: (i) concentration (possibly isotopic composition), (ii) chemical speciation and (iii) localization. The control of all the three is essential for the cellular metal homeostasis [1,2].

The lecture discusses the state of the art of the of the mass spectrometry-based analytical methodologies for metallomics. Particular attention is given to global approaches aimed at a simultaneous comprehensive speciation analysis of one or several elements. The topics discussed will include metalloproteomics and metallometabolomics, especially in the context of the recent advances in high resolution (Fourier Transform) electrospray mass spectrometry. Trends in the other fields of analytical metallomics: multielement microanalysis, high-resolution multicollector ICP MS and laser ablation - ICP MS and nanoSIMS imaging will be briefly overviewed.

Mounicou, S., Szpunar, J., Lobinski, R., (2009) Chemical Society Reviews, 38 (4), pp. 1119-1138.
Lobinski, R., Becker, J.S., Haraguchi, H., Sarkar, B., (2010) Pure and Applied Chemistry, 82 (2), pp. 493-504.

IL4

Exploration of brain iron uptake and turnover in rodents using stable isotope techniques

Walczyk, Thomas

Brain iron (Fe) accumulation is a hallmark of Alzheimer's and Parkinson's disease while brain iron influx is supposed to be marginal in adulthood once brain development is complete. To study brain iron homeostasis *in vivo*, we have developed protocols for tracing brain iron influx and brain iron balance by continuous feeding of stable isotope tracers (^{54}Fe, ^{57}Fe and ^{58}Fe) to rats and mice. In a series of long-term feeding experiments we could show that iron transfer from diet into brain is significant even for the healthy adult brain. Brain iron uptake was found to exceed iron efflux which points to iron accumulation to occur in the mammalian brain over life-time even under non-pathological conditions. Intervention studies targeting diet showed that brain iron influx is dependent on dietary iron intake despite the rather small fraction of whole body iron present in brain. Intestinal iron absorption was also found to determine brain iron influx when the feed was supplemented with zinc. Zinc is known to compete with iron for intestinal uptake and to suppress iron absorption. Observations point to a possible role of iron nutrition in the aetiology of neurodegenerative disorders that needs to be further explored.

you1

Evaluation of Fe metabolism for deep sea organisms based on isotope signature

Yamagata, Yuko; Tanaka, Yu-ki; Chen, Chong; Toyofuku, Takashi; Hirata, Takafumi

Fe is one of the most important inorganic nutrients for almost all plants and animals. For marine organisms, Fe isotope ratios ($\delta^{56}Fe/^{54}Fe$) became higher than those found in terrestrial organisms. These results suggest higher absorption efficiency of Fe for marine organisms, possibly due to lower iron abundance in the seawater. In this study, to investigate the Fe metabolism for marine organism at the Fe-enriched environment, the $\delta^{56}Fe$ of two closely related snails (scaly-foot and Gigantopelta aegis) at the deep-sea hydrothermal vent were measured using a multiple collector ICP-mass spectrometer (MC-ICP-MS). The resultant $\delta^{56}Fe$ values of Gigantopelta aegis was about 1‰ lower than the environment, indicative of lower Fe absorption same as terrestrial organisms. In contrast, there were no significant difference in the measured $\delta^{56}Fe$ values between the scaly-foot and Fe in seawater. This can be due to higher absorption efficiency for scaly-foot, possibly to produce scales made of iron sulfide on their foot. The Fe isotope signatures obtained here revealed clear differences in the absorption efficiency of dietary Fe at the unique area, hydrothermal vent.

you2

High-precision isotopic analysis of Cu via multi-collector ICP-mass spectrometry in blood serum of liver transplant recipients

Lauwens, Sara; Costas-Rodríguez, Marta; Van Vlierberghe, Hans; Vanhaecke, Frank

High-precision isotopic analysis of Cu in human biofluids has recently been shown valuable for diagnosis of diseases affecting Cu metabolism, such as end-stage liver disease (ESLD) and hepatocellular cancer [1].

In this work, the Cu isotopic composition was studied in blood serum of patients with ESLD before and after liver transplantation. For this, Cu isotopic analysis in serum samples of 32 liver transplant recipients was carried out *via* multi-collector ICP-mass spectrometry (MC-ICP-MS) after acid digestion of the serum and chromatographic isolation of the target element. The Cu isotopic composition of blood serum in patients with ESLD was fractionated in favour of the lighter isotope. After transplantation, a generalized normalization in the Cu isotopic composition towards the reference range was found when the patients recovered normal liver function.

As the Cu isotopic composition provides valuable information in a clinical context, also the extent of diurnal variations in the serum Cu isotopic composition was investigated. For this, serum samples collected at different time points during a day from 5 healthy volunteers were analysed. No diurnal variations were found.

[1] M. Costas-Rodríguez, J. Delanghe and F. Vanhaecke, TrAC Trends in Analytical Chemistry 76, 182-193 (2016).

IL5

Toxicological characterization of arsenolipids: insights from cells, flies, worms and mice

Schwerdtle, Tanja

Because of the lack of toxicological data, so far no risk characterization exists for arsenolipids, which are frequently present in seafood. Here we present a toxicological characterization of arsenic-containing hydrocarbons (AsHCs), arsenic-containing fatty acids and related metabolites. The studies in human liver, bladder and brain cells indicate a defined, massive cellular toxicity especially of AsHCs. AsHCs exerted toxicity in a concentration range similar to arsenite, but via different toxic modes of action. In the fruit fly, Drosophila melanogaster, AsHCs, in contrast to arsenite, caused late developmental toxicity and reached the brain of the flies. Investigations in the worm Caenorhabditis elegans additionally demonstrate high developmental toxicity of AsHCs. In summary, both in vitro and in vivo approaches provide strong evidence that AsHCs are neurotoxic and show developmental toxicity, indicating that risks to human health related to the presence of arsenolipids in seafood cannot be excluded. To further characterize these risks to human health, we started recently to investigate the toxicity of arsenolipids after oral intake in mice. Preliminary results suggest that mice metabolize AsHCs similarly to humans. The quantification of the arsenic species in the organs of mice will enable us to identify target organs for arsenolipid-induced toxicity in higher mammals.

you3

Arsenolipids: A journey through the human gut

Chavez-Capilla, Teresa; Maher, William; Foster, Simon

Arsenolipids are present in seaweed, fish and crustaceans and comprise a wide range of lipid soluble species including arseno-hydrocarbons, arseno-fatty acids and arseno-phospholipids. The use of dietary supplements as health remedies over recent decades, has led millions of people worldwide to increase their consumption of fish oil. Although fish oil has potential benefits, it is one of the main sources of arsenolipids in the human diet, with concentrations of arsenic from 0.2 to 16 mg kg^{-1} oil. To date, little is known about general arsenolipid metabolism, but recent publications have shown that arseno-hydrocarbons and arseno-fatty acids are toxic to human cells. In this study, the degradation of arseno-hydrocarbons, arseno-fatty acids and arseno-phospholipids through human metabolism was evaluated. The *in vitro* gastrointestinal digestion of arseno-hydrocarbon and arseno-fatty acid standards, as well as of the arsenolipids present in krill oil and hijiki seaweed, revealed that arseno-hydrocarbons and arseno-fatty acids are likely to enter the liver unchanged. Arseno-phospholipids, however, degrade to more toxic inorganic arsenic species. The subsequent exposure of human liver cells to arseno-hydrocarbons and arseno-fatty acids provided insight into the main metabolic products of these arsenolipids after detoxification in the liver.

you4

A novel arsenolipid biosynthesised by Dunaliella tertiolecta under controlled culturing conditions

Glabonjat, Ronald; Raber, Georg; Jensen, Kenneth; Zangger, Klaus; Guttenberger, Nikolaus; Ehgartner, Josef; Duncan, Elliott; Foster, Simon; Maher, William; Francesconi, Kevin

Lipid-soluble arsenicals, also called arsenolipids, have attracted significant interest in the last decade owing to their ubiquitous presence in many marine organisms and their currently unknown biosynthesis and possible biological role. We chose to investigate these processes in the arsenolipid-rich green alga, *Dunaliella tertiolecta,* and uncovered during our analyses an arsenolipid fundamentally different from all previously identified ones. This compound constituted ca 30-80 % of all arsenic lipids in *D. tertiolecta* grown under various laboratory culture conditions; the remaining lipid-arsenic mainly consisted of known arsenic-containing hydrocarbons and -phospholipids. To elucidate the chemical structure of the new lipid, we isolated the compound and utilised a variety of analytical approaches including NMR spectroscopy and the coupling of HPLC to elemental and molecular mass spectrometry. We also performed a range of chemical derivatisations on the natural compound, and chemically synthesised several model compounds. Based on these experiments, we propose a structure for the new arsenolipid, and discuss its significance in biosynthesis and arsenic cycling.

IL6

Nanostructured metallodrugs: new challenges for analytical chemistry

Montes, Maria; Turiel, Daniel; García, Jenifer; Bettmer, Jörg; Blanco, Elisa; Llopis, Juan; Sánchez, Cristina

For management of iron deficient anemia (IDA) oral iron supplementation is normally recommended using ferrous iron Fe(II) salts that are inexpensive and the iron is well absorbed. However, recently, these compounds have been found responsible to induce undesirable changes to bacteria of the colon and to increase pro-inflammatory signaling of the gut epithelium enhancing systemic infection rates. As alternative to ionic Fe species, different approaches have been taken. In particular, Fe from nanocompounds, including Fe-ferritin, have shown to be more efficiently absorbed than the ionic metal species in animal models without any detectable accumulation in the gastrointestinal tract or other tissues.

However, the application of these particles in clinical studies is going to be conditioned by the development of adequate analytical tools that permit the monitoring of the fate of such formulations in complex biological matrices. In this regard, this presentation will illustrate the design and evaluation of new platforms to permit the sensitive monitoring of iron and other chemotherapeutic drugs in cell cultures and animal models using ICP-MS based strategies. The suitability of the proposed strategies will be tested to the evaluation of nanostructured iron and iron/platinum treatments.

you5

Towards quantitative high throughput single cell LA-ICP-MS: microarraying of single cells and calibration standards via piezo based non-contact dispensing

Loehr, Konrad; Wellhausen, Robert; Panne, Ulrich; Jakubowski, Norbert

Analysis of single cells via LA-ICP-MS is a technique with great potential, however manual targeting of single cells is laborious and therefore microarraying of cells looks promising. In this work, we investigate the potential of a commercial non-contact piezo dispenser arraying system (S3, Scienion AG, Berlin), equipped with a novel technology for single-cell isolation called CellenONE™ (Cellenion, Lyon). Usually if one aims to create a microarray of single cells via spotting a suitably diluted cell suspension, one will observe a Poisson-distributed cell number per spot. CellenONE™ overcomes this problem by controlling the number of cells optically in the piezo dispense capillary (PDC) via image recognition to obtain true single cell arrays. We will present the figures of merit of the customized and optimized setup. As a model system we use THP-1 cells stained with two dyes, mDOTA-Ho (CheMatech, Dijon), and Ir-DNA intercalator (Fluidigm, San Francisco) and used LA-ICP-MS (NWR213, ESI, Portland; ElementXR, ThermoScientific, Bremen) to measure Ho and Ir signals from single cells. Both metallo-dyes will be quantified by matrix matched calibration at single cell resolution after Wang et al. (Anal. Chem. 2014, 86, 10252−10256). We believe that this novel approach opens new ways for automated quantitative single cell LA-ICP-MS.

you6

Single cell analysis of arsenic-containing drugs -Implicating the design of more effective arsenic drugs with better intracellular uptake

Zhou, Ying

Arsenic trioxide (As2O3) has long been used in clinic for the treatment of Acute Promyelocytic Leukemia (APL). Exploration of intrinsic mechanism of As2O3 in the treatment of leukemia will facilitate the development of new efficient arsenic drugs. Single-cell analysis more precisely represents cell-to-cell variations and improve the understanding of the fundamental biological principle. Here, a novel strategy using cisplatin as a viability dye together with conjugating lanthanide tags to marker proteins was developed to examine the cytotoxicity of As2O3 in single leukemia cells via single cell ICP-MS (SC-ICP-MS)1. A positive correlation was observed among the intracellular arsenic contents and the ratios of cell apoptosis and cell death in leukemia cells. We further explored the cytotoxicity of a new arsenic-based anticancer agent, ZIO-101, which is in clinical phase I/II clinical trials. Compared with As2O3, ZIO-101 causes more G2/M cell cycle arrest and apoptosis. ZIO-101-treated cells contains more than two-fold arsenic than As2O3-treated cells. A positive link between arsenic uptake and cytotoxicity of arsenic-containing drugs suggests that the therapeutic efficiency of arsenic-containing drugs is improved by enhancing the intracellular uptake of arsenic in cancer cells.

Reference:

1. Zhou, Y., Li, H. Y., Sun, H. Z., Chem. Commun., 2017, 53, 2970-2973.

L1

Dual-Band Luminescent Nanoparticles toward Integrated Therapy and Imaging Platform

Sun, Lingdong; Yan, Chunhua

Dual-band luminescent nanoparticles, with emission in visible and near infrared simultaneously, are investigated as an integrated platform for therapy and imaging triggered with NIR light. A core/shell structure, with $NaGdF_4$:Yb,Er and $NaGdF_4$:Nd,Yb as core and shell parts, was responsible for UC visible emissions and downshifting NIR emissions, respectively. Conjugated with rose Bengal molecules, the visible emissions in green were used to trigger singlet oxygen generation *in vitro* and *in vivo* photodynamic treatments. Benefit from the NIR emission, an imaging route to track the location of the LNPs during therapy was developed. In addition, these nanoparticles showed much lower heating effect under continuous laser irradiation for a relatively long time. These conjugates demonstrated a novel detect-to-treat modality.

L2

Complementary bioimaging to investigate changes in the phospholipid distribution in lung tissue after instillation of nanoparticles

Dietrich, Dörthe; Vennemann, Antje; Wiemann, Martin; Sperling, Michael; Karst, Uwe

The uptake of cerium oxide nanoparticles (CeO2-NP) e.g. from diesel exhaust via inhalation may pose a health hazard for humans. As CeO2-NP elicit lung inflammation and fibrosis, knowledge about both, their distribution in the lung and their biological effects is needed. Here we combined the imaging techniques micro x-ray fluorescence analysis (µXRF), laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) and matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) to study the distribution of CeO2-NP and changes of phospholipid distribution in the lung.

CeO2-NP were intratracheally instilled into rat lungs and cryosections were prepared 3 and 90 days post application. Cerium distribution pattern as well as the distribution of naturally occurring elements such as phosphorus were depicted by means of µXRF and LA-ICP-MS. Cerium distribution changed over time from a largely even to a more concentrated distribution pattern. Alterations of the phosphorus distribution were spatially linked to the cerium distribution, especially at high doses and after longer incubation times.

MALDI-MS experiments suggest that zones with increased phosphorus concentration were co-localized with elevated levels of phospholipids. Phospholipids were identified via exact mass and fragmentation experiments.

Results show that combining MALDI-MS and elemental imaging techniques can provide new insights into local changes caused by nanoparticles in tissues.

L3

Multi-element rapid detection using time-of-flight mass spectrometry for bioimaging and single cell analysis

Borovinskaya, Olga; Bussweiler, Yannick

The capabilities of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to visualize and quantitatively characterize distribution of essential and toxic elements in biological specimens is of interest for many biological, medical and nanotoxicological studies. The recent advances in low aerosol dispersion LA chambers significantly increased the speed and lateral resolution enabling imaging down to sub-cellular level. Besides the laser sampling, detection of metallomes in individual cells from suspensions is very attractive due to preservation of information on cell diversity and heterogeneity. Both measurement approaches, however, are associated with detection of very narrow signal peaks (1-100 ms) and limit conventional analysis using sequential mass spectrometers to only a few isotopes. The advantages of alternative simultaneous time-of-flight-based MS with full element coverage have been recently demonstrated for high performance 3D LA-bioimaging [S. J.M Van Malderen *at al*., Anal. Chem., 2017, 89, 4161-4168]. We propose a new bioimaging approach realized by high level synchronization of LA and TOFMS with every pixel representing spatially resolved individual laser pulse. We demonstrate its performance with the latest commercial rapid LA technologies on selected applications from medical and nanotoxicological studies and show the first results from ICP-TOFMS for the analysis of single cells in suspensions.

you7

Investigation of mechanisms of the ZnO nanoparticles uptake in edible plants by single particle ICP MS and HPLC - ICP MS / ESI FT MSn

Wojcieszek, Justyna; Jiménez-Lamana, Javier; Ruzik, Lena; Asztemborska, Monika; Jarosz, Maciej; Bierla, Katarzyna; Szpunar, Joanna

The aim of this work was the development of analytical methodologies using mass spectrometry (MS) to study, on the molecular level, the fate zinc oxide nanoparticles (ZnO NPs) taken up by edible plants (*Lactuca sativa* L. and *Raphanus sativus* L.). Single particle ICP-MS (SP-ICP-MS), a valuable tool for characterization of nanoparticles in suspensions at environmentally relevant concentrations, was used to probe the speciation of zinc in its dissolved and/or nanoparticulated forms.

Results showed that most ZnO nanoparticles underwent dissolution, releasing zinc ions. This information is essential from the point of view of food safety taking into account that the presence of dissolved ions can play an important role in metal cytotoxicity. The presence of zinc ions resulted in the formation of new species through their binding with organic molecules naturally present in plants or induced by metal stress. High-performance liquid chromatography coupled to high resolution ESI FT MS^n was used for identification of zinc species, originated from oxidation of ZnO NPs present in plants. In addition, the speciation of zinc complexes in plants cultivated in the presence of ZnO NPs and soluble zinc salt ($ZnCl_2$) was compared.

Acknowledgement: Project financially supported by the National Science Centre, Poland (grant no 2015/18/M/ST4/00257).

IL7

Toxicology of tellurium explored by speciation and identification of tellurometabolites

Ogra, Yasumitsu

Tellurium (Te) is an element belonging to the same group on the periodic table as oxygen, sulfur, selenium, and polonium, i.e., group 16, and is widely used in industry because it has unique physicochemical properties. In addition, the accident at the Fukushima Daiichi Nuclear Power Plant in Japan has resulted in the discharge of artificial radionuclides, including ^{129m}Te, ^{132}Te, ^{131}Iodine(I), ^{132}I, ^{134}Cesium (Cs), ^{136}Cs, and ^{137}Cs, into the environment. Although Te is a non-essential element in animals and plants, it is expected to be metabolized to organometallic compounds having a carbon-Te bond in living organisms exposed to inorganic Te compounds. Thus, the speciation and identification of tellurometabolites are expected to contribute to the depiction of the metabolic chart of Te. Speciation by elemental mass spectrometry and identification by molecular mass spectrometry coupled with separation techniques have significantly contributed to the discovery of tellurometabolites in animals and plants. In this presentation, the recent advances in the biology and toxicology of Te revealed by the speciation and the identification of unknown tellurometabolites are overviewed.

you8

Mapping protein targets of bismuth- and silver- based antimicrobials enables in-depth deciphering their molecular mechanisms

Wang, Hai-Bo; Hongzhe, Sun

The rapid emergency of antibiotic-resistant bacteria but dwindling antibacterial pipeline pose an enormous threat to human health. With broad antimicrobial spectrum and less chance to develop resistance, metal-based antimicrobials, e.g. silver and bismuth, have been widely used historically. Albeit the antibacterial activities of silver and bismuth have been studied for decades, the modes of action of them remain obscure owing to the lack of appropriate techniques to explore its molecular targets. We have previously developed an approach namely GE-ICP-MS (gel electrophoresis hyphenated with ICP-MS) and separated bismuth-binding proteins in H. pylori successfully. Herein, we found the clinical antibiotic-resistant bacterium, *Burkholderia cepacia complex (BCC)*, being susceptible to bismuth drugs and uncovered over 20 bismuth-binding proteins in *BCC* via GE-ICP-MS. We further extended the one dimensional method into two dimensional and mapped out over 30 silver-binding proteins in *S. aureus* for the first time. The details of the in-depth deciphering of their molecular mechanisms will be discussed. Our studies revealed that bismuth and silver based metallodrugs are promising drugs to tackle the bacterial infection via a multi-targets based strategy and the identified proteins are potential targets for the rational design of new antimicrobials.

you9

Distribution of arsenic and its species in human milk

Stiboller, Michael; Raber, Georg; Lenters, Virissa; Gjengedal, Elin Lovise Folven; Eggesbø, Merete; Francesconi, Kevin A.

The toxicity of arsenic depends on its chemical form, with inorganic arsenic being highly toxic to humans whereas organic arsenic compounds are generally considered much less so, and thus risk assessment of arsenic exposure must consider the type of arsenic compound. Furthermore, because risk assessments need to include the most vulnerable persons within a population, such as newborns and infants, estimation of the arsenic species in human milk is needed. As part of the Norwegian HUMIS-NoMIC birth-cohort study on the effects of environmental toxicants on the neuropsychological development in children, we aimed to determine trace levels of arsenic and arsenic species in human milk samples by using ICPMS and HPLC/mass spectrometry. Herein, we describe a novel sample preparation procedure developed to measure the distribution of arsenic in both the aqueous and lipid phases of human milk, and report preliminary data on the types of arsenic species present in 297 milk samples from the HUMIS-NoMIC study.

L4

Impact of chronic Ag exposure on intracellular Zn homeostasis in a fish intestinal cell line

Stewart, Theodora; Mottaz, Hélène; Maret, Wolfgang; Hogstrand, Christer; Schirmer, Kristin

Health of intestinal barrier function is central to disease prevention and Zn plays an important role in its maintenance. As fish are early indicators for aquatic ecosystem health, understanding compromised barrier function has significant implications for environmental toxicology and aquaculture. Acute Ag exposure of the fish intestinal cell line RTgutGC was recently shown to disrupt Zn homeostasis. However, long-term chronic effects are poorly understood. RTgutGC was grown on transwell inserts and apically exposed to 1 µM Ag_T over the course of two weeks. Uptake of Ag occurred within 24h, coinciding with an up regulation of the Zn exporter ZnT1 at 3h and 24h and metallothionein (MTb) at 24h and 72h. Following up regulation of ZnT1, decrease in total intracellular Zn was measured at 72h. Expression of ZnT1, MTb, and intracellular Zn recovered to control levels after 1 week. No cytotoxic effects or impact to barrier tightness were observed. Long-term chronic Ag exposure caused temporally defined Zn dyshomeostasis, which was addressed quickly through up regulation of MTb, ZnT1, and likely sequestration of intracellular Ag, opening new questions regarding the significance of intracellular metal species dynamics, which are currently being addressed through a combination of Zn specific probes, LA-ICP-MS, and STXM.

L5

Metalloproteomic and metabolomic analyses reveal the competing mechanism of gallium with iron in Pseudomonas aeruginosa

Wang, Yuchuan; Han, Bingjie; Xie, Yanxuan; Zhang, Zhen; Li, Hongyan; Sun, Hongzhe

Gallium-based compounds have shown great potential in fighting against bacterial infections, either being used alone or as antibiotics adjuvants. The antibacterial property of Ga^{3+} is attributed to its chemical similarity to Fe^{3+}, resulting in down-regulated iron uptake; however, the exact cellular targets of Ga^{3+} are still largely unknown. By using a home-made fluorescent probe Ga^{3+}-*TRACER* that specifically targeting on cellular Ga-binding proteins, we observed that *Pseudomonas aeruginosa* PAO1 grown under iron-depleted medium could be stained with intense blue fluorescence by Ga^{3+}-*TRACER*, while the bacterium grown under iron-rich medium could no longer be lighted up, providing strong evidence of Ga^{3+} targeting on Fe-binding proteins in the bacterium. Ga-binding proteins in *P. aeruginosa* were subsequently separated and identified by combined use of Ga^{3+}-IMAC and Ga^{3+}-*TRACER*-2DE. Moreover, we observed common metabolomic features between Ga-treated and iron deficient *P. aeruginosa* by GC-MS-based metabolomic profiling. Our study provides a detailed understanding of the competing mechanisms of gallium with iron in infectious bacteria through an integrative omics point of view.

L6

Selenium reduced the level of mercury and promoted it to bind with Selenoprotein P in serum from methylmercury-poisoned rats

Li, Yu-Feng

Selenium (Se) has been found to antagonize the toxicity of methylmercury (MeHg) when co-administered, but the application of Se to treat methylmercury (MeHg)-poisoning is less studied. In this study, MeHg-poisoned rats were treated with selenite every other day for 30 days. Blood samples were collected and the concentrations of Se, Hg and MeHg in serum were measured. The Hg, Se binding selenoproteins in serum samples was separated and the levels of both Hg and Se binding to selenoproteins (GPx, SelP and SeAlb) were quantified. It was found that Se reduced the level of Hg in serum in MeHg-poisoned rats. Both Hg and Se (73% of Hg and 93.6% of Se) were mainly bound to SelP in serum. Se promoted more Hg to bind with SelP in MeHg-poisoned rats, suggesting that SelP in serum plays an important role in fighting against the toxicity of MeHg and Se treatment could help to promote the health status of MeHg-poisoned rats by reducing the levels of Hg in the blood.

you10

Characterizing neurotoxic effects of arsenolipids applying various in vitro models

Witt, Barbara; Meyer, Sören; Ebert, Franziska; Francesconi, Kevin A.; Schwerdtle, Tanja

Arsenolipids are lipid-soluble organoarsenicals, predominantly present in seafood. Feeding studies on *Drosophila melanogaster* with arsenic-containing hydrocarbons (AsHCs), a subgroup of arsenolipids, indicated late developmental toxicity and an accumulation of AsHCs in the fruit fly's brain.

In the present study, we investigated the neurotoxic potential of selected AsHCs using various *in vitro* models. First, the toxic profile was characterized in differentiated neurons assessing effects on cell viability, mitochondrial activity and cellular bioavailability. AsHCs exerted cytotoxic effects in a low concentration range comparable to the toxic reference arsenite. In contrast to arsenite, however, AsHCs reduced the mitochondrial membrane potential and massively disturbed the neuronal network. A large cellular arsenic accumulation was observed following incubation with AsHCs. To investigate neurodevelopmental toxicity, studies were carried out in pre-differentiated neurons. Cytotoxicity was 2-fold higher in pre-differentiated neurons compared to differentiated neurons, although the accumulation behaviors were similar concerning cellular bioavailability. Additionally, studies on neurite outgrowth showed that AsHCs have strong effects on the development of the neuronal network.

Currently, we are conducting studies using a co-culture of human neurons and astrocytes. First studies indicated a reduced toxicity by AsHCs in this co-culture, despite showing substantial effects in the respective monocultures implying potential protective effects.

IL8

Developing the Next Generation of Reference Materials for Proteomic and Metalloprotein Measurements

Davis, Clay

Proteomics is one of the predominant “omics” fields that focus on both the targeted identification and quantification of candidate biomarkers comprised of peptide and/or protein constituents, as well as the non-targeted spectral profiles for the identification of molecular marker signatures from various instrumental platforms. These proteomic and metabolomics data and spectral profiles are used to discriminate pheno- or enviro-typical differences (disease processes, histopathological changes, drug effects, etc.) associated with biological specimens, and used to classify healthy and/or non-treated samples from diseased and/or treated biological samples.

As has been frequently observed, analytical data from instrumental platforms can be more variable than the actual proteomic changes attributable to biological treatment and/or condition. Most laboratories create in-house QC materials in an attempt to control and normalize intra-laboratory variability, but typically the supply is limited and not intended to serve inter-laboratory assessments. NIST is focuses on developing a large supply stable QC materials – including a suite of materials used to establish definitive levels of analytes in differential based studies – that could be used as harmonization materials within the fields of both metabolomics and proteomics.

L7

IDMS-based quantification of metal-containing proteins with clinical relevance

Gleitzmann, Julia; Wätzig, Hermann; Swart, Claudia

Metalloproteins play an important role in vital functions. As many of them are important markers for diseases they are particularly relevant in medical diagnosis. Therefore, it is necessary to develop primary reference methods for the quantification of these metalloproteins that are traceable to the SI. One method of choice is species-specific isotope dilution mass spectrometry (SS-IDMS), in which the proteins are quantified via their metal content with inductively coupled plasma mass spectrometry (ICP-MS).

To perform SS-IDMS the protein of interest has to be separated from all other interfering compounds in biological matrix, well characterized reference materials have to be available and adequate spike material is needed consisting of the native protein containing the metal in an isotopically enriched form.

The investigations presented focus on the Cu-containing proteins superoxide dismutase and ceruloplasmin and Fe-containing ferritin. Commercially available native proteins were characterized in-house for the use as references in IDMS. Spike materials were produced from the native proteins and used in the quantification of the proteins in biological matrices such as erythrocytes, serum and cerebrospinal fluid.

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L8

Laser Ablation Imaging Using Triple Quadrupole ICP-MS as a Tool for Biological Studies

Müller, Larissa; Kutscher, Daniel; Rottmann, Lothar; McSheehy Ducos, Shona

Visualization of the distribution of trace elements is a key factor for the understanding of metabolic or other functional processes in biological systems. It is well understood that trace elements are involved in many biological functions, for example as active centers in proteins, or acting as catalysts. Besides naturally occurring trace elements, also artificial introduction of element containing probes or the cellular up-take of nanoparticles are under investigation.

In recent years, laser ablation imaging has been established as a promising new tool to visualize trace elements, both naturally present in a sample or used as a surrogate for cell characteristics. However, low concentrations and small sampling areas (required to increase lateral resolution) challenge ICP-MS as a detection system with respect to detection sensitivity (equivalent to image contrast) and stability over long analysis time.

In this presentation, the benefits of using triple quadrupole ICP-MS for laser ablation imaging will be shown using different sample types and focusing on biologically relevant analytes such as phosphorous or selenium.

IL9

Renal handling of heavy metals and its implications in renal toxicity

Himeno, Seiichiro; Fujishiro, Hitomi

Proximal tubule of the kidney is the target of toxic heavy metal compounds including environmental pollutant cadmium and antitumor agent cisplatin. Although Cd and cisplatin cause deleterious effects on S1 and S3 segment of proximal tubule of the kidney, respectively, the mechanism of segment-specific transport and toxicity of metals remain unclear. We developed an experimental system using cultured cell lines derived from S1, S2, and S3 segments of proximal tubule and a trans-well culture system. This allowed us to determine the apical and basal transport of metals in each segment-derived cells. We found that the Cd taken up in the S1 cells could be released into the apical side (lumen of proximal tubule) to some extents and then re-absorbed in S3 cells from apical side. This kind of dynamic handling of metals between each segment of proximal tubule cells may occur in other metal compounds. Utilization of S1, S2, and S3-derived cells also enabled the in vitro evaluation of endocytosis efficiency of the proteins filtered through the glomerulus as well as the expression of novel renal damage markers such as Kim-1 and clusterin upon exposure to metal compounds.

L9

Identification of molecular targets of different chemical forms of nickel in human skin cells by mass spectrometry

Jiménez Lamana, Javier; Szpunar, Joanna; Lobinski, Ryszard

Despite nickel is classified as carcinogenic under Regulation (EC) No1272/2008 and nickel allergy is common worldwide, the molecular mechanisms of nickel toxicity are still unknown. It has been suggested that primary effects of nickel could result from the interaction with proteins, but the Mode of Action and the specific subcellular targets of different chemical forms of nickel still have to be elucidated.

In the first step of this work, the cytotoxicity of different Ni compounds was studied as a function of their concentrations and incubation times and the nickel content in the different subcellular compartments of human skin cells (keratonocytes) was determined by ICP-MS with the highest values found in cytosol. Then, the analytical strategies were developed in order to characterize the molecular targets of different chemical forms of nickel in cells cytosol.

The results obtained by SEC-ICP-MS and 1D-GE followed by LA-ICP-MS for the characterization of Ni binding in the cell cytosols will be shown and compared. Finally the characterization and identification of Ni-binding molecules by HPLC coupled to high resolution ESI-FT-MS will be presented.

Acknowledgements

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L10

On the track of trafficking gold nanoparticles: Speciation changes in human cytosol

Matczuk, Magdalena; Legat, Joanna; Timerbaev, Andrei; Jarosz, Maciej

Gold nanoparticles (AuNPs) are widely researched as multifunctional theranostic agents, having the capability to exert anticancer efficacy by interacting and altering tumor cells. Unfortunately, the knowledge of the events involved in the cellular processing of AuNPs is still limited. Recently, we have confirmed that in human serum AuNPs are forming the protein corona [1,2], and based on this event, it can be assumed that AuNPs enter the cell being covered with plasma proteins and as such are possibly underwent further compositional alterations. The aim of this contribution is on unraveling these speciation changes using methodology based on coupling capillary electrophoresis to ICP-MS. In the reported experiments, AuNPs, transformed into the protein-conjugated forms in real human serum, are then subject to the action of human cytosol fortified (or not) with reductive and complexing agents (characteristic to cancer cytosol environment). This approach allowed us to discover that only under cancer cytosol conditions the degradation of the serum-induced corona occurs, followed by conversion of the released Au species into the high molecular-weight forms.

[1] Metallomics, 2015, 7, 1364−1370

[2] Electrophoresis, 2016, 37, 2257–2259

Financial support of the National Science Centre, Poland (grant no. 2015/17/B/ST4/03707) is acknowledged.

IL10

A Metallomic Approach to Study the Interaction of Inorganic Oxide Nanoparticles with Biological Systems in Nanotoxicity Studies

Bartczak, Dorota; Goenaga-Infante, Heidi

A number of reports have highlighted the need to develop reliable strategies for measurement of the physical and chemical changes that nanoparticles (NP) undergo when they encounter biological matrices and link these changes to their potential toxicity. The implementation of approaches involving NP capping with ligands to reduce their toxicity is of increasing interest but again, testing the effect of nanoparticle surface modifications on the bioactivity of coated NP is challenging and often required NP characterisation in the presence of a complex biological matrix.

This lecture will discuss the challenges encountered when using a multi-method platform (including AF4 hyphenations, elemental and molecular MS, TEM, FT-IR and PTA for nanoparticle or nanoparticle-ligand characterisation and label free impedance spectroscopy for cell toxicity measurements) for the characterisation of the interactions, modifications and uptake of inorganic oxide nanoparticles in a HepG2 cell toxicity model. Examples of these challenges will be illustrated through (*i*) measurement of the impact of surface modification of ZnO nanoparticles coated with different ligands (including proteins) on their bioactivity in a HepG2 model and (ii) real- time characterisation of silica nanoparticles and their interactions with cell media and cell components in lysates of exposed HepG2 cells.

L11

Single-cell analysis by ICP-MS/MS as fast tool for cellular bioavailability studies of metal species

Meyer, Sören; Lopez-Serrano, Ana; Jakubowski, Norbert; Schwerdtle, Tanja

Cellular uptake is one basic information for a toxicological *in vitro* characterization of compounds e.g. metal species. For ICP-MS determination which has become the gold standard in elemental quantification, cells have commonly be digested by microwave technology to get a measurable solution. This approach is time consuming and usually lots of cells are needed. For this purpose, we tested a novel approach based on single-cell analysis by time-resolved ICP-MS/MS to monitor the total content of metals at a single-cell level. The most important benefit of this method is the less complex sample preparation by just re-suspending the cells for the ICP-MS/MS measurement.

As cellular model system adenocarcinoma lung cells (A549) have been chosen and exposed to arsenite and Cd^{2+} as representatives of toxic metal species. Several exposure times were investigated to study uptake kinetics at single-cell levels. Finally, results of cellular uptake determined *via* single-cell ICP-MS/MS analysis were compared with the commonly used digestion protocols and were in good correlation. Therefore, our single-cell ICP-MS/MS approach is a promising tool for a fast and material-saving *in vitro* characterization of metal species to investigate their cellular bioavailability.

L12

Single Quadrupole and Triple Quadrupole ICP-MS for Single Particle Analysis of TiO_2 Particles

Bettmer, Jörg; Candás Zapico, Silvia; Montes Bayón, Maria

Titanium dioxide is considered as a safe and inert material that have entered various application areas. Besides its use in paintings, photocatalysts, cosmetics, a TiO_2 material is permitted as food additive (E171). Due to its brightness and high refractive index E171 can be found in sweets, chewing gums, etc.. Recently, discussions about adverse effects of TiO_2 have raised, as its amount entering environmental and biological systems is steadily increasing.

In order to better estimate the effects of TiO_2 analytical strategies are urgently needed. Besides total Ti determinations, the characterisation of TiO_2 particles has become a challenge for analytical chemists. ICP-MS based approaches consist of techniques like field flow fractionation coupled to ICP-MS and single particle ICP-MS. The aim of this presentation is to evaluate the potential of ICP-MS equipped with a triple quadrupole (ICP-TQ-MS) for the analysis of single particles of TiO_2. Comparative studies with single quadrupole technology will show the influence of spectral interferences on the analytical figures of merit. The analysis of different consumer products will demonstrate the applicability to the characterisation of TiO_2 particles.

The authors gratefully acknowledge Thermo Fisher Scientific, Bremen, for the loan of the iCAP TQ ICP-MS.

L13

Quantification of Silver Nanoparticles at Single Cell Level by Mass Cytometry

Lopez-Serrano, Ana; Baumgart, Sabine; Jakubowski, Norbert; Haase, Andrea; Luch, Andreas; Grützkau, Andrea

A novel technique for real time analysis of multi-parameter assays of single cells is mass cytometry which is applied here for the first time to quantify AgNPs at single cell level. Mass cytometry based on inductively coupled plasma time of flight mass spectrometry (CyTOF) uses multiple element tags (lanthanides) that are attached to antibodies for specific identification of cell phenotypes and is applied here in a proof of principle experiment to measure the uptake of AgNPs by differentiated THP-1 cells exposed to two different AgNPs concentrations (0.1 and 1 mg L^{-1}) for incubation times of 4 and 24 h. Methodical parameters were optimized and applied for subsequent quantitative analysis. CyTOF measurements allowed the quantitative analysis of AgNPs by simultaneous detection of immunophenotypic markers of THP-1 cells, such as CD45, HLA-DR, CD66b, etc. For calibration, an AgNPs suspension was measured and the Ag signal intensity directly converted into the number of AgNPs per cell. Results reveal that the uptake of AgNPs by THP-1 depends on both AgNPs exposure concentration and incubation time. Up to 125±6 AgNPs per cell were detectable at the highest concentration and longer exposure time. Results were validated by analysis of digested cells using ICP-MS.

L14

Improving Drug Therapies using Single Cell ICP-MS

Michel, Jörg; Amable, Lauren

It is estimated that in 2017, over 1.6 million new cancer cases will be diagnosed. Cisplatin and other platinum-based analogs, remain a mainstay in the clinic treating a wide range of tumors including ovarian, lung, testicular, bladder, as well as others. Frequently the patient initially responds to chemotherapy but when the tumor returns, patients are resistant to further platinum-based chemotherapy. With new research, we are taking a cellular step closer to understanding how cisplatin chemotherapy, and other metal-based cancer drugs, can be developed to improve their success rates. The use of single cell ICP-MS (SC-ICP-MS) is featured, enabling researchers to see how effective a cancer drug is being targeted to each and every cancer cell. Key learnings:

· Single cell ICP-MS analysis methodology

· Quantifying single cell uptake with Cisplatin

· Novel targets for increasing Cisplatin uptake

· Overcoming current challenges with drug uptake analysis

L15

ICP-MS based single cell analysis and its application to the study of element masses and distribution patterns in single cells

Feng, Weiyue; Wang, Hailong; Wang, Meng; Wang, Bing; Zheng, Lingna; Chai, Zhifang

Cellular heterogeneity resulting from stochastic expression of genes, proteins, and metabolites is an inherent condition of cell populations. The heterogeneity of individual cells can dramatically influence cellular decision making and cell fate. So far our knowledge about how the variation of endogenous metals in individual eukaryotic cells is limited.

Herein, ICP-MS equipped with a high efficiency cell introduction system (HECIS) has been developed as a method of single-cell ICP-MS (SC-ICP-MS) in our laboratory. In SC-ICP-MS analysis, ICP-MS operates in a time resolved mode so that the frequency of signals is directly related to the number concentration of cells and the intensity of signals is related to intracellular amounts of elements. We have successful achieved the method for single-cell analysis of Mn, Fe, Co, Cu, Zn, P, and S in human cancer cell lines (HeLa and A549) and normal human bronchial epithelial cell line (16HBE). SC-ICP-MS analysis showed marked differences of the masses of elements either in individual cells nor among the various cell lines. Because of the high sensitivity of the method, subpopulations of the elements could be found in the cell populations, demonstrating that SC-ICP-MS is able to unravel the extent of variation of endogenous elements in individual cells.

you11

Synthesis and Functionalisation of Gold Nanoparticles with Biogenic Amines

Mattern, Annabelle

There is a large number of potential applications for nanoparticles in medicine e. g. their combination with biomolecules. Previous work by Gasiorek et. *al* [1] showed the activation of histamine receptors by gold nanoparticles which were functionalised with a histamine derivative. Based on these results in a first step a ligand was synthesised based on a bifunctional linker that was connected with a biomolecule. Finally the new ligand system was attached to nanoparticles. Gold nanoparticles were used during this work because they showed great biocompatibility and furthermore, they were easily synthesised in order to fine-tune selective sizes and spherical shapes. Mercaptoacids with different chain lengths proved to be suitable linker molecules. The biogenic amines used were dopamine and noradrenaline. Moreover, the functionalised nanoparticles were synthesised in different sizes and diverse solvents. They were successfully tested at the Institute of Veterinary Physiology and Biochemistry, Justus Liebig University Giessen (research group of Prof. Martin Diener) for their ability to enhance ligand-receptor interaction by multivalency. The talk will present the results of this investigation.

1. Gasiorek, F. et *al*., *Organic & biomolecular chemistry* **13,** 9984–9992 (2015).

IL11

Understanding the interaction of living systems with engineered metal nanoparticles by synchrotron radiation-based techniques

Chen, Chunying

Many nanomaterials are promising in biological detection, diagnosis, and therapy for diseases and have shown great potential for biomedical applications. Therefore, the toxicity of nanomaterials becomes an increasing concern. Nanotoxicology is an emerging field to characterize and categorize the interactions of nanoscale materials with biologic systems, and consider the potential health and environmental effects caused by engineered nanomaterials.

It is important to obtain a better understanding of the uptake, trafficking, pharmacokinetics, clearance, and role of nanomaterials in biological systems, so that their possible undesirable effects can be avoided. Chemical speciation, dynamics and kinetics of nanomaterials in biological systems are extremely necessary since we have very limited knowledge. Synchrotron radiation, which is highly polarized, tunable, and concentrated over a small area, has been proved to play an indispensable role for nanotoxicology studies. As an example, in our study, the combination of μ-SRXRF and microbeam X-ray absorbance near edge structure (μ-XANES) can simultaneously provide information about the subcellular distribution and chemical species of metal-containing nanomaterials of interest. In this talk, we will summarize the recent progresses for the cellular trafficking and transformation of nanomaterials in biological systems.

L16

Hyphenated capillary electrophoresis ICP/MS: a promising technique to boost the metallomics toolbox.

Hagege, Agnes; Huynh, Suong; Vidaud, Claude

Bone is one of the major organs in which uranium accumulates and the interactions between uranium and the bone organic matrix are likely to be of considerable importance. The strong affinity for uranium of two proteins involved in bone turnover has even been demonstrated, suggesting the role of these proteins in the accumulation of uranium.

Obtaining reliable thermodynamic data describing protein-uranium interactions is currently the subject of numerous publications. The spectroscopic methods have proved their interest in this field. However, these methods do not always have the required sensitivities and the sample volumes required limit studies to proteins available in large quantities. In this context, a coupling between capillary electrophoresis and ICP/MS was used. Its interest lies in the possibility of studying protein/metal interactions from small volumes (50µL). The challenge is to maintain the integrity of the species up to the detection point. Both contact and separation conditions are essential if the actual distribution of uranium is to be reflected.

We demonstrate the determination of affinity constants of uranium from only 4nmol of protein. We also show the possibility to prove the presence of several interaction sites (<2h) and the occurence of ternary species (~10min) in the presence of partner proteins.

L17

Sulphur-containing peptides - Detection, Identification and Quantification

Raab, Andrea

The two sulphur containing amino acids cysteine and methionine are part of the amino acid sequence of most proteins. In addition one or both are present in a number of important biological peptides, like glutathione.

So far the proteomic community does rarely make use of the presence of sulphur or other heteroatoms for quantification of proteins or peptides resulting from tryptic digests. Quantification of proteins is so far mostly a relative quantification compared to a “standard” sample and not absolute. The parallel use of elemental and molecular detection techniques (ICP-MS plus ES-MS) in combination with separation techniques should enable absolute quantification of sulphur containing peptides and proteins including mass balance. To test this approach a mass balance of sulphur-containing peptides of garlic was done including species identification and an attempt was made to quantify BSA and casein via their sulphur containing peptides after tryptic digest of the proteins. The results of these were compared to estimation of the protein amount via Bradford assay and total sulphur determination in the non-digested protein and digested protein samples.

L18

Binding constants for copper binding to metallothionein: Solving very complicated problems using ESI mass spectrometry

Stillman, Martin; Scheller, Judith; Heinlein, Lina; Hartwig, Andrea

Copper and zinc are essential cofactors in many metalloenzymes. Both excess copper and zinc become harmful if not sequestered appropriately in the cell. Metallothionein (MT), has been proposed as key player in zinc and copper homeostasis within the cell. The underlying mechanisms by which MT sequesters and transfers these metals, and how it subsequently achieves its proposed biological function remain unknown. Using electrospray ionization mass spectrometry (ESI-MS), circular dichroism (CD) and emission spectroscopy we report the binding properties of Cu(I) to MT1a. The 20 relative Kf-values for the binding of 1-20 Cu(I) to the 20 cysteine MT, were obtained from computational simulation of the experimental mass spectral data. For Cu(I) binding to apo-MT, these data identified the formation of three sequential but different clusters as a function of Cu(I) loading, providing the first overall sequence for Cu(I) binding in terms of domain specificity. Under cooperative binding at pH 7.4, a series of four clusters form: Cu_4S_{CYS6}, followed by Cu_6S_{CYS9}, then a second Cu_4S_{CYS6}, and finally Cu_7S_{CYSx} (x= up to 11). Using a cysteine modifier, we were able to confirm that Cu_6S_{CYS9} formed solely in the N-terminal b-domain and the existence of the presumed Cu_4S_{CYS6} cluster in the a-domain.

L19

Peptide analysis of selenoproteins produced after intravenous injection of 82Se enriched selenite or selenomethionine in mice

Furuta, Naoki; Tashiro, Yuki; Saito, Kei; Suzuki, Kazuhiro; Nishida, Sho

Selenium (Se) is one of the essential trace elements for mammals. ^{82}Se, one of the stable isotopes of Se, was used for tracer experiments. ^{82}Se-enriched selenite (Se(IV)) or selenomethionine (SeMet) was injected into mice fed with Se-adequate diets (Se-adequate mice) or Se-deficient diets (Se-deficient mice), and the liver and muscle were collected at 6 hours after injection. Soluble selenoproteins were collected from the liver and muscle, and separated by anion-exchange chromatography and size-exclusion chromatography coupled with inductively coupled plasma mass spectrometry (ICPMS). Specific protein was fractionated and tryptic digestion was carried out in polyacrylamide gel. Produced peptides were separated by capillary reversed-phase chromatography-ICPMS. Se level of selenoproteins increased when administrated with SeMet than when administrated with Se(IV) in mice[1]. We found that after Se(IV) injection, only selenocysteine (SeCys) was incorporated into selenoproteins, whereas both SeCys and SeMet were incorporated into the selenoproteins after SeMet injection. Se-containing peptides were fractionated and the sequence of amino acids will be investigated by using Electrospray Ionization-MS/MS. The difference of Se metabolism after Se(IV) injection and SeMet injection will be presented.

[1] Y. Suzuki, Y. Hashiura, T. Sakai, T. Yamamoto, T. Mastsukawa, A. Shinohara
and N. Furuta, Metallomics, 2013, **5**, 445–452.

L20

What is hidden in the goji berries? A response from hyphenated techniques

Wojcieszek, Justyna; Kwiatkowski, Piotr; Ruzik, Lena

The nutritional potential of goji berries (*Lycium Barbarum*, L.) as a good source of trace metals in the form of highly bioaccessible compounds is presented. Despite a large number of studies have focused on total concentration of trace metals from plant material, it is still known a little about their bioavailability for human organism. Due to the lack of appropriate information, the aim of the present study was to optimize extraction procedure of selected metal complexes with bioligands from goji berries. It is necessary to find methods of efficient extraction that allow us to get the knowledge about which kind of bioligands create complexes with metals.

To obtain information about metal complexes present in goji berries, extraction treatment using different solutions was performed. Extracts of berries were analysed by LC and CE with mass spectrometry detection (ICP MS, ESI MS). Identified compounds are probably responsible for better bioaccessibility of analyzed elements to human organism from goji berries. Evaluation of the extracted amount of element, not only its total concentration, is highly important due to the fact, that only a part from total content of metal is absorbed by the human body.

IL12

Method development for metal detection at cellular levels

Jakubowski, Norbert; Traub, Heike; Herrmann, Antje; Löhr, Konrad; López-Serrano Oliver, Ana; Mueller, Larissa

An overview about different analytical approaches will be presented of how to detect metals in individual biological cells by use of ICP-MS. For this purpose, we are using different sample introduction systems for ICP-MS for detection, imaging and quantification of metals at cellular levels.

By use of laser ablation, we have studied the up-take by and distribution of nanoparticles in single cells. Recently we have developed staining techniques to measure protein and DNA content of cells and identifying the cell status by immunoassays using metal-tagging of antibodies. New research based on cell arrays will be shortly discussed.

Using pneumatic nebulization and microdroplet generation, we have also studied the up-take of nanoparticles and toxic metals as well as essential elements in single cells using different ICP-MS mass spectrometric concepts (sector field instrument, triple-quad instrument, time of flight (CyTOF) instrument).

The different ICP-MS based methods will be compared concerning their analytical figures of merit and their strengths and weaknesses will be evaluated.

IL13

Complementary Imaging Techniques for Metallomics

Karst, Uwe

Imaging methods have become apparent as important tools in many application areas of the Metallomics field, including studies on the distribution and the effects of (metallo)pharma-ceuticals in the body, on the role of nutrients in plants, animals and humans and on toxic effects of compounds and nanoparticles. To further improve the information gained from imaging experiments, the combination of complementary imaging techniques to solve complex questions has strongly increased in recent years. Combinations of chemical imaging methods including MALDI-MS, µXRF or LA-ICP-MS or of chemical *in vitro* methods with medical *in vivo* imaging methods including CT or MRI provide valuable additional information.

Within this lecture, strategies and examples for the combined use of several chemical and medical imaging techniques are presented. These include the analysis of iodine and gadolinium containing CT and MRI contrast agents in human and animal tissue samples. Requirements for sample preparation and quantification will be discussed as well as possibilities and remaining challenges in this field. While the medical imaging techniques allow a 3-dimensional *in vivo* analysis, the rich chemical information particularly when using molecular and elemental mass spectrometry, the possibility for unambiguous quantification and a superior lateral resolution are advantages of the chemical methods.

IL14

Networks of molecular mechanisms cooperate in resistance against anticancer metal drugs

Berger, Walter; Kowol, Christian; Heffeter, Petra; Keppler, Bernhard K.

Metal drugs are a mainstay of systemic anticancer therapy at the disseminated state. Nevertheless, the developments and novel approvals in the field of oncology have been massively hampered by the propensity of cancer cells to rapidly acquire resistance against these highly active compounds. Thereby the resistance phenotypes can synergistically orchestrate impeded drug delivery into the malignant tissues, reduced cancer cell uptake or enhanced efflux primarily via ATP-binding cassette transporters, altered drug metabolism and detoxification as well as activation of cellular survival and repair pathways. Several examples of molecular networks underlying failure of anticancer metal drugs and suggestions for their circumvention will be discussed. Hence we have recently shown that epidermal-growth factor (EGFR)-mediated DNA repair and survival signals might cooperate with those of another receptor tyrosine kinase (Met) in acquired resistance against arsenic trioxide (ATO). As a second example, genome-wide gene expression data are used to dissect the molecular mechanisms underlying resistance against the ruthenium(III) compound KP1339 currently in clinical testing. Serum protein interaction and active albumin-targeting are represented as two of several prodrug strategies not only to reduce adverse effects but also to minimize vulnerability by resistance mechanism including ABC transporter-mediated drug efflux.

IL15

Systems approach for revealing the role of metals in medicine

Sun, Hongzhe; Wang, Haibo; Li, Hongyan; Zhou, Ying; Koohi-Moghadam, Mohamad

Metal compounds have long been used in medicine and healthcare. Metal- and metallodrug-protein interactions play a crucial role for metallodrugs. It is critical to identify metal-protein interactions at a proteome-wide scale which are difficult due to diversity of such interactions (1,2). We developed a system approach consisting of continuous-flow gel electrophoresis and inductively-coupled plasma mass spectrometry, LA-ICP-MS, IMAC, and fluorescence to identify metal-associated proteins using bismuth antiulcer drug as an example (3). We have identified metal-associated proteins as well as to quantify the metals for fast metallome/proteome-wide profiling of metal-binding proteins.

We further established a bioinformatic method which allows potential metal-binding proteins to be searched and new drug lead was discovered based on virtual screening. We also show that the systemic metallomic approach represents a powerful tool for metals medicine including pharmacology of metallodrugs.

We thank the Research Grants Council of Hong Kong, Innovative Technology Fund, the University of Hong Kong for financial support.

[1] H. Sun, Z.F. Chai, *Ann Rep Prog Chem A Inorg Chem* **2010,** *106*, 20-38.

[2] X. S. Sun, C.N. Tsang, H. Sun, *Metallomics* **2009,** *1*, 25-31.

[3] Y.C. Wang, L.G. Hu, H. Li, H. Sun et al, *Chem Sci* **2017**, in press.

L21

On the molecular mechanism of action of organometallic anticancer drugs

Gerner, Christopher; Meier, Samuel M.; Neuditschko, Benjamin; Kreutz, Dominique

Despite organometallic anticancer drugs are routinely used in clinical practice, the molecular mechanisms of action are not very well understood. However, this knowledge would be required for a more appropriate choice of drugs based on individual parameters as aimed for in personalized medicine. Proteome profiling of cell responses to various drugs has taught us that a given drug may exert different effects on different kinds of cancer cells, most probably dependent on the metabolic cell state affecting redox and ROS homeostasis as well as DNA repair capabilities. Here we suggest that the determination of the functional status of mitochondria in cancer cells in addition to mitonuclear stress signals upon drug treatment using both proteomics and metabolomics may represent a valid strategy to investigate molecular mechanisms of drug action.

you12

Analysis of cisplatin uptake in sensitive and resistant individual cells by single-cell-ICP-MS

Corte Rodríguez, Mario; Álvarez-Fernández García, Roberto; Blanco González, Elisa; Bettmer, Jörg; Montes Bayón, María

One of the main limitations in cancer therapies using platinum metallodrugs like cisplatin is the development of intrinsic or acquired resistance to the drug. Side resistance is caused by multifactorial and not completely understood mechanisms, but one of the most important ones is related to a reduced intracellular accumulation of the drug[1]. Thus, intracellular concentration could be considered as a biomarker of cisplatin resistance. Bulk analysis methods of Pt that are based on cell mineralization and total Pt quantification in samples containing several million cells will mask individual cell heterogeneities that can be critical. Thus, single cell analysis is of great importance in order to correlate drug uptake with other biological parameters like cell cycle or mRNa content. Thus, here we will present the development and characterization of a single-cell analysis method using ICP-MS and a new sample introduction system by means of a microflow concentric nebulizer fitted to a total consumption spray chamber to analyze Pt content. The methodology will be applied to the evaluation of the comparative uptake of Pt species in cisplatin -resistant and -sensitive cell lines of human ovarian carcinoma (A2780 and A2780cis).

[1] L. Galluzzi et al., *Cell Death and Disease*, 5 (2014)

L22

Cu isotope ratio variations in mice suffering from liver disease induced by common bile duct ligation

Costas Rodriguez, Marta; Devisscher, Lindsey; Hastuti, Agustina A.M.B.; Van Campenhout, Sanne; Van Vlierberghe, Hans; Vanhaecke, Frank

Serum Cu isotopic composition is altered in liver disease patients. However, the actual variations in the serum Cu isotopic composition accompanying different liver features and the factors governing the isotopic variability remain unclear. High-precision Cu isotopic analysis was performed using multi-collector ICP-mass spectrometry in samples from mice with cholestatic liver disease induced *via* common bile duct ligation (CBDL). CBDL is used to induce secondary biliary cirrhosis (due to long-term bile duct obstruction) in rodents. CBDL-operated mice exhibit signs of cholestasis after 1 week of the surgical intervention, fibrotic changes after 3 weeks and secondary biliary cirrhosis after 6 weeks. The mice were sacrificed at 2, 4, 6 and 8 weeks after the surgical intervention in order to evaluate the distribution of the Cu isotopes in the body upon progressing cholestasis-induced secondary biliary cirrhosis. Sham-operated mice that underwent the same procedure, but without ligation of the bile duct, were used as controls. Total body Cu became enriched in the ^{63}Cu isotope with the progression of the disease. At the earliest stage (*i.e.* after 2 weeks), liver and kidney were the most affected organs, while after 8 weeks, the whole body Cu isotopic composition of the CBDL-mice was significantly altered.

you13

Evaluation of the changes in the net bone volume through the calcium isotopic signatures for CKD and diabetic rat

Tanaka, Yu-ki; Hirata, Takafumi

Isotopic signatures of Ca for biological samples can become the new proxy for the evaluation of Ca exchange between bone and serum, which directly reflects the bone volume. Due to the large differences of Ca isotope ratio between serum and bone (>0.3‰), the enhancement of bone resorption can cause the modification of Ca isotope ratio for serum or urine. To investigate the validity of the Ca isotope signatures as a biomarker, we have measured the Ca isotope ratios ($^{44}Ca/^{42}Ca$) for bone and serum samples collected from rats with chronic kidney disease (CKD) or diabetes mellitus (DM), together with the control rats.

The resulting $^{44}Ca/^{42}Ca$ values of bone and serum varied significantly among the groups. For the bones of the CKD and DM rats, the $^{44}Ca/^{42}Ca$ values were 0.1‰ and 0.3 - 0.6‰ lower than those for the control rats, respectively. Moreover, the measured Ca isotope ratios were positively correlated with the bone mineral density of femur of the rats. These results suggest that the Ca isotope ratios for serum can be used to detect the change in the relative amount of Ca released from bones against that incorporated into bones.

IL16

The influence of RAPTA-T on the tumor microenvironment.

Dyson, Paul

We show that RAPTA-T, a compound undergoing preclinical evaluation, enhances tumor vascular function by decreasing blood vessel tortuosity and dilation, while increasing the coverage of endothelial cells by pericytes and vessel perfusion within tumors. This in turn significantly reduces the interstitial fluid pressure and increases oxygenation in the tumor. As a result of these changes to the tumor microenvironment, pre-treatment of RAPTA-T, followed by the application of cytotoxic agents such as cisplatin, leads to increased drug uptake in the tumor and enhanced efficacy of the combined chemotherapy overcoming resistance in chemoresistant tumors. We use a number of imaging methods to elucidate these effects and to establish the mechanistic basis of RAPTA-T.

L23

Distinctly enhanced anticancer activity in vivo by albumin-targeted platinum(IV) prodrugs

Kowol, Christian; Heffeter, Petra; Mayr, Josef; Groza, Diana; Galvez, Luis; Koellensperger, Gunda; Berger, Walter; Keppler, Bernhard

A long term goal in anticancer drug research is to increase the tumor-specific drug delivery. A very elegant way is the selective binding of anticancer drugs to the Cys34 thiol group of serum albumin in the blood stream, which results in accumulation at the tumor side due to the enhanced permeability and retention (EPR) effect. Consequently, we synthesized a series of oxaliplatin- and cisplatin-containing maleimide-functionalized platinum(IV) complexes. As reference compounds succinimide analogues were prepared, unable to bind thiols. The interaction of the compounds with albumin was studied by RP-HPLC and the behavior in serum over 24 h with SEC-ICP-MS. In addition, the reduction kinetics using ascorbic acid as a model compound were studied and revealed strong differences between cisplatin- and oxaliplatin-releasing complexes. Also with regard to the anticancer activity *in vivo*, profound differences between cisplatin- and oxaliplatin-releasing compounds were found. Subsequent analysis of collected tissue and serum samples indicated that this might be due to higher stability of the oxaliplatin-releasing platinum(IV) drugs in the body. Based on these data, a novel oxaliplatin-releasing lead compound with strongly improved anticancer activity compared to oxaliplatin was selected for further (pre)clinical development.

L24

Multicellular spheroids as models and tools in anticancer metallodrug research

Jakupec, Michael

Conventional cultures of adherent tumor cell lines are extremely simplified models not properly reflecting essential features of real tumors. Multicellular spheroids grown from tumor cell lines more closely resemble tissues in their three-dimensional structure, increased cell-cell contacts and quiescent cell fractions, longer diffusion paths and increased penetration barriers as well as gradients of physicochemical parameters (such as O_2, pH, redox potential). Effects of compounds can be studied in comparison with two-dimensional cultures with regard to cytotoxicity, expression of specific factors on different levels (qPCR, Western blotting, immunohistochemistry), impact on cellular ultrastructure (TEM) and drug distribution (NanoSIMS). The crucial question of increased predictive power for the efficacy of anticancer metallodrugs in vivo is subject of current investigations. Invasive growth (which defines malignant tumors) can be better mimicked in the three-dimensional model, and the influence of substances on the cellular capacity of invading a surrounding semisolid milieu (consisting of extracellular matrix components) can be studied by means of a spheroid invasion assay. Moreover, co-culture systems are being established to mimic the interplay with the tumor stroma and cells of the immune system, which might be of relevance for the anticancer activity of metal compounds.

you14

Understanding the pharmacological behavior of the anticancer drug Triapine and its biologically active iron complex

Pelivan, Karla; Miklos, Walter; Van Schoonhoven, Sushilla; Heffeter, Petra; Koellensperger, Gunda; Gille, Lars; Berger, Walter; Kowol, Christian R.; Keppler, Bernhard K.

With the aim to target the iron dependence of tumor cells, several iron chelators have been developed for anticancer chemotherapy. Regarding the clinical practice, Triapine, one the most prominent chelators, showed promising activity against hematological diseases, but ineffectiveness against several solid tumors. The underlying reasons are still vague and might be based on its sparsely investigated pharmacological behavior. Consequently, novel analytical tools have been developed to get more insights into Triapine and its biologically active iron complex.

First, *in vitro* stability of the compounds was examined and drug uptake was quantified in cell culture. For distribution experiments *in vivo*, drug levels were determined in samples collected from treated Balb/c mice. Notably, Triapine and its iron complex revealed a highly different behavior. Triapine showed low protein binding in cytosol and serum whereas fast adduct formation was observed for Fe-Triapine. Moreover, in accordance to clinical data, basically no renal excretion was found for Triapine, in contrast to the iron complex which was effectively excreted *via* urine. Remarkably, no Fe-Triapine was detected in cytosolic extracts or serum after Triapine treatment.

Taken together, novel insights about Triapine and its iron(III) complex were obtained which will help to improve the design of anticancer thiosemicarbazones.

L26

In vivo evaluation of serum binding, tissue distribution and anticancer activity of bismaleimide-containing oxaliplatin prodrugs after short- and long-time treatment

Heffeter, Petra; Groza, Diana; Hermann, Gerrit; Schueffl, Hemma; Dhery, Vineet; Mayr, Josef; Legin, Anton; Keppler, Bernhard; Berger, Walter; Koellensperger, Gunda; Kowol, Christian

Therapy with platinum compounds is limited by strong side effects, resistance development and insufficient tumor accumulation. To overcome these drawbacks we aimed to enhance tumor-targeting of oxaliplatin via binding to albumin. Thus, the first bis-maleimide-containing platinum(IV) drug KP2156 was synthesized and investigated in vivo in comparison to its corresponding succinimide derivative KP2157. With regard to in vivo anticancer activity, KP2156 displayed significantly higher anticancer activity against CT26 colon carcinoma than KP2157. To gain more insights into tissue distribution and pharmacokinetic of the drugs, serum, urine and tissue samples were collected at several time points after drug treatment and analyzed by ICP-MS. These analyses revealed that oxaliplatin and KP2157 were rapidly excreted from the body, while KP2156 is characterized by a prolonged plasma half-life time and tumor accumulation. Moreover, preliminary data indicate that the KP2156 anticancer activity (based on upregulation of the DNA damage marker pH2A.X) occurs in a biphasic manner with a peak after 24h and 14 days. This is of interest as NanoSIMs measurements indicated cellular drug uptake via vesicles already 24h after drug application, which indicates that maleimide functionalization of platinum(IV) drugs leads to a depot effect resulting in distinctly enhanced therapeutic activity compared to free oxaliplatin.

L27

Ruthenium arene complexes for G-quadruplex DNA recognition

Terenzi, Alessio; Mokesch, Stephan; Hager, Laura A.; Kieler, Claudia; Dinhof, Carina; Berger, Walter; Keppler, Bernhard K.

B-DNA remains the traditional target for metallo-based anticancer drugs used in clinics nowadays. There has been considerable progress in DNA targeting drugs, especially considering non-conventional DNA motifs. G-rich sequences, for example, are able to form four-stranded structures organized in stacked guanine tetrads. These structures, called G-quadruplexes (G4s), are not randomly distributed within the human genome, but are overrepresented in telomeres and in cancer related gene promoters.[1]

Properly designed metal complexes are reported to effectively interact with G4 structures.[2] With the aim to combine the anticancer properties of half-sandwich complexes with those of the ligand scaffold (originally designed to inhibit topoisomerase activity),[3] we synthesized 1,3-dioxoindane-2-carboxamide ligands and their respective ruthenium(II) arene complexes. The ligand has a pendant naphthyl-group to allow for π-interaction with G-quartets, while the ruthenium center may bind to guanine after ligand exchange. Two spacers with differing length were introduced to investigate the importance of flexibility. Furthermore, the chloride ligand was exchanged for pyridine, introducing a potential light-activation switch.

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IL17

The power of ICP-MS based bioimaging and speciation analysis to study mineral ion transport and functionality in plants

Husted, Søren

I will present results to demonstrate the scientific potential of metallomics in plant science. This includes bioimaging by Laser Ablation (LA) to study mineral ion transport across cellular barriers and speciation analysis to study the roles of mineral ions in photosystem II (PSII). A LA-ICP-MS based method for bioimaging of ions was developed. Using conventional protocols we observed that diffusible ions were lost during sample preparation. A new procedure was developed to preserve ions in their native cellular environment. To demonstrate the potential of these developments, we analyzed mutants unable to synthesize the nicotianamine (NA). Mutants defective in NA synthesis accumulated more Zn and Mn relative to the wild type (WT) in specific tissue regions. Relative to Zn and Mn, Fe showed a different tissue compartmentation and I will show that these differences are linked to their molecular speciation. Metals, including Mn, exert essential functions in chloroplasts, where they act as cofactors in the electron transport chain. I will present a LC-ICP-MS based method that enables analysis of metal binding in catalytically active PSII supercomplexes. Proper separation and characterization of Mn binding to PSII complexes were only achieved after critical selection of buffers, detergents and stabilizers to maintain complex integrity.

L28

Mobilization of iron by phytosiderophores in the rhizosphere of wheat

Puschenreiter, Markus; Gruber, Barbara; Wenzel, Walter; Schindlegger, Yvonne; Hann, Stephan; Schenkeveld, Walter D. C.; Kraemer, Stephan M.; Oburger, Eva

Iron is a micronutrient for higher plants, but its bioavailability in soils is typically extremely low. Plants have developed different strategies for increasing the solubility of iron(III) in the root-near soil (i.e. the rhizosphere). Most plants solubilize iron by the release of protons as well as weakly complexing and/or reducing root exudates; this mechanism is called strategy I. Grasses however have developed a very efficient iron mobilization strategy (i.e. strategy II), which is based on the release of phytosiderophores (PS), i.e. non-proteinaceous amino acids, that form strong complexes with iron, but also with other micronutrients, such as Zn or Cu. In rhizobox studies we could show that the phytosiderophore (PS) release rates from wheat roots are much smaller than previously determined in nutrient solution culture, suggesting that the carbon and energy investment is also lower than initially assumed. Based on experimental data we calculated that during the investigated period (21-47 days after germination), PS release initially exceeded Fe plant-uptake 10 times but significantly declined after about 5 weeks after germination. On the other hand, the efficiency of Fe mobilization is limited by competing metals, by microbial degradation and by adsorption of PS. However, we could demonstrate that there is a time and concentration-dependent “window of iron uptake”. Furthermore, the microbial degradation (determined using in-house synthesized 13C-enriched 2’-deoxymugineic acid), we were able to follow the partitioning of DMA-derived 13C into the microbial (biomass &13CO2) and soil matrix (liquid & solid phase) pool. Microbial decomposition is less effective in bulk soil compared to the rhizosphere, suggesting that PS release close the root tip into soil spots containing a non-adapted microbiome contributes to more efficient Fe scavenging.

L29

Hyperaccumulation of manganese in a submerged plant is mediated by epiphytic bacteria

Harada, Emiko; Asayama, Takuma; Tsuji, Kousuke; Hasegawa, Hiroshi

Egeria densa (Brazilian waterweed) is a submerged, freshwater perennial macrophyte native to South America. Many aquatic plants are considered to work as biosorbents for water purification to extract toxic chemicals including heavy metals. To assess the biosorbent activity of *E. densa*, plants were collected monthly from a circular drainage area in Lake Biwa basin and the Mn concentrations of the plants were analyzed. Mn concentrations in these plants were generally above those of terrestrial hyperaccumulators, and were markedly higher in spring and summer than in autumn. Mn concentrations were much lower in plants incubated in hydroponic medium at various pH levels with and without Mn supplementation than in field-collected plants. The precipitation of Mn oxides on the leaves was determined by variable pressure scanning electron microscopy-energy dispersive X-ray analysis (VPSEM-EDX) and Leucoberbelin blue staining. Several strains of epiphytic bacteria with Mn-oxidizing activity were isolated from the field-collected plants. High Mn concentrations in *E. densa* were mediated by the production of Mn oxide in biofilms on the leaf surfaces. These findings provide new insights into plant epidermal bacterial flora that affect metal accumulation in plants. The potential use of the aquatic plants in Mn phytomining will be discussed.

L30

Splicing isoform of NjZNT1 expressed in the zinc hyper accumulator Noccaea japonic encodes full active zinc transporter

Nishida, Sho; Tanikawa, Ryoji; Fujiwara, Toru; Furuta, Naoki

The Zrt/Irt-like protein (ZIP) family of transporters has been shown to play a critical role on the transport of divalent metal ions in several plants. Recent genome-wide transcriptome analyses have suggested that ZIP genes are involved in metal accumulation in the metal hyperaccumualtor species. Previously, we identified the alternative splicing isoform of *ZNT1* (a homolog of *Arabidopsis ZIP4*), which is specifically expressed in the zinc hyperaccumulator *Noccaea* species and not detectable in the non-accumulator relative species. Here we report the functional analysis of *Noccaea japonica ZNT1* (*NjZNT1*) splicing isoform. Sequence analysis revealed that the splicing isoform has the main open-reading frame encoding N-terminal truncated NjZNT1. The truncated NjZNT1 complemented yeast zinc transporter ZRT1, and also the *Arabidopsis* transgenic plants overexpressing the truncated NjZNT1 showed increased zinc accumulation in roots compared to untransformed plants, establishing that truncated NjZNT1 acts as a zinc transporter. By contrast, the transgenic plants overexpressing untruncated NjZNT1 (general type of ZNT1/ZIP4) showed no significant change in zinc accumulation, suggesting that the untruncated form has less or no zinc transport activity. Our results suggest that the splicing isoform of *NjZNT1* encoding full active zinc transporter was acquired in the process of evolution into zinc hyperaccumulator.

L31

Identification of palladium species following the uptake and metabolism of Pd nanoparticles by Sinapis alba L.

Kinska, Katarzyna; Jiménez-Lamana, Javier; Bierla, Katarzyna; Godin, Simon; Kowalska, Joanna; Krasnodebska-Ostrega, Beata; Szpunar, Joanna

The increased use of nanoparticles (NPs) of elements with low natural distribution, such as palladium, leads to their increasing emission and spreading in the environment. The uptake and metabolism of palladium NPs by plants has not been thoroughly investigated so far; the presentation discusses the development of analytical methods suitable to study these processes in the model plant *Sinapis alba* L. cultivated in the presence of PdNPs.

The optimized mild enzymatic digestion, followed by the analysis by single particle inductively coupled plasma mass spectrometry (SP-ICPMS), allowed the confirmation of the presence of palladium NPs in all plant tissues as well as their size distribution and number concentration and the concentration of dissolved Pd. The results confirmed the ability of the plant to translocate intact PdNPs to aboveground plant organs. The observed partial dissolution of palladium has stimulated the second part of the study aimed at the identification of Pd molecular targets in the plant tissues. Palladium compounds extracted from plant organs were fractionated by size exclusion chromatography (SEC) with ICP MS detection and identified by HILIC chromatography coupled to high resolution ESI Orbitrap MS^n.

The study was supported by the National Science Centre (NCN), Poland, Grant No. 2014/15/N/NZ8/00326.

L32

Investigation into the coumarin-mediated mechanism of iron acquisition from alkaline soil into plants

Baune, Matthias; Kang, Kyounglim; Schenkeveld, Walter D. C.; Kraemer, Stephan M.; von Wirén, Nicolaus; Weber, Guenther

Due to the low iron solubility in alkaline soils, plants have evolved different iron acquisition strategies, which are either based on ferric iron reduction (strategy I) or complexation by phytosiderophores (strategy II). Recently, a prominent role of coumarins for iron acquisition under conditions of iron deficiency has been discovered, but details of the respective mechanism remain unclear. Since coumarins may act as iron-binding ligands but also as reductants, various reaction sequences are possible for iron mobilization, resulting in different iron species and modified coumarins.

In order to identify such species in root exudates and rhizosphere soil solutions, we applied several analytical techniques in parallel, namely capillary electrophoresis and liquid chromatography coupled to mass spectrometry (CE- and LC-MS), and electrochemistry - mass spectrometry (EC-MS). The latter is particularly useful for the detection of labile and short-lived redox intermediates, which do not survive separations. In addition to the detection of expected iron-coumarin chelates (1:2 and 1:3 stoichiometry) our results highlight the fact that redox interaction of coumarins with iron minerals produces oxidized coumarins, incl. more hydroxylated (i.e., more reactive) species. Consequences of such catalytic oxidation to iron acquisition are discussed.

IL18

Supramolecular Self-assembled Metallacages for Biomedical Applications: New Insights

Casini, Angela

A specific and attractive area of Supramolecular Coordination Chemistry is the self-assembly of M_2L_4 (M = metal, L = ligand) metallacages, which can enclose a wide range of small molecules within their cavity, including drugs. In this context, we investigated fluorescent Pd_2L_4 cages as drug delivery systems for the anticancer drug cisplatin, which proved to be active in cancer cells, while showing low ex vivo toxicity in healthy rat liver tissue.[1] The obtained Pd(II) metallacages showed fluorescence properties due to the used ligand system.[3]

Recently, with the idea of achieving targeted delivery of the metallacages to tumor cells, we reported for the first time on the bioconjugation of exo-functionalized self-assembled Pd_2L_4 cages to peptides.[5] The obtained bioconjugates were identified by high-resolution mass spectrometry. This report aims at providing an overview of our studies in the area of self-assembled metallacages as drug delivery systems, with particular emphasis to strategies to overcome possible challenges for biomedical applications and future perspectives.

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L33

The role of caspase 8 induction and disruption of ER homeostasis in the sensitivity towards the GRP78 inhibitor KP1339/IT-139

Schoenhacker-Alte, Beatrix; Mohr, Thomas; Pirker,, Christine; Kryeziu, Kushtrim; Kuhn, Paul-Steffen; Buck, Alicia; Hofmann, Thilo; Gerner, Christopher; Hermann, Gerrit; Koellensperger, Gunda; Keppler, Bernhard K.; Berger, Walter; Heffeter, Petra

The ruthenium drug and GRP78 inhibitor KP1339/IT-139 has already demonstrated promising anticancer activity in a phase I clinical trial. In order to elucidate the mechanisms underlying increased sensitivity to KP1339 treatment, 23 cell lines were screened to allow selection and comparison of KP1339-sensitive and low-responsive models. For this panel, the biochemical response to KP1339 was analyzed using whole genome expression arrays. These data indicated that sensitive cell lines were characterized by “response to chemical stimuli” and “regulation of cell death”, whereas low-responsive cells preferentially activated pathways controlling cell cycle, DNA repair, and metabolism. Subsequent cell culture experiments confirmed that, while low-responsive cells executed cell cycle arrest in G2 phase, pronounced apoptosis induction via activation of caspase 8 was found in sensitive cells. This cell death induction by KP1339 was based on a unique disruption of the ER homeostasis by depletion of key cellular chaperons including GRP78 in combination with enhanced treatment-mediated protein damage. Taken together, this study indicates the unique and multi-facetted mode of action of KP1339 and supports patient selection for future phase II investigations.

you15

Anti-Tumour Complex Dirhodium(II) Tetraacetate and its Interactions with Glutathione and Human Metallothionein

Wong, Daisy

A main concern of modern drug development is maximizing drug efficacy. Metal-bearing drugs can be hindered by the body's defense against toxic metals. Metallothioneins (MT) are a ubiquitous class of thiol-rich, metal-binding proteins involved in physiological metal homeostasis (Zn(II), Cu(I)), as well as heavy metal detoxification. MT utilizes its thiol rich structure to hyperchelate multiple metal ions, including metal anticancer drugs. This can result in developed drug resistance, rendering treatment ineffective. The focus of this presentation is on recently published data involving dirhodium tetraacetate, a potential anti-tumour compound. Electrospray ionization mass spectrometry and spectroscopy show the MT beta domain binds readily to dirhodium tetraacetate, causing the tetraacetate ligands to dissociate, disrupting the active drug structure. New data also describe glutathione binding to dirhodium tetraacetate, the latter being a prominent cellular antioxidant and heavily involved in metabolism.

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IL19

Study of Organic Reactions with ICP-MS/MS

He, Qian; Wei, Chao; Xing, Zhi; Zhang, Sichun; Fang, Xiang; Zhang, Xinrong

ICP-MS is a tool originally for elemental and isotopic analysis. After years of efforts by several groups including the contribution of our group, ICP-MS has been a powerful tool now applicable successfully to proteins and DNAs analysis. In my opinion, however, potential of ICP-MS is far from being fully explored. It is still important to expand the new application of ICP-MS. Recently we found that ICP-MS/MS might be a useful tool for rapid screening of Cu^{+}-intermediates in the azide–alkyne cycloaddition reaction. Once the intermediates formed in the reaction cell, they would be separated using the mass analyzer and detected immediately. This study provided not only a tool for azide–alkyne cycloaddition reaction, but also for other organic reactions catalyzed by metal(I) ions. In our second study, the single-atom metal catalysts were produced from ICP source, and then mixed with methane in dynamic reaction cell. The higher molecular weight hydrocarbons were catalytically produced by converted methane, including ethane, ethylene, and benzene etc. The results demonstrated that ICP-MS/MS platform assures stable production of various homogeneous single-atom ions for high throughput screening catalysts, and would also be potential applied for the discovery of new catalysts in methane conversion.

you16

Studies on Characterization and Bioavailability of Iron Oxide Nanoparticles for the Treatment of Iron Deficiency Anaemia

García-Fernández, Jenifer; Jakubowski, Norbert; Sánchez, Cristina; Llopis, Juan; Bettmer, Jörg; Montes-Bayón, María

Iron deficiency anaemia is one of the most common and widespread nutritional disease in the world. Oral iron supplementation represents an effective way of treating this pathologic disease due to its well absorption and safety in most of cases. However, latest trials suggest that it also can induce severe toxicity in the gastrointestinal tract as well as digestive intolerance due to production of a variety of highly reactive oxygen species (ROS) as a consequence of possible non-absorbed iron salts. In order to minimize these side effects, iron nano-dispersions mimicking ferritin core have been synthesized.

In this study, tartrate-modified coated-iron oxide nanoparticles (NPs) have been synthesized according to a precipitation method of a Fe^{3+} salt in basic medium [[1]]. With the aim of evaluating the lability of these NPs in acidic medium (similar to stomach acidic conditions), specific assays were conducted and total Fe was quantified in nanoparticulate and soluble fractions. In addition, intestinal and cell uptake studies as well as iron NPs stability within cells is quantitatively conducted by using a newly developed HPLC-ICP-MS method.

[[1]] D.I.A. Pereira et al., *Nanomedicine: Nanotech. Biol. Med.*, **10**, 1877 (2014).

you17

Interaction of medically promising gold nanorods with human serum proteins examined by CE-ICP-MS

Legat, Joanna; Matczuk, Magdalena; Scaletti, Federica; Messori, Luigi; Timerbaev, Andrei; Jarosz, Maciej

Due to their unique optical properties gold nanorods (AuNRs) present one of the most promising metal-based nanomaterials in biomedical applications, including drug delivery and photothermal therapy. Their mode of action in living organisms greatly depends on the protein corona formed on their surface after intravenous administration. Therefore, it is essential to investigate the surface binding of serum proteins which may influence biodistribution and toxicity of AuNRs.

Our study focused on exploring the interactions between differently functionalized AuNRs (surface PEG-ylated with introducing various terminal groups) and human serum proteins under simulated physiological conditions. The combined CE-ICP-MS technique was used to separate the intact nanorods and gold–protein conjugates at biocompatible conditions and to detect gold species at physiologically relevant concentrations. It was proven that the type of terminal groups on AuNRs surface has a crucial effect on protein-mediated transformations. The second important parameter is the applied dose of nanorods [1]. The reported results would facilitate the future development of AuNRs for application in cancer therapy.

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IL20

Medical diagnosis based on natural isotope ratio variations of essential mineral elements in human biofluids?

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There is increasing evidence that diseases also affecting the metabolism of essential mineral elements exert an influence of the isotopic composition of these elements in human body fluids measurable using multi-collector ICP-mass spectrometry (MC-ICP-MS). Research carried out at UGent has, e.g., demonstrated that liver disease gives rise to a lighter isotopic composition of serum Cu (lower $\delta^{65/63}$Cu value). Within the first months after successful liver transplantation, the serum Cu isotopic composition becomes normalized. Oral squamous cell carcinoma tissue, on the other hand, shows a heavier isotopic composition (higher $\delta^{65/63}$Cu value) than the neighboring healthy tissue. Iron deficiency and iron deficiency anemia shift the isotopic composition of Fe in serum towards higher $\delta^{56/54}$Fe values (heavier isotopic composition). This opens possibilities for “isotopic diagnosis” of diseases that might otherwise only be established at a more progressed state or via more invasive approaches. In this new field, the factors driving these changes in the isotopic composition still need to be unraveled. We rely on both *in vivo* experiments with lab animals and *in vitro* approaches with cell lines to obtain a more profound insight into the isotope fractionation accompanying physical and biochemical processes in the body.

L34

Characterization of metals profiles and homeostasis in serum during the progression of Alzheimer's disease

Callejon-Leblic, Belen; Garcia-Barrera, Tamara; Gómez-Ariza, Jose-Luis

Metal dyshomeostasis is closely related to Alzheimer's disease; therefore, the characterization of metals profiles is of interest in relation to neurodegenerative pathogenesis. An analytical approach, based on nondenaturing precipitation of proteins, has been optimized for the fractionation of high molecular mass (HMM) and low molecular mass (LMM) metal-species from serum, which were subjected to multielemental analysis by inductively coupled plasma mass spectrometry (ICPMS). In addition, metal content was analyzed after size-fractionation of species and then, inter-element and interfraction ratios computed. These methodologies were applied to healthy controls, Alzheimer's disease (AD) and mild cognitive impairment (MCI) patients in order to study the progression of dementia. Some metals, such as Mn, Li and V allow discriminating between controls and diseased subjects, both AD and MCI, but no differences were found between these two clinical stages. In addition, it was noted the important role that low molecular mass fractions of Fe, Cu, Cu, Al and Co in the pathogenesis of Alzheimer. Finally, correlation analysis indicated that these metal abnormalities can be interrelated, participating in common processes as oxidative stress, altered homeostasis and uptake into brain, as well as impaired glucose metabolism.

L35

Multimodal imaging of multicellular tumor spheroids by MALDI-MS and high- resolution LA-ICP-MS

Theiner,Sarah; Van Malderen, Stij; Holzlechner, Matthias; Svirkova, Anastasiya; Van Acker, Thibaut; Legin, Anton; Keppler, Bernhard K.; Marchetti-Deschmann, Martina; Vanhaecke, Frank; Koellensperger, Gunda

Three-dimensional multicellular tumor spheroids are an attractive in vitro assay to screen potential anticancer drug candidates in a high throughput manner for further animal and clinical evaluation, as they mimic the complex tumor micro-environment.
Herein, we present an imaging approach based on matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) for lipid analysis and laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) for mapping of phosphorus and platinum in HCT116 tumor spheroids upon treatment with platinum-based compounds. The heterogeneous morphology of the spheroids was reflected in the distribution pattern for certain lipid classes as well as in the elemental accumulation.
Platinum was mainly accumulated in the necrotic core and the outer rim of proliferating cells. A low dispersion LA-ICP-MS setup was used to obtain high-resolution images and to enhance the speed of analysis. The high-resolution of ~2.5 µm and the Pt/P ratio enabled to eliminate intracellular space and to compensate for differences in cell density.

L36

Tin- and gold complexes with antioxidant pendants - candidates for selective anticancer agents

Milaeva, Elena; Shpakovsky, Dmitry; Tyurin, Vladimir; Gracheva, Yulia; Antonenko, Taisia; Kharitonashvili, Elena

This study is focused on a novel approach to design hybrid metal-based physiollogically active compounds with dual modes of action – prooxidative activity of metal and antioxidative activity of 2,6-dialkylphenol group. The synthesis and anti/prooxidant activity and cytotoxicity studies of novel Sn and Au organometallic/coordination compounds are presented and discussed.

The biological activity has been studied in *in vitro, ex vivo, in vivo* experiments in lipid peroxidation and mitochondria-associated processes, by using neurons, liver homogenates and in enzymatic reactions (*xanthine oxidase*, *lipoxygenase, gluthathione reductase, thioredoxine reductase*).

Thus, we can conclude that the combination of two physiologically active moieties in a complex molecule is a promising approach to find the novel hybrid therapeutic agents with opposed biological mode of action.

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L37

'Gold-finger' domains formation by organometallic gold compounds: strategies to design PARP-1 inhibitors for cancer treatment

Wenzel, Margot; Meier-Menches, Samuel; Casini, Angela

Gold complexes have raised attention in recent years as new promising anticancer agents. Among their possible pharmacological targets, zinc finger (ZF) proteins occupy an important place, being involved in a wide range of functions which are essential for cell growth and development and having direct implications in health and disease. Among ZF proteins, PARP-1 is believed to be involved in resistance and DNA repair mechanisms upon platinum-based drugs administration.

Our group recently reported on the ability of Au complexes to inhibit PARP-1. The hypothesized mechanism of inhibition is displacement of Zn^{2+} from the ZF by gold ions leads to decreased protein activity, and to formation of the so-called ‘‘gold-finger’’ (GF) domain.

Within this framework, we studied the reactivity of a new series of gold(III) complexes with different ZF domains *via* various techniques, including high-resolution mass spectrometry and quantum mechanics/molecular mechanics. The influence of the gold oxidation state, the ligand type, as well as the ZF coordination sphere in GF formation provided useful insights into the possible design of new gold complexes selectively targeting PARP-1 ZF domains for possible applications as therapeutic agents in cancer treatment.

L38

Restoring cellular sensitivity to platinum-based drugs by targeted inhibition of STAT3

Kiakos, Konstantinos; Jakupec, Michael; Valentine, Helen; Lee, Moses; Hartley, John A.; Keppler, Bernhard

The transcription factor signal transducer and activator of transcription 3 (STAT3) is responsible for the regulation of a wide range of genes implicated in cancer proliferation, survival, angiogenesis and metastasis. Constitutive activation of STAT3 is prevalent in many cancers and has been identified as a key mediator of chemoresistance. VS-43, a novel direct STAT3 inhibitor, is currently being developed as a single-agent and in combination with DNA damaging platinum-based chemotherapy. VS-43 selectively inhibits the tyrosine (705) phosphorylation of STAT3, in a dose-, cell density- and time-dependent manner. We have previously reported the chemosensitisation of DU145 and A549 cells to cisplatin by VS-43. We have extended the studies in paired cell lines of sensitive and multifold resistant phenotypes, to cisplatin and oxaliplatin. VS-43 efficiently inhibits the pSTAT3 levels of the two resistant sublines, both harbouring increased levels of activated STAT3 compared to the parental, drug-sensitive cells and induces apoptosis. We determined by combination index analysis that VS-43 acts synergistically in combination with the platinum drugs in the respective resistant cell lines and enhances the levels of apoptosis. VS-43 impairs the ‘unhooking’ of the platinum-induced DNA interstrand crosslinks, and potentiates the DNA damage response, providing a mechanistic insight for the observed resensitisation.

L39

Oxaliplatin reacts with DMSO only in the presence of water. Impact on drug combination studies

Varbanov, Hristo; Ortiz, Daniel; Höfer, Doris; Menin, Laure; Galanski, Markus; Keppler, Bernhard; Dyson, Paul

Cisplatin ($[Pt(NH_3)_2Cl_2]$), carboplatin ($[Pt(NH_3)_2(CBDCA)]$) and oxaliplatin ([Pt(DACH)(Ox)]) are amongst the most effective and widely used anticancer drugs, which continue to be investigated in numerous preclinical and clinical studies[1].
Herein, we show that despite being stable in pure water and in pure DMSO, oxaliplatin reacts rapidly in a mixture of the two solvents, forming mono-DMSO adducts. Furthermore, the reactivity of the clinically applied Pt(II) drugs in different water/DMSO and PBS/DMSO mixtures, as well as the nature of the species formed were thoroughly investigated by means of MS, NMR and RP-HPLC techniques[2]. The outcome of these studies provides new insights into the solution chemistry of second and third generation platinum drugs and would be of high relevance for further formulations or combination studies where mixtures of water and DMSO are in use. In this light, examples for interactions between non-platinum drugs and oxaliplatin taking place under physiologically relevant conditions (PBS, pH 7.4, 37 °C) in the presence of DMSO are discussed.

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IL21

Metal-tagging strategy for PTMs Analysis

Liang, Yong; Tang, Nannan; Yang, Limin; Wang, Qiuquan

Protein post-translational modifications (PTMs) are important processes of life, being responsible for functional diversification of a protein. After the development of chemoselective and biospecific element/isotope-tagging strategy for targeted protein biomarkers, we have been recently interested in the analysis of PTMs. I will talk about two examples of what we have done in this area. One is 'specific and absolute quantification of phosphotyrosine' via a Ga-tagging and Tyr-phosphatase mediated strategy; the other is 'imaging and quantification of cell surface sialic acids' through a metabolism-based click-mediated platform.

you18

Elemental labelling for addressing peptides and proteins post-translational modifications: the formation of cysteine sulfenic acid

Sharar, Mona; Linscheid, Michael; Montes-Bayon, Maria

The formation of cysteine sulfenic acid (SA) is considered a transient state for thiol oxidation in living organisms that can either be reduced back and bind with different residues such as glutathione (-GSH) or can be over-oxidized by reactive oxygen species (ROS) leading to the formation of sulfinic and sulfonic acids. As any disturbance in oxidation is correlated to age-related diseases such as cancer and Alzheimer's disease, the detection of SA transient state formed a sensor for such redox- mediated events.Herein, we provide a new strategy for the highly sensitive and specific detection of SA in peptides and proteins using alkyne β-ketoester (KE) previously linked to a lanthanide (Ln)-containing chelator (Ln-DOTA, where DOTA is 1,4,7,10-tetraazacyclododecane-1,4,7,10- tetraacetic acid).SA was generated by hydrogen peroxide (H_2O_2) to mimic oxidative events produced in living cells by ROS and was detected by the prepared compound Ln-DOTA-KE. Molecular mass spectrometry (ESI-MS) and inductively coupled plasma (ICP-MS) have been used to monitor the formation of SA linked to Ln-DOTA-KE. Isotopic dilution analysis (IDA) was used for the absolute quantification of labelled SA in protein samples using an isotopically enriched Nd tracer. Satisfactory quantification results were observed for SA labelling with the Ln-DOTA-KE.

you19

Novel antibody tagging strategy using lanthanide loaded NHS-DOTA-ester for the application in highly selective LA-ICP-MS-based immunoassays

Herrmann, Antje Jutta; Jakubowski, Norbert; Schwab, Karima; Lauer, Dilyara; Theuring, Franz; Mueller, Larissa

Using metal tagged antibodies for multiplex immunoassays is an important aspect in the research field of bioanalytics. Various tagging strategies have been published, however the formed antibody conjugates were described only rudimentarily. We present an inexpensive, simple and fast DOTA-NHS-ester tagging strategy and the characterization of the conjugates. The monoclonal anti-beta-actin-antibody was used as model system and was modified with the bifunctional ligand DOTA-NHS-ester complexing monoisotopic lanthanide ions. In contrast to the antibody tagging with other tags based on maleimide chemistry the DOTA-NHS-ester reacts with unprotonated amino groups instead of free sulfhydryl groups. The great advantage of this is that a partially reduction of the antibody is not required and therefore the antibody remains intact. The characterization of the conjugates by different analytical techniques demonstrates that no antibody fragments are formed. A mean tagging degree of 8 tags/antibody was determined. The applicability of this tagging strategy is shown in first results of an Alzheimer's disease study in a mouse model. We have chosen three different NHS-DOTA-ester tagged antibodies for multielement LA-ICP-MS based bioimaging of mice brain tissue. The artificial introduced lanthanides as well as naturally occurring elements were analyzed simultaneously and differences between transgenic and wild-type animals are presented.

you20

Deeper insight into Fe(III) and Al(III) binding to the shuttle protein serum transferrin using ESI mass spectrometry and circular dichroism

Ott, Dorothee; Hartwig, Andrea; Stillman, Martin J.

Serum transferrin is a well-known single chain, iron-binding protein. This protein plays a key role in metal transport, primarily Fe^{3+} ions, in the blood stream. There are two binding sites, named the N-lobe and C-lobe. Sequestering Fe^{3+} is particularly important because of the possible formation of reactive oxygen species via Fenton`s reaction. Many studies have provided information on the transport of other metal ions for example Al^{3+}, In^{3+} and Ga^{3+}. Our research focuses on the competitive binding of Fe^{3+} and Al^{3+} to serum transferrin. There is considerable ongoing discussion about the toxicological impact of aluminum on humans. It has been recognized that excessive exposure towards this light metal can cause serious neurotoxicological effects. The objective of our study is to answer the question: In which way does aluminium compete with iron for the two binding sites of serum transferrin and thus negatively impact iron homeostasis? We investigated the binding mechanisms, binding site preferences and kinetics of Al^{3+} and Fe^{3+} binding to human serum transferrin both alone and in competition using circular dichroism and UV visible absorption spectroscopy. The gentle ionization method of ESI mass spectrometry was used extensively to study the metallation reactions, protein-metal structures and dynamics.

you21

Gadolinium in human brain - LA-ICP-MS to quantify the distribution of gadolinium in different brain regions

Fingerhut, Stefanie; Ann-Christin, Niehoff; Sperling, Michael; Jeibmann, Astrid; Paulus, Werner; Niederstadt, Thomas; Allkemper, Thomas; Heindel, Walter; Holling, Markus; Karst, Uwe

Due to its paramagnetic properties resulting from seven unpaired f-electrons, Gd is frequently applied in magnetic resonance imaging examinations. Due to the acute toxicity of free Gd^{3+}, ligand ions based on polyaminocarboxylic acids are used to create thermodynamically stable linear or macrocyclic complexes. The highly water soluble Gd-based contrast agents (GBCAs) are known to be excreted fast and unmetabolized, mostly via the kidneys. Nevertheless, recent studies showed that Gd traces persists not only in animal but also in human brain.

To reveal the distribution of Gd within the brain of patients after GBCA administration, a laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) method for the localization and quantification of trace amounts of Gd was developed. Cryosections with a thickness of 10 µm were ablated line wise using a 213 nm Nd:YAG-laser with a spot size of 50 µm and a scan rate of 100 $\mu m \cdot s^{-1}$. For quantification, matrix-matched standards based on gelatin were prepared achieving limits of quantification (LOQ) of 7 $ng \cdot g^{-1}$. Thus, Gd was quantified in different brain regions even two years after the last GBCA-administration, contributing to a better understanding of the deposition of Gd in the body, which is currently under discussion.

you22

LA-ICP-MS imaging experiments on snap frozen tissue sections using a cooled ablation stage

Amirkhalili, Shahin; Limbeck, Andreas; Bonta, Maximilian

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a rapid and accurate analytical method for direct elemental analysis of solid samples in their native state. In the last few years this technique has been used in many different medical and biological studies specifically for imaging of metal distributions in tissue sections. [1]

Recently we have shown that snap frozen samples without fixation definitely provide the most accurate way for analysis of metal distributions in tissue samples. [2] Nevertheless, conventional LA-ICP-MS instrumentation is operated at room temperature, thus thawing of the frozen samples is required prior to measurement. This step could introduce an additional error, since trace element distributions might be altered.

In this work a Peltier cooled ablation stage is proposed, which allows analysis of element distributions in frozen tissue samples. With this setup thawing of the sample could be completely circumvented, thereby all problems associated with sample melting are avoided. Applicability of this approach has been demonstrated by the analysis of cryo-cut tissues with 10 µm thickness at a temperature of -10 °C. Obtained results were compared with the findings derived via conventional measurement of thawed and dried cryo-cut tissue samples, benefits as well as observed drawbacks will be discussed.

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you23

Efficient separations of organometallic anticancer agents in serum samples using coated capillaries for CE-ICP-MS analysis

Holtkamp, Hannah U.; Movassaghi, Sanam; Morrow, Stuart J.; Kubanik, Mario; Jamieson, Stephen M. F.; Hartinger, Christian G.

Metal-based anticancer agent development can be improved by the advance of screening methods that quickly and efficiently characterise their behaviour after administration. A study with organoruthenium complexes and HCT116 tumour cells demonstrated that accumulation is not always correlated with cytotoxic activity [1]. We contrast the cellular accumulation rates of both cisplatin and RAPTA-C at 4, 24, 48 and 72 h time intervals, measuring both the intra- and extracellular metal concentration *via* ICP-MS and then determine speciation *via* CE-ICP-MS analysis. Protein binding behaviour of metal based anticancer agents, particularly with the proteins albumin and transferrin, is considered an important influence on their transportation, selectivity, and efficacy. CE analysis using silica capillaries can be challenging due to protein interactions with the charged silanol groups. We optimised a capillary coating method using poly(vinyl pyrrolidone) (PVP), which demonstrated considerable advantages compared with polybrene, for the efficient separation and detection of metal complex-protein adducts. The use of the internal standard tris(acetylacetonato)cobalt(III) further improved the reproducibility of electropherograms and detection limits.

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you24

In vitro investiagions on oxaliplatin – a comparison study of resistant and sensitive cells

Luis Gálvez; Michaela Schwaiger; Sonja Hager, Petra Heffeter; Christian R. Kowol ;Walter Berger; Gunda Koellensperger

Oxaliplatin was successfully introduced in 2000 in the treatment of colorectal cancer where cisplatin showed to be inactive. However, oxaliplatin, like cisplatin, is not efficient in patients where resistance towards the drug is present. This resistance is correlated to a decreased intracellular drug accumulation and/or increased drug efflux mediated by thiol-containing metabolites among other resistance mechanisms [1]. In this work, we investigated key metabolic differences between sensitive and resistant cells of the cancer line HCT116, employing for that a heart-cut 2D-LC-Orbitrap which led to a wider coverage of the intracellular metabolome compared to the conventional 1D-LC [2][3]. In parallel, the drug uptake was studied by monitoring the platinum signal by FI-ICP-MS.

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you25

DNA or Protein - Capillary Zone Electrophoresis-Mass Spectrometry Rapidly characterizes Metallodrug binding Preferences

Artner, Christian; Holtkamp, Hannah U.; Hartinger, Christian G.; Meier, Samuel M.; Keppler, Bernhard K.

It is generally accepted that the cytotoxic mode of action for platinum(II) anticancer drugs is based on their binding ability to DNA [1]. In a similar way, DNA was believed to be the primary target for early ruthenium-based complexes as well [2]. However, in recent years, the therapeutic effect for ruthenium-based drug candidates is considered to be caused by interaction with proteins rather than DNA, although the exact mechanisms of action are often unknown and direct target not yet identified. A fundamental question in the process of metallodrug discovery is whether the metal complex binds preferentially to DNA or proteins which cannot be specified by classical screening assays on a molecular level so far.

We developed a novel capillary zone electrophoresis-mass spectrometry approach that allows the simultaneous characterization and quantification of the binding preferences of metal-based anticancer drugs to DNA (oligonucleotide) and protein in a competitive experiment. Moreover, we resolved metalation of DNA and protein at single-residue resolution.

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IL22

Bioanalytical Studies in the Development of Anticancer Metallodrugs

Hartinger, Christian

The design of novel metal-based cancer chemotherapeutics was for a long time driven by the DNA-targeted mode of action of platinum complexes, while the reactivity to biomolecules other than DNA has often been associated with side effects. In recent years, many exciting new metal complexes with novel modes of action have been reported and their anticancer activity was linked to selective protein interaction that may lead to improved accumulation in the tumor, higher selectivity and/or enhanced antiproliferative efficacy. The protein-targeted nature of new generations of metallodrugs has driven the development of new methods to characterize these interactions, confirm the hypothesized modes of action or identify new, previously unexplored biological targets and pathways. This is essential information to promote new metallodrugs to clinical development [1,2].

This presentation will cover the development of new methods to study the modes of action of anticancer metallodrugs at the molecular level. Their application will be discussed for selected examples.

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L40

Tuning the metabolism of cisplatin

Gailer, Juergen

Numerous *in vivo* studies have shown that the sever toxic side-effects of intravenously administered cisplatin can be significantly reduced by the co-administration of sulfur-containing 'chemoprotective agents'. Using a metallomics approach, a likely biochemical baiss for these potentially useful observations was only recently uncovered and appears to involve the reaction of chemoprotective agents with cisplatin-derived Pt-species in human plasma to form novel platinum-sulfiur complexes (PSC's). The structure of two PSC's will be presented. These findings suggest that the identification of an optimal chemoprotective agent to ameliorate the toxic side-effects of cisplatin represents a feasible research strategy to transform cisplatin into a safer and potentially more effective anticancer drug.

L41

Molecular basis of platinum based drugs' otoxicity-an in vitro study on inner ear cells

Perde-Schrepler, Maria; Valcan, Angela; Virag, Piroska; Brie, Ioana Carmen; Gurzau, Eugen S.; Gurzau, Anca E.; Maniu, Alma Aurelia; Fischer-Fodor, Eva

Cisplatin, Carboplatin and Oxaliplatin are highly effective chemotherapeutic agents; however, side effects have been associated with their use, such as progressive, irreversible neurosensorial hearing loss. The mammalian copper membrane transporter 1 (CTR1) plays a significant role in the cellular cisplatin uptake and their cytotoxicity is directly related to their cellular uptake. We compared the toxicity of these drugs when administered *in vitro* to HEI-OC1 cells derived from the cochlea (from Charles River Laboratory), with or without pre-treatment with an inhibitor of CTR1. The same molar concentrations of platinum compounds had different toxic effects on HEI-OC1 cells; the blockage of CTR1 led to a decreased toxicity for all the compounds. The platinum cellular uptake, measured by atomic absorption spectrometry, was dose and time- dependent for all three compounds and correlated with the toxicities of the substances, while the expression of ABCB1 multidrug resistance protein was not always correlated to the dose. Different patterns of Poly-ADP-Ribose Polymerase 1 (PARP-1) activation were observed in HEI-OC1 exposed to the three platinum-based drugs. The CTR1 inhibition reduced significantly the uptake of all the compounds; thus we emphasize the possibility of administration of CTR1 and PARP-1 inhibitors during cisplatin therapy as otoprotective strategy.

L42

Unravelling the Reactivity of Gold-based Metallodrugs with Zinc Finger Domains and G-Quadruplex DNA by Mass Spectrometry

Meier, Samuel Matthias; Casini, Angela; Wenzel, Margot

Organometallic gold-based compounds are promising scaffolds for the design of both anticancer agents and of chemical probes for imaging. Among the several families being discovered, cyclometallated gold(III) and gold(I) bis-N-heterocyclic carbenes (NHCs) target zinc finger domains of PARP-1 or telomeric G-quadruplex (G4) DNA, respectively. Notably, PARP-1 is an enzyme that marks DNA mismatches for repair. Telomeric G4s are guanine-rich DNA secondary structures at chromosomal end that are associated with cancer immortality by over-expressing telomerase, whose activity can be inhibited by stabilizing G4s.

It emerges structural variations of gold compounds dictate their reactivity towards biomolecules, but also the binding sites on target biomolecules. For example, the cyclometallated gold(III) compound [$Au^{III}Cl_2$(*C,N*-2-benzylpyridine)]Cl is shown to selectively target the $CysHisCys_2$ zinc finger motif of PARP-1 over transcription factor-like zinc finger domains (Cys_2His_2 motif). Moreover, the gold(I)-based bis-NHC [Au^{I}(9-methylcaffein-8-ylidene)$_2$]BF_4 selectively and potently stabilizes telomeric G4 DNA over duplex DNA. Thus, the reactivity and selectivity of different organometallic compounds were probed by electrospray mass spectrometry using mixtures of biologically relevant structural domains. The results show promise to identify drug targets for gold compounds and to characterize metallodrug speciation in biological systems.

IL23

Bioinorganic Chemistry of Amyloidogenic Peptides

Faller, Peter

Amyloid plaques are a halllmark of Alzheimer's disease (AD). They consist mainly of the aggregated peptide amyloid-β (Aβ). Amyloid plaques contain high amount of metal ions (Cu, Z, and Fe). Copper and zinc ions are bound directly to Aβ. In vitro Cu-Aβ complexes are able to catalyze the production of hydrogen peroxide and hydroxyl radicals, and could hence contribute to the oxidative stress observed in AD.

Monomeric Aβ has no well defined 3D structure, and hence is a so called "intrinsically disordered protein". Even after Cu(I)- and Cu(II)-binding Aβ is still disordered. Different forms with different coordination chemistry of both Cu(I) and Cu(II) complexes have been proposed. This raised the question of the relation between the structure of the Cu-Aβ and the reactivity, in particular concerning the production of reactive oxygen species (ROS).

Recent advancements in the coordination chemistry, the mechanistic understanding of the ROS production and metal-exchange reactions by Cu-Aβ with biomolecules will be reported.

L43

The ß-site Amyloid precursor protein cleaving enzyme beta-secretase (BACE1) modulates intracellular copper homeostasis

Multhaup, Gerhard; Liebsch, Filip

The beta-secretase (BACE1) initiates processing of the amyloid precursor protein (APP) into Aβ peptides. BACE1 is classified as unique amongst aspartic acid proteases through its single transmembrane sequence (TMS) that links the ectodomain to a short C-terminal cytosolic tail. The literature has described BACE1 as a copper binding protein and the native stoichiometry of BACE1 as monomeric, dimeric, and/or oligomeric. The cytosolic domain of BACE1 interacts with CCS, the copper chaperone of Cu/Zn-superoxide dismutase SOD1. We found that full-length BACE1, independent of the subcellular localization, exists as trimers in human cells by using two different methods, single-molecule fluorescence and *in vitro* cross-linking experiments with photo-activatable unnatural amino acids. Trimerization is driven by the BACE1 transmembrane sequence, which encompasses amino acids 458-478, and harbors a conserved sulfur-rich $M_{462}xxxC_{466}xxxM_{470}$ motif with a low-affinity Cu(I) binding site. While physiological significance of copper binding to BACE1 had remained uncertain, we find that cytosolic copper levels are regulated by endogenous BACE1, similarly to the copper-transporting ATPase Atp7b, which exports copper out of the cells, e.g., hepatic copper into the bile. Cys466 in the center of the BACE1 TMS is the critical residue that accounts for the modulation of cytosolic copper concentrations. Typically, Atp7b exports copper from the cytoplasm into the lysosomal lumen for further exocytosis. As Atp7b in renal cells, BACE1 could maintain cytosolic copper levels in brain by transporting copper to the ER, Golgi, TGN, endosomes, and/or lysosomes. In summary, the results from biochemical and biological assays suggest a role for endogenous BACE1 in intracellular copper compartmentalization by transferring cytosolic copper to intracellular compartments with leaving the overall cellular copper content unaltered. Thus, our results provide novel insight into the atypical interactions between copper and BACE1 and its non-enzymatic activities.

L44

Extracellular Zn2+ is essential for amyloid ß1-42-induced cognitive decline in the normal brain and its rescue

Takeda, Atsushi

Brain amyloid-$\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) accumulation is considered an upstream event in pathogenesis of Alzheimer's disease. Accumulating evidence indicates that other neurochemical changes potentiate the toxicity of this constituively-generated peptide. Here we report that the interaction of $A\beta_{1\text{-}42}$ with extracellular Zn^{2+} is essential for in vivo rapid uptake of $A\beta_{1\text{-}42}$ and Zn^{2+} into dentate granule cells in the normal rat hippocampus. The uptake of both $A\beta_{1\text{-}42}$ and Zn^{2+} were blocked by CaEDTA, an extracellular Zn^{2+} chelator, and by Cd^{2+}, a metal that displaces Zn^{2+} for $A\beta_{1\text{-}42}$-binding. Furthermore, we examined the impact of $A\beta_{1\text{-}42}$ drawing extracellular Zn^{2+} into the cell upon in vivo LTP at perforant pathway-dentate granule cell synapses, where the recording area was locally pre-injected with $A\beta_{1\text{-}42}$. $A\beta_{1\text{-}42}$-induced attenuation of LTP was rescued by co-injection of $CdCl_2$. $A\beta_{1\text{-}42}$ injected into the dentate granule cell layer of rats induced rapid memory disturbance that was also rescued by co-injection of $CdCl_2$. The present study supports blocking the formation of Zn-$A\beta_{1\text{-}42}$ in the extracellular compartment as an effective preventive strategy for Alzheimer's disease.

L45

Identifying specific metalloproteomic changes in dementia with Lewy bodies using HPLC-ICP-MS

McAllum, Erin; Hare, Dominic; Finkelstein, David

Biologically-relevant metals have been implicated in neurodegeneration, stretching back nearly 100 years to when iron was first identified to be abnormally distributed in the Parkinson's disease brain. Metals have subsequently been associated with multiple neurodegenerative diseases, yet most studies have focussed primarily on measuring changes in metal levels and not the relationship between metals and the biochemical factors that determine their neurological function. Thus, understanding the relationship between metals and their protein ligands is essential to elucidate how metal imbalances participate in neuropathology. Online hyphenation of HPLC to ICP-MS offers a relatively simple and effective means of assessing metal-protein binding of soluble proteins, allowing identification of discrete changes that may be masked by measurement of total metal levels. We combined chromatography and element-specific detection to profile soluble metalloproteins in dementia with Lewy bodies (DLB), the second most common form of dementia. In the disease-affected entorhinal cortex, metal levels were not universally altered in DLB compared with controls; rather, changes were associated with specific copper-binding metalloproteins. No changes were observed in unaffected brain regions. Identification of these metalloproteins will allow investigation of how their altered binding of metals may contribute to disease, potentially leading to targeted therapies correcting aberrant metalloprotein function.

IL24

The case for imaging and speciation of metals in neurodegenerative disorders

Collingwood, Joanna

Disrupted metal ion homeostasis is indicated in many neurodegenerative disorders, with numerous reports of metal ions and metal-binding proteins present in altered concentrations in particular cells or brain regions, and associated with neuropathological hallmarks. Better mechanistic understanding of the processes responsible for these observed changes is required to utilize these observations for improved diagnosis and treatment.

Techniques that permit the spatial distribution and the chemical and mineral state of transition metals in brain tissue, and technique applications, are considered **[1]**. Examples include X-ray spectromicroscopy with its high spatial and energy resolution **[2],** and SQuID magnetometry which enables evaluation of mineralized content in tissues **[3]**. Speciation of metals and minerals in the brain, to gain understanding of the processes responsible for disease-specific differences in distribution and form, could support earlier clinical diagnosis in future. The minerals serve as endogenous contrast agents in MRI, and non-invasive clinical measures of metal ion status will be important to facilitate therapies to alleviate metal-associated toxicity.

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L46

Imaging of Intracellular Fatty Acids by a Single Element Labeling

Shimura, Mari; Shindou, Hideo; Szyrwiel, Lukasz

Fatty acids are taken up by cells and incorporated into complex lipids, such as neutral lipids and glycerophospholipids. Fatty acids have been well studied by mass spectrometry, which has indicated their essential relationship to cellular functions and diseases. However, intracellular imaging of fatty acids and their derivatives has not been successful due to labeling difficulties with large fluorescence molecules and insufficient resolution by conventional methods. Here, we developed a method for labeling fatty acids with bromine (Br) and applied scanning X-ray fluorescence microscopy (SXFM) to obtain intracellular elemental mapping data with submicrometer resolution. Mass spectrometry showed that cells took up Br-labeled fatty acids and metabolized them, mainly, into glycerophospholipids in CHO cells. Most Br signals accumulated in the perinuclear region. Br signals colocalized likely at the endoplasmic reticulum and the Golgi, and less at mitochondria. Higher resolution revealed the distribution of numerous Br spots (< φ1 μm) in the cytoplasm. The current method enabled successful visualization of intracellular Br-labeled fatty acids. Single-element labeling combined with SXFM technology will provide a new tool to identify dynamic changes in fatty acids.

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L47

High-resolution LA-ICP-MS imaging of lanthanide-based (hybrid) labels with low-dispersion aerosol transport systems

Van Malderen, Stijn J. M.; Van Acker, Thibaut; Buckle, Tessa; Vanhaecke, Frank

Laser ablation-ICP-mass spectrometry (LA-ICP-MS) is a tool for elemental imaging which enables labelling strategies based on metal tags. Although the image resolution of LA-ICP-MS (~1 µm) still falls short of that obtained in confocal fluorescence microscopy (CFM) immunohistochemistry, LA-ICP-MS has attractive features: high multiplexing potential (>100 antibody tags simultaneously), low background (especially for lanthanide tags) and a dynamic range of $>10^5$. In order to establish a direct link between CFM and mass spectrometry, a hybrid imaging label containing a Cy5 fluorescent dye and a ^{165}Ho-coordinating DTPA chelate was coupled to the CXCR4 targeting peptide Ac-TZ14011, and was administered intravenously in tumor-bearing mice. Five µm thick sections of excised brain, tumor, and liver were imaged using a 193 nm ArF* Analyte G2 LA-system, equipped with the ARIS (Aerosol Rapid Introduction System), coupled to an XSeries 2 ICP-MS unit. ARIS enables a pixel acquisition rate $>10^2$x higher than conventional systems. The localization and receptor affinity of the tracer in mouse tumor cells observed by CFM was reflected by the LA-ICP-MS image. Furthermore, cellular localization of a (^{165}Ho-)hybrid-GFP-Cetuximab-antibody label in stained EGFR-overexpressing MDAMB468 cells was evaluated by CFM and LA-ICP-MS imaging using a 2 µm beam width.

L48

Quantitative Bioimaging by LA-ICP-MS for Studying the Migration of Silver from Silver-coated Endoprostheses

Sperling, Michael; Großgarten, Mandy; Niehoff, Ann-Christin; Jeibmann, Astrid; Paulus, Werner; Karst, Uwe

Osseous defects, which result from the removal of a tumor, are reconstructed with mega-endoprostheses. Especially in case of oncological patients, post-operative infection rates of up to 35% occur. To circumvent bacterial infection, silver is used as implant coating due to its antimicrobial properties. However, silver also affects human cells when exceeding a cytotoxic concentration level. Our present work focusses on laser ablation-based elemental bioimaging of brain as possible target organ of patients with silver-coated endoprostheses. In detail, thin brain sections of a patient with three silver-coated endoprostheses are investigated. Quantification of silver in five brain areas is performed using matrix-matched standards, revealing a hundredfold increased silver concentration compared to native brain tissue. Additionally, high resolution LA-ICP-MS is performed to allocate the silver deposits to relevant cell types stained with a gold-labelled secondary antibody. Overlays of the elemental distribution images of both 107Ag and 197Au enable the evaluation of the correlation of silver signals to marked cell type. Besides an accumulation in endothelial cells of blood vessels, silver can be co-localized to neurons and glial cells, demonstrating its ability to cross the blood-brain-barrier. Thus, LA-ICP-MS empowers to obtain an improved understanding of transport, distribution, and deposition of silver in biological tissues

L49

A Novel Absolute Quantitative Imaging strategy of Iron, Copper and Zinc in Biological Tissues by Isotope Dilution Laser Ablation ICP-MS

Feng, Liuxing

In this study, we reported a novel quantitative imaging strategy for biological thin section based on ID-LA-ICP-MS. To distribute the enriched isotope spikes on tissue section homogeneously, a "border" was built just around the section by using water-repellent material. In this way, the spike droplet with specific amount was confined to stay on the tissue for isotope exchange. To obtain optimal ID-LA-ICP-MS conditions, laser energy, LA scan speed, material of "border" and different isotope exchange conditions including isotope exchange time and solvent of spike, were investigated. The *in-house* standard, which was prepared by homogeneous mouse brain of control group, was used to validate the ID-LA-ICP-MS approach and good agreement with the bulk analysis was achieved. After method validation, the quantitative imaging of Fe, Cu and Zn in mouse brain of Alzheimer’s Disease (AD) were measured by the improved methodology. Assessment of the method for quantitative imaging of AD mouse brain was undertaken by comparison of the LA-ICP-MS data with that obtained by micro-XRF. Moreover, comparative analysis of elements distribution and immunohistochemical markers in AD mouse brain was also carried out to demonstrate that the proposed methodology is robust to investigate the correlation of biomarker heterogeneity and elements distribution.

L50

An instrumental approach to improving trace metal determinations for metallomics analysis

Spence, Bill; Green, Damon

As the field of Metallomics evolves, then the instrumentation required to accurately determine the role that low level metals and nano-particles play in life sciences must also evolve. In this paper a selection of novel applications are discussed to demonstrate how the latest developments in analytical hardware can enable the measurement of sub-cellular and single-micron distribution / concentration of trace elements. In particular, the use of laser ablation (LA) instrumentation coupled with ICP-MS detection will be described for these applications.

Challenges in this LA-ICP-MS approach include accurate quantification and system optimisation parameters such as mass spectrometer type, ablation sample chamber design, spectrometer dwell time, scan speed, laser spot size etc.

Work derived from ongoing collaborative research projects using ICP-TOF-MS (Time of Flight), with selected academic institutions will be presented to illustrate leading trends in this field.

L25

Ruthenium-based drug interactions with lipid turnover in cancer cells revealed by correlative NanoSIMS and TEM imaging

Legin, Anton; Schintlmeister, Arno; Eckhard, Margret; Reipert, Siegfried; Jakupec, Michael; Wagner, Michael; Keppler, Bernhard

Background. KP1339 is a ruthenium-based investigational anticancer drug that has successfully accomplished the first stage of clinical trials showing activity in gastro-intestinal neuroendocrine and other solid tumors with minor side effects. The mechanisms lying behind its cytotoxicity in cancer cells are incompletely understood. **Methods**. The localization of KP1339 in treated colon cancer cells was analyzed by nano-scale secondary ion mass spectrometry (NanoSIMS) and correlated to transmission electron microscopy (TEM) images, based on an approach previously established on platinum-containing samples (*1*). **Results**. Ruthenium sensitivity achieved with a cesium ion source was sufficient for negative secondary ion detection and allowed to visualize the localization within different subcellular compartments. Correlative distribution analyses revealed ruthenium accumulation in voluminous organelles morphologically identified as autophagosomes. Drug-induced phospholipidosis (abnormal accumulation of multi-lammellar bodies inside lysosomal structures) was observed as a consequence of KP1339 treatment, with specific traits that will be discussed. **Conclusions**. Conspicuous effects of KP1339 on the metabolism of lipid membranes were demonstrated paving new ways for the research of lipid turnover. **Acknowledgment**. This work was supported by the Austrian Science Fund (FWF) project P27749.

1. A. A. Legin *et al.*, *Chem Sci*, **7**, 3052-3061 (2016).

L51

Importance of Reference Measurement Procedures in Diagnostic of Alzheimer's Disease

Swart, Claudia; Köllensperger, Gunda; Goenaga-Infante, Heidi; Raab, Andrea

Neurodegenerative diseases such as Alzheimer's disease are one of the major challenges for the health care systems with currently over 6 million affected people in the European Union. This number will likely double in the next 20 years due to the aging population. According to epidemiological data, only half of the patients are currently identified, and then often only in advanced stages of the disease. One of the main reasons might be the lack of accuracy of the results for the biomarkers used, namely β-amyloid peptide 1-42, total τ-protein, hyperphosphorylated τ-protein and their ratios in cerebrospinal fluid (CSF). It has been demonstrated that in the prodromal stage the accuracy of single biomarkers is only about 85% leading to a considerable amount of false positive/negative diagnoses. In the EMPIR project ReMIND reference measurement procedures are developed to improve the comparability and reliability of the determination of existing biomarkers. Furthermore, the influence of certain metals and related metalloproteins on the progress of the disease is investigated to identify potential new biomarkers in blood, serum and CSF.

Acknowledgement: This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

L52

A New Approach to Microns-Resolution Trace Element Mapping at PPM Sensitivity for Metallomics

Yun, Wenbing; Lewis, Sylvia; Stripe, Benjamin; Lau, SH; Lyon, Alan; Reynolds, David; Spink, Richard

Synchrotron micro x-ray fluorescence (microXRF) is a powerful chemical analysis technique that enables ultratrace-level analysis (e.g. sub-ppb) at resolutions from microns-scale to tens of nanometers. In recent years, the technique has become vital particularly the emerging field of metallomics. Laboratory-based microXRF has also developed, but is bottlenecked by the brightness of laboratory x-ray sources and the limited efficiency of available x-ray optics.

We have developed a patented breakthrough laboratory x-ray analytical microscope, the AttoMap, which provides capabilities comparable to synchrotron microXRF systems for tissue and cellular-level mapping of trace elements and nanoparticles in tissue. A key enabling technology of the AttoMap is its patented x-ray source featuring a target comprised of fine microstructured metal x-ray emitters embedded in a diamond substrate. The design of the target enables rapid thermal dissipation and linear accumulation of x-rays. Used in combination with the source is our proprietary x-ray optics, which concentrates the high brilliance of the source onto a small focal spot.

Here we will present recent results from the x-ray analytical microscope. Applications include high throughput mapping of nanoparticle drugs in tissue, trace elements in mutant and hyperaccumulating plant species, and metal concentrations diseased tissue.

L53

Tracking arsenic binding proteins in live leukemia cells by an organoarsenic probe

Li, Hongyan; Hu, Xuqiao; Koohi-Moghadam, Mohamad; Yang, Xinming; Sun, Hongzhe

Arsenic trioxide is a highly effective drug to treat acute promyelocytic leukemia (APL), and has also shown significant promise against many other tumors. However, the molecular mechanism of arsenic therapy is not well understood. The PML fusion proteins in APL (PML-RARα) has been thought originally to be the target of arsenic trioxide, recent studies suggest that a number of proteins might also serve as targets of arsenic trioxide. Comprehensive identification of these proteins is essential for understanding therapeutic efficacy of the drug.

Here we develope a new organoarsenic probe that can rapidly enter cells to target arsenic binding proteins, leading to significant fluorescent enhancement upon photoactivation of the arylazide of the probe. Upon UV activation, the probe is anchored to the labelled proteins, allowing downstream protein identification *via* proteomic approach. Using this probe, a numbers of arsenic binding proteins were identified in both NB4 and HL60 cells. Subsequently these proteins were functionally categorized by Gene Ontology (GO) enrichment analysis, providing insights into molecular mechanism of action of arsenic trioxide.

Acknowledgments: This work is supported by the Research Grant Council of Hong Kong (17304614, 703913) and the University of Hong Kong.

L54

Natural Fe isotope fractionation in an intestinal Caco-2 cell line model

Florez, Maria R.; Anoshkina, Yulia; Costas-Rodríguez, Marta; Grootaert, Charlotte; Van Camp, John; Delanghe, Joris; Vanhaecke, Frank

An improved insight into Fe isotope fractionation effects in the human body is required for enabling full exploitation of whole blood/serum Fe isotopic analysis for assessing an individual's Fe status. Fe isotope ratio data obtained from an *in vitro* enterocyte model were used to study the isotope fractionation effects accompanying Fe uptake and transport fluxes through differentiated Caco-2 cells. Bi-cameral experimental setups were optimized and Fe ascorbate was offered to the cells as a source of bioavailable Fe. Under optimized conditions, low blank levels and suitable experimental repeatability and reproducibility were attained. Fe isotopic analysis was performed in cells, apical and basal solutions *via* multi-collector ICP-mass spectrometry. After 24 hours of Fe exposure, isotope fractionation effects were marked, with apical solutions enriched in the heavier Fe isotopes, while cells and basal solutions exhibited lighter Fe isotopic composition. Results show that both Fe uptake and transport mechanisms are accompanied by Fe isotope fractionation in favor of the light isotopes. The results obtained are in good agreement with the conclusions from previous *in vivo* and *ex vivo* studies, suggesting the validity of *in vitro* cell culture models as complementary tools to study Fe pathways through intestinal cells.

IL25

Mechanisms of Zinc Metallostasis in Bacterial Pathogens

Giedroc, David

First-row late *d*-block metals from Mn to Zn play varied and distinct roles in cellular metabolism. In bacterial pathogens, metalloregulation of transcription underscores physiological adaptation to host-mediated transition metal starvation and toxicity, required to maintain metal homeostasis. In zinc (Zn) homeostasis, a pair of metal-sensing transcriptional repressors regulate the transcription of metal uptake and efflux transporters, where Zn allosterically activates or inhibits DNA operator-promoter binding. We will discuss metal-mediated allostery in multi-domain transcriptional regulators that maintain Zn homeostasis in the Gram-positive respiratory pathogen, *Streptococcus pneumoniae*. These are the Zn uptake regulator is the multiple antibiotic resistance repressor (MarR)-family protein, adhesin-competence repressor (AdcR), while the efflux regulator is a novel tetracycline repressor (TetR) family protein, streptococcal zinc activator (SczA). We will also describe recent efforts to understand the global physiological response to host protein (calprotectin)-mediated Zn starvation in *Acinetobacter baumannii.* Part of this response involves the upregulation of the expression of a G3E P-loop superfamily GTPase, ZigA, which we hypothesize functions as a Zn metallochaperone under conditions of extreme Zn starvation. The results of our efforts to identify target(s) of ZigA-dependent activity using a number of complementary ‘omics approaches will be discussed. Supported by the US NIH (GM118157).

L55

Structure and Function of Heme Transport Proteins in Corynebacterium glutamicum

Aono, Shigetoshi; Muraki, Norifumi

Heme uptake machinery of Corynebacteria including *Corynebacteria glutamicum* and *Corynebacterium diphtheriae* consists of heme binding/transport proteins, HtaA and HtaB, and the ABC-type heme transporter HmuTUV. In this work, we have studied the structural and functional relationships of HtaA and HtaBin *Corynebacterium glutamicum*.

HtaA consists of two homologous conserved regions (CRs) in the N- and C-terminal regions that are responsible for heme binding. We have determined the crystal structures of the N-, and C-terminal CR of HtaA (HtaA-N and HtaA-C, respectively) and HtaB at the resolution of 2.0, 1.3, and 1.7 Å, respectively. HtaA-N consists of 11 beta strands and two short alpha helices and accommodates one heme molecule with Tyr58 located in the first a helix as the heme axial ligand. Tyr58 forms a hydrogen bond with His111. A heme propionate forms hydrogen bonds with Ser54 and Tyr201. Heme is accommodated in an open pocket formed by hydrophobic amino acid residues.

HtaA-C and HtaB show similar global structures to HtaA-N. The key residues for heme-binding and recognition including the axial ligand of heme and residues involved in the hydrogen bonding interactions with heme are conserved among HtaA-N, HtaA-C, and HtaB.

L56

Inorganic Complexes for Applications in Biology: Mn-Complexes as SOD mimics from Design to Evaluation in Cells

Policar, Clotilde; Mathieu, Emilie; Bernard, Anne-Sophie; Quévrain, Elodie; Delsuc, Nicolas; Lai, Barry; Seksik, Philippe; Masliah, Joelle

Oxidative stress is known to be involved in inflammation and in inflammatory bowel diseases (IBD) for which antioxidative defenses are weakened. In particular, superoxide dismutases (SOD), redox active metalloproteins that protect the cell against oxidative stress, are under expressed or in an inactive form. SOD mimics are low molecular weight complexes able to reproduce the superoxide dismutation activity of SOD. [1-4] In this talk, we will describe the study of a Mn complexe SOD mimic (Mn1) mimicking superoxide dismutase (SOD), an using an approach in inorganic cellular chemistry.[5] After incubation of Mn1 in intestinal epithelial cells (HT29-MD2), we have combined the investigation of Mn1 intracellular speciation using mass spectrometry and of its quantification and distribution using electron paramagnetic resonance and spatially resolved X-ray fluorescence with evaluation of its biological activity. DNBS-induced colitis in mice was used to investigate its activity in vivo. Interestingly, we have shown that Mn1 exerts an intracellular antiinflammatory activity, remains at least partially coordinated, with diffuse distribution over the whole cell, and functionally complements mitochondrial MnSOD.

L57

Synthetic studies for bioremediation enzymes

Chavez, Ferman

Bioremediation enzymes containing His_3 metal binding sites include gentisate 1,2-dioxygenases (GDO), salicylate 1,2-dioxygenase (SDO), 3-hydroxyanthranilate 3,4-dioxygenases (HAD), and 1-hydroxy-2-naphthoate dioxygenase (HNDO). Their reactivities include scission of aromatic rings. These enzymes are known to reversibly nitric oxide. In our efforts to synthesize structural and functional models for these enzymes, we have synthesized [Fe(T1Et4iPrIP)$(OTf)_2$] (T1Et4iPrIP = tris(1-ethyl-4-isopropyl-imidazolyl) phosphine) which reversibly binds nitric oxide to afford [Fe(T1Et4iPrIP)(NO)(THF)(OTf)](OTf) (**1**), the first example of a 6-coordinate $\{FeNO\}^7$ *S* = 3/2 complex containing a linear Fe-N-O group and exhibiting the highest v(NO) for compounds in this class (1831 cm^{-1}). DFT studies reveal an enhanced degree of ß electron transfer from the π*(NO) to the iron d orbitals accounting for the large stretching frequency. Reaction of **1** with 2 equiv water affords [Fe(T1Et4iPrIP)(NO)(H_2O)]$(OTf)_2$ (**2**) which is more electron rich and has a lower v(NO) (1791 cm^{-1}). Model studies for the enzyme 2,4'-dihydroxyacetophenone dioxygenase (DAD) will also be included. DAD has a nonheme iron coordinated to 3 His ligands and is capable of cleaving alkyl groups. We will discuss the reactivities of model compounds related to DAD.

L58

Structure and Function of Non-Covalent Weak Interaction in Blue Copper Protein

Kohzuma, Takamitsu; Yamaguchi, Takahide; Sakai, Chihiro

Noncovalent weak interactions play important roles in biological systems[1]. In particular, such interactions in the second-coordination shell of metal ions in proteins may modulate the structure and reactivity of the metal ion site in functionally significant ways.

Recently, we have demostrated the perturbation of weak non-covalent interaction on the structure and properties of copper site in a blue copper protein. The weak interaction around a copper coordinated histidine (His81) imidazole ring in the second coordination sphere of a blue copper protein, pseudoazurin significantly modulates the properties of pseudoazurin (Paz). The π-π interaction between His81 and aromatic amino acids substitutant at Met16 position remarkably enhance the axial character at the active site.[2] The introdcution of aliphatic amino acids demonstated the significant decreasing of the axial componet. Here we also would like to add the unique structure and properties of Met16Gly, and would like to report more detailed and general features of non-covalent weak interaction in blue copper protein.

1. S. K. Burley and G. A. Petsko, Science, 229, 23 (1985).

2. T. Yamaguchi, K. Akao, A. Takashina, S. Asamura, M. Unno, R. K. Szilagyi, T. Kohzuma, *RSC Adv*., **6**, 88358-88365 (2016).

you26

Computational and spectroscopic studies toward design of chlorophyll derivatives for photodynamic therapy

Zhang, Angel; Stillman, Martin

Photodynamic therapy (PDT) uses light, molecular oxygen, and a photosensitizer to selectively destroy cancerous cells by the production of singlet oxygen. Compared with traditional cancer treatments like chemotherapy and radiation therapy, PDT has a higher degree of selectivity and fewer side effects. The efficacy of PDT depends on a number of factors, one being the depth of light penetration through tissue, with the optimal wavelength for tissue penetration ranging from 700 to 900 nm. Porphyrin-based photosensitizers are promising due to their tunable long wavelength absorption between 600 and 800 nm, their tumor localizing properties, and their ability to generate singlet oxygen following absorption of light. Chlorophylls are naturally occurring chlorins that exhibit strong absorption bands within this optimal wavelength range. This suggest that chlorins can be designed with optimal PDT properties. Absorption and magnetic circular dichroism (MCD) spectral data were obtained for a range of synthetic chlorins and computational studies were carried out to provide further insight into their electronic structure. Computational methods provided an understanding of the spectroscopic data. A significant result is that these methods could also be used to predict the electronic structure of porphyrins before actual synthesis, allowing the tuning of molecular designs.

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POSTER 1

A new analytical strategy for quantification of gene-specific methylation: application to cisplatin-sensitive and cisplatin-resistant ovarian cancer cell lines analysis

Blanco-González, Elisa; Espina, Marta; Iglesias-González, Tamara; Montes-Bayón, María; Sierra, L. María

The most widely studied epigenetic modification of the human genome is the methylation of cytosine. Aberrrant DNA methylation has been observed in many different malignancies and is frequently described as global hypomethylation combined with local hypermethylation. Specifically, transcriptional silencing of distinct DNA repair and apoptosis associated genes by hypermethylation has been linked with cisplatinum resistance in numerous cancers, including ovarian. Therefore, the determination of the methylation status of a selected gene could be a potential biomarker to predict and monitor the response to chemotherapy treatment with cisplatin.

In this work, we present a new analytical strategy for quantification of the methylation status of specific genes, which is based on the hybridation capture of the sequence of interest from enzymatic fragmented genomic DNA by using biotinylated oligonucleotides and streptavidin-coated magnetic beads. The captured sequence can be released by simple denaturalization for subsequent analysis of their methyl cytosine content by HPLC-UV/VIS [1]. The developed methodology was applied to study promoter methylation status of four selected genes involved in apoptosis and three DNA repair genes in cisplatin-sensitive and cisplatin-resistant ovarian carcinoma cell lines.

[1] T. Iglesias, M. Espina, M. Montes-Bayón, L. M. Sierra and E. Blanco-González. Anal. Bioanal. Chem. 407 (2015)2423–2431

POSTER 2

Determination of Fe natural isotopic composition in yeast and plants by MC-ICP-MS - focus on sample preparation

Kwiatkowski, Piotr; Berail, Sylvain; Barre, Julien; Baltrons, Oriol; Szpunar, Joanna; Donard, Olivier; Mari, Stephane; Dirick, Leon

The measurement of stable isotopes natural composition by MC-ICP-MS is now an emerging tool for metallomics.
In particular, it can be applied to better understand Fe uptake mechanisms of different organisms. However, MC-ICP-MS requires careful sample preparation including critical purification steps. This work presents the optimization and validation of sample preparation protocol for Fe isotopic analysis in yeast and plants by MC-ICP-MS.

Saccharomyces Cerevisiae yeast is well-characterized for its Fe acquisition. It shares some similarity to plant Fe uptake mechanisms and was thus chosen as biological model for the method development. *Arabidopsis thaliana* plants (seeds and leaves) were also investigated.

For the sample mineralization procedure, a hot-block and a High Pressure Asher (HPA) were compared; no significant difference in Fe recovery was observed. Then, Solid Phase Extraction (SPE) purification using ion-exchange resin was applied. All these steps were evaluated in terms of Fe recovery and concomitant elements separation using ICP-QMS analysis.

Iron recovery after the samples preparation and purification was higher than 90%. Iron was completely separated from concomitant elements (Cr, Ni, Mn, Zn and alkali ones). The results of $\delta^{56}Fe$ and $\delta^{57}Fe$ measurements by MC-ICP-MS in samples prepared following the developed procedure will be presented.

POSTER 3

Metallomic and metabolomic study of liver from mice Mus spretus fed with salicornia

Ramírez-Acosta, Sara; García-Barrera, Tamara; Abril, Nieves; Gómez-Ariza, José Luis

Salicornia is a halophytic plant that grows in salty marsh areas. Marshes usually receive high inputs of pollution from industrial and urban areas and can accumulate different amounts of essential and toxic metals. This plant is used for free living organisms for feeding, such as mice *Mus spretus* and transfer its metallic load through the digestive tract to blood stream and tissues, where metals form metalloproteins with important biological functions [1]. A metallomic approach based on the use multidimensional chromatography, including size exclusion chromatography (SEC) and anion exchange liquid chromatography (AEC) with ICP-MS detection was applied to liver from free-living *Mus spretus* mice feeded with *Salicornia europaea* and *Salicornia bigalovii* during several weeks. In addition, non-targeted metabolomics analysis based on direct infusion mass spectrometry (DI-ESI-QqQ-TOF-MS) was applied polar and non polar liver extracts to know potential changes of metabolic profiles and metalhomeostasis along the time.

POSTER 4

Use of an Inline Autodilution Method to Eliminate Species Interconversion for LC-ICP-MS Based Applications

Quarles, Derrick; Field, Paul M.; Sullivan, Patrick; Kim, Hwan; Wiederin, Daniel R.

Depending on the species present, the influence of a metal ion or metal complex within an environment or biological system can be essential or toxic. It is well established methods exist in the literature on how to differentiate and detect essential and toxic species. Typically, a gas chromatography (GC) or liquid chromatography (LC) system is coupled to an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) instrument. While these instruments continue to improve, there is still one major issue that continues to be debated: Is the integrity of the sample maintained from sample preparation to final analysis.

Species interconversion during sample collection and/or preparation prior to analysis has to be carefully monitored. Typical LC-ICP-MS experiments can be lengthy, resulting in hours of analysis time. Additionally, to minimize matrix loading and ensure good separation, samples typically need to be diluted. Not only can the simple process of diluting the samples effect metal species, the effect can vary with time. This study investigates whether inline autodilution immediately prior to the column at time of analysis can mitigate species interconversion and maintain the integrity of the original sample (i.e., no species interconversion). A comparison of traditional sample preparation versus inline dilution for arsenic species (AsB, DMA, MMA, As III, and As V) in urine will be presented.

POSTER 5

Body distribution and excretion of selenium in rats fed the fish-extract containing selenonein

Anan, Yasumi; Kato, Yu; Yoshida, Masato; Ogra, Yasumitsu

Selenium is an essential micronutrient because it forms the active center of selenoenzymes such as glutathione peroxidases and thioredoxin reductase. Selenium is present as various chemical forms in foods. The nutritional significance and metabolism of selenium is dependent on forms ingested in animals. Recently, selenoneine (2-selenyl-*N,N,N*-trimethyl-L-histidine) was identified in marine fishes as a novel and major selenometabolite. To evaluate the bioavailability and metabolism of selenoneine in mammals, we administered selenoneine which was extracted from tuna meat orally to male Wistar rats. The selenium concentration in the liver of the rats fed selenoneine was preferentially increased at 24 hr after administration in comparison with that of rats fed selenomethionine. LC-ICP-MS analyses showed that selenoneine was distributed as a intact form in the liver suparnatant. On the other hand, a little amount of selenoneine was utilized to synthesize selenoproteins in liver and serum. Selenonene was also detected in urine, and the amount of selenosugar, a major urinaly selenometabolite in mammals, was not increased after the administration of selenoneine. These results suggested that selenoneine may be hard to be metabolized and assimilated compared with other seleno-amino acid derivatives in mammals.

POSTER 6

Comparison Of Elemental Concentration In Rice Grains By Xrf, Icp-Ms And Icp-Oes

Nicoloso, Fernando; Gomes Farias, Júlia; Carey, Manus; Meharg, Andrew

Polished market rice (159 samples) was purchased from retailers from 4 countries: Argentina, Brazil, Paraguay and Uruguay. All samples were analyzed by three multi-element analysis methods, i.e., x-ray fluorescence spectrometry (XRF), inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). Values determined by those methods were compared with certified values of NIST samples. Comparing the values determined by XRF and those determined by ICP-MS and/or ICP-OES, there was a slight difference between the samples, with the range of 70–120% except for P. For elements such as arsenic, which overall shows in ultralow concentration in grains, an alternative digestion with low acid concentration may be a good alternative that ensure accurate results. Compared to ICP-MS and ICP-OES, XRF can be used to precisely and efficiently analyze many elements such as Ca, Mg, Mo and P so it may become the most effective means of analysis in environmental studies in the near future. Besides the high correlation between the analyzes and equipment for the same element, when tested for correlation between different elements (i.e. As and P, As and Cd, As and Co, As and Rb and Ca and Mg) the same response pattern was observed among the tested equipment; being the only negative correlation observed between As and Cd, with the others being positive. These data are highly relevant once they confirm the accuracy of data under different methodologies and institutes/locations (UK and Brazil). In addition this study brings a good perspective for analyzes that have a less environmental impact and are ultimately possible for developing institutions.

POSTER 7

Complementary use of enzymatic digestion and tandem mass spectrometry to investigate adducts of mercury species and proteins

Strohmidel, Philipp; Sperling, Michael; Karst, Uwe

Mercury is a ubiquitous pollutant and its toxicity is known to depend strongly on its species. Human exposure to different species of mercury can be traced back to diverse sources, for example consumption of seafood for methylmercury ($MeHg^+$) and thiomersal containing vaccines for ethylmercury ($EtHg^+$).

Organomercuric compounds in biological matrices are usually bound to sulphur containing compounds, for example cysteine containing proteins. To understand toxicological effects, it is necessary to conserve the molecular information of mercury-protein-adducts during analysis. To gain even more information on the binding site of the mercury species, enzymatic digestion of protein adducts can be carried out prior to analysis.

In this study, adducts of the blood proteins human serum albumin (HSA) and hemoglobin (Hb) with $MeHg^+$ and $EtHg^+$ were investigated. The protein adducts were digested using different enzymes and the obtained peptides were separated by RP-HPLC, which was hyphenated to an electrospray MS, operating in tandem MS mode. This approach allows to distinguish between adducts of $MeHg^+$ and $EtHg^+$ and reveals the binding sites of organomercury in HSA and Hb. The method proved to be suitable for HSA in blood plasma and Hb in hemolysate.

POSTER 8

Determination of selected organoarsenic SPME/GC-MS in aquatic samples

Czaplicka, Marianna; Jaworek, Katarzyna

A solid microextraction stationary phase coupled with gas chromatography with mass spectrometry was developed for the determination of selected organoarsenic compounds in aquatic environment. For the analysis by gas chromatography, analyte was derivatized with 1,3 – propanedithiol. The cyclic dithiaarsenoline formed was extracted from the sample matrix in the liquid phase by solid – phase microextraction. The optimized solid – phase microextraction conditions employed a 65 µm polydimethylsiloxane divinylbenzene fiber, derivatization reaction temperature of 60ºC and fiber equilibration time 40 min. The methods were applied to determine an organoarsenic compounds in mining water and waste water from metallurgy of non ferrous metals industry.

POSTER 9

ICP-MS/MS-Based Analysis of the Human Serum Ionome

Konz, Tobias; Migliavacca, Eugenia; Dayon, Loïc; Bowman, Gene; Oikonomidi, Aikaterini; Popp, Julius; Rezzi, Serge

Ionome is defined as the complete set of elements of a specific biological compartment. Inductively coupled plasma triple quadrupole mass spectrometry (ICP-MS/MS) offers the possibility to simultaneously analyse a broad range of biological relevant elements by combining different gas modes with augmented selectivity. We have developed and validated a high-throughput method for the quantification of 29 elements in minimal-volume of serum from human blood samples. The method exhibits remarkable performance in terms of recovery, sensitivity and robustness. We have deployed this method for the analysis of 120 serum samples from elderly adults. The biological variability of the generated ionomic profiles was inspected as a function of age and gender by using an unsupervised dimensionality reduction technique. We observed significant effects of gender on the human ionome marked by higher circulating levels of calcium, phosphorous and copper in the serum of female subjects. Although within a relatively small range, age could be associated with circulating zinc concentration. The dataset was complemented by several clinical chemistry readouts including ceruloplasmin and cholesterol. This validated ionomic method complements other available phenomics approaches by offering a mean to inspect biological variations of a broad set of elements in relation with nutrition and health.

POSTER 10

Laser ablation inductively coupled mass spectrometry for immunoassay

Wang, Meng

An immunoassay is a biochemical test that measures the concentration of a biomolecule using an antibody-antigen reaction .Inductively coupled plasma mass spectrometry (ICP-MS) has been proven to be a powerful elemental detection technique and emerges as a promising detector for immunoassay. This ICP-MS-based immunoassay has great multiplexing potential because more than 100 isotopes are theoretically available for antibody labeling. One inherent limitation when using elemental labeling reagents is that only limited metal atoms can be attached to an antibody molecule, thus restricting immunoassay sensitivity. The use of nanoparticle as element labeling in ICP-MS analysis will increase the signal response and provide more information with low levels of target proteins because each label can contain thousands of atoms. Here we developed a new competitive immunoassay of ferritins in this paper using lanthanide nanoparticle labeling and LA-ICP-MS analysis.

POSTER 11

Structural elucidation of pyoverdines produced by Pseudomonas putida KT2440 and Pseudomonas taiwanensis VLB120

Baune, Matthias; Karen, Scholz; Weber, Günther; Hayen, Heiko

Pyoverdines are the siderophores of *Pseudomonas*, which is a genus of widely-distributed, pathogenic bacteria. The unknown structure of the pyoverdines of the biotechnologically-relevant strain *P. taiwanensis* VLB120 was elucidated using HPLC coupled to high-resolution mass spectrometry (HRMS). Sample preparation was performed using a novel solid-phase extraction procedure requiring reduced sample material as compared to previous methods. Chromatographic separation was performed via hydrophilic interaction liquid chromatography (HILIC) with gradient elution. The chromatographic procedure enables separation of pyoverdines according to their individual structure and in some cases even separation according to the central ions of pyoverdine-metal-complexes. It is noteworthy that no apo-pyoverdines were detected but only the respective iron(III)- and aluminum(III)-complexes. For structure elucidation two fragmentation techniques - collision-induced dissociation and *Higher-energy collisional dissociation*n - was applied. In *P. putida* KT2440 the well-known pyoverdines G4R and G4R A were identified. For *P. taiwanensis* VLB120 no pyoverdines were described before and three new pyoverdines were identified. Peptide sequencing by MS/MS provided the sequence Orn-Asp-OHAsn-Thr-AcOHOrn-Ser-cOHOrn. Of particular interest is the presence of OHAsn, which has not been reported as PVD constituent before.

POSTER 12

Study on Accumulation for Rare Earth Elements in the callus of Athyrium yokoscense by SR-XRF analysis.

Naoko, Hirose; Goto, Fumiyuki; Hokura, Akiko

A fern, *Athyrium yokoscense*, is widely known as a hyper accumulating plant for heavy metals. There, we have focused attention on callus of fern, *A. yokoscense*, to recovery of rare earth elements (REEs) as phytomining technology. The purpose of this study is to elucidate REEs accumulation in callus of fern *Athyrium yokoscense* by utilizing X-ray spectroscopy.

The calluses were agitated with a solution containing REEs during a certain period. After washing, the sample was freeze-dried and subjected to XAFS analyses and μ-XRF imaging.

It was found that the callus of *A. yokoscense* accumulated various REEs with a range from 5,000 to 20,000 ppm during 7 days and that heavy REEs preferentially were accumulated. The REEs L_3-edges EXAFS date revealed that the accumulated REEs could be bound to carboxylate ions or phosphate ions in the calluses. Furthermore, the elemental mapping at a cellular level showed that light REEs were accumulated throughout the callus, whereas heavy REEs were relatively distributed in the near-surface region of calluses. We assumed that light and heavy REEs were accumulated into calluses via selective absorption processes.

POSTER 13

The use of D2 as a collision gas in ICP/MS for the accurate determiation of selenoproteins

Pak, Yongnam

Recent development of technology in ICP/MS using collision cell enabled accurate determination of Selenium by removing interference from Ar dimers. However, introduction of H2 as a collision gas can produce unexpected reaction products causing interferences for the accurate determination of Se, especially in using isotope dilution technique. Matrix such as Br can interfere on m/z 80 and 82 by producing BrH in case of using H2 collision gas. In this report, D2 is employed instead of H2 to remove interferences on m/z 80. Isotope 78Se can be spiked to pair with 80Se, which shows highest signal(abundance) among Se isotopes. Though other isotopes such as 76Se and 77Se can be used, 78Se has the advantage of larger signal and consequently better precision. This technique has been applied to the accurate determination of selenoproteins in Korean human blood serum using on line isotope dilution.

Affinity Chromatography combined with ICP/MS has been used for the separation and detection of selenoproteins. Post column isotope dilution is an accurate and absolute technique for determining selenoproteins. Cancer patients and normal person groups are compared in selenoprotein species for the search of using them as a possible biomarker.

POSTER 14

The use of single cell (SC)-ICP-MS to monitor metal incorporation into yeast and bacterial cells

Álvarez-Fernández García, Roberto; Corte-Rodriguez, Mario; González-Quiñonez, Nathaly; Manteca-Fernández, Ángel; Montes-Bayón, Maria; Bettmer, Jörg

Selenium is an essential micronutrient for humans. Selenium-enriched yeast is a common form of Se used to supplement the dietary intake. The optimization of the Se-incorporation during the yeast growth and the characterization of the final products in terms of the selenium chemical forms present is requested by regulatory agencies. For this aim, the development of suitable analytical methods that allow to obtain rapid information on the success of the biotechnological process is highly demanded. In this work, we propose the use of ICP-MS for Se detection fitted with a high-efficient sample introduction system to permit individual cell introduction. The use of triple quadrupole technology (SC-ICP-TQ-MS) that improves the interference removal allowing the measurement of elements like Se or P with high accuracy will be illustrated.

Following the same analytical strategy, the work will be extended to bacterial cells. Streptomyces are industrial bacteria characterised by their complex development that includes hyphae differentiation and sporulation. Spore dormancy and germination is one of the most critical developmental stage. Recent experiments demonstrate that germination correlates with cytosolic cooper concentration. Thus, the SC-ICP-MS methodology will be further applied to the evaluation of the Cu uptake by individual spores of Streptomyces.

POSTER 15

Using Triple Quadrupole ICP-MS for the Analysis of Trace Elements in Biological Systems

Kutscher, Daniel; McSheehy Ducos, Shona

Metals and other heteroatoms play important roles in living beings. Their presence or absence may affect the actual function of a biological system, metabolic process, but also lead to pathological conditions. For the analysis of trace elements, techniques like inductively coupled plasma mass spectrometry (ICP-MS) in conjunction with dedicated sampling devices like chromatography or laser ablation (LA) have been applied in biomedical studies. Hyphenation of these techniques allow investigation of the association of given elements to biomolecules; or the spatial distribution within organs or tissues. However, in many cases, spectral interferences (polyatomic or isobaric) are obstacles on the way of either unveiling interesting correlations or quantifying them. Triple quadrupole ICP-MS is a tool that allows to effectively remove all interferences typically observed in ICP-MS through effective reactive chemistry in the collision/reaction cell system. It therefore allows to reach much lower limits of detection, that in turn allow to get more meaningful data when studying interactions of trace elements in biological systems.

This presentation will show dedicated examples on how TQ-ICP-MS can aid in in such studies, for example, by eliminating interferences on analyte ions such as Phosphorous, Sulfur or Titanium and others in relevant related applications.

POSTER 16

Complementary Bioimaging of a Gd-based contrast agent in mouse heart tissue after myocardial infarction

Buchholz, Rebecca; Wildgruber, Moritz; Karst, Uwe

The use of Gd-based contrast agents for MRI examination is already popular in everyday clinical practice. Since most of these contrast agents are systemically, it is of great importance to discover target-specific contrast agents to detect early clinical changes before they lead to severe diseases. The recent project constitutes the interface between the clinical examination and analytical chemistry.

B6 mice with differently progressed myocardial infarction were examined with MRI after intravenous administration of Gadofluorine P, a novel Gd-based contrast agent. After the examination tissue sections were prepared and the samples were analyzed with two complementary imaging techniques.

Laser ablation with inductively coupled plasma – mass spectrometry was used to quantify Gd spatially resolved in the mouse heart tissue with an external calibration of matrix-matched standards. Enrichment between factor 2 and 7 was obtained in infarctional compared to healthy tissue.

Matrix-assisted laser desorption/ionization – mass spectrometry was used to confirm the distribution of Gadofluorine P on a molecular level and to visualize tissue characteristics by endogenous molecules like heme *b* or phosphatidylcholine.

The combination of complementary imaging techniques used in this project is a powerful tool to complement the results from the MRI examination and quantify the enrichment in infarctional tissue.

POSTER 17

Distribution of phosphorus and uranium in kidney of rats exposed to uranyl acetate

Takeda, Shino; Numako, Chiya; Oikawa, Masakazu; Terada, Yasuko; Kokubo, Toshiaki

The decommissioning work of nuclear reactors following the nuclear accident in Japan has proceeded and raised increasing concerns about the dangers posed by internal exposure to uranium. The kidney is known as the critical target of uranium exposure. Uranium is considered to act as uranyl ion in the body and have a high affinity for phosphate group. In the present study, the distribution of phosphorus and uranium in the kidney was examined in Wistar male rats exposed to uranyl acetate (0.5 or 2 mg/kg, s.c.) by *in situ* determination of elements performed by micro-PIXE (particle induced X-ray emission) and micro-SR-XRF (X-ray fluorescence spectrometry using high energy synchrotron radiation). One day after administration at the higher dose, colocalization of uranium and phosphorus was observed in the proximal tubules in the inner areas of the outer stripe of the outer medulla, whereas the colocalization was not detected in the case of the lower dose. Uranium concentrated areas with phosphorus were still detected on day 15 post-administration. The time course of localized quantity of uranium, chemical status of uranium, and elemental composition after uranium exposure will be presented.

POSTER 18

Investigation of novel therapies for Wilson's disease by means of LA-ICP-MS

Müller, Jennifer-Christin; Zibert, Andree; Schmidt, Hartmut; Zischka, Hans; Sperling, Michael; Karst, Uwe

Wilson's disease (WD) is a rare genetic disorder of the copper metabolism leading to copper accumulations in various organs, predominantly in liver and brain. Patients need a lifelong therapy with chelating agents to increase the copper excretion and obtain a negative copper balance. However, the established drug for this purpose, D-penicillamin, can cause severe side effects, like bone marrow toxicity. Moreover, it leads to neurologic deterioration in up to 20% of the patients with neurological symptoms. Because of these side effects, the development of novel therapies is essential.

In this study, elemental bioimaging was used to investigate the copper removal efficiency of two new drugs, tetrathiomolybdate and methanobactin. Tetrathiomolybdate is currently tested in a clinical Phase II trial and shows a significantly lower risk of neurologic deterioration. Methanobactin is able to treat acute liver failure in animal studies. The distribution of copper, iron and zinc in rat liver was successfully analyzed by means of laser ablation-inductively coupled plasma-mass spectrometry with a spatial resolution down to 4 µm. Quantification of these elements in samples treated with different WD drugs was realized with matrix matched gelatin standards. A limit of detection of 1.1 µg/g for copper was achieved.

POSTER 19

LA-ICP-MS revealing gadolinium distribution and correlation with other elements in human brain sections after macrocyclic gadolinium-containing contrast agent administration

El-Khatib, Ahmed H.; Radbruch, Helena; Trog, Sabrina; Neumann, Boris; Linscheid, Michael W.; Schellenberger, Eyk; Jakubowski, Norbert

Introduction: Macrocyclic gadolinium based contrast agents (GBCA) that are known for their very good safety profiles are currently under investigation for their depositions in certain tissues e.g. bone and brain.

Method: Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been used to map the depositions of gadolinium in human histologic brain sections and to correlate these with other elements following the administration of macrocyclic GBCAs.

Post mortem tissue sections of the dental nuclei of three patients were measured: One patient was injected with a GBCA (macrocyclic Gadovist®) 2 weeks before dead, one received Gadovist® 4 weeks before dead and one was without GBCA application as control.

Results: High Gd signals were found in the dentate nucleus of the patient that died 2 weeks following GBCA administration. A section of the patient with Gadovist® administration 4 weeks before dead showed minimal Gd signals slightly above the control sections without GBCA application. The measurements revealed the co-location of high amounts of Gd, Fe, Cu, and P indicating a related accumulation mechanism of these elements.

Conclusion: The study demonstrates the capability LA-ICP-MS to image GBCA-derived Gd in histological sections of human brain and to study correlations with other elements.

POSTER 20

Mapping Fe and Se at clinically relevant concentrations by LA-ICP-MS: quantification and uncertainty estimation

Douglas, David; Goenaga-Infante, Heidi

The dysregulation of iron has been identified in the development and progression of Alzheimer's Disease; demonstrated by metal co-localisation with increased expression of APP (amyloid precursor protein) and Aβ (amyloid plaques). An abundance of accessible cellular iron can result in the non-apoptotic, peroxidation-driven, cell death pathway: ferroptosis. Thus understanding the antioxidant protection that proteins such as glutathione peroxidase can provide is paramount. Specifically of interest is the seleno-cysteine containing GPx4 (the most widely expressed isoform in brain) and the role that other selenium containing compounds have to play in protection. [1]

However, quantitative imaging of Se and Fe, simultaneously, by LA-ICP-MS [2] (a necessity for anatomically resolved information) in micro-sections of tissue presents many analytical challenges given that they are present at concentrations from low ppb to hundreds of ppm, respectively. Here we present LA-ICP-MS instrumental developments to tackle these challenges. Also, we will discuss strategies and associated challenges for matrix matched standard preparation with focus on material stability, homogeneity and measurement uncertainty evaluation.

[1] D. Xiubo, W. Chao and L. Qiong, *Curr. Top. Med. Chem.*, 2016, **16**, 835-848

[2] D. N. Douglas, J. O'Reilly, C. O'Connor, B. L. Sharp and H. Goenaga-Infante, *J. Anal. At. Spectrom*, 2016, **31**, 270-279

POSTER 21

Molecular Speciation and Intracellular localization of Fatty Acids labeled with a Single Element

Szyrwiel, Lukasz; Shimura, Mari; Shindou, Hideo

Fatty acids homeostasis impacts on the basic cellular function and diseases. Analysis of their uptake and metabolism into various lipids *e.g.* glycerophospholipids, neutral lipids have been well studied by chromatography and mass spectrometry. However, intracellular imaging of fatty acids and their derivatives has not been successful due to labeling difficulties with large fluorescence molecules and insufficient resolution by conventional methods. In this study, we developed a method for labeling fatty acids with a single element and applied for ICP-MS, LC-MS, and Scanning X-ray fluorescence microscopy (SXFM) to obtain intracellular elemental mapping data with submicrometer resolution [1]. Labeling fatty acids with a single element is useful technique to understand cellular dynamics of fatty acids by both molecular speciation and localization.

[1] Shimura M, *et al.,* Imaging of intracellular fatty acids by scanning X-ray fluorescence microscopy. ***FASEB J***. 30, 2016.

POSTER 22

Biological production of gold nanoparticles using unicellular alga, Pseudococcomyxa simplex

Takanashi, Kazuki; Hokura, Akiko; Yamagishi, Fumitaka

Many studies have described the biosynthesis of nanomaterials using living cultures of microalgae over the past several years. We have found that the unicellular algae, *Pseudcoccomyxa simplex,* accumulated several metals such as Au and Ag in the cell. However, the molecular details of such accumulation remain unclear. In this study, we studied gold accumulation and gold nanoparticle (Au-NP) synthesis by *P. simplex*.

Pseudcoccomyxa simplex was cultivated in culture media and homogenized by ultrasonic breaking. The water-soluble materials were obtained as the extracted fraction. The fraction was added to a solution containing a certain amount of gold as tetrachloroauric acid at several pH. The chemical form of gold in the solution was determined by Au L_3-XAFS analysis. The solution was also subjected to UV-vis spectrometry to evaluate the optical characteristic of Au-NP synthesized.

The living cells of *P. simplex* accumulated Au at ca. 10,000 ppm (dry weight). The gold in the living cell was reduced to Au(0) immediately and Au-NP was clearly confirmed by SEM images.

The Au(+3) as tetrachloroauric ion added to the extracted fraction was also reduced to Au(0) under controlled conditions. The absorption around 540 nm in UV-vis spectra showed the formation of monodisperse Au-NP.

POSTER 23

The behaviour of zero-valent iron nanoparticles and their interactions with Cd2+ in wastewaters investigated by single particle ICP-MS

Vidmar, Janja; Oprckal, Primoz; Milacic, Radmila; Mladenovic, Ana; Scancar, Janez

Nanoremediation with zero valent iron nanoparticles (nZVI) exhibits great potential for the efficient removal of various contaminants from wastewaters. However, the fate and behaviour of nZVI after their use in nanoremediation have not been thoroughly investigated and should be followed carefully to ensure that nZVI do not present a risk to living beings and the environment. In our study, an SP-ICP-MS method was optimised for sizing and quantification of iron nanoparticles in wastewater matrices, and applied to follow the aggregation and sedimentation of nZVI at different iron loads during the nanoremediation procedure. The interaction of nZVI with Cd^{2+} and their ability to remove this pollutant from wastewaters was also investigated by quantifying Cd^{2+} which was distributed between the dissolved fraction or attached to the residual nZVI with the use of SP-ICP-MS. Results showed that Fe loads as low as 0.25 g L^{-1} nZVI were able to effectively remove Cd^{2+} from the effluent wastewater and after seven days of settling, a negligible concentration of nZVI remained dispersed in the effluent wastewater. This indicates that use of nZVI in nanoremediation under the described conditions does not present an environmental nano threat.

POSTER 24

A novel strategy for the production and characterization of isotopically-enriched copper albumin: Towards development of reference methodology to validate measurements in Alzheimer's research

Del Castillo, M.Estela; Nunez, Susana; Goenaga-Infante, Heidi

Albumin (ALB), the most abundant protein in human blood plasma, is able to bind a variety of essential and toxic metal ions, including Cu(II), Zn(II), Ca(II) and Cd(II). Recent studies have pointed out that metal ions, such as Cu ions, play a key role in the developing of neurodegenerative diseases (e.g. Alzheimer's disease, AD). In order to protect the body from oxidative stress, metal ions are bounded by metalloproteins, and transported from the blood stream to the brain across the blood-brain barrier. This controlled permeation of metals may be affected in AD. Therefore, the role of metalloproteins as potential biomarkers of AD should be investigated.

Under the frame of the EU REMIND project, efforts have been undertaken to develop and validate methodology for the accurate determination of Cu bound to ALB in serum, CSF and brain using species-specific isotope dilution mass spectrometry (IDMS). In this regard, the strategy used to produce and characterize an isotopically labelled ^{65}Cu-ALB spike will be discussed here for the first time. Reference methodology for ALB-metal species in matrices relevant to AD research will be invaluable for the provision of reference values to clinical trials. Also, to help validation of measurements performed in AD models.

POSTER 25

Ultra-trace analysis of Tc using solid phase extraction and isobaric dilution analysis

Schlatt, Lukas; Clases, David; Sperling, Michael; Karst, Uwe

The unstable ^{99m}Tc has ideal properties to be used in scintigraphies, due to the emission of a gamma quant during nuclear transition to its ground state ^{99g}Tc. Along with radiological investigations, other anthropogenic effects have led to significantly increased concentrations of Tc in the environment. Sensitive and selective quantification methods have to be developed to investigate the concentration of Tc in a variety of samples.

The quantification of Tc is hampered since no stable isotopes are available and a lack of respective standards. A novel method called isobaric dilution analysis (IBDA) can be used for the internal calibration of Tc. Unlike isotope dilution analysis (IDA), where an isotopically enriched solution of the analyzed element is used as a spike, IBDA uses the isotope of another element with a spectral overlap as an internal standard. For conventional inductively coupled plasma-mass spectrometry (ICP-MS) instruments, it is not possible to distinguish these two elements and a quantification is therefore feasible when response factors are evaluated carefully.

In this study, trace amounts of Tc are extracted from water samples and then detected using ICP-MS. Quantification is achieved with Ru as an isobaric internal standard.

POSTER 26

Genetic analysis of survival of Salmonella enterica serotype Enteritidis in egg white; impact to iron restriction and lysozyme

Ghareeb, Abdulameer; Andrews, Simon

This work is to determine the role of three distinct gene clusters (*dgoRKADT*, uxuAB-*uxaC* and SEN1433-6) related to in utilisation of hexonates in the survival of *Salmonella enterica* serovar Enteritidis (*S*E) in egg white and low iron conditions, these genes are strongly induced during exposure SE to egg white. Nine of the putative promoter regions of interest were tested for expression by generation of *lacZ* fusions. Activities were variable, with highest observed for SEN1436 (encoding for putative dehydratase) and SEN2977. The SEN1436-*lacZ* fusion was selected for the study of environmental regulation. Upon exposure to egg white (EW), SEN1436 expression was induced up to 60 fold. However, egg-white filtrate (EWF; lacking proteins >10 kDa) and iron had no marked effect on expression. This result suggests that EW proteins are responsible for the induction of the hexonate genes during EW exposure. Experiments involving exposure to isolated EW proteins showed that that major EW protein affecting SEN1436 expression is lysozyme (14 fold) with little effect of ovotransferrin. Interestingly, low levels of EW (2.5%) completely inhibited growth of *S*E, and this effect was reversed by addition of iron suggesting that iron restriction is the dominant anti-microbial activity of EW with respect to *S*E.

POSTER 27

Characterization of metal profiles in strawberry samples during treatment with different post-harvest controlled athmospheres

Ramírez-Acosta, Sara; Arias-Borrego, Ana; García-Barrera, Tamara; Gómez-Ariza, Jose Luis

In recent years, the consumers' interest for strawberries has grown due to its beneficial health effects because of the antioxidant and anticancer properties and also as rich source of polyphenols, vitamins and minerals (Fe, Mg, P, K, and Mn). Fruit nutritional quality is affected by exogenous factors such as environmental parameters, ripening season and pre-harvest and post-harvest treatments [1], [2]. The major challenge of post-harvest technology is to try that fresh product gets the consumer with a similar quality that harvest time and, if possible, with improved organoleptic and nutritional properties. Strawberry post-harvest techniques often focus on the use of controlled atmospheres (CA) (storage with low O_2 and high CO_2 concentrations) [2]. The aim of this study is to investigate the mineral profiling of strawberry fruit treated with different post-harvest controlled atmospheres. An analytical approach, based on non-denaturing precipitation of high molecular mass (HMM) and low molecular mass (LMM) metal species was optimized for the fractionation of these molecules in strawberries tissues, which were subjected to multielemental analysis by inductively coupled plasma mass spectrometry (ICP-MS). This methodology was applied to strawberries with different post-harvested treatments in order to study their differences in metals profiling associated to metabolomic expression

POSTER 28

Characterization of metals profiles in serum, urine and bronchoalveolar lavage fluid from lung cancer patients using ICP-QQQ-MS.

Callejón-Leblic, Belén; García-Barrera, Tamara; Pereira-Vega, Antonio; Gómez-Ariza, José Luis

Lung cancer (LC) is the most cause of cancer-related deaths worldwide [1,2], and it is well known that elements play essential roles in biological processes activating or inhibiting enzymatic reactions. To this end, an analytical method based on ICP-QQQ-MS was optimized for eleven elements in order to characterize the metal profile in fluids of LC patient. In addition, an analytical approach based on non-denaturing precipitation of proteins has been optimized for the fractionation of high molecular (HMM) and low molecular mass (LMM) metal species from serum to distinguish between species because it affects biological activity.

There are a lot of studies about the determination of trace elements in blood, urine and lung tissues in relation to the metal distribution in LC patients, but only a few are referred to bronchoalveolar lavage fluid (BALF), and the combined information about different fluids has to be still stablished.

In this work, multi-elemental determination in serum, urine and BALF samples was performed to know the metal distribution. Partial Least Square Discriminant Analysis (PLS-DA) and Mann-Whitney U test were carried out to establish significant differences between groups. Finally, Spearman´s test indicated that these metal abnormalities can be interrelated, participating in processes such as oxidative stress.

POSTER 29

Copper and nickel enhanced zinc-induced neurotoxicity: cross talk of neurometals and pathogenesis of vascular dementia

Kawahara, Masahiro; Kato-Megishi, Midori; Tanaka, Ken-ichiro

Zinc (Zn^{2+}) is accumulated in the synaptic vesicles and co-released with glutamate during the neuronal excitation. Excess Zn released into the synaptic clefts during transient global ischemia is believed to play central roles in the pathogenesis of vascular dementia. We have investigated the coordination of other metal ions including Cu^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, and Al^{3+} in Zn-induced neurotoxicity using immortalized hypothalamic neurons (GT1-7 cells). Although the co-existence of Al^{3+} attenuated Zn-induced neurotoxicity, sub lethal concentrations of Cu^{2+} markedly exacerbated Zn-induced neurotoxicity. The co-administration of Cu^{2+} and Zn^{2+} significantly increased the expression of genes related to the endoplasmic reticulum's stress response, including CHOP, GADD34, and ATF4. Furthermore, sodium pyruvate, an energy substrate, attenuated the neurotoxicity. Similar findings were also observed by Ni^{2+}. Based on our findings, we hypothesize that Cu^{2+} interacts with Zn^{2+} in the synaptic clefts to synergistically promote neuronal death and significantly influence the pathogenesis of vascular dementia. Further research about metal-metal interactions in their neurotoxicity may enlighten the precise roles of metals in neurodegenerative diseases.

POSTER 30

Element-labeled immunoassay combined with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for highly sensitive transferrin receptor determination

Alonso-Garcia, Javier; Blanco-Gonzalez, Elisa; Montes-Bayon, María

Alterations in the cellular mechanisms of iron regulation may cause an imbalance and ultimately result in disease. In the particular case of cancer cells, upregulation of iron importers and downregulation of iron exporters and storing molecules may rather increase the bioavailable iron, while favouring the proliferation and survival of tumour cells. The transferrin receptor is a membrane glycoprotein whose only clearly defined function is to mediate cellular uptake of iron from a plasma glycoprotein, transferrin. Iron uptake from transferrin involves the binding of transferrin to the transferrin receptor, internalization of transferrin within an endocytic vesicle by receptor-mediated endocytosis and the release of iron from the protein [1]. Highly sensitive analytical methods are required to perform transferrin receptor quantification in biological samples.

In this vein, a new analytical strategy based on inductively coupled plasma-mass spectrometry (ICP-MS) combined with antibody labeling in a sandwich assay format is proposed in this work for transferrin receptor determination. The developed methodology involves two transferrin receptor antibodies, one of them biotinylated and the other one labeled with a lanthanide chelate that we used for monitoring by ICP-MS. Such strategy permits transferrin receptor determination at very low levels in cancer cells.

POSTER 31

Multiplex PCR in combination with gel electrophoresis-mass spectrometry (GE-ICP-MS): A powerful tool for the determination of gene copy number varitations and gene expression.

Fernández Asensio, Alejandro; Iglesias González, Tamara; Espina Fernández, Marta; Cotarelo Fernández, Ana; Blanco González, Elisa; Sierra Zapico, Luisa Maria; Montes-Bayón, María

Multiplex PCR has nowadays a broad range of applications, in particular if the simultaneously amplified sequences can be also simultaneously quantified. In this regard, the use of multiplex real-time PCR (qPCR) has become the method of choice for multiplex gene expression analysis or gene copy number variations (CNVs) during the last few years. However, such determinations require the use of different fluorescent labels for the different amplified sequences which limits the use of the technique to the amplification of three sequences. In this regard, the use of the coupling between gel electrophoretic separations (GE) with inductively coupled plasma detection (ICP-MS) detection allows the label-free determination of simultaneously PCR-amplified sequences (multiplex) by monitoring the P present in the backbone of the DNA. The quantitative dimension can be obtained by using inorganic phosphate standards with known concentration as well as the calibration of the GE system in terms of the separation as function of the bp of the fragments. The suitability of the proposed strategy has been evaluated for addressing two different situations: determination of copy number variations and gene expression in DNA obtained from cell cultures of ovarian cancer after cisplatin exposure.

POSTER 32

Oral supplementation with low strontium doses improve bone quality in adult rats

Ji, Xiang; Nur Singh, Nadia; Ren, Minqin; Osipowicz, Thomas; Walczyk, Thomas

Strontium (Sr) ranelate is a potent drug for osteoporosis treatment. But Sr is also part of our diet. In a dose response study, we have supplemented the feed of male, healthy rats (6-8 months; n=20 per group) with Sr lactate for 24 wks (0, 35, 70 and 150 mg Sr/kg body weight per day). Tibiae and vertebrae were harvested after sacrifice. Sr concentration in serum and bone increased linearly with dose and was deposited mainly in newly formed bone but also in old bone. Sr was found to exert a positive effect on bone at doses as low as 16% of the effective therapeutic Sr dose of the drug in rats (increased trabecular thickness, osteoid thickness, trabecular volume, bone formation rate and mineral apposition rate obtained from histomorphometric measurements, and increased modulus and hardness measured by nanoindentation test, $p<0.05$). Our finding indicates that low Sr doses could serve as a cost-effective strategy for osteoporosis prevention during adulthood.

POSTER 33

Red Meat Consumption Triggers Serum Non-Transferrin Bound Iron (NTBI) Formation in Humans

Yang, Dongxiao; Paik, Merlene; Walczyk, Thomas

Iron (Fe) can catalyse formation of hydroxyl radicals in the body, a mechanism that has been linked to various degenerative disorders. Transferrin acts not only as the main Fe transport protein in serum but also shields Fe from reaction. Using stable isotope techniques, we developed a novel assay for analysis of non-transferrin bound iron (NTBI) in serum. By preserving NTBI at the time of blood sampling and correction of Fe leakage from transferrin into serum during analysis, we achieved a higher accuracy as in previous assays. Serum NTBI has been reported so far in response to Fe supplement intake but not for regular meals. Here, we have measured the effect of a standardized meal high in bioavailable iron (beef stew and rice) on serum NTBI concentration in apparently healthy, male Chinese (24.4 ± 4.0 a; n=11) after an overnight fast. Serum NTBI was 0.03 ± 0.15 µmol/L before meal intake and increased to an average of 0.14 ± 0.24 µmol/L two hours after test meal intake ($p < 0.05$). Possible risks associated with high meat consumption as a trigger of iron induced oxidative stress demand further investigations.

POSTER 34

Total trace element analysis in biological matrices by ICP-MS.

Griffin, Elizabeth; Raab, Andrea; Koss, David; Platt, Bettina; Feldmann, Jörg

Alzheimer's disease (AD), the most common cause of dementia, is the most prevalent neurodegenerative disease worldwide. It is characterised pathologically by amyloid plaques and neurofibrillary tangles in the brain, but evidence suggests that metal ions are also involved in the development and progression of the disease. The aim of this work was to develop a method for reproducible total element determination by ICP-MS in brain, using a metrological approach for later application of the method to AD affected brains and controls. Results showed that brain is a highly heterogeneous tissue with regard to spatial element distribution. There is therefore a requirement for extensive homogenisation of tissue before sub-samples are used for quantification, and either use of the whole organ, or determination of the element content in precisely the same location of a specific brain region when samples of different origin are to be compared in a study. The developed method was applied first to mouse brains, being the major animal model for AD research. The element content of brains from different mouse models was compared.

POSTER 35

Bioaccumulation of Cd and Pb in vegetables and transfer through the trophic chain

Vela, Doris

We examined the bioaccumulation of Cd and Pb in tomatoes, carrots and lettuces produced by organic and conventional farming (mg/kg using AOAC 999.11 method) and the transfer of them through the trophic chain using Drosophila melanogaster.

Accumulation of Pb in vegetables by organic (tomatoe 0.58; carrots 2.13; lettuce 5.62) is higher than by conventional (tomatoe 0.49; carrots 1.44; lettuce 4.15). Accumulation of Cd in tomatoes is higher in organic (0.69) than in conventional (0.57), for carrots and lettuce there are an increment in conventional (0.72; 1.01 respectively) respect to the organic (0.46; 0.69 respectively).

Flies fed with tomatoe (10.1 organic, 11.05 conventional) and carrot (11.1 organic, 8.7 conventional) show higher accumulation of Pb than flies fed with lettuce (2.1 organic and 3.5 conventional). Accumulation of Cd in flies fed with tomatoe is also higher (2.45 organic, 2.0 conventional) than carrots (0.85 organic, 1.12 conventional) and lettuce (0.35 organic, 0.77 conventional).

Bioaccumulation of Pb and Cd in vegetables and flies exceeded the safety limits given by the CODEX. Heavy metals could be transferred through the trophic chain since the vegetables to the humans or animals by feeding, this evidence the risk of metals bioaccumulation for the human health and ecosystems.

POSTER 36

Distribution and excretion of arsenic in mice after oral administeration of arsenolipid

Kobayashi, Yayoi; Suzuki, Noriyuki; Ogra, Yasumitsu; Hirano, Seishiro

It is known that marine organisms and seafood contain arsenic-containing lipids (arsenolipids), and they are sources of daily intake of arsenic in humans. It was recently reported that some arsenic-containing hydrocarbons (AsHC) caused deleterious effects to hepatic and bladder cell lines (Meyer S, et al., Metallomics, 2014, 6, 1023-1033). However, very little is known about the metabolism and toxicity of arsenolipids in mammals. Thus, we administrated synthesized AsHC360 ($C_{19}H_{42}AsO$), one of the toxic AsHC, orally into mice and compared the tissue distribution and excretion of AsHC360 with those of dimethylarsinic acid (DMA^V). Approximately 57% and 77% of the dose were excreted in urine in one day after the treatment with AsHC360 and DMA^V, respectively. On the other hands, approximately 8% of the dose was excreted in feces in one day in both groups. It was found that % dose recovered in the tissues was higher in AsHC360-treated group than in DMA^V-treated group; in liver (approximately 2.6 times), kidneys (approximately 2.6 times), and brain (approximately 6 times). Arsenic accumulation in brain in AsHC360-treated group may suggest that AsHC360 is transported across the blood brain barrier.

POSTER 37

Profiles of trace elements associated to biomolecules in hepatic cytosols of two freshwater fish species

Dragun, Zrinka; Krasnici, Nesrete; Filipovic Marijic, Vlatka; Ivankovic, Dusica; Erk, Marijana

The aim of this study was to define and compare the distributions of five trace elements (essential: Cu, Fe, Se, Zn; non-essential: Cd) among cytosolic biomolecules of different molecular masses isolated from livers of European chub (*Squalius cephalus*) and brown trout (*Salmo trutta*). These two fish species are often applied as bioindicators of environmental contamination in different types of fluvial ecosystems. European chub were sampled in the lowland river Sutla and brown trout in the karstic river Krka in Croatia, in autumn seasons of 2009 and 2015, respectively. To obtain the elemental profiles, metals bound to cytosolic biomolecules were separated by size exclusion high performance-liquid chromatography and subsequently analysed by high resolution-inductively coupled plasma-mass spectrometry. Obtained profiles indicated certain differences between two species. Both Cd and Cu were associated to metallothioneins in both species, but only in trout their binding to high molecular mass biomolecules (>100 kDa) was evident. Binding of Fe and Se to certain very low molecular mass biomolecules (<10 kDa) was observed only in trout, whereas distribution profiles of Zn were comparable in both studied species. The results of this study provide the possibility for better understanding of metal/metalloid fate in the cells.

POSTER 38

Speciation of nickel in cocoa infusions by the use of monolithic chromatography - post-column ID-ICP-MS and Q-TOF-MS

Peeters, Kelly; Zuliani, Tea; Zigon, Dusan; Milacic, Radmila; Scancar, Janez

Nickel (Ni) is considered to be a potentially toxic element for humans. Its levels in foodstuffs are normally low (below 0.2 mg kg^{-1}), but sensitive individuals may develop allergy to Ni as a result of dietary consumption. Cocoa contains relatively high Ni concentrations (around 3 mg kg^{-1}). The bioavailability of Ni, its role in the flavour of food and its potential impact on human health depends primarily on its chemical forms. However, there is a lack of information about Ni speciation in cocoa. In the present work Ni species were separated on a weak convective interaction media diethylamine (CIM DEAE) monolithic chromatographic column and quantified by the post-column isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS). The Ni binding ligands in the separated fractions were identified “off line” by quadrupole time-of-flight mass spectrometry (Q-TOF MS). As a product of the glucose fermentation, gluconic acid was identified as the most important LMM-Ni binding ligand in cocoa infusions. Ni^{2+} and, in most of samples analysed, traces of Ni-citrate were also identified in the cocoa infusions.

POSTER 39

Synthesis and evaluation of antibacterial novel chitosan derivatives as inhibitors for biomaterial stainless steel corrosion in simulated body fluid solution

Hassan, Nazly; Omer, A. M.; Ebrahim, Asmaa; Tamer, T. M.

New O- amine functionalized chitosan derivatives were obtained from the coupling of O- amine functionalized chitosan with 4-dimethyl amino-benzaldehyde and N-methyl-2-pyrrolidone. The structure of the prepared innovative compounds was characterized using different techniques. The activity of the derivatives against different bacteria strains, displays inhibition activity against these bacterial strains. As biomaterials, self-assembled monolayers (SAMs) of these compounds were formed on the surface of 316L stainless steel samples. The obtained SAMs were investigated as corrosion inhibitors using electrochemical methods in simulated body fluid solution (SBF). An inhibition efficiency as high as 65 - 76% was achieved for monolayers prepared at 100 ppm.

KEY WORDS: Chitosan derivatives, antibacterial, biomaterial, SAMs,316L stainless steel, corrosion inhibition, SBF

POSTER 40

Cisplatin vs. organometallic Au(III)compound: New insights into their mechanisms of action using an ex vivo model

Spreckelmeyer, Sarah; Estrada-Ortiz, Natalia; van der Zee, Margot; Prins, Gerian; Stürup, Stefan; de Graaf, Inge A. M.; Groothuis, Geny; Casini, Angela

Cisplatin is widely used as chemotherapeutic agent. Unfortunately, there is still lack of knowledge about its mechanisms of accumulation in cells and tissues. So far, the OCT2 and CTR1 have been hypothesized to be involved in the nephrotoxicity of anticancer Pt drugs. However, since most studies were carried out on cancer cell lines, up to now it has been impossible to fully elucidate the mechanisms of Pt(II) uptake and efflux. Similarly, limited knowledge is available of the new generation of anticancer metal-based compounds, such as Au complexes.

Here, we report our results on the mechanisms of uptake as well as toxicity of cisplatin in comparison to a cytotoxic Au(III) compound, using an *ex vivo* tissue model, named precision cut kidney slices (PCKS). The viability of rat PCKS was assessed via ATP/protein determination and the metal content via ICP-MS measurements. Our results show no correlation between metallodrug +/- cimetidine treated samples. Nonetheless, a correlation between the viability of treated kidney samples and the drug concentration was detectable.

Based on these findings, OCT are not involved in the accumulation mechanisms of the studied metallodrugs. Additionally, this PCKS *ex vivo* model represents a valuable further methodological development in the biological evaluation of metallodrugs.

POSTER 41

Development of Gold N-heterocyclic carbene complexes with increased selectivity towards cancer cells and reduced toxicity in precision cut kidney slices

Estrada Ortiz, Natalia; Guarra, Federica; de Graaf, Inge A. M; Marcheti, Lorella; de Jager, Marina H; Groothuis, Geny M. M.; Gabbiani, Chiara; Casini, Angela

A novel series of Au(I), Pt and Pd N-heterocyclic carbene (NHC) complexes was synthesized and characterized by NMR, crystal X-ray diffraction and mass spectrometry. The cytotoxic activities of the compounds were tested in 4 human cancer cell lines and their toxicity in healthy tissue was determined using precision cut kidney slices (PCKS) as a tool to determine the potential selectivity towards cancer cells and reduced side effects in healthy organs of the Au complexes. All the evaluated gold compounds presented cytotoxic activity towards the cancer cells in the low micro molar range. However, Pd and Pt complexes displayed scarce activity against the evaluated cell lines. Interestingly, the mixed Au(I) NHC complex - (ter-butylethynyl)-1,3-bis-(2,6-diisopropylphenyl)-imidazol-2-ylidene gold(I), bearing an alkynyl moiety as ancillary ligand, showed high cytotoxicity in cancer cells in vitro, while being barely toxic in healthy rat kidney tissues. The obtained results open new perspectives towards the design of mixed NHC-alkynyl gold complexes for chemotherapeutic applications with low toxicity in healthy tissues, giving us a led compound to derivatize and further study the mechanisms behind its activity.

POSTER 42

Impact of linker modification on the in vitro and in vivo anticancer activity of novel albumin-targeting Platinum (IV) drugs

Schüffl, Hemma; Mayr, Josef; Groza, Diana; Galvez, Luis; Lahnsteiner, Marianne; Koellensperger, Gunda; Berger, Walter; Kepller, Bernhard K.; Kowol, Christian R.; Heffeter, Petra

Cancer treatment is often associated with serious side effects due to lack of tumor specificity. In order to enhance the drug delivery to the malignant tissue, albumin is a promising drug carrier. Thus, we have recently developed the first platinum(IV) derivatives of oxaliplatin containing a maleimide moiety, which is reacting with the thiol group of albumin. Aim of this study was to investigate the impact of different linkers on the anticancer activity of the complexes in cell culture and in vivo and to correlate their activity with the albumin uptake of the cancer cells. To this end, as a first step the albumin uptake kinetic of several murine cancer cell models was established by flow cytometry (using FITC maleimide) and correlated with drug uptake as well as long- and short-term anticancer activity (by clonogenic and MTT assay, respectively). To assess the in vivo anticancer activity Balb/c mice with subcutaneous CT-26 tumors were used. These tests revealed that while the cell culture behavior of the compounds was rather similar, distinct differences in the in vivo anticancer activity were observed. Overall, this indicates that the linker system has to be carefully selected and evaluated in order to find the ideal drug candidate.

POSTER 43

Metal N-heterocyclic carbenes (NHCs) as antitumor drugs: synthesis and biological activity tests

Guarra, Federica; Gabbiani, Chiara; Biver, Tarita; Busto, Natalia; García, Begoña

Cisplatin and other platinum drugs currently used in the treatment of cancer may have many drawbacks such as systemic toxicity, related resistance and tolerance mechanisms. Therefore, many efforts are being spent in the development of new metal based antitumor drugs. Among these, metal N-heterocyclic carbenes (NHCs) turned out to be particularly promising. NHCs manifest similar donor properties to phosphines, thus affording very stable complexes. In addition, the imidazolium salt precursors are more easily sinthesized than similarly functionalized phosphines.

Herein, we present the synthesis of a series of gold-NHCs and of and silver precursor. These complexes are commonly known to target proteins, yet more recent studies also consider the interaction of gold compounds with dsDNA or G-quadruplexes. In particular, our designed complexes aim at combining gold or silver inhibition of TrxR with the intercalating activity of the NHC ligand. In this frame, the novel compounds were chemically characterized and different biochemical, biophysical and spectroscopical methods were used to characterize their interaction with target proteins, an oligonucleotide and CT-DNA. Furthermore, preliminary *in vitro* studies on solid tumor cell lines were performed.

Reference:

Oehninger L., Rubbiani R., Ott I., *Dalton Trans, 2013, 42, 3269- 3284*.

POSTER 44

Multifunctional peptide-drug conjugates for cancer therapy

Hager, Sonja; Conibear, Anne C.; Mayr, Josef; Klose, Matthias H. M.; Keppler, Bernhard K.; Kowol, Christian R.; Becker, Christian F. W.; Heffeter, Petra

Improvement of anti-cancer therapy can be achieved by targeting cancer-cell specific features, thereby, increasing the specificity of cancer therapy and decreasing damage to normal cells. Integrin $\alpha_v\beta_6$ is a receptor involved in cell adhesion and is frequently upregulated in cancer cells compared to healthy cells. After selecting a peptide ligand reported to bind specifically to the integrin β_6, we have synthesized a suite of multifunctional molecules comprising the integrin-targeting peptide in combination with cytotoxic platinum(IV) prodrugs and fluorescent or biorthogonal tags joined with flexible linkers. For controlled *in-vitro* testing, we established an integrin β_6-expressing cell line from the non-expressing colon carcinoma cell line SW480 by transfection. Fluorescence and viability experiments with this established cell model showed significant increase in binding, uptake and cytotoxicity of the construct in the integrin β_6-expressing compared to the parental cell line. This versatile and highly controlled approach to synthesizing labeled peptide-drug conjugates has the potential to target potent cytotoxic drugs specifically to cancer cells, reducing side-effects and the doses required for effective treatment.

POSTER 45

Tackling Adverse Effects in Cancer Therapy: Evaluation of Poly(organo)phosphazene-based Metallodrug Conjugates

Schönhacker-Alte, Beatrix; Hackl, Carmen; Klose, Matthias; Henke, Helena; Legina, Maria; Jakupec, Michael; Berger, Walter; Keppler, Bernhard K.; Brüggemann, Oliver; Teasdale, Ian; Heffeter, Petra; Kandioller, Wolfgang

Due to the major limitations (adverse effects and rapid resistance development) of systemic therapy for late stage patients, there is still an urgent need of more effective and better tolerated drugs. Since cisplatin and its descendants have so far failed to overcome these limitations, other metals especially in combination with new drug delivery systems are coming into the focus of research. Therefore, organoruthenium and -rhodium complexes were synthesized and analyzed in cell culture and in vivo. Despite promising in vitro activity, these testings revealed unselective reactivity in vivo, leading to local adverse effects at the application site. To improve the performance, the organometallic complexes were attached to biodegradable poly(organo)phosphazene macromolecules resulting in conjugates with increased aqueous solubility. Subsequently, the anticancer activity and organ distribution of the new conjugates was evaluated again, revealing beneficial influence of this macromolecular prodrug system by a significant reduction of adverse effects compared to the free metallodrugs. Noteworthy, there was a dramatic impact of the attached compound on the organ distribution of the novel conjugates. Together, our data indicate that despite structural similarities conjugation of different organometallic compounds to the identical polymer leads to completely different pharmacological behavior both in vitro and in vivo.

POSTER 46

Inhibition of Lysozyme Fibrillation by Gold Nanorods and Nanoparticles

Wang, Bing; Feng, Weiyue

Amyloid fibrillation has been implicated in many neurodegenerations, dialysis-related amyloidosis, type II diabetes and more than 30 other amyloid-related diseases. Nanomaterials as potential inhibitors of amyloid fibrillation have attracted increasing interests. In the present study, the effects of gold nanorods (AuNRs) and nanoparticles (AuNPs) on amyloid fibrillation were investigated using hen egg white lysozyme (HEWL) as a model system. Our results indicated that AuNRs and AuNPs, especially AuNRs, present significant inhibitory effects on HEWL amyloid fibril formation during all the kinetic processes, from nucleation to elongation and equilibration stages. The stronger adsorption capacity of HEWL on AuNRs surface is the key mechanism of inhibition of HEWL amyloid fibrillation. Furthermore, AuNRs lead to more stable a-helix conformation and hydrophobic microenvironment of aromatic side groups in HEWL molecules, which facilitate the system to form small amorphous aggregates rather than oligomer, profibril or mature fibril.

POSTER 47

Polyoxometalates structure/function features of P-type ATPases inhibitors

Gumerova, Nadiia; Breibeck, Joscha; Al-Sayed, Emir; Bijelic, Aleksandar; Rompel, Annette; Krivosudsky, Lukas; Fraqueza, Gil; Fuentes, Juan; Aureliano, Manuel; Tanuhadi, E.

Polyoxometalates (POMs) mechanisms of action as anti-cancer and anti-bacterial activities are, at least in part, due to the inhibition of P-type ATPases. POMs interactions with these membrane ion pumps can rapidly induce changes in ion homeostasis with implications in, for example, calcium accumulation and concomitantly ROS production and cell death. In the present communication we analyzed the effects of several Nb-, V- and W-based POMs in the activity of Ca^{2+}-ATPase from sarcoplasmic reticulum vesicles and in the activity of Na^{+}/K^{+}-ATPase from basal membrane of epithelial skin. It was observed that the different POMs inhibits the Ca^{2+}-ATPase activity with IC_{50} values from nM to mM range of concentrations, showing different types of inhibition from non-competitive (V_{10}, Nb_{10}) to mixed type (TeW_6, P_2W_{18}) inhibitors. However, the most potent Ca^{2+}-ATPase inhibitor (Se_2W_{29}) (IC_{50} = 0.3 μM) among the POMs studied has no effects in the Na^{+}/K^{+}-ATPase from basal membrane of the skin epithelia. It was observed that P_2W_{18} IC_{50} values of inhibition, about 30, 70 and 400 times lower (IC_{50} = 0.6 μM) than V_{10} (15 μM), Nb_{10} (35 μM) and TeW_6 (~ 200 μM), respectively, can be correlated with specific structural features, that can lead to a better understanding of POMs applications as anti-tumoral and anti-bacterial metal based drugs.

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Acknowledgments

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POSTER 48

Silver Nanoparticles (SNP): Antibacterial properties and intervention in copper turnover in mice

Ilyechova, Ekaterina; Rozhkova, Natalia; Orlov, Iurii; Sosnin, Ilya; Sankova, Tatiana; Puchkova, Ludmila

SNP are one of the most perspective compounds used in biomedicine due to their antibacterial activity.

However, SNP dissociate with formation of silver ions (Ag+) that are isoelectronic to Cu+ and can be transported using the same pathways, incorporated in the active centers of Cu-containing proteins and disturb them, *i.e.* demonstrate an ecotoxic effect.

New SNP were synthesized by chemical reduction and characterized by spectroscopy, diffractometry, electron microscopic methods. The SNP were 35-nm size, spherically shaped, and demonstrated antibacterial activity against *E.coli*.

The ability of SNP to interfere with mammalian copper metabolism was tested in mice. After SNP injections for 7 days Ag+ were unevenly distributed in the mice body, accumulated mainly in liver and excreted through bile and urine. Ag+ was revealed in cytosol, mitochondria, and Golgi complex, where ceruloplasmin (main copper transporting protein) is metallized. In serum Ag+ were bound with ceruloplasmin and alfa2-macroglobulin, decreased sharply serum copper concentration, oxidase activity, but did not changing ceruloplasmin protein concentration. Cancellation of the SNP injections led to copper concentration and serum oxidase activity restoration in a week, but the silver level in tissue remained unchanged.

The possibilities and limitations of SNP application as a potentially antitumor agent are discussed.

POSTER 49

Comparative characterisation of the ferrous iron transport systems EfeUOB and FeoABC in E. coli

Al-Aidy, Salem; Andrews, Simon

Iron is an essential element for bacterial growth. However, it is a dangerous metal because it has the ability to catalyse reactive oxygen species through Fenton reaction. The oxidation status of iron in environment is determined by pH and oxygen; poorly soluble ferric with high pH and O_2, more soluble ferrous by low pH and O_2. This study explores the differences in activities of the two ferrous-iron transporters (Feo and Efe) of *E. coli* in their responses to H_2O_2. *E. coli* mutants devoid of iron-transport systems were employed along with inducible plasmid (pBAD), carrying either *efeUOB* or *feoABC*. Results showed that H_2O_2 enhances ^{55}Fe uptake for *efeUOB* transformants whereas provision of exogenous catalase caused strong inhibition. In contrast, FeoABC dependent iron-uptake was enhanced by catalase but inhibited by H_2O_2. The iron uptake results were found to correlate with the growth phenotypes of EfeUOB and FeoABC-dependent *E. coli*, where FeoABC dependent low-iron growth was inhibited by peroxide whereas EfeUOB-dependent low-iron growth was enhanced by peroxide. In conclusion, EfeUOB and FeoABC are ferrous-iron transporters that appear to be required under distinct conditions relating to peroxide abundance; this requirement would thus explain the need for two alternate ferrous-iron uptake systems in *E. coli*

POSTER 50

Different arsenic and zinc species in Russula pumila and Inocybe napipes

Sácký, Jan; Leonhardt, Tereza; Borovicka, Jan; Braeuer, Simone; Goessler, Walter; Santrucek, Jiri; Kotrba, Pavel

The high concentrations of metal(loid)s accumulated in their sporocarps indicate that certain fungi substantially contribute to the cycling and environmental sequestration of these elements, including As and Zn. Two ectomycorrhizal species, *Russula pumila* and *Inocybe napipes* were identified as accumulating remarkably high concentrations of arsenic (As), with the concentrations of up to 400 and 1300 mg As kg^{-1} dry mass, respectively. Both species can also accumulate significant amounts of zinc (Zn; up to 850 and 1200 mg kg^{-1}, respectively). Size exclusion chromatography (SEC) revealed that nearly 90 % of As and Zn is in the extracts from *R. pumila* sequestered with 6 kDa, cysteinyl-rich peptides, resembling major Zn-binding RaZBPs of *Russula atropurpurea*, whose isoforms are also expressed in *R. pumila*. In contrast, SEC analyzes of the extracts from phylogenetically distant *I. napipes* identified virtually all As, like the majority of Zn, eluting unbound to peptidaceous ligands; the HPLC-ICPMS analyzes identified these As species as dimethylarsinic acid. Our data reveal that *R. pumila* and *I. napipes* employ distinct strategies for intracellular handling of the excess sporocarp As and Zn.

This work was supported by the Czech Science Foundation (16-34869L) and the Austrian Science Fund (FWF I2352-B21).

POSTER 51

In vivo LTP is impaired by picomolar Aß1-42 in the presence of extracellular Zn2+, but no by Aß1-40

Kobuchi, Shuhei; Sasaki, Miku; Tenpaku, Munekazu; Tamano, Haruna; Takeda, Atsushi

Amyloid-β (Aβ) accumulation plays an upstream role in Alzheimer's disease (AD) pathogenesis and Aβ oligomers can induce synapse dysfunction that contributes to cognitive decline in the pre-dementia stage. In the present study, in vivo perforant pathway long-term potentiation (LTP), which was recorded using a recording electrode attached to a microdialysis probe, was unaffected by perfusion with 1000 nM Ab_{1-42} in artificial cerebrospinal fluid (ACSF) without Zn^{2+}. However, LTP was attenuated under pre-perfusion with 5 nM Ab_{1-42} in ACSF containing 10 nM Zn^{2+}, recapitulating the concentration of extracellular Zn^{2+}, but not with 5 nM Ab_{1-40} in ACSF containing 10 nM Zn^{2+}. Ab_{1-40} and Zn^{2+}, unlike the case of Ab_{1-42} and Zn^{2+}, were not taken up into dentate granule cells, consistent with lower affinity of Ab_{1-40} for Zn^{2+} than Ab_{1-42}. Ab_{1-42}-induced attenuation of LTP was observed even with 500 pM Ab_{1-42} in the presence of 10 nM Zn^{2+}. These results indicate that extracellular Zn^{2+} is required for Ab_{1-42}-induced impairment of LTP, a cellular mechanism of memory in the rat normal brain.

POSTER 52

Involvement of intracellular Zn2+ signaling in LTP at perforant pathway-CA1 pyramidal cell synapse

Nishio, Ryusuke; Tamano, Haruna; Suzuki, Miki; Takeda, Atsushi

Physiological significance of synaptic Zn^{2+} signaling was examined at perforant pathway-CA1 pyramidal cell synapses. In vivo long-term potentiation (LTP) at perforant pathway-CA1 pyramidal cell synapses was induced using a recording electrode attached to a microdialysis probe and the recording region was locally perfused with artificial cerebrospinal fluid (ACSF) via the microdialysis probe. Perforant pathway LTP was not attenuated under perfusion with CaEDTA (10 mM), an extracellular Zn^{2+} chelator, but attenuated under perfusion with ZnAF-2DA (50 μM), an intracellular Zn^{2+} chelator, suggesting that intracellular Zn^{2+} signaling is required for perforant pathway LTP. Even in rat brain slices bathed in CaEDTA in ACSF, intracellular Zn^{2+} level, which was measured with intracellular ZnAF-2, was increased in the stratum lacunosum-moleculare where perforant pathway-CA1 pyramidal cell synapses were contained after tetanic stimulation. These results suggest that intracellular Zn^{2+} signaling, which originates in internal stores/proteins, is involved in LTP at perforant pathway-CA1 pyramidal cell synapses. Because the influx of extracellular Zn^{2+}, which originates in presynaptic Zn^{2+} release, is involved in LTP at Schaffer collateral-CA1 pyramidal cell synapses, synapse-dependent Zn^{2+} dynamics may be involved in plasticity of postsynaptic CA1 pyramidal cells.

POSTER 53

Memory loss via excess activation of AMPA receptor is induced by Zn2+ influx, but not by Ca2+ influx

Murakami, Taku; Nakada, Hiroyuki; Hisatsune, Marie; Tamano, Haruna; Takeda, Atsushi

The present study was examined the idea that maintained LTP and memory are lost by either increase in intracellular Zn^{2+} in dentate granule cells or increase in intracellular Ca^{2+} to clarify significance of the increases induced by excess synapse excitation. Maintained LTP and memory were impaired after high K^+ injection into the dentate gyrus of the hippocampus, but rescued by co-injection of CaEDTA, which blocked high K^+-induced increase in intracellular Zn^{2+}, but not high K^+-induced increase in intracellular Ca^{2+}. When AMPA was locally injected in the same manner, AMPA also impaired maintained LTP and the impairment was rescued by co-injection of CaEDTA, which blocked AMPA-induced increase in intracellular Zn^{2+}, but not AMPA-induced increase in intracellular Ca^{2+}. The present study indicates that increase in Zn^{2+} influx into dentate granule cells through AMPA receptors loses maintained LTP and memory. Regulation of Zn^{2+} influx into dentate granule cells is more critical for not only memory acquisition but also memory retention than that of Ca^{2+} influx.

POSTER 54

Metal selective DNA-binding by a zinc sensor protein from an open ocean cyanobacterium

Mikhaylina, Alevtina; Ksibe, Amira; Marks, Eleanor; Scanlan, David J.; Blindauer, Claudia

Cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* are major contributors to global CO_2 fixation. Understanding their zinc metabolism is potentially critical for understanding controls on marine primary production due to low oceanic zinc concentrations and the predicted requirement of zinc for carbonic anhydrase and other key metabolic enzymes.

Analysis of zinc homeostasis in the model oligotrophic strain *Synechococcus* sp. WH8102 suggests zinc uptake is regulated by a predicted metal sensing Zur protein which controls expression of at least four genes thought to be involved in zinc acquisition: *znuA*, *znuB*, *znuC* (encoding a high-affinity zinc uptake system) and *bmtA* (encoding metallothionein).

Given that predicting the metal specificity of proteins is not straightforward we cloned, over-expressed in *E. coli*, and purified the predicted *Synechococcus* sp. WH8102 Zur protein to establish which metal ions this sensor protein responds to. The isolated protein was characterised by ICP-OES and ESI-MS, and the specificity of its response assessed using electrophoretic mobility shift assays with several predicted promoter regions in the presence of a range of different metal ions. We demonstrate that *Synechococcus* sp. WH8102 Zur binds DNA in its dimeric form, and all lines of evidence indicate that Zn^{2+} is indeed the sensed species.

POSTER 55

Novel type of Zn-binding peptide confirmed in several Zn-accumulating fungi of the genus Russula

Leonhardt, Tereza

Nearly half of the Zn in the ectomycorrhizal, Zn-accumulating fungus *Russula atropurpurea* (up to 1.1 g Zn kg^{-1} sporocarp dry weight) is complexed with ZBPs, a novel class of Zn binding peptides. Other closely related species, *R. viscida*, *R. ochraleuca*, and *R. pumila*, also show a marked Zn accumulation trait (up to 350 mg Zn kg^{-1}); however, whether these species can handle excess Zn by using ZBP homologs remained unknown. We thus examined the sporocarps of these species for the presence of ZBPs on both protein and gene level. Size exclusion chromatography of sporocarp cell extracts revealed that the majority of the sporocarp Zn was sequestered by 6-8 kDa peptides, resembling the RaZBPs of *R. atropurpurea*, and these also contained cysteinyl residues as confirmed by fluorescent labeling with a sulfhydryl-specific dye. Homologous PCR screening for RaZBP orthologs resulted in obtaining the corresponding coding sequences of ZBPs in all three species, and the respective peptides were back-tracked to the sporocarp protein extracts by mass spectrometry. Our results suggest that the closely related *Russula* spp. share a novel type of Zn-binding peptide that appears to contribute to the Zn-accumulation trait.

POSTER 56

Study of metals interactions and metabolic changes caused by cadmium exposure of mouse Mus musculus. Protective effectof Selenium

Baya Arenas, M del Rocio; Rodriguez Moro, Gema; Garcia Barrera, Tamara; Gomez-Ariza, Jose Luis

Cadmium is a widespread, highly toxic, environmental pollutant derived from natural and industrial sources, which is known to be accumulated in the human body. Once incorporated to organism cadmium readily gets liver, kidneys, and gastrointestinal tract, producing serious harmful effects that cause poor bone mineralization, anemia, retarded growth, developmental abnormalities and carcinogenic effects in humans[1–3] and experimental animals, such as mice. Moreover, Cd has long half-life in the body, and recently, Cd exposure has been associated with several endocrine effects[4] For this reason is of great interest to evaluate the effects of controlled exposure of *Mus musculus* to Cd and Se.In this research, 40 specimens of *Mus musculus* (10 per group) were exposed during 15 days to oral (Se) and subcutaneous (Cd) administration of different doses of Se/Cd. Metals (Cd, Se, Zn, Mn, Co, Fe, As, Pb, Cu) were measured by ICP-MS in different organs of exposed *Mus musculus* (liver, kidney, brain, lung and testis) and serum to study metals homeostasis and interactions and their accumulation was found in some metabolically active organs.Also, a study of the metabolites in the serum by GC-MS was carried out, which confirm the existence of 29 altered metabolites belonging to different metabolic pathways.

POSTER 57

Study of the homeostasis of metals in mice Mus musculus under arsenic, cadmium and mercury exposure to evaluate metal toxicity. Antagonistic interaction with selenium

Rodríguez-Moro, Gema; García-Barrera, Tamara; Navarro-Roldán, Francisco; Gómez-Ariza, José Luis

Metals have a central role in biological systems, regulating numerous cellular processes, and, alternatively, having toxic or deleterious effects on the metabolism. Hence, the study of metal-induced changes in cellular metabolic pathways is crucial to understanding the biological response associated with environmental issues. Arsenic (As), Cadmium (Cd) and Mercury (Hg) are toxic metals of environmental significance with harmful effects on man. On the other hand, it is well known that Selenium (Se) presents antagonistic interactions with numerous elements. In the present work, *Mus musculus* mice were exposed over a period of 10 days to the action of toxic metals: As, in the form of $NaAsO_2$; Cd, in the form of $CdCl_2$; and Hg as $HgCl_2$. Furthermore, antagonistic interactions with Se were evaluated in this experiment. The total metals concentrations were analysed by ICP-QQQ-MS in different tissues: liver, kidney, heart, testis, brain and blood serum to evaluate the impact of these contaminants on global homeostasis on living organism.

The results provide information about elements distribution, interactions, homeostasis and metabolic disturbing that reveals the potential of combined metallomic and metabolomic approaches in environmental exposure experiments.

POSTER 58

Understanding the copper resistance of Rhizobium tropici CIAT 899 both in free living and symbiosis

Elizalde Díaz, José Pedro; Davalos Rodriguez, Araceli; Leija Salas, Alfonso; Hernandez Delgado, Georgina; Hernandez Lucas, Ismael; Garcia de los Santos, Alejandro

R. tropici CIAT 899 is a symbiotic nitrogen fixing α-proteobacteria used in biofertilizers. In crop fields this bacteria is exposed to high concentrations of copper present in chemical fertilizer and fungicides used in the agriculture. The genetic basis of its copper resistance remains unexplored. Its genome predicts the presence of genes coding for two Cu-P_{1B} type ATPases (CopA1/A2), two multicopper oxidases (CueO1/O2) and one Cu-sequestering protein CopC. The contribution of these genes to the copper resistance of this bacterium was assessed comparing the median lethal concentration values (LC_{50}) of wild type and mutant strains growing in different copper concentrations. Only the LC_{50} of *copA1* (0.17mM) and *copA2* (0.84mM) mutants were significantly different from the LC_{50} of the wild type strain (1.16mM). Beans plants inoculated with copA1 mutant and grown in the presence of toxic concentration of copper showed a drastic reduction in number of nodules, dry weight of plants and nodules, as well as low nitrogenase activity. Histological analysis of plant revealed H_2O_2 accumulation and peroxidative damage of membrane lipids. These growth defects were not observed in plants inoculated with wild type strain or *copA1* mutant complemented with the *copA1* gene.

POSTER 59

Understanding the uptake of copper in rhizobia

González Sánchez, Antonio; Cubillas, Ciro Alberto; Davalos, Aracelí; Miranda Sánchez, Fabiola; Bañuelos Vázquez, Luis Alfredo; Brom Klanner, Susana; García De Los Santos, Alejandro

Copper (Cu) is an essential cofactor of enzymes catalyzing redox reactions in crucial biological processes. In Gram-negative bacteria the uptake of Cu is an unexplored component of a finely regulated trafficking network. The characterization of a mutant of *Rhizobium etli* CFN42, a facultative symbiotic diazotroph of common bean plants, with enhanced $CuCl_2$ tolerance led us to the identification of *ropAe* gene coding for a putative outer membrane β-barrel channel. RT-qPCR analyses indicated that *ropAe* maintains a basal transcription level, even under copper overload, facilitating the entrance of copper to the cell. Under copper limitation, the transcription of *ropAe* increases, allowing the bacterium to survive when copper is scarce. The disruption of *ropAe* did not increase the resistance to other divalent metals suggesting that RopAe is not their main entrance.

Preliminary competition experiments for nodule occupancy when both strains were co-inoculated 1:1 on bean roots growing under high-copper conditions the wild type strain was outcompeted by *ropAe* mutant strain. A maximum likelihood phylogenetic analysis revealed that RopA proteins are highly conserved in the *Rhizobiales* order but poorly conserved in other alpha proteobacteria and unrelated to the best-studied outer membrane β-barrel channel of other bacteria.

POSTER 60

Ruthenium(II) Arene NSAID Complex: Synthesis, Antiproliferative Activity Against Cancerous Cell Lines and Inhibition of Cyclooxygenase

Mandal, Poulami; Malviya, Novina; Mukhopadhyay, Suman

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of molecules which have been found to be active against cancer cells with chemopreventive properties by targeting cyclooxygenase (COX-1 and COX-2) and lipoxoygenase (LOX), commonly upregulated (particularly COX-2) in malignant tumors. Arene ruthenium(II) complexes with a pseudo-octahedral coordination environment containing different ancillary ligands have shown remarkable activity against primary and metastatic tumors. Herein we report the synthesis of novel Ruthenium(II)-arene complex using diclofenac as chelating agent. Complex **1** has shown promising antiproliferative activity against three different cell lines with GI_{50} values comparable to adriamycin. At the concentration of 50 μM, complex **1** has also shown effective inhibition of cyclooxygenase and lipooxygenase enzyme, indicating a possible correlation between inhibition of COX and/or LOX and anticancer property.

POSTER 61

Strontium (Sr) accumulation in woody plants: production of Ca/Sr containing grains in the stem bark

Harada, Emiko; Yokoyama, Miki; Sho, Misako; Kimura, Hiromi; Mori, Takuya; Inada, Kazuyuki; Takenaka, Chisato; Tomioka, Rie; Hokura, Akiko; Terada, Yasuko; Mizuno, Takafumi

The uptake and accumulation of Sr in trees have a strong impact on the fate and distribution of radionuclide in the forest. We here investigated the localization of stable isotope of Sr in *Salix* sp, *Eleutherococcus innovans*, and *Chengiopanax sciadophylloides*, that were reported as Sr accumulating woody plants. The plants were harvested in the field and the stem parts were mainly used for the further analysis. Sr, Ca, Fe, Mn and Zn were accumulated in stem bark than stem wood. Synchrotron radiation-based micro X-ray fluorescence analyses were performed and Ca/Sr containing grains were observed in the idioblasts in woody stem bark. The grains, isolated by the generation of protoplasts of stem bark of a willow (*S. miyabeana*), were revealed to the crystal druse. To elucidate the mechanism of the production of Ca/Sr grains, RNA-seq was performed by using stem barks of a willow tree in different developmental stages cultivated in hydroponics. The processed sequencing reads were mapping to the reference genome of *Populus trichocarpa.* GO annotation identified genes that were more enriched in woody stem bark than in young stem bark and involved in Ca homeostasis. Our study identified several candidate genes responsible for the Ca/Sr accumulation in woody plants.

POSTER 62

Structure and Function of Side-Loop in a Blue Copper Protein, Pseudoazurin

Nodoka, Funakubo; Yamaguchi, Takahide; Kohzuma, Takamitsu

Pseudoazurin (PAz) from *Achromobacter cycloclastes* is a member of blue copper protein. PAz functions as an electron donor to nitrite reductase (NiR) and nitrous oxide reductase (N_2OR) in denitrifying bacteria. PAz has a copper ion coordinated by Cys78, His80, and Met86 in a front-loop region and His40 located at a back-loop region with distorted tetrahedral geometry. Recently, DFT calculation of the electronic structure of PAz was performed to know the role of weak interaction in the second-coordination sphere of blue copper protein showing unique spectroscopic properties. An amino acid, Asn9 in the side loop region was required for the computational reproduction of the active site structure. The X-ray crystallographic analysis of PAz demonstrates the amido side chain of Asn9 residue has a hydrogen bonding with the side chain of Lys38. The hydrogen bonding between Asn9 and Lys38 seems to be important skelton for maitaining the active site structure. Here we would like to report the role of side loop involving Asn9 by the mutation of Asn9 to Phe. The structure and properties of Asn9Phe and related studies to add more knowledge of weak interaction in blue copper protein also will be discussed.

POSTER 63

Structure and Properties of a Side-Loop Variant, Met16Gly Pseudoazurin

Yamaguchi, Takahide; Tamaoki, Saori; Kohzuma, Takamitsu

Outer sphere interactions of protein regulate the structure and properties of metal active sites. The effects of hydrogen bonds have been evaluated in past a few decades. Non-covalent weak interactions among the amino acid resides are also important on the formation of outer sphere protein matrix. Earlier studies of us demonstrated the interaction between Met16 in second coordination sphere and His81 as inner sphere ligand regulate the spectroscopic and electrochemical properties of type 1 Cu site of pseudoazurin. However, the effects of such a weak interaction on the electron transfer reaction is still unclear. We thereby investigated the electron transfer from newly established Met16Gly variant to nitrite reductase in order to demonstrate the effects due the elimination of S-π interaction. And the X-ray crystal structure analysis of Met16Gly variant led us the hypothesis about an unnoticed role of “side-loop” in outer sphere of type 1 Cu site.

POSTER 64

Exploring the biotransformation of metal-bearing extraterrestrial and terrestrial materials by the extreme thermoacidophile Metallosphaera sedula

Kölbl, Denise

The thermoacidophilic archaeon *Metallosphaera sedula*, is capable of colonizing and mobilizing metal-rich environments as it thrives on the extraction of different metals from a mineral phase.
As a consequence of this lifestyle, *M.sedula* developed strategies not only to survive the deleterious impact of metals on normal cellular function, but also to utilize them as an energy source. Investigations of the metal-extracting abilities of *M.sedula* incubated with synthetic Martian Regolith Simulants (JSC 1A; P-MRS; S-MRS; MRS07/52) which were modelled after a variety of analysed Martian soils, were carried out in regard to extraterrestrial and terrestrial biomining applications. Observations with energy-dispersive X-ray spectroscopy (EDS) revealed the presence of sphere-like particles on the mineral phase which were absent under abiotic conditions. Furthermore, electron paramagnetic resonance spectroscopy (EPR) was applied to investigate *M.sedulas* specific spectral shifts and signatures on Martian Regolith simulants which are clearly distinguishable from abiotic simulants. These preliminary results allow us to catch a glimpse into an archaeon-mediated biotransformation of (extra)terrestrial minerals, indicating that these polyextremophiles are capable to gather fingerprints of (extraterrestrial) life under extreme conditions and simultaneously emphasize the role of chemolithotrophs in the geobiochemical cycle of earth.