Abstracts

Organizers

Analytical Spectrometry Group

University of Oviedo

www.metalloffomics2013.com
METALLOMICS WITH DNA: FROM MOLECULAR MACHINES AND SENSORS TO INTRACELLULAR DRUG DELIVERY

Itamar Willner¹

¹ Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel
Email: willnea@vms.huji.ac.il

Metal ions and metal nanoparticles interact with nucleic acids and yield hybrid materials of new functional properties that are implemented in different scientific disciplines. This topic will be addressed with several examples.

1. Sequence-specific nucleic acids stabilize Ag⁰ nanoclusters (NCs) exhibiting tunable fluorescence properties. The nucleic acid/Ag NCs hybrids are deposited on graphene oxides, and the resulting matrices are used for the fluorescence, multiplexed, analysis of DNA [1].

2. Metal nanoparticles (NPs) are assembled on DNA machines (tweezers, three-ring catenanes). The programmed cyclic mechanical arrangement of metal NPs is accomplished by the DNA machines, resulting in interparticle plasmonic coupling dictated by the nanostructures of the NPs. By the integration of metal NPs and fluorophores with the DNA machines, switchable surface-enhanced fluorescence and fluorescence quenching are dictated by the DNA machines [2].

3. The hemin/G-quadruplex acts as catalytic nucleic acid (DNAzyme) that mimics the functions of horseradish peroxidase. Its use as catalytic amplifying label for bioanalytical applications will be discussed. Specifically, its use as an optical label for the analysis of DNA [3] and the multiplexed analysis of nucleic acid targets [4] will be demonstrated. Also, the use of hemin/G-quadruplex DNAzyme as an electrocatalyst for the detection of DNA will be discussed [5].

4. Metal-ion-dependent DNAzymes hybridized with their substrates act as capping units for the entrapment of substrates (drugs) in the pores of mesoporous SiO₂ nanoparticles (MS-SiO₂-NPs). In the presence of appropriate metal ions (e.g., Mg²⁺, Zn²⁺), the pores are unlocked, leading to the release of the pore-entrapped substrates. By the nano-engineering of the DNAzyme sequences, the activation of the DNAzymes by cell biomarkers (e.g., ATP), using cooperative aptamer-ATP interactions, was achieved. The use of these “smart” materials for the controlled release of doxorubicin (an anti-cancer drug) and the selective destruction of cancer cells was demonstrated [6].

References

CYTOMETRY AND ATOMIC MASS SPECTROMETRY CONVERGE IN SINGLE CELL DEEP PROFILING OF THE HUMAN IMMUNE SYSTEM

Scott Tanner¹²

¹ Department of Chemistry, University of Toronto, Canada
² DVS Sciences Inc., Markham, Ontario, Canada
Email: sd.tanner@utoronto.ca, scott.tanner@DVSsciences.com

Fluorescence flow cytometry helped to define the cell subsets of the immune system. Advances in instrumentation and reagents increased the number of simultaneous measurements to 8 and more, which enabled the distinction and characterization of rare immune subsets and stem cells. The addition of intracellular staining led to the examination of signaling networks and, recently, stratification of patients correlated with clinical outcome. The potential for further advances has been stymied by the physical and spectral limitations of fluorophores.

Mass Cytometry, which uses antibody tags incorporating stable heavy element isotopes combined with detection using an application-specific enactment of Inductively Coupled Plasma Mass Spectrometry, has opened the floodgates to permit 30-50 parameter measurements in single cells, at up to 1000 cells per second. We will review the technology through the eyes of advances made in the reagents and hardware in recent years.

Already the technology has offered dramatic new insights into the operation and function of the human hematopoietic hierarchy, shown novel application for the screening and mechanistic understanding of drug candidates, and foresees improved prognostic and diagnostic application in the clinic. We will report on our work, and the work of others, in profiling the signaling and functional responses of the suite of cell populations in human bone marrow, the revealing of unappreciated levels of organization in virus-specific memory T cell compartments, and massively multiplexed single-cell kinase inhibitor profiling.

We speculate that deep profiling of the individual immune state, both static and in response to stimulation, could be more beneficial to the maintenance of health, and better guide the recovery from perturbations from this state, than static predictive markers.
IRON IN HEALTH AND DISEASE: 
THE ROLE OF THE HEPcidIN/FERROPORTIN AXIS

Sophie Vaulont¹

¹ INSERM, U1016, Institut Cochin, Faculté de Médecine Cochin Port Royal, CNRS, UMR8104, Paris, 75014, Université Paris Descartes, Sorbonne Paris Cité, Paris, France
Email: sophie.vaulont@inserm.fr

Iron is an essential biometal employed by almost all cells as a cofactor for fundamental biochemical activities, such as oxygen transport, energy metabolism and DNA synthesis. In mammals, body iron homeostasis is complex and depends on the regulated absorption of dietary iron by mature enterocytes of the duodenum and iron recycling by macrophages, which supply most of the serum iron through recovery of the metal from senescent erythrocytes. These two fundamental processes are regulated by the iron-dependent hormone hepcidin, a 25-aminoacid peptide produced mainly by the liver, that allows iron adaptation according to the body iron needs. The circulating peptide acts to limit gastrointestinal iron absorption and serum iron by binding to ferroportin, a transmembrane iron exporter, thereby inducing its internalization and subsequent degradation, leading to decreased export of cellular iron. Complete hepcidin deficiency in mice leads to progressive iron accumulation with predominant iron overload in tissues and iron sparing of the macrophages. Conversely, transgenic animals constitutively expressing the hepcidin gene display iron deficiency anemia.

In recent years, there has been important breakthrough in our knowledge of hepcidin regulation that has also implications for understanding the physiopathology of human iron disorders. Different aspects of hepcidin regulation will be considered in this presentation, including regulation by the iron status (the BMP6/HJV/SMAD pathway) and the infection/inflammatory pathway.

In human, dysregulation of hepcidin is involved in the pathogenesis of a spectrum of iron disorders. Most of the iron overload syndromes known to date (hereditary hemochromatosis and secondary iron overload such as β-thalassemia) imply a reduction of hepcidin secretion. In contrast, excessive hepcidin expression causes hypoferremia and contributes to the anemia of inflammation (commonly observed in patients with chronic infections, malignancy, trauma, and inflammatory disorders) and genetic anemia called IRIDA (iron-refractory iron deficiency anemia).

The emergence of hepcidin as the pathogenic factor in most systemic iron disorders should provide important opportunities for improving their diagnosis and treatment. If further investigations are awaited concerning the molecular regulation and interaction of hepcidin and ferroportin to expand our understanding of iron disorders, there is no doubt that targeting the hepcidin-ferroportin axis constitutes an interesting alternative therapeutic for human application.
SPECIFICITY IN METAL-SENSING:
SELECTIVITY AS A COMBINED FUNCTION OF A SET OF SENSORS

C.J. Patterson¹, R. Pernil¹, S.J. Dainty¹, B. Chakrabarti¹, C.E. Henry¹, C. Hess¹, V.A. Money¹, A.W. Foster¹, N.J. Robinson¹

¹ Department of Chemistry, School of Biological and Biomedical Sciences, Department of Mathematics, Durham University, DH1 3LE, UK
Email: nigel.robinson@durham.ac.uk

High-fidelity, metal-sensing is thought to control the buffered concentration of each metal inside cells which, in turn, is thought to be vital in the control of metal-protein speciation (Nature 2009 460: 823-830, Nature 2008 455: 1138-1142).

Over the decades we have discovered four classes of DNA-binding, metal-sensing, transcriptional regulator in the model organism Synechocystis PCC 6803. These sensors include Zn(II)-responsive de-repressor ZiaR (PNAS 1998 95: 10728-10733, Mol Microbiol 1993 7: 177-187), Zn(II)-responsive co-repressor Zur (PNAS 2012 109: 95-100), Ni(II)-responsive de-repressor InrS (J Biol Chem 2012 287: 12142-12151) and Co(II)-responsive activator CoaR (J Biol Chem 1999 274: 25827-25832). What allows each of these sensor-proteins to recognise and respond to their cognate metal but not to the other inorganic elements?

For Ni(II) (J Biol Chem 2012 287: 12142-12151), for Zn(II) (manuscript in preparation), but not for Co(II) (Metallomics 2013 5: 352-362), metal-selectivity matches the relative affinities of the complement of metal-sensors within the cell. These findings have implications for the nature of the buffered pools of metals available to proteins. They illustrate the merit of a metallomics approach in which metal-selectivity is considered in the context of a cellular system.
THERAPEUTIC AND DIAGNOSTIC METAL COMPLEXES IN MEDICINAL INORGANIC CHEMISTRY

Chris Orvig¹

¹ Medicinal Inorganic Chemistry Group, Department of Chemistry and Faculty of Pharmaceutical Sciences, University of British Columbia, 2036 Main Mall, Vancouver, BC, V6T 1Z1, Canada
Email: orvig@chem.ubc.ca

The role of metal complexes as therapeutic and diagnostic agents is burgeoning due to interest from many academic and industrial concerns; the current value of medicinal inorganic chemistry is in excess of $10^9. Cis-platin, for example, is the archetypal inorganic drug containing as it does, no atoms of carbon. Principles in the design of metal compounds as drugs will be discussed in detail with examples from work in the speaker’s research laboratories presented to illustrate these principles. These examples are taken from our group’s work in insulin-enhancing vanadium compounds, diagnostic and therapeutic radiopharmaceuticals, and multifunctional agents for neurodegenerative diseases and malaria, as well as antimalarial, antibiotic and antiosteoporotic compounds.
THE 10 YEARS HISTORY OF METALLOMICS AND FUTURE PERSPECTIVE

Hiroki Haraguchi¹

¹ Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Japan
Email: haraguch@gmail.com

In 2001, the primary reports about the DNA sequences in human genome were published in Nature and Science, from the Human Genome Project and the Celera Genomics, respectively. In the same year, the publication of the journal “Proteomics” was announced from the VCH Publishing Co. These news inspired me to create “Metallomics” as bio-metal science. Thus, in 2002, I proposed “Metallomics” as the new scientific field in the invited lectures on the occasion of the local seminar and the International Symposium on Bio-Trace Elements held in Japan; the titles of those lectures were “A Challenge to Pico-World and Metallomics: A New Frontier of Trace Element Chemistry” and “Trace Element Speciation for Metallomics”, respectively. In 2004, according to the backgrounds mentioned above, my paper entitled “Metallomics-Integrated Biometal Science” was published in J. Anal. At. Spectrom. [1]. In 2007, the present author also organized the first International Symposium on Metallomics (ISM 2007) held in Nagoya, followed by the 2nd in Cincinnati in 2009, and 3rd in Munster in 2011. Furthermore, in 2009, the journal “Metallomics” was launched from RSC. In 2010, the IUPAC Technical Report “Metallomics: Guidelines for terminology and critical evaluation of analytical chemistry approaches” was published in Pure Appl. Chem. [2]. Through these events and publication activities, “metallomics” has been spreading over last ten years as the interdisciplinary scientific field in academia.

References
METALLOMICS: THE MEANINGS BEHIND

Ryszard Lobinski¹

¹ Laboratoire de Chimie Analytique Bio-inorganique et Environnement (LCABIE), UMR5254, CNRS, Hélioparc, 2, av. Pr. Angot, 64053, Pau, France
Email: ryszard.lobinski@univ-pau.fr

The term metallomics, coined by Haraguchi [1], reflected the trend in life sciences to study biomolecules globally using a large scale approach. It also claimed for the recognition by the life sciences community of the largely neglected, however essential, component of a biological system, that of metal ions and their species. The quest for this information opened a challenging area of elemental speciation analysis as predicted in a seminal paper: Metallomics – a New Frontier in Analytical Chemistry in the first 2004 ABC issue by Szpunar [2].

In the meantime, metallomics has become a fashionable term and a sort of umbrella for any research involving trace metals in a biological systems and, sometimes, even beyond that. The etymology of the term which evokes, simultaneously, a metal or metalloid, a large-scale approach, and a biological connotation, often seems to be ignored. However, two trends proper to the very essence of the term are paving their way: (i) the correlation of a metal concentration fingerprint (which can be determined accurately in a cell or tissue at any moment of the organism’s development) with the genome of this organism, and (ii) analysis for large sets of metal complexes with biomolecules, usually proteins and metabolites and their correlation with the system biological conditions.

Electrospray MS, already the basis of analytical proteomics and metabolomics, and inductively coupled plasma (ICP) mass spectrometry, allowing quantitative trace element analysis, have naturally become the linchpin techniques for metallomics [3]. Their couplings with separation techniques, such as multidimensional electrophoresis and chromatography, offer a versatile and powerful platform for the large scale acquisition of information on metal complexes with biomolecules. The progress in analytical methodology, bioinformatics, and molecular biology unite the conditions for fascinating metallomics research.

References
TOOLS FOR METALLOMICS

Gary M. Hieftje¹, Steven J. Ray¹, Alexander W.G. Graham¹, Elise A. Dennis¹, Christie G. Enke², David W. Koppenaal³, Charles J. Barinaga³

¹ 800 East Kirkwood Ave, Bloomington IN 47405
² Department of Chemistry, University of New Mexico, Albuquerque, NM 87131, USA
³ Pacific Northwest National Laboratory, Richland, Washington 99352, USA
Email: hieftje@indiana.edu

“Distance-of-flight mass spectrometry” (DOFMS) is a new sort of mass spectrometry that is similar in architecture to time-of-flight mass spectrometry (TOFMS), one of the most common types used in metallomics investigations. Both TOFMS and DOFMS have no upper mass range, so are attractive for biomolecule analysis, and both feature very high repetition rates, so can be employed for detection of species that have been separated by high-speed chromatography or electrophoresis. In DOFMS as in TOFMS, ions are accelerated to a mass-dependent velocity. In TOFMS, the mass-to-charge ratio (m/z) of those ions is then determined from the time they reach a fast detector positioned at the end of a field-free region. In contrast, in DOFMS, ions are not allowed to emerge from the field-free region but are pushed sideways onto an array of detectors stationed part way down the region. A single detector is therefore not required to do all the work, and high-speed electronics are not needed. The result is a broader dynamic range and simpler instrumentation. In one new implementation of DOFMS, collection surfaces are substituted for the detector array, so mass-separated biomolecular ions can be accumulated and studied by other methods. In a second extension, DOFMS principles are applied to TOFMS, so higher resolution can be obtained.
HIGH-RESOLUTION GENOME-WIDE SCAN OF THE YEAST IONOME

David E Salt¹

¹ University of Aberdeen
Email: david.salt@abdn.ac.uk

To balance the demand for uptake of essential elements with their potential toxicity living cells have complex regulatory mechanisms. Here, we describe a genome-wide screen to identify genes that impact the elemental composition (“ionome”) of yeast *Saccharomyces cerevisiae*. Using inductively coupled plasma - mass spectrometry (ICP-MS) we quantify Ca, Cd, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S and Zn in 11,890 mutant strains, including 4,940 haploid and 1,127 diploid gene deletion strains, and 5,798 gene over expression strains. We developed novel analytical methods and statistical procedures, and designed and deployed a publically accessible database at www.ionomicshub.org to allow fully searchable public access to the data. We identified 1,065 gene deletion strains and 446 gene over expression strains with an altered ionome. Disruption of protein metabolism or trafficking has the highest likelihood of causing large ionic changes, with gene dosage also being important. Gene over expression produced more extreme ionomic changes, but over expression and loss of function phenotypes are generally not related. Ionomic clustering revealed the existence of only a small number of possible ionomic profiles suggesting fitness tradeoffs that constrain the ionome. Clustering also identified important roles for the mitochondria, vacuole and ESCRT pathway in regulation of the ionome. Network analysis identified hub genes, novel members of ionomic networks and organelle cross talk.

References

VASCULAR CALCIFICATION AND BONE DEMINERALIZATION

Jorge B. Cannata-Andía¹, Pablo Román-Álvarez¹, José Luis Fernández-Martín¹

¹ Bone and Mineral Research Unit, Hospital Universitario Central de Asturias, RedinRen del ISCIII, Instituto Reina Sofía de Investigación, Universidad de Oviedo, Spain.
Email: cannata@hca.es

Vascular calcification, bone loss and increased fracture risk are age-associated disorders. Several epidemiological studies have suggested a relationship between vascular calcification, impaired bone metabolism and increased mortality. So far, this relationship had been under-estimated as osteoporosis and vascular calcification have been considered non-modifiable disorders of aging. Recent data suggest that this association is not simply an artefact of age, stressing that the co-incidence of vascular calcification with low bone activity and osteoporosis could be biologically linked.

During the development of vascular calcification, the transition of vascular smooth muscle cells towards an osteoblast-like phenotype promotes the release of the vesicular structures and mineralization within these structures is promoted by several players, including those related to mineral metabolism, like phosphorus, calcium or parathyroid hormone, which influence either the supersaturation within the structure or the expression of osteogenic factors. However, an intriguing question is whether the presence of vascular calcification impacts bone metabolism, thus demonstrating true crosstalk between these tissues.

Evidence is now emerging, suggesting that some inhibitors of the Wnt pathway, such as secreted frizzled Proteins 2 and 4 and Dickkopf related protein-1 may play a role linking vascular calcification and bone loss. An additional important question to answer, from the patient’s perspective, is whether or not progression of vascular calcification can be prevented or restricted and whether altering this progression we can efficiently impact patients’ outcomes. Much evidence suggests that the control of the chronic kidney disease-mineral and bone disorder components, particularly serum phosphorus, are the main targets to maintain normal bone turnover and protect against vascular calcification.
BIOIMAGING OF METALS CAN UNRAVEL THE PATHOGENS HOST DYNAMICS WITH REGARDS TO METAL HOMEOSTASIS IN MAMMALIAN ORGANISMS

Dagmar S. Urgast¹, Andrea Raab¹, Joanna Potrykus², Alistair J.P. Brown², Sarah Hill³, Heidi Goenaga-Infante³, Joerg Feldmann¹

¹ TESLA_Trace Element Speciation Laboratory Aberdeen, Chemistry, University of Aberdeen, AB24 3UE, Scotland, UK
² Aberdeen Fungal Group, School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK
³ LGC London, Teddington, UK
Email: j.feldmann@abdn.ac.uk

Mammalian hosts defend themselves against microbial infection by limiting the availability of some micronutrients, a phenomenon known as nutritional immunity. Iron is one such essential micronutrient that is critical for microbial growth and virulence and zinc and manganese are other elements which are regulated to defend the host for bacterial infection for example as shown for Staphylococcus aureus liver infection [1]. Fungal infections pose a considerable threat worldwide, yet to date, nutritional immunity has not been examined during fungal infection, and the dynamics of iron availability within local host niches are poorly defined.

In this lecture the fungal infection of rats by Candida albicans is followed by using elemental and molecular bioimaging using MALDI and laser ablation ICPMS. The renal iron landscape changes dramatically during disease progression. Iron accumulates in the renal medulla partly as a result of perturbed red blood cell recycling in the spleen by red pulp macrophages. Paradoxically, the increased iron loading in the renal medulla is accompanied by nutritional immunity in the renal cortex as local iron exclusion zones emerge around fungal foci. This localized nutritional immunity deprives Candida albicans of iron. Our study underpins the role of the kidney in systemic iron homeostasis and affords a unique glimpse into pathogen-host dynamics in situ during disease progression. To investigate the kinetics and the dynamics in the system is a bioimaging technique which makes the time scale visible. This can be done with using multiple isotopes and using a laser ablation multi-collector approach. The proof of concept will be presented in second part of the lecture [2, 3].

References
METAL TRAFFICKING AND PROTEINS IMPORT IN MITOCHONDRIA

Lucia Banci¹

¹ University of Florence, CERM, Faculty of Chemistry, Via Sacconi, 6 50019 Sesto Fiorentino (FI)Italy
Email: banci@cerm.unifi.it

Cellular processes commonly require the concerted action of biomolecules: Each must have suitable conformations, be located in the proper cellular compartment and able to interact with each other in the correct mode. Their characterization therefore requires a comprehensive knowledge of all the players, of their properties and of their interactions, many of which are transient as the process must proceed sequentially. Notably, metal transfer from metal transporters to the final recipient proteins occurs through a series of transient protein-protein interactions, where metal transfer correlates with metal affinity gradients among the various proteins, with kinetic factors contributing to the selectivity of the processes [1]. NMR approaches are extensively exploited to describe such cellular pathways at atomic resolution [2]. Examples will be presented for pathways responsible for copper trafficking in the cell and folding of the involved proteins, with a particular focus on mitochondria. Protein folding and maturation are highly concerted processes requiring multiple steps and often involving interactions with facilitating-proteins which guide the nascent protein to its mature state in the functional cellular compartment(s) [3]. Data will be discussed within an integrated framework where, from single structures to protein complexes, processes are described in their cellular context within a molecular perspective.

References

Abstracts

Invited Lecture  IL 08  Wednesday, 10th July 2013, 10:00  Room 13

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION OF SE⁰ NANOPARTICLES IN RATS FOLLOWING PER ORAL ADMINISTRATION

Erik H. Larsen¹, Sonia Pereira¹, Katrin Loeschner¹
¹ National Food Institute, Technical University of Denmark
Email: Ehlar@food.dtu.dk

Elemental selenium nanoparticles (Se⁰-NPs) were dosed daily per os to rats at three dosage levels plus controls for 28 days (N=6 per group). Selenite (Se(IV)) was dosed as positive control. The results showed that Se⁰-NPs and Se(IV) were distributed in tissues and organs by the same pattern. This triggered interest about which chemical form(s) of Se existed in circulation and in the biological material. HPLC-ICP-ID-MS analyses demonstrated that the predominant chemical form of Se in blood plasma was selenoprotein-P, irrespective of which dosage form was used. The existence of Se⁰ in rat tissues was demonstrated by Se⁰-selective derivatization with sulphite to form the selenosulfate anion, which was determined by anion exchange HPLC-ICP-MS. The fraction of Se⁰ in liver tissue ranged between a few per cent in controls up to one third of the total Se content depending on the dosage level of Se-NPs or Se(IV). The equal uptake and distribution of Se⁰-NPs and Se(IV) and the existence of selenoprotein P in rat’s blood plasma showed that Se⁰-NPs were highly bioavailable. The detection of Se⁰ in liver tissues however, did not imply that the pristine Se⁰-NPs were bioavailable. TEM imaging was unable to detect any nanoparticles of similar size as those used as for the rat study. The low excreted concentration of Se in urine was present primarily as one selenosugar.
METALLOPROTEOMES AND METAMETALLOMICS

Wolfgang Maret¹

¹ King’s College London
Email: wolfgang.maret@kcl.ac.uk

Metalloproteomes are dynamic. Their interpretation in a biological context requires metametallomics, an approach that considers the interaction of the environment with the system under investigation. Intrinsically, dynamics are linked to variable gene expression and protein turnover under different physiologic states, genetic variability, and factors that control metal ion homeostasis. Extrinsically, dynamics depend on how nutritional and toxic exposures change metal ion homeostasis. A definition of all these conditions is critical in quantitative metalloproteomics [1]. How metal ions are controlled in biological systems depends on metal buffering and muffling, i.e. the transport of metal ions. For zinc, a complex system of zinc homeostatic proteins controls zinc redistribution to supply about 3000 human zinc proteins with zinc at the right place and at the right time. Remarkable aspects of zinc biochemistry are the presence of zinc(II) ions in cellular vesicles, their release from cells and within cells, and the use of zinc(II) ion fluctuations in biological control and communication [2,3]. The proteins regulating metal ions and those being regulated by metal ions defy the traditional definition of metalloproteins: they may not have integer metal/protein stoichiometries and they may contain different types of metals or no metal at all. Different criteria are needed for the definition of metalloproteins with transient metal binding and coordination dynamics.

References

INTEGRATION AND APPLICATION OF MULTIPLE OMICS TECHNOLOGIES FOR BIOLOGICAL EFFECTS OF NANOMATERIALS

Chunying Chen¹, Yufeng Li², Zhifang Chai²

¹ CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology, Beijing 100190, China.
² Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China.
Email: chenchy@nanoctr.cn

Nowadays, nanotechnology has experienced a rapid development and many different nanoparticles are incorporated in many products, such as medicine, sunscreens, cosmetics, paints, electronic devices and water purification systems, due to their unique properties. 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. In this talk, we will focus on the uptake, accumulation and metabolism of a couple of metallic or metal-containing nanomaterials, such as gold nanoparticles and nanorods, quantum dots, iron oxides nanoparticles, and endohedral metallofullerenes in biological systems. We will present a summary of currently available data on the fate and toxicity of these metallic or metal-containing nanoparticles based on animal and cell studies. The state-of-the art analytical methodology and tools are playing important roles in the study of nanotoxidology and nanobiology by taking advantages of absolute quantification, high sensitivity, excellent accuracy and precision, low matrix effects and non-destructiveness. 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. We used this combined approach to investigate quantum dot (QD) uptake and biotransformation by ingestion in the natural feeding environment and the subsequent fate and behavior of QDs in C. elegans. The μ-XANES technique was used for the analysis of the physicochemical changes of chemical species in vivo. Finally, future directions regarding integration and application of multiple omics technologies for biological effects of nanomaterials are also discussed.

References
METALLODRUGS

Bente Gammelgaard¹

¹ University of Copenhagen
Email: bente.gammelgaard@sund.ku.dk

Metal-based drugs have been used since the early days of civilization. Apart from the more obvious use as diagnostics and radiotherapeutic agents, they are mainly used as anticancer agents [1]. Since the fortuitous discovery of cisplatin, this drug substance used alone or in combination therapy, has proven high efficacy against various types of cancer. However, severe side effects and resistance has led to the development of second generation platinum-based agents as well as an increasing interest in alternative metal-based drugs. While the discovery of cisplatin was a result of serendipity, rational design of metal-based drugs has become increasingly important and a vast number of new compounds with alternative metals such as Ru, Au, Pd, Ga, Re, etc. have been synthesized for investigation of anticancer properties or other therapeutic effects. So far, this has resulted in registration of only few new platinum-based drugs and a few other compounds entering clinical trials. Although many of the designed compounds exhibit outstanding cytotoxic properties, they may not be very suitable as drug substances owing to poor water solubility, low bioavailability or large systemic side effects. This has led to development of drug delivery systems that meet these challenges [2]. Examples of these are modification of the ligand in the coordination complex of the drug substance, use of the pro-drug design and use of nanocarriers. To characterize the behavior of new drug substances in vitro and in vivo, efficient analytical methods are demanded. Examples of characterizations of drug substances and drug delivery system with focus on the analytical angel will be given.

References

POTENTIAL ROLE OF METALLOTHIONEINS IN EYE DISEASES

Miguel Coca-Prados¹,², Hector Gonzalez-Iglesias¹, Lydia Alvarez¹, Montserrat García¹, Carson Petrash¹, Alfredo Sanz-Medel³

¹ Instituto Oftalmológico Fernández-Vega, Avda. Dres. Fernandez-Vega, 34, 33012, Oviedo, Spain
² Yale University School of Medicine, New Haven, Connecticut 06510, USA
³ University of Oviedo, C/Julián Claveria, 8, 33006, Oviedo, Spain
Email: Miguel.Coca-Prados@yale.edu

Metallothioneins (MTs) are cytosolic zinc-ion-binding proteins with a wide range of functions, including neuroprotection, cellular zinc homeostasis, and defense against oxidative damage and inflammation. The human eye is enriched in MT proteins and in multiple MT isoforms that may contribute to antioxidant defense mechanisms. Zinc is a main regulator of gene and protein MT expression. We have recently applied bioanalytical techniques to address key questions on zinc homeostasis, including the stoichiometry of zinc binding sites per MT molecule, the fate of zinc tracers (³²Zn and ⁶⁸Zn) in MT proteins during the activation by exogenous zinc (⁶⁸ZnSO₄) and cytokines, and the concentration of MTs in human eye cells. We found that exogenous zinc induced a potent expression of newly synthesized MTs and a strong inhibition of proinflammatory cytokines. After exposure to zinc or proinflammatory cytokines, a stoichiometry transition of MTs from Zn⁶Cu¹-MT to Zn⁷-MT was observed, suggesting that the MTs may interact more effectively with ROS, decreasing the potential oxidative damage. With aging and in disease, MT levels decrease resulting in a zinc release, being cytotoxic to the cell. This situation will lead to increased oxidative stress, cytotoxicity by free zinc, and inflammation, suggesting that the zinc-metallothionein cycle becomes impaired. We propose a working model under the “Zinc-Metallothionein redox cycle” to regenerate, and enhance the antioxidant function of MTs.
SPECIATION AND METALLOMICS - POWERFUL METHODS TO STUDY METALLOPROTEIN REGULATION IN BIOMEDICAL RESEARCH

Joseph Caruso¹, Julio Landero A. Figueroa¹, Kavitha Subramanian², George Deepe²

¹ Department of Chemistry, University of Cincinnati, Cincinnati, OH, USA 45221
² Division of Infectious Diseases, University of Cincinnati, Cincinnati, OH, USA 45221
Email: joseph.caruso@uc.edu

Metallomics studies extend to various trace metal species and their interactions with each other in animals or plants. In our laboratories the metallomics approaches involve combinations of chromatography, elemental mass spectrometry and molecular mass spectrometry, and much of our recent work has been dedicated to studying up or down regulation of metalloproteins as agents to control fungal diseases.

Macrophages (MΦ, activated white blood cells) and their importance to human life as 'pathogen sensors' play important roles in elimination of pathogens. Not as well known are the effects of metal ions, metal species and metalloproteins on the ability of particular macrophages to do their critical work as the body's first line of pathogen defense.

In this presentation, the metallomics approach allows us to study up or down regulation of zinc metalloproteins using Zn speciation methods as we develop a viable model (see figure) to explain how zinc assists the macrophage in eliminating a lung pathogen - *Histoplasma capsulatum, Hc*. We utilize LC-ICPMS in tandem with ESIMS to search the Zn proteome in the macrophage and in the Hc. Results to date are unambiguous regarding specific isoforms of metallothioneins (MT) in depriving Hc of Zinc leading to its elimination. Based on this and other data we have gathered, the model shown will be presented and discussed.
ICP-MS BASED BIOANALYSIS: FROM PROTEINS TO DNAs

Guojun Han¹, Zhi Xing¹, Sichun Zhang¹, Xinrong Zhang¹

¹ Tsinghua University, Beijing 100084, China
Email: xrzhang@tsinghua.edu.cn

Inductively coupled plasma mass spectrometry (ICPMS) has great advantages in high-sensitive and selective multi-element detection and accurate isotope ratio measurement. With elemental tags, ICP-MS allows multiple and absolute analysis of biologically important molecules. Since it was proposed in 2001, ICP-MS based immunoassays have been successfully applied to the quantification of proteins with various strategies such as flow injection ICP-MS, LA-ICP-MS and single particle ICP-MS.

Recently, we have demonstrated elemental labeling methodologies for quantitative DNA assays by ICP-MS. One-step homogenous DNA assay was realized by using single nanoparticle ICP-MS detection with gold nanoparticle (AuNP) probes. [1] Furthermore, by designing DNA probes with enriched isotopes, we developed an absolute quantification of multiplex DNA assays. Our ongoing works will investigate nucleic acid post-modifications such as DNA methylation. [2]

Acknowledgments
The authors gratefully thank to the financial supports by 973 (2013CB933800) and NSFC (No. 21027013) Programs.

References
[1] Han, GJ; Xing, Z; Dong, YH; Zhang, SC; Zhang, XR, Angew. Chem. Int. Ed. 2011, 50 (15), 3462-3465
MOLECULAR MECHANISMS OF METAL SPECIES INDUCED TOXICITY

Tanja Schwerdtle¹, Franziska Ebert¹, Julia Bornhorst¹, Uwe Karst¹, Mojtaba S. Taleshi², Kevin A. Francesconi²

¹ University of Muenster
² University of Graz
Email: Tanja.Schwerdtle@uni-muenster.de

Inorganic arsenic (iAs) is classified as human carcinogen. However, its toxic modes of action are still to be elucidated. Our in vitro studies clearly indicate that human metabolism strongly contributes to iAs induced toxicity. iAs and its metabolites affect common but also different cellular targets, pointing out that after iAs ingestion and subsequent metabolism, DNA integrity is likely affected by different mechanisms and therefore very effectively; this might facilitate the carcinogenic process. Arsenosugars and arsenolipids are yet not well toxicologically characterized. In contrast to arsenobetaine, arsenosugars and arsenolipids are extensively biotransformed by humans to a multitude of arsenic metabolites; some of these are believed to be strongly toxic. Our intestinal and cellular bioavailability studies as well as the toxicological characterization of these arsenicals strongly reveal that arsenosugars and arsenolipids cannot be categorized as non-toxic to humans. Therefore risks to human health related to the presence of these organoarsenicals in seafood cannot be excluded.

In industrial countries dietary ingestion of manganese (Mn) is well above its estimated average requirement. Mn overexposure is known to cause adverse neurological effects in humans, which are yet mechanistically not understood. By the use of in vitro blood-brain and blood-CSF barrier models we have recently shown that after Mn species ingestion the blood-CSF barrier is likely to be the major route for Mn species into the brain.

Our toxicological characterization of organic mercury (Hg) species in various mammalian brain cells identified the inhibition of the DNA-damage-induced signaling reaction poly(ADP-ribosyl)ation as most sensitive toxicity endpoint. The observed inhibition of this essential DNA-repair related signaling reaction in human astrocytes by exposure-relevant thiomersal or MeHg⁺ concentrations indicates that these Hg species exert indirect genotoxic effects, which might contribute to their neurotoxicity in humans.
AN INTEGRATED APPROACH FOR IDENTIFICATION OF METALLODRUG BINDING PROTEINS IN CELLS

Hongzhe Sun¹, Ligang Hu¹, Cheuk-Nam Tsang¹, Yau-Tsz Lai¹

¹ Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong
Email: hsun@hku.hk

The effect of metals in biology effects is double-edged. Metal ions operate, on one hand, as cofactors for around 40% enzymes, on the other hand, they also exhibit toxic effects. Some metal ions, although being not essential, have been widely used in human healthcare as either therapeutic agents or diagnosis agents. To understand the molecular mechanism of a metallodrug, it is crucial to identify metal-binding proteins at a proteome-wide scale [1]. We used an integrated approach consisting of gel electrophoresis and inductively coupled plasma mass spectrometry, LA-ICP-MS, IMAC and bioinformatic approach to identify metal-associated proteins using bismuth antiulcer drug as an example [2,3]. Using continuous-flow gel electrophoresis in combination with ICP-MS, we developed a comprehensive and robust strategy to readily identify metal-associated proteins as well as to quantify the metals for fast metallome/proteome-wide profiling of metal-binding proteins.

Acknowledgements

This work was supported by the RGC of Hong Kong (N_HKU75209 and 7046/12P) and the University of Hong Kong.

References

LASER ABLATION-BASED BIOIMAGING WITH SIMULTANEOUS ELEMENTAL AND MOLECULAR MS: TOWARDS SPATIALLY RESOLVED SPECIATION ANALYSIS

Uwe Karst¹, Christina Herdering¹, Olga Reifschneider¹, Christoph A. Wehe¹

¹ University of Münster, Germany
Email: uk@uni-muenster.de

A simultaneous elemental and molecular mass spectrometric imaging approach based on laser ablation sampling has been developed. A 213 nm frequency-quintupled Nd:YAG laser is used to ablate material from tissue slices of animal or human origin. The generated aerosol is transported by a carrier gas, which consists of nitrogen or argon and may contain helium as additive. The gas flow is separated by a T-piece into two streams, which are directed into the ICP-MS and the atmospheric pressure chemical ionization (APCI)-MS, respectively. This way, elemental and molecular information is gathered in parallel. For tissue slices with a thickness between 5 and 10 µm, quantitative ablation can be achieved, which is important to apply quantification strategies for ICP-MS based on external, matrix-matched element standards. LA-APCI-MS provides the complementary molecular information on the detected species. A spatial resolution of 10 µm was obtained. Several pharmaceutical applications have been developed based on this approach: Iodinated contrast agents for X-ray tomography in rat liver tissue slices were identified and quantified using the combined technique. While pseudomolecular ion and fragment ion information is obtained by LA-APCI-MS, the total iodine signal at m/z = 127 is detected in a spatially resolved way by LA-ICP-MS.

References

INNOVATIVE ANALYTICAL STRATEGY USING ION-MOBILITY SHIFTING ADDITIVE FOR ISOBARIC SELENIUM COMPOUND IDENTIFICATION IN SELENOMETHIONINE STANDARDS BY IMS

Christopher Kune¹, Johann Far¹, Gauthier Eppe¹, Edwin De Pauw¹

¹ University of Liège, Belgium
Email: c.kune@doct.ulg.ac.be

Selenium (Se) is a trace element which is both essential and toxic depending on its concentration and its chemical form. Selenomethionine (SeMet) is one of the widely used selenium standard during Selenium speciation studies. This work was focused on the elaboration of an analytical strategy for the detection and the structural elucidation of an isobaric Se interference, which is found in standard solutions of SeMet by high resolution mass spectrometry (R(m/Δm) > 20.000). The structural elucidation of these compounds requires the isolation of the respective parent ion. Nevertheless, the mass difference between SeMet and its interference is less than 0.02Da which is well below the window selection of conventional techniques in mass spectrometry (Quadripole, ion trap). The empirical formula and double bound equivalent (DBE) of these ions suggest different tridimensional structures which lead to a discrimination depending on the ion mobility. This separation is observed, both in gaseous and liquid phase, by Ion Mobility Spectrometry (IMS), Capillary Electrophoresis (CE) and Liquid Chromatography (LC) which are hyphenated to mass spectrometry as detector. The separation efficiency of these ions by IMS and CE is improved by using specific shifting agents (18-Crown-6 Ether) selective to only one of these ions. This strategy has successfully separated the two isobaric ions present and leads to the structural elucidation of the isobar contaminant of SeMet.
METALLOMICS IS A POWERFUL TOOL IN PLANT SCIENCE

Søren Husted¹, Daniel Persson¹, Jan K. Schjoerring¹

¹ University of Copenhagen, Faculty of Science, Department of Plant and Environmental Sciences, Frederiksberg C, Copenhagen, Denmark
Email: shu@life.ku.dk

In this talk I will present some of our most recent publications to demonstrate the width and scientific potential of ICP-MS based metallomics. These examples include i) the role of molecular Zn speciation in controlling the mechanisms of Zn hyperaccumulation in plants and ii) examples will be given on how a transgenic approach was applied to induce a change in Fe and Zn speciation to increase human bioavailability of Fe and Zn in rice. Nicotianamine (NA) is an important ligand for Zn and Fe in plants. Typical Zn hyperaccumulators have elevated expression of nicotianamine synthase (NAS) relative to non-accumulators. Suppression of NAS genes by RNAi interference resulted in a strong decrease in NA and a marked decrease in translocation of Zn-NA. Speciation analysis showed that 97% of total Zn was found in complexes of <1000 Da and that these were dominated by NA, followed by traces of small peptides. Fe deficiency is the most widespread micronutrient related disorder in humans. Thus, in order to improve the Fe status it is important to improve the Fe bioavailability of grains. Transgenic rice in which NAS genes were up-regulated was used to increase NA 10-20 fold. Analysis of the grains showed only a marginal difference in the total Fe, but a marked ligand exchange in the transgenic lines was found, where Fe complexed to inositol phosphates with low bioavailability was exchanged with bioavailable Fe-NA complexes. The improved bioavailability was confirmed in anemic mice.

References
HYPHENATED TECHNIQUES LC - ICP MS AND LC - ESI-LTQ ORBITRAP MS IN INVESTIGATION OF METALS SPECIATION IN PISUM SATIVUM AND PLANTAGO ALMOGRAVENSIS

Paulina Flis¹, Laurent Ouerdane¹, Ryszard Lobinski¹, Louis Grillet², Stéphane Mari², Tomás Grevenstuk³

¹ CNRS/University of Pau, LCABIE UMR 5254, Hélioparc, Pau, France
² Biochimie et Physiologie Moléculaire des Plantes AGRO-M/INRA Montpellier, France
³ University of Algarve, Faculty of Sciences and Technology, Institute, IBB/CGB, Faro, Portugal
Email: pflis@op.pl

The investigation of metals speciation in plants aims at the prediction of their bioavailability and usage this knowledge for nutritional and toxic studies. However, to obtain the information about the metals bioavailability it is necessary to understand better plants physiological processes like detoxification, homeostasis and also uptake, transport and accumulation of essential and nonessential elements. In order to control these processes plants have evolved several mechanisms at the cellular level including production of proteins, peptides, organic acids and amino acids that are complexing metals such as iron, zinc, copper or aluminium. To date, however, analytical information on molecular forms of metabolites involved in intake, transport and storage of these elements are very limited. The lack of this knowledge is caused by different obstacles that emerge during the work with complex biological matrix. The main issues are low concentrations, various labilities, unstability and high diversity of complexes present in plants. The solution of these problems is in hyphenated techniques based on high-resolution separation by chromatography or electrophoresis with sensitive detection by elemental and molecular MS [1, 2].

In this study online coupling of size exclusion column (SEC) and hydrophilic interaction column (HILIC) to either a collision cell ICP MS or an ESI-LTQ Orbitrap MS instruments has been chosen to achieve speciation analysis in xylem and post-phloem of Pisum Sativum and leaf and root water extracts of Plantago almogravensis. A model plant - Pisum Sativum and an aluminium hyperaccumulating species - Plantago almogravensis were great objects to study metal metabolism in plants saps. Concerning chromatographic techniques, size exclusion chromatography and hydrophilic interaction chromatography has been chosen because they seem to be the most suitable for these kind of applications. The results that have been already obtained using these chromatographic techniques are very satisfying especially for avoidance of metal complexes degradation during the analysis [3]. A collision cell ICP MS and an ESI-LTQ Orbitrap MS instruments has been used because they are giving the great potential for element specific and molecule specific detection, respectively. The high sensitivity of collision cell ICP MS and an ESI-LTQ Orbitrap MS/MS instruments allows detection of different complexes at the low concentration levels. Additionally, the advantage of using ESI-LTQ Orbitrap MS/MS instruments is their high spectral resolution. The comparison and combination of data obtained by coupling LC in parallel with ICP MS and ESI MS/MS allowed us to identify several novel metal complexes such as iron, zinc or copper - nicotianamine complexes in Pisum Sativum. We
Abstracts

also have observed iron - malate - citrate complexes with different malate - citrate ratio in the green pea saps and mixed complexes iron - aluminum - citrate in *Plantago almogravensis*.

References

SPIKE PRODUCTION FOR QUANTIFICATION OF METALLOPROTEINS VIA IDMS

Julia Gleitzmann¹, Claudia Swart¹, Andrea Raab², Jörg Feldmann², Hermann Wätzig³

¹ Physikalisch-Technische Bundesanstalt, Bundesallee 100, 38116 Braunschweig, Germany
² Trace Element Speciation Laboratory, College of Physical Sciences - Chemistry, University of Aberdeen, Aberdeen, United Kingdom
³ Institut für Medizinische und Pharmazeutische Chemie, Technische Universität Braunschweig, Braunschweig, Germany
Email: julia.gleitzmann@ptb.de

Metalloproteins are important participants in biological pathways. About 30 % of the whole proteome is represented by proteins which contain metal ions often in the active center. As many of them are important markers for diseases, they are particularly relevant in medical diagnosis. Examples are hemoglobin as a marker for anaemia or e.g. transferrin or ceruloplasmin (CER) for deficiency diseases. For precise and reliable analysis of patient samples, it is necessary to develop primary reference measurement procedures for the quantification of these metalloproteins with results traceable to the SI. A very promising approach is the use of species specific isotope dilution mass spectrometry (IDMS) using inductively coupled plasma MS (ICP-MS) coupled to high performance liquid chromatography (HPLC) for the separation of the investigated protein from the biological matrix. The investigations focus on two copper containing proteins: superoxide dismutase (SOD) and CER. For the use of ID-ICP-MS species specific isotopically enriched spike material is necessary. The preparation of this spike material is based on demetallation of commercially available SOD followed by remetallation of the apo-protein with copper and zinc ions enriched in one isotope, resp. Dialysis is used for the demetallation of SOD. Various dialysis solutions with different pH values as well as different methods for the remetallation are tested. Produced spike material is analysed by ICP-MS and ESI-MS.

References
A NEW GENERATION OF REFERENCE MATERIALS FOR METALLOPROTEIN BIOMARKERS

Clay Davis¹, Guillaume Ballihaut¹, Yoana Nuevo Ordonez¹

¹ National Institute of Standards and Technology, Chemical Sciences Division, 331 Fort Johnson Rd, Charleston, SC 29412
Email: clay.davis@nist.gov

As the emerging metallomics community strives to fully understand the role of metals in biological and clinical systems, NIST has developed a core program to solve some of the unique measurement problems and to be at the forefront with regard to benchmarking chemical measurements and developing new reference materials important to the healthcare sector. Despite numerous routine clinical measurements of metal containing biomarkers, there are relatively few certified reference materials suitable for higher order measurements which establish traceability of clinical diagnostic results despite these values being utilized for disease diagnosis and treatment protocols.

Reference material production for bio-markers, clinical diagnostics, disease states, and disease profiling represents a host of new challenges. Reference materials have typically been produced by pooling healthy donors across multiple genders and races and are not particularly useful in clinical research due to the diluting of clinical data and uniqueness of the expression of certain proteins and/or biomarker of interest due to particular disease states.

The final piece in the development of the new generation of materials is the incorporation of reference data specifically linked to a material and/or sample. NIST is incorporating reference mass spectra and other associated data for proteins, peptides, metabolites, etc. in order to provide quantitative and qualitative data across instrument platforms.
DUAL-ISOTOPE PROCEDURE FOR THE LABELLING AND CODIFICATION OF LIVING ORGANISMS

J. Ignacio Garcia Alonso¹, Isabel Carames Pasaron¹, Gonzalo Huelga Suarez¹, Mariella Moldovan Feier¹

¹ Department of Physical and Analytical Chemistry, University of Oviedo
Email: jiga@uniovi.es

The labelling of living organisms is one of the oldest techniques for population studies. The use of rings to mark birds in combination with capture-recapture methods has been employed since the XVIII century. Nowadays there is a need for labelling techniques which allow also the codification of different marks to assess a variety of population studies in one single experiment. For example, in the study of restocking efficiency of river fishes it would be ideal to have a differential mark for naturally reared and restocked fishes. Also, in the study of the dispersion of seeds to study the effects of global warming on plant populations, different plants should be marked with different labels to reduce the uncertainty of the study.

In this communication we present a dual isotope labelling procedure which allows the codification of different marks by changing the molar ratio between two different enriched isotopes of the same element. The method is based on the administration of a dual enriched isotope mixture to the test specimen and the measurement of the molar fraction ratios by ICP-MS. The method has been shown to be transgenerational for the labelling of fishes and the isotopic signature injected to the female fish is preserved in the fish otoliths of the juveniles. Also, the method was employed successfully for the labelling of plants and plant seeds.
COMBINING FASP-ASSISTED TRYPTIC DIGESTION, OFFGEL-IEF FRACTIONATION, LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY FOR Pt-PROTEINS ANALYSIS

Irene Moraleja San José¹, Estefanía Moreno Gordaliza¹, Mª Luz Mena Fernández¹, Mª Milagros Gómez Gómez¹

¹ University Complutense of Madrid, Faculty of Chemistry, Avenida Complutense s/n, Madrid, 28040, Spain
Email: irene_msj@hotmail.com

To achieve the greatest number of protein identifications by liquid chromatography and tandem mass spectrometry (LC-MS/MS) analyses, samples typically must be fractionated in multiple dimensions. Fractionation strategies for proteomics studies can occur either before or after protein tryptic digestion. OFFGEL isoelectric focusing (IEF) is an attractive method for both protein and peptide fractionation [1], with sample recovery in solution phase, which makes this technique compatible with LC-MS/MS. The aim of this work is to develop a methodology which allows the separation and digestion of cytosolic proteins from target organs of rats treated with different Pt-based drugs, for their identification by nLC-ESI-LTQ-MS/MS. The Pt-binding proteins have been trypsin-digested by the filter-aided sample preparation method (FASP)[2], where the reagents employed along the process are eliminated before the following step, minimizing the risk of Pt loss. The use of denaturing and reducing conditions both in the OFFGEL separation (at protein and peptide level), and digestion step has been evaluated. The feasibility of thiol free reactants such as the phosphines TBP and TCEP as an alternative to DTT or BME has been studied.

References
NEW STRATEGIES FOR THE QUANTIFICATION OF SERUM FERRITIN AND Fe:FERRITIN-RATIO AS BIOMARKERS FOR IRON METABOLISM DISORDERS

Tobias Konz¹, María Montes-Bayón¹, Alfredo Sanz-Medel¹

¹ Department of Physical and Analytical Chemistry, University of Oviedo, c/ Julian Claveria 8, 33006, Oviedo, Spain.
Email: konztobias@uniovi.es

Ferritin is the predominant iron storage protein in humans. It is composed of 24 subunits forming a hollow sphere and a mineral core which contains a variable amount of up to 4500 Fe$^{3+}$ ions per protein. Although ferritin is mainly located intracellularly, a small fraction can be found in serum which is correlated to the total body iron stores. Alterations in ferritin are commonly seen in clinical practice, often reflecting perturbations in iron homeostasis or metabolism [1]. However, common methodologies allow only the quantification of the protein shell and do not provide information about the ferritin bound Fe. Therefore, it remains unclear if the Fe-ferritin ratio remains constant in different pathologies and if this could be a more specific biomarker of iron metabolic disorders. For this purpose we have developed an analytical strategy to address the absolute concentration of serum ferritin and ferritin bound Fe using elemental mass spectrometry (ICP-MS). Ferritin determination is based on the use of the specific Ru-labeled antibody and the separation of the antigen-antibody complex separation by means of magnetic microparticles. Those particles can be directly introduced into the ICP-MS and the Ru is quantified using IDA (isotope dilution analysis) without the need of specific calibrating solutions. In a second approach, a multidimensional sample preparation strategy was applied for the absolute quantification of ferritin-bound Fe by post-column IDA.

References
CAP-LC AND QQQ-ICPMS, FOR DETECTING PHOSPHORUS AND SULFUR IN DNA-PROTEIN CROSS-LINKS

Julio Landero-Figueroa¹, Jiawei Gong¹, Morwena Solivio¹, Joseph Caruso¹, Edward Merino¹

¹ McMicken College of Art & Sciences, Department of Chemistry, University of Cincinnati, Cincinnati, OH, USA 45221-0172
Email: julio_landero80@yahoo.com

Along with the oxidation of guanidine, the ROS driven reactions between proteins and DNA (cross-links) generate the most common damages that nucleic acids can suffer. These are important reactions because if they remain unrepaired, permanent mutations or replication stops are formed leading to cytotoxicity. However, they are not well studied, mainly because of the lack of good analytical procedures with sufficient detection capabilities. Techniques, based on ultra-trace level detection of S and P, are good, but they are not without interferences. The state-of-the-art for interference removal is to use the newer QQQ-ICPMS approach by passing $^{31}\text{P}^+$ in Q1, passing to Q2 operating in the reaction mode and adding O$_2$, therefore generating $^{47}\text{PO}^+$ with Q3 and leaving the usual NOH$^+$, etc. interferences behind. Monitoring $^{47}\text{PO}^+$ results a signal absent from polyatomic interferences. The lower detection levels, when compared with those from cells using the collision or energy discrimination modes, are a major plus for the QQQ-ICPMS. In conjunction with the high resolving power of capillary liquid chromatography, the new QQQ-ICPMS technology was applied to study a DNA-Protein cross-link, by following both $^{47}\text{PO}^+$ and $^{48}\text{SO}^+$ in the intact complex and in its enzymatic digestion products. A good assignment of S containing peptides was possible and the peptide link to the DNA oligonucleotide was identified. Complementary MS methods are used to identify the cross-link.
IRON (Fe) TRANSPORT IN PLANTS: WHEN SPECIATION IS CRUCIAL TO REVEAL NEW BIOLOGICAL FUNCTIONS

Louis Grillet¹, Catherine Curie¹, Stéphane Mari¹, Laurent Ouerdane², Paulina Flis², Marie-Pierre Isaure², Ryszard Lobinski²

¹ Laboratoire de Biochimie et Physiologie Moléculaire des Plantes, BPMP, UMR5004, CNRS/INRA/UMII/SupAgro, place Viala, Montpellier, France.
² Institut Pluridisciplinaire de Recherche sur l’Environnement et les Matériaux, UMR5254, CNRS/université de Pau et des pays de l’Adour, Pau, France.
Email: mari@supagro.inra.fr

Seeds represent a major source of essential transition metals (Fe, Zn, Cu) for human nutrition. Understanding the mechanisms controlling seed loading are of crucial importance in this perspective. Seeds of grain legumes represent an interesting model to study Fe transport since it is easy to obtain the sap that feeds the embryo (embryo sac liquid). The analysis of Fe speciation in this fluid is of potential high importance to further understand how Fe is transported to the embryo and thus to biochemically dissect the whole process. This study started with the chemical analysis of the embryo sac liquid sampled from developing pea (Pisum sativum) seeds, using liquid chromatography coupled to mass spectrometry. This approach revealed that Fe is delivered to the embryo as ferric-citrate-malate complexes. With these data we have been able to further dissect the whole machinery of Fe uptake by isolated embryos and discover a new Fe transport strategy. To dissociate the ferric complexes and take up Fe, embryos efflux massive amounts of ascorbate that chemically reduces Fe before its membrane transport. These results have illustrated how the study of Fe speciation by analytical chemistry has allowed to identify a novel mechanism of Fe transport in plants paving the way for future work on the molecular characterization of this new Fe transport process, which could be a promising target for biofortification programs.
INVESTIGATION OF IRON-SPECIES DERIVED FROM ORGANIC ACIDS IN PLANTS
BY HILIC-MS AND CYCLIC VOLTAMMETRY

Günther Weber¹, Nicolaus von Wirén²

¹ Leibniz-Institut f. Analytische Wissenschaften - ISAS - e.V., Otto-Hahn-Str. 6b, 44227 Dortmund, Germany
² Leibniz-Institut f. Pflanzengenetik u. Kulturpflanzenforschung, Corrensstr. 3, 06466 Gatersleben, Germany
Email: guenther.weber@isas.de

Organic acids of the TCA cycle (e.g. citrate, malate) are prominent candidates for iron binding in plants, but only few reports are available on the exact stoichiometry and redox state of respective iron complexes. Unequivocal identification is hindered by the relatively low stability of some iron species with respect to separation and detection procedures, and by the high complexity of iron-ligand equilibria, involving polynuclear and mixed-ligand complexes. Moreover, complex stoichiometry and stability depend on pH and the metal to ligand ratio. Hence, analytical results may differ depending on the plant species, plant compartment, etc.

Here, results are shown for iron species derived from organic acids (mainly citric and malic acid as ligands), originating from different plant species (maize, barley, Arabidopsis) and different plant compartments (xylem, phloem, leaves). HILIC-MS is used as main analytical method, and cyclic voltammetry is employed as complementary technique for elucidating the redox behavior of iron species. Several stoichiometries are found, incl. stable di- and trinuclear complexes and mixed-ligand complexes. The dependence of species stoichiometry on their biological origin is discussed, and analytical problems for labile species are highlighted.
CADMIUM-INDUCED RESPONSES IN AN ACCUMULATOR PLANT SPECIES

Rebeca Fernández¹, Daniel Fernández-Fuego¹, Ana Bertrand¹, Aida González¹

¹ University of Oviedo, Department of Biology of Organisms and Systems, Catedrático Rodrigo Uría, s/n, 33006 Oviedo, Spain
Email: fernandezfrebeca@uniovi.es

Some plant species can survive in highly metal-polluted soils, toxic for most other plants. The mechanisms underlying metal detoxification in these plants are still unknown, especially in the case of accumulators and hyperaccumulators. The objective of this work was studying the biochemical and physiological mechanisms involved in the responses to Cd of Dittrichia viscosa (L.) Greuter, an accumulator plant species commonly found in metal-polluted areas of Asturias. We compared two clones with contrasting origins: DV-A, metallicolous, which accumulated up to 1,300 mg Cd kg⁻¹ dry wt. in shoots (Fernández et al., 2008) and DV-W, non-metallicolous. Both clones exceeded hyperaccumulation levels when cultured in vitro and Cd induced the production of phytochelatins (PCs). They also showed a high Cd tolerance and accumulation in hydroponics, but clone DV-A was more tolerant than DV-W at the highest Cd doses tested due to a better efficiency of antioxidant enzymes (Fernández et al., 2013). In these conditions Cd exposure induced PC synthesis, especially in DV-W, and affected organic acids levels. Finally, ultrastructural localization studies shown that most of the Cd accumulated was kept in the cell wall. Thus, retention of Cd in the cell wall was the major metal-induced response conferring Cd tolerance in D. viscosa, although PCs and organic acids might also participate in Cd detoxification once it enters into plant cells.

Acknowledgments
Project funding by CTM2011-29972.

References
Cd ACCUMULATION IN THE HYPERACCUMULATING PLANT ARABIDOPSIS HALLERI AND ITS NON ACCUMULATOR RELATIVE ARABIDOPSIS LYRATA

M.-P. Isaure¹, Huguet¹, C.L. Meyer², N. Verbruggen², G. Sarret³

¹ LCABIE, IPREM UMR5254, Université de Pau et Pays de l’Adour & CNRS, Hélioparc, 2 av P Angot, 64053 Pau cdx9, France
² LPGMP, Université Libre de Bruxelles, Brussels, Belgium
³ ISTerre UMR 5275, Université Joseph Fourier & CNRS, Grenoble, France
Email: marie-pierre.isaure@univ-pau.fr

Although cadmium is highly toxic some higher plants are able to cope with its toxicity and grow on contaminated soils. This capacity is poorly understood and Cd hyperaccumulators are unique materials to get insights on the processes involved in tolerance and accumulation (1). Arabidopsis halleri is one of the few Cd hyperaccumulators and is a relative of Arabidopsis lyrata, which is non tolerant and non accumulator, thus facilitating the comparison between both species. It is known that an enhanced metal uptake from soils to roots, followed by an enhanced xylem loading and transport from the roots to the shoot, and an efficient metal unloading are involved in hyperaccumulation. However, the processes are still misunderstood at the tissue and cell levels. In this context, synchrotron radiation based techniques, and particularly micro-focused X-Ray Fluorescence (µXRF) and X-ray Absorption Spectroscopy (XAS) appear as powerful tools to determine the localization and the chemical forms of metals in plants (2). This work presents the application of µXRF and Cd XAS to determine the distribution and speciation of Cd in A. halleri and A. lyrata. The distribution at the cell level is also investigated using micro-beam and the organic components of the cells are studied using synchrotron-based micro Fourier Transform Infrared spectromicroscopy (SR-µFTIR). Results revealed distinct mechanisms of sequestration between the hyperaccumulating and the non accumulating species.

References
DIRECT IMPLICATION OF GLUTATHIONE IN THE TOLERANCE OF ARABIDOPSIS THALIANA PLANTS TO MERCURY

Juan Sobrino-Plata¹², Sandra Carrasco-Gil¹, Luis E. Hernández¹, Carolina Escobar⁵, Ana Álvarez-Fernández³, Javier Abadía³

¹ Universidad Autónoma de Madrid
² Universidad de Castilla la Mancha
³ Estación Experimental de Aula Dei-CSIC

Email: juan.sobrino@uam.es

Glutathione (GSH) is considered a fundamental component of metal homeostasis because its dual role as antioxidant and precursor of phytochelatins (PCs), important metal ligands in plant cells (1). Many aspects of GSH metabolism regulation under heavy metal stress remain to be elucidated. This was studied in two allele g-glutamylcysteine synthetase (g-ECS) mutants of Arabidopsis thaliana with reduced GSH content relative to the wild type (Col-0): cad2-1 (30 %) and rax1-1 (45 %); and cad1-3 mutant unable to produce PCs. Leaves of mutants and Col-0 Arabidopsis were infiltrated and incubated for 24 and 48 h with Cd and Hg (0, 3 and 30 µM), and biothiols were analysed by HPLC and HPLC-ESI-TOFMS (2). PCs were detected in Col-0, cad2-1 and rax1-1 plants exposed to Cd, whereas PCs only accumulated in Col-0 and rax1-1 treated with Hg. PC-Hg complexes were subsequently only detected in Col-0 and rax1-1. We were unable to detect GSH-Hg complexes even in cad1-3 plants under Hg stress, where GSH concentration augmented remarkably relative to Col-0 (2). The highly Hg-sensitive enzyme GSH reductase was more affected in cad2-1 mutants than in the rest of plants, suggesting that a certain threshold GSH-depletion caused higher cellular toxicity. The different behaviour of cad2-1 and rax1-1 implies differences in g-ECS expression, which is under study by Western-blot immunodetection. Our results highlight the importance of adequate GSH metabolism to minimize the toxic effects exerted by Hg.

References
IDENTIFICATION OF TELLURIUM METABOLITE IN THE SELENIUM ACCUMULATOR PLANT, GARLIC EXPOSED WITH TELLURATE

Yasumi Anan¹, Miyuki Yoshida¹, Saki Hasegawa¹, Maki Tokumoto¹, Laurent Ouerdane², Ryszard Łobiński²³, Yasumitsu Ogra¹

¹ Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan
² CNRS-UPPA, Laboratoire de Chimie Analytique Bio-inorganique et Environnement, UMR5254, Pau, France
³ Faculty of Chemistry, Warsaw University of Technology, 00-664, Warszawa, Poland

Email: ananya@ac.shoyaku.ac.jp

Tellurium (Te) is a member of the group 16 element same as sulfur (S) and selenium (Se), and is widely used in industry because of its unique chemical and physical properties. Although Te is expected to be metabolized at least in part via the same pathway as S and Se in organisms, the precise metabolic pathways had not been known in organisms, particularly in plants. In this study, we analyzed the accumulation and metabolites of Te in Se accumulator plant, garlic exposed to sodium tellurate. The accumulation of Te in garlic was increased being dependent on the concentration of tellurate exposed to garlic without any apparent adverse effects. Analyses by HPLC-ICP-MS equipped with a multi-mode gel filtration column showed that the water-soluble fraction of the garlic leaves contained at least three Te-containing metabolites. To identification of these metabolites, the fraction containing each metabolite was purified and subjected to HPLC-ESI-MS-MS. The MS spectra obtained by ESI-MS-MS indicated that one of the metabolites was Te-methyltellurocysteine oxide (MeTeCysO). The chromatographic behavior of synthetic MeTeCysO matched with that of the Te-containing metabolite in garlic. Another was assigned as cysteine S-methyltellurosulfide by HPLC-ESI-MS-MS. We firstly presents that garlic can assimilate tellurate, an inorganic Te compound, and tellurate is transformed into a Te-containing amino acid, the so-called telluroamino acid.
NEW ASPECTS ON THE MECHANISM OF BIOLOGICAL HYDROXYAPATITE FORMATION IN PATHOLOGICAL CONDITIONS

F. Grases¹, O. Söhnel, M. Zelenková, A. Costa-Bauzá¹

¹ Laboratory of Renal Lithiasis Research, University Institute of Health Sciences Research (IUNICS), University of Balearic Islands, Mallorca, SPAIN.
² University of J.E.Purkyně, Faculty of Environmental Studies, Ústí n.L., Czech Republic.
Email: fgrases@uib.es

We aimed to establish detailed morphology of the structureless amorphous hydroxyapatite (HAP) phase in three different samples: non infectious phosphate renal calculi, aortic valve HAP deposits and synthetic concretions formed in simulated body fluid. The objective is to improve our understanding of the formation mechanism of these concretions.

Several cross-sections of those samples were examined with atomic force microscope. Both 2- and 3-dimensional images of their structure and nanoscale elastic modules maps were obtained. Scanning and transmission electron microscopy were also used.

In renal calculi the amorphous HAP phase consists of 2 distinctly different morphologic forms of hydroxyapatite: separate and/or intergrown columnar crystals, and spherical agglomerates with diameters in the range 150-300 nm consisting of spherulites approximately 10 nm in diameter. The columnar crystals are irregularly disseminated in the stone interior. Organic matter is almost distributed throughout the stone interior.

Valve calcific deposits consist of closely arranged elongated needle and plate like particles (crystals) of 30 to 70 nm in diameter and irregularly disseminated areas of organic substrate.

Synthetic HAP concretions formed in simulated body fluid developed as hemispheres attached to the wall of the recipient. The hemispheres with diameter between 50 and 200 μm were composed of closely connected round spherical and elliptical objects of diameter varying from 70 to 120 nm with surface layer composed of tightly packed spherical objects of diameter 25-30 nm.

These results demonstrate that two different situations of biological HAP formation in pathological conditions can be distinguished: HAP formation in a free liquid media (urine or plasma) and HAP formation in confined situations (valve interior or cavities inside the renal calculi).

In liquid solution HAP spheres correspond structurally to the expected ultrafine structures resulting from the accumulation of particles formed by aggregation of ionic cluster (Posner’s clusters Ca₉(PO₄)₃ or [Ca₃(PO₄)₂]ₙ) by perikineti coagulation followed by sedimentation through surface nucleation. These structures can be observed in synthetic HAP spheres obtained in vitro and in some zones of renal calculi in direct contact with urine. It is important to emphasize that if these colloidal structures develop in the plasma, probably would be eliminated through the liver.

In confined situations, phosphatic phase consists of needle (columnar) and plate-like particles (crystals) of 30 to 70 nm in diameter. These crystals are nucleated on organic substrate and their growth is controlled by diffusion of building units through stagnant liquid. Preferentially formed precursors of HAP, dicalcium, octacalcium and/or amorphous calcium phosphate are later...
Abstracts

transformed into biological HAP. This mechanism would explain the formation of the compact phosphatic phase which constitutes aortic valve deposits and also some parts of the non-infectious phosphate renal calculi integrated by columnar crystals.
INVolVEMENT OF Zn\textsuperscript{2+} SIGNAL IN DENTATE GRANULE CELLS IN OBJECT RECOGNITION MEMORY

Haruna Tamano\textsuperscript{1}, Taisuke Ogawa\textsuperscript{1}, Shunsuke Takada\textsuperscript{1}, Naoto Oku\textsuperscript{1}, Atsushi Takeda\textsuperscript{1}

\textsuperscript{1}School of Pharmaceutical Sciences, University of Shizuoka, Yada 52-1, Suruga-ku, Shizuoka, Japan
Email: tamano@u-shizuoka-ken.ac.jp

Zn\textsuperscript{2+} is released from glutamatergic neuron terminals in the hippocampus and may serve as a signal factor [1]. We have demonstrated that dentate gyrus long term potentiation (LTP) and object recognition memory are impaired after intraperitoneal injection of clioquinol, a membrane-permeable zinc chelator into young rats, which transiently decreases synaptic Zn\textsuperscript{2+} levels in the hippocampus, especially in the dentate gyrus [2, 3]. To pursue the role of Zn\textsuperscript{2+} signal in dentate granule cells in object recognition memory, in the present study, the role of synaptic Zn\textsuperscript{2+} signal was examined focusing on the perforant pathway-dentate granule cell synapses in the dentate gyrus molecular layer. In vivo dentate gyrus long-term potentiation (LTP) was affected under the local perfusion of the recording region (the dentate granule cell layer) with 0.1 mM ZnAF-2DA, a membrane-permeable zinc chelator, but not with 1-10 mM CaEDTA, a membrane-impermeable zinc chelator, suggesting that intracellular Zn\textsuperscript{2+} signal in dentate granule cells is required for dentate gyrus LTP. To examine the role of Zn\textsuperscript{2+} signal in dentate granule cells in recognition memory, ZnAF-2DA (100 pmol, 0.1 mM/1 μl) was injected into the dentate molecular layer. When rats were subjected to object recognition test 1 h after injection, the recognition memory was affected. The present study demonstrate that intracellular Zn\textsuperscript{2+} signal in the dentate gyrus, probably in dentate granule cells is required for object recognition memory.

References

INVOLVEMENT OF ENDOPLASMIC RETICULUM (ER) STRESS IN ZINC INDUCED NEUROTOXICITY

Masahiro Kawahara¹, Dai Mizuno¹, Hironari Koyama², Keiko Konoha², Susumu Ohkawara², Yutaka Sadakane³

¹ Department of Bio-Analytical Chemistry, Faculty of Pharmacy, Musashino University, Tokyo 202-8585, Japan.
² Department of Analytical Chemistry, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, Miyazaki 882-8508,
³ Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science, Mie 513-8670, Japan
Email: makawa@musashino-u.ac.jp

Excess zinc (Zn) causes neuronal death following transient global ischemia and plays a central role in the pathogenesis of vascular-type dementia. We have investigated the molecular mechanism of Zn-induced neurotoxicity by using GT1-7 cells (immortalized hypothalamic neurons), which are more susceptible to Zn compared to other neuronal cells. Using the screening system for substances that prevent Zn-induced neurotoxicity, we found that neither agonists or antagonists of neurotransmitters including glutamate, GABA, did not influence Zn-induced death of GT1-7 cells. However, carnosine (ß-alanyl histidine) and histidine protected against Zn-induced neurotoxicity. Our analysis using the DNA microarray revealed that expressions of several genes were affected after Zn exposure. These genes include metal-related genes (metallothionein, zinc transporter 1), endoplasmic reticulum (ER)-stress related genes (GADD34, GADD45, p8), calcium-related genes (activity-related cytoskeleton protein (Arc)). We have demonstrated that coexistence of carnosine or histidine inhibited the expressions of GADD34, p8, and Arc, meanwhile they did not influence the expression of metal-related genes. Furthermore, dantrolene, which prevents Ca²⁺ release from ER, attenuated Zn-induced neurotoxicity, and thapsigargin, which increases intracellular Ca²⁺ by blocking influx into ER, enhanced Zn-induced neurotoxicity. Our results suggest that ER stress and calcium dyshomeostasis may underlie the molecular mechanism.

References

ZINC IN AGE-RELATED MACULAR DEGENERATION

Imre Lengyel¹

¹ Department of Ocular Biology and Therapeutics, UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK
Email: i.lengyel@ucl.ac.uk

Age-related macular degeneration (AMD) is the most prevalent cause of irreversible visual impairment in the elderly in the developed world. While some genetic, environmental, and behavioural risk factors contributing to the development of AMD are known, our understanding of the biochemical basis of deterioration at the RPE/choroid interface is quite incomplete. The modest success of oral zinc supplementation in AMD suggests that zinc homeostasis is vital for RPE/choroid function and failure of homeostasis contributes to disease. This in turn suggests that understanding zinc balance/imbalance is key to understand how the disease develops and progresses. We now have several lines of evidence for how zinc can affect the RPE/choroid complex, from the regulation of cellular responses to molecular interactions involving zinc.
A COMPARISON OF INTERNAL STANDARDS IN LA-ICP-MS IMAGING EXPERIMENTS ON TISSUE SECTIONS

Maximilian Bonta¹, Hans Lohninger¹, Andreas Limbeck¹

¹ Vienna University of Technology, Institute of Chemical Technologies and Analytics, Getreidemarkt 9/164-IAC, A-1060 Vienna, Austria
Email: max_bonta@hotmail.com

Laser ablation inductively coupled mass spectrometry (LA-ICP-MS) is increasingly used for the elemental mapping of inorganic and heteroelement containing organic analytes on biological samples (e.g. tissue sections). To increase the reliability of the obtained results (images) internal standards have to be used to compensate for changes in material desorption by the laser as well as altering instrument conditions such as change of plasma conditions and detector drift during measurement time. In the past carbon had often been used as internal standard; however the suitability is controversial and has been questioned [1]. Therefore novel methods for signal standardization have been developed. The use of gold layers on the sample surface for internal standardization has been proposed by Konz et al. [2]. In this work the optimization of gold standardization for quantitative imaging analysis of tissue samples is reported. Method development and result evaluation have been carried out with samples using printed inkjet patterns on paper and other substrates as an alternative for tissue sections. Inkjet patterns offer the possibility to create reproducible samples suitable for reliable method comparison; additionally, sample complexity is reduced in contrast to tissue samples as the number of monitored trace elements can be minimized.

References
BIOIMAGING IN METAL BASED ANTICANCER DRUG DEVELOPMENT AND THERAPY

Alexander E. Egger¹, Sarah Theiner¹, Bernhard K. Keppler¹, Christian G. Hartinger¹, Petra Heffeter², Christoph Kornauth³, Guenther Bayer³

¹ Institute of Inorganic Chemistry, University of Vienna, Waehringer Strasse 42, 1090 Vienna, Austria
² Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria
³ Institute of Clinical Pathology, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria
Email: alexander.egger@univie.ac.at

Among platinum based drugs in clinical cancer therapy, oxaliplatin exhibits fewer side effects than cisplatin and further improvements are expected by ruthenium compounds. In order to optimize novel complexes, knowledge on the spatially resolved distribution of the metal in tissues is important. This enables tracking of the compound on its way to the target tissue (e.g. tumor) or on its elimination route (e.g. kidney). Hyphenation of laser ablation (LA) to inductively coupled plasma-mass spectrometry (ICP-MS) is a powerful tool for this purpose and the use of matrix matched standards allows quantification [1, 2].

Mice treated with sodium trans-[tetrachloridobis(1H-indazole)ruthenate(III)] showed a homogenous distribution of ruthenium within the liver and a functional division in kidney: Ru is 3-times enriched in the cortex than in the medulla. Correlation with the corresponding histology revealed a spatial resolution of at least 70 µm. A rare, but painful complication in administration of oxaliplatin is extravasation as the toxic platinum compound remains in subcutaneous tissue and - in high concentrations - causes inflammation eventually followed by tissue necrosis requiring surgical intervention. Imaging of the resectate allowed differentiation between muscle, fatty tissue and connective tissue. The concentration determined in connective tissue was 30-50 ppm and in muscle 5-10 ppm and may be used for correlation with the extent of damage observed by the pathologist.

References
QUANTITATIVE IMAGING OF IRON IN BIOLOGICAL TISSUE BY ISOTOPE DILUTION LASER ABLATION ICP-MS

Liuxing Feng¹, Jun Wang¹, Jennifer O’Reilly², Heidi Goenaga-Infante²

¹ National Institute of Metrology, Beijing, China
² LGC Limited, Queens Road, Teddington, Middlesex, UK TW11 0LY
Email: fenglx@nim.ac.cn

Traceable methods for the determination of total metal concentrations, as well as the regional spatial distribution (quantitative imaging), in diseased tissues compared to normal tissues are important to understand the pathogenesis and potential treatment of neurodegenerative diseases. Calibration strategies for quantitative imaging of trace elements in biological tissues have been reported earlier [1, 2]. However, their validation still remains a challenge due to the lack of suitable certified reference materials and of primary methods for quantitative elemental imaging. In this work, a methodology based on isotope dilution calibration with LA-ICP-MS is, for the first time, developed for the accurate quantitative imaging of iron in thin sections of biological tissue. A novel approach was developed to determine the exact mass of spike ($^{57}$Fe) added to the tissue section. Different isotope exchange conditions and their effect on the achievement of isotopic equilibration were investigated. Assessment of the accuracy of the developed method for quantitative iron imaging of brain tissues by LA-ID-ICP-MS was performed by analysis of bovine liver NIST SRM (SRM 1577c), immobilised in gelatin. Total Fe analysis of gelatin slices by ICP-MS was also carried out and the Fe concentrations obtained using bulk and LA-ICP-IDMS analysis were found to agree well. Finally, the feasibility of the developed LA-ID-ICP-MS method was applied to the quantitative imaging of iron in rat brain section.

References
GHOST - A GST HYDROGEL SYSTEM FOR SPECTROSCOPIC CHARACTERIZATION AND RAPID NMR STRUCTURE DETERMINATION OF SMALL PROTEINS

Jens Loebus¹, Silke Johannsen¹, Eva Freisinger¹

¹ Institute of Inorganic Chemistry, Winterthurerstr. 190, 8057 Zurich, Switzerland
Email: Jens.Loebus@aci.uzh.ch

Obtaining structural and functional information of proteins rapidly and at low-costs is essential in the ever-growing fields of structural biology. While in recent years great progress has been made on the computational side of the process, experimental workflows still heavily depend on manual labour. Working with small (2-9 kDa), cysteine-rich (relies on the formation of a hydrogel, surprisingly allowing the selective detection of properties of the target protein fused to the GST-tag. By employing a Zn₆Cys₆ metal cluster containing plant metallothionein domain denoted γ-Ec-12 fused to the GST tag, we compared metal binding properties as well as NMR solution structures of the GST fusion protein and the isolated γ-Ec-1 domain. Next to the decrease of time needed to prepare a suitable sufficiently concentrated sample, i.e. from 2 weeks to 3 days, we observed an improvement in the NMR solution structure for the GST-γ-Ec-1 fusion protein compared to the untagged, isolated metallothionein. Expanding our investigation to other metal binding proteins, i.e. Neclu_MT1, we validated the GHOST system while conducting metal binding experiments.

References
STRUCTURAL BASIS FOR THE TRANSCRIPTIONAL REGULATION OF HEME HOMEOSTASIS

Shigetoshi Aono¹, Hitomi Sawai¹, Masaru Yamanaka¹, Hiroshi Sugimoto², Yoshitsugu Shiro²

¹ Okazaki Institute for Integrative Bioscience, 5-1 Higashiyma, Myodaiji, Okazaki 444-8787, Japan
² RIKEN/SPring-8 Center, 1-1-1 Kouto, Sayo-cho, Hyogo 679-5148, Japan
Email: aono@ims.ac.jp

Numerous lactic acid bacteria including *Lactococcus lactis* acquires heme molecules as an exogenous source of heme to establish an aerobic respiratory chain. As free heme molecules show cytotoxicity, heme homeostasis should be regulated strictly. A transcriptional regulator HrtR senses and binds a heme molecule as its physiological effector to regulate the expression of a heme-efflux system responsible for heme homeostasis in *L. lactis*, but its regulatory mechanisms are not clear. To elucidate the molecular mechanisms of how HrtR senses a heme molecule and regulates the gene expression for the heme efflux system, we determined the crystal structures of apo-HrtR/DNA complex, apo-HrtR, and holo-HrtR at a resolution of 2.0, 2.8, and 1.9 Å, respectively. These structures revealed that HrtR is a member of TetR family transcriptional regulators. Arg46 and Tyr50 in pairs play a crucial role for the specific DNA-binding using a hydrogen-bonding and a CH-π interactions with the DNA bases. HrtR adopts a unique mechanism for its functional regulation upon heme-sensing. Heme-binding to HrtR causes a coil-to-helix transition of the α4 helix in the heme-sensing domain, which triggers a structural change of HrtR to dissociate from the target DNA for derepression of the genes encoding a heme efflux system. HrtR uses a unique heme-sensing motif with bis-His (His72 and His149) ligation to the heme that is essential for the coil-to-helix transition of the α4 helix upon heme-sensing.

References
METALLOMICS APPROACHES TO UNDERSTAND THE METABOLISM AND TOXICITY OF METAL-BASED NANOMATERIALS IN VIVO

Weiyue Feng1, Bing Wang1, Meng Wang1, Yuliang Zhao1, Zhifang Chai1

1 CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, and CAS Key Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China.
Email: fengwy@ihep.ac.cn

As a wide range of new types of nanomaterials (NMs) have been synthesized, more and more nanotechnology-related consumer products are coming into people's life, it is an urgent need to understand the absorption, distribution, metabolism, excretion (ADME) profiles of NMs in vivo and clarify the potential health and safety impacts as a result of their novel physicochemical properties. In recent years, we use metallomics related methods to detect the distribution, accumulation, the passage across natural biological barriers, and the final fate of metal-based NMs in vivo. We show that the high biological and chemical activity of nanosurface may initiate coatings or reactions by various biomolecules in the complex and dynamic biological systems, inducing agglomeration/aggregation, dispersion, dissolution, phase transformation, protein adsorption, etc. of NMs, thus result in the alteration of the bio-chemical activities of the NMs, and finally influence the transport, fate, and toxicity of NMs in vivo.
QUANTIFICATION OF ZnO NANOPARTICLE UPTAKE, DISTRIBUTION AND DISSOLUTION WITHIN INDIVIDUAL HUMAN MACROPHAGES

Simon A. James¹, Martin D. de Jonge¹, Manoj Sridhar¹, Paul F. A. Wright¹², Terry W. Turney¹, Bryce N. Feltis², Terence W. Turney²³, Jing Fu¹²³

¹ Australian Synchrotron and CSIRO
² RMIT University
³ Monash University
Email: simon.james@synchrotron.org.au

The usefulness of zinc oxide nano-particles has led to their wide distribution in consumer products, despite only a limited understanding of how this nano-material behaves within biological systems. From a nano-toxicological viewpoint, the interaction of ZnO nano-particles with cells of the immune system are of great interest as these structures are readily phagocytized and can promote cellular dysfunction. In this study, rapid scanning x-ray fluorescence microscopy was used to assay the number of ZnO nano-particles associated with ~1000 individual THP-1 monocyte derived human macrophages. These data showed that nano-particle-treated cells endured a 400% elevation in total Zn levels, 13-fold greater than the increase in Zn effected by application of an equitoxic concentration Zn(II)Cl₂. Intriguingly, even after excluding the contribution of internalized nano-particles, Zn levels in treated cells were still increased ~200% above basal. Using ZnO nano-particles doped with Co, we were able to monitor NP composition in situ and observed particle breakdown appeared to be cell mediated. As dissolution of ZnO nano-particles is believed to underlie the cytotoxicity of these objects we utilized a strategy combining ion beam milling, x-ray fluorescence and scanning electron microscopy to probe the distribution and composition of ZnO nano-particles throughout the cellular interior. Our results demonstrated that correlative photon and ion beam imaging techniques can provide high-resolution imaging and statistically powerful information on the biology of metal oxide nano-particles at the single cell level. This approach promises ready application to broader studies of phenomena at the interface of nanotechnology and biology.
FROM ZINC PROTEIN STRUCTURE TO ZINC AFFINITY - WHAT HAVE WE LEARNED ABOUT ZINC PROTEOME?

Agnieszka Drozd¹, Anna Miloch¹, Tomasz Kochanczyk¹, Artur Krezel¹

¹ University of Wrocław, Faculty of Biotechnology, Laboratory of Chemical Biology, Tamka 2, 50-137 Wrocław, Poland
Email: krezel@biotech.uni.wroc.pl

Zn(II) is one of the most widespread metal cofactors in biology serving multiple roles. Although is suggested by computational methods that up to 10% of human genes encodes zinc proteins there is still unknown number of zinc transient regulatory and interprotein sites in the metalloproteome [1]. Not only zinc proteins utilize different structural scaffolds to serve their function in the cell, but also the coordination environment differs depending on protein function and destination. Here, we summarize recently gained knowledge regarding the correlation between structural properties and affinity of protein to Zn(II) emphasizing on the first and the second coordination sphere. Our recent study on PDLIM1 showed that secondary interactions in C-terminal tail of common LIM domain can help in stabilization of the zinc-binding sites, which contributes to Zn(II) affinity, protein folding and can participate in interactions with other macromolecules [2]. We demonstrate for the first time that naturally occurring substitution of Cys and His residues to Asp and Glu has significant impact on LIM domain stability and functionality. We also unveiled the unique stabilization of protein-protein interaction by zinc hook motif in Mre11 of DNA repair complex [3]. Moreover new experimental methods used for the determination of affinities of highly stable motifs from zinc metalloproteome are presented and discussed.

Acknowledgements
We thank FNP (FOCUS F1/2010), MNiSW (IP2011 026971, IP2012 018272) for support.

References
GENOME-WIDE TRANSCRIPTOMIC RESPONSE TO ZINC OVERLOAD IN THE YEAST SACCHAROMYCES CEREVISIAE EXPRESSING OR NOT A PLANT DEFENSIN

Oriane Mith¹, Laurence Marques¹, Pierre Berthomieu¹, Pierre Delobel², Isabelle Sanchez², Bruno Blondin²

¹ B&PMP INRA, Montpellier SupAGro, Oriane MITH, Montpellier, 34060, France
² SPO-INRA, Montpellier SuAgro, Montpellier, 34060, France
Email: oriane.mith@supagro.inra.fr

Our group is interested in unravelling the molecular and cellular bases of zinc tolerance. Previous results have highlighted a role for plant defensins in conferring zinc tolerance at a cellular level in yeast but also in plants. This result is quite new because defensins were only known as antimicrobial peptides and had never been shown to interfere with metal ions. A role of plant defensins in zinc tolerance thus revealed an interesting new function for these proteins. This role needs to be better understood and we hypothesize that metal ions such as zinc could play a yet unexplored role in the mode of action of plant defensin. Zinc is an essential metal that contributes to the structure and/or function of many proteins. It is also not highly toxic, but a zinc overload is deleterious for the cell. A wealth of knowledge is available on the genome-wide response and molecular process operating in yeast cells under zinc depletion. On the other hand, little is known about the damages caused by zinc overload. We are interested in understanding how the disorders triggered by zinc excess could be alleviated by the over-expression of plant defensins. For this purpose, we performed a genome-wide transcriptional profiling analysis using yeast as a model system. We studied the expression of a plant defensin in combination or not with a zinc overload to investigate how the defensin interferes with the metabolic processes and cellular components to confer zinc tolerance.

References
METAL (Ni, Co) SENSING AND SIGNAL TRANSDUCTION BY CnrX FROM CUPRIAVIDUS METALLIDURANS CH34

Jacques Coves¹

¹ Institut de Biologie Structurale de Grenoble, 41 Rue Jules Horowitz, 38 027 Grenoble, France
Email: jacques.coves@ibs.fr

CnrX is the membrane-anchored periplasmic sensor of the CnrYXH complex that contributes to regulate Co and Ni resistance in C. metallidurans CH34. This resistance is induced by the specific release of the ExtraCytoplasmic Function sigma factor CnrH from the CnrYX complex upon sensing of increasing amount of Co or Ni in the environment. We have determined the high-resolution structures of the sensor domain (CnrXs) under the Ni-, Co-, and Zn-bound forms as well as in the apo-form and established the structural basis of metal sensing by CnrX [1]. The Zn-bound form represents the resting state of the complex. While the Zn ion is pentacoordinated in a N3O2 sphere, Ni or Co ions recruit the only methionine (Met123) residue as a sixth ligand to switch on the sensing mechanism. This active site thus offers an original N3O2S1coordination sphere for Ni or Co where S stands for the thioether sulfur of Met123. We have used a series of spectroscopic techniques to characterize the metal-binding sites of CnrXs [2]. We have recently demonstrated that Met123 plays a crucial role in metal selectivity and affinity and, is a key player of the signal transduction as the M123A-CnrX derivative is no longer able to propagate the signal of the metal binding on the sensor protein in the CnrYXH complex [3]. The mechanism deduced from these results is in agreement with the concept of metal selectivity and allosteric switching developed for the DNA-binding metal-responsive transcriptional regulators.

References
[3] Trepreau et al., 2013, JACS, in revision
DNA-binding proteins under starved cells (Dps) have been proposed to have an important role on DNA protection from reactive oxygen species (ROS) by two mechanisms: DNA binding and/or iron storage. This work is focused on the characterization of Dps from the radiation resistant organism Deinococcus radiodurans (Dr). Although this organism has two Dps: Dps1 and Dps2, it is an organism with low-iron content, therefore the cellular function for these proteins is not understood. Our goal is to characterize the DrDps mechanisms for iron storage/release and DNA interaction/protection and correlate this with their cellular function. The iron kinetic studies were performed for both DrDps on iron oxidation using both oxygen and hydrogen peroxide and on iron reduction, using sodium dithionite as a reductant. Both DrDps have a long N-terminal tail compared with other Dps, such as the Escherichia coli homologous protein. DrDps1 has on this region positively charged residues that could to be involved on DNA binding, as proposed for other Dps. In order to characterize the DNA-Dps complex, we have used gel-shift assays and Atomic Force Microscopy. Furthermore, in vivo co-localization of each DrDps and DNA has been performed using fluorescence microscopy. Although both DrDps have a conserved structure, our results, from the different studies, indicated that each DrDps have a specific cellular function.

References
EVALUATION OF QUANTITATIVE PROBES FOR WEAKER Cu(I) BINDING SITES COMPLETES A SET OF FOUR CAPABLE OF DETECTING AFFINITIES FROM NANO- TO ATTOMOLAR

Anthony Wedd¹, Zhiguang Xiao¹, Saumya Udagedara¹

¹ University of Melbourne
Email: agw@unimelb.edu.au

Copper plays essential roles in biology, but abnormal interactions are damaging. Reliable quantification of copper-protein interactions will underpin molecular understanding of copper nutrition and toxicity. We have previously established two high affinity probes Bathocuproine disulfonate (Bcs) and Bicinchoninic anion (Bca) capable of in vitro quantification of Cu(I) binding with affinities from pico- to atto-molar concentrations (ref 1). Quantitative probes are required for Cu(I) binding of lower affinity for proteins and peptides typically associated with neurodegenerative diseases. The present work evaluates two classic Fe(II) ligands Ferene S (Fs) and Ferrozine (Fz) as quantitative probes for Cu(I). Both react with Cu(I) quantitatively to yield well-defined complex anions \([\text{CuI(Fs)}_2]^3-\) (\(\lambda_{\text{max}} = 484 \text{ nm}, \varepsilon = 6,700 \text{ cm}^{-1} \text{ M}^{-1}\)) and \([\text{CuI(Fz)}_2]^3-\) (\(\lambda_{\text{max}} = 470 \text{ nm}, \varepsilon = 4,320 \text{ cm}^{-1} \text{ M}^{-1}\)). Formation constants \(\beta_2\) were determined by two approaches: direct metal titration and ligand competition. They provided estimates that consolidated the affinities of the two probes to a unified standard: \(10^{15.1} \text{ M}^{-2}\) for Fz and \(10^{13.7} \text{ M}^{-2}\) for Fs; ref 2). The four ligands Bcs, Bca, Fz and Fs in combination form a set of versatile probes capable of detecting and differentiating an extended spectrum of Cu(I) binding affinities from nano- to atto-molar concentrations. Selected examples of quantification of weaker Cu(I) binding in proteins and peptides are provided, including an amyloid-\(\beta\) peptide.

References
IN VIVO DISTRIBUTION AND SPECIATION OF COBALT AND SILVER TAKEN UP BY A MICRO-ALGA REVEALED BY SYNCHROTRON X-RAY SPECTROSCOPY TECHNIQUES

Thomas LEONARDO¹,², Corinne RIVASSEAU¹, Emmanuel FARHI², Christophe DEN AUWER³

¹ CEA, IRTSV, Laboratoire de Physiologie Cellulaire Végétale, Grenoble, France
² Institut Laue-Langevin, Grenoble, France
³ Université de Nice Sophia-Antipolis, Processus Chimiques et Radiochimiques dans l'Environnement, Nice, France
Email: thomas.leonardo@cea.fr

A new micro-alga species which is highly radio-resistant and strongly accumulates radionuclides has recently been discovered in a nuclear environment [1,2]. Thanks to its properties, a biotechnology based on this alga is being developed for the clean-up of nuclear effluents. We aim at understanding the mechanisms involved in the accumulation of metallic radionuclides, particularly silver and cobalt which are the main gamma emitting metals present in effluents issuing from nuclear facilities. As part of this project, we study the sub-cellular localization and the in vivo speciation of these metals, once incorporated by the micro-alga. Metals were localized using synchrotron micro X-ray fluorescence. Elemental maps of algae exposed to several metallic ions concentrations enabled to identify specific sub-cellular accumulation compartments for each metal. Additionally, the chemical forms of accumulated ions were determined by synchrotron X-ray absorption spectroscopy. The obtained spectra, fitted with theoretical models, informed on the ligands involved in the metal chelation as well as on redox reactions carried out by the alga under certain conditions. Transmission electron microscopy observations and X-ray diffraction measurements corroborated these findings. Altogether, these data gave a good insight into the mechanisms underlying the alga's metal uptake. They will help optimizing the biotechnological clean-up process based on the alga that is currently under development.

References
ELEMENTAL BIOIMAGING OF NANOSILVER-COATED PROSTHESES IN BONE TISSUE BY MEANS OF LA-ICP-MS

Franziska Blaske¹, Christoph A. Wehe¹, Olga Reifschneider¹, Michael Sperling¹, Uwe Karst¹

¹ University of Muenster, Institute of Inorganic and Analytical Chemistry, Corrensstraße 28, 48149 Muenster, Germany
Email: franziska.blaske@uni-muenster.de

Silver is known to exhibit bactericidal properties. Therefore elemental silver is integrated into surgical prostheses. While the release of ions is assumed to reduce infections, the behavior and fate of silver in living organisms is not fully understood. To find out about possible long-term effects of the respective prostheses the development of analytical methods for silver is required. Elemental bioimaging by means of laser ablation coupled to inductively coupled plasma mass spectrometry (LA-ICP-MS) is a capable method for the investigation of metals in biological samples. Its high sensitivity and good detection limits permit the determination of elements in low concentration ranges. In this project, nanosilver-coated prostheses were investigated. Explanted tissue sections containing the medical device were analyzed using LA-ICP-MS. Method development was challenging considering the inhomogeneity of the examined samples including metal, tissue or embedding media with their different physical properties. With this approach, visualization of the surface was achieved and the distribution of different metals was investigated. Phosphorous and sulfur were determined as well.

References
CADMIUM STRESS LOCALIZATION IN THE UNICELLULAR GREEN ALGA
CHLAMYDOMONAS REINHARDTII AT SUBCELLULAR LEVEL BY ICP-MS AND
SYNCHROTRON-BASED TECHNIQUES

Florent Penen¹, Marie-Pierre Isaure¹, Dirk Schaumlöffel¹, Ivo Bertalan², Dirk Dobritzsch²-³

¹ Université de Pau et des Pays de l’Adour/CNRS UMR 5254, LCABIE/IPREM, Hélioparc, 2 av. du
   président Angot 64053 Pau, France
² Martin-Luther-Universität Halle-Wittenberg, Institute for Biology, Weinbergweg 22, 06120 Halle
   (Saale), Germany
³ Martin-Luther-Universität Halle-Wittenberg, Institute for Biochemistry/Biotechnology,
   Weinbergweg 22, 06120 Halle (Saale), Germany
Email: florent.penen@univ-pau.fr

Cadmium is a toxic metal released in environment by industrial activities (Cd-Ni batteries, anti-
corrosion treatment or phosphate fertilizer production). Already used in phytoremediation [1], the
unicellular freshwater green alga Chlamydomonas reinhardtii has been shown to be a relevant model
to study metallic stress, and particularly Cadmium stress, in photosynthetic organisms [2]. In order to
bring out tolerance mechanisms against cadmium stress, wild type and mutant strain of C. reinhardtii
have been cultivated mixotrophically with different cadmium concentrations. As thiolated peptides
were found to be involved in cadmium tolerance [3], the chosen mutant strains can synthetize
thiolated peptides in their chloroplast. The aim of the experiments was to determine cadmium
toxicity and cadmium speciation and localization at the cellular and subcellular levels. Firstly,
cadmium impact on cells health has been studied by measuring cell growth and chlorophyll
production. Secondly, cadmium distribution between extracellular fraction, bound to cell-wall
fraction and intracellular fraction has been determined by ICP-MS after various extractions. Finally,
cadmium localization (elemental cell imaging) and speciation have been made by Synchrotron µ-X ray
Fluorescence (µXRF) and µ-X ray Absorption Near Edge Structure (µXANES), respectively.

References
   Management, GIWRM 2012.
VISUALIZATION OF INTRACELLULAR ELEMENTS BY SCANNING X-RAY FLUORESCENCE MICROSCOPY

Mari Shimura¹

¹ Dept. of Intractable Diseases, Research Institute, National Center for Global Health and Medicine 1-21-1 Tokyo 162-8655, Japan
Email: mshimura@ri.ncgm.go.jp

Minerals and metals are essential for a healthy body, and the concentrations of these elements have been suggested to change in various cellular conditions and the diseased state. However, the distribution of these intracellular elements was hard to be visualized. We describe the development and use of a scanning X-ray fluorescence microscope (SXFM) system at SPring-8 (Harima, Riken) and an accompanying analytical method (elemental array). We demonstrate that a SXFM can reliably determine the cellular distribution of multiple elements with a high spatial resolution. Visualizing intracellular elements and understanding their kinetics may provide greater insight into cellular kinetics and disease etiology, and may help to identify preventions against diseases and potential therapies.

References
IMAGING OF HETERO-ELEMENTS AND METALS IN SINGLE CELLS BY ICP-MS

Norbert Jakubowski¹, Heike Traub¹, Ulrich Panne¹, Kaori Shigeta², Daniela Drescher³, Janina Kneipp³

¹ BAM Federal Institute for Materials Research and Testing, Richard Willstaetter-Str. 11, D-12489 Berlin, Germany
² Institute for Environmental Management Technology, 16-1 Onogawa, Tsukuba 305-8569, Japan
³ Department of Chemistry, Humboldt University zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany
Email: norbert.jakubowski@bam.de

Cellular heterogeneity that arises from stochastic expression of genes, proteins and metabolites is a fundamental principle of cell biology, but metal analysis in single cells has been beyond the capability of the quickly growing metallomic technology. In our research we want to measure and image the metal heterogeneity of essential elements (metals and hetero-elements) in single diseased or healthy cells together with the distribution of biomarkers using metal labeled antibodies. For this purpose we have grown fibroblast cells on microscopic slides and after fixation we have ablated them by a scanning 213 nm laser ablation system with the smallest laser spot size of 4 (8) µm only. Interactions of nanoparticles (Au, Ag) with cells together with natural element distributions in single cells are measured using different incubation concentrations and times. First results will be presented.
TOWARDS A BETTER UNDERSTANDING OF Pt ANTI-CANCER DRUGS: SUB-CELLULAR PARTITIONING AND COMPLEXATION

Tamer Shoeib¹, Eslam M. Moustafa¹, Aref Zayed², Helen Reid², Barry L. Sharp²

¹ The American University In Cairo, New Cairo Egypt
² Loughborough University, Loughborough UK
Email: T.Shoeib@aucegypt.edu

The complexation of the Pt-based anti-cancer drugs with ligands other than DNA is believed to be one of their major cellular sinks reducing their therapeutic effects and increasing toxicity. The total fate of Pt in human cell populations following treatment with cis- or oxaliplatin (and combination treatments) is presented. Work on three cell models showed the sub-cellular Pt distribution to be ~70% localized in the cytosol, ~17% in the membrane and membrane localized fraction, ~9% in the nucleus and ~4% in the cytoskeleton. The first ever reported in vivo sub-cellular Pt fractionation data is presented which closely mirror the in vitro results and indicate the feasibility of applying our methods in a clinical environment. Mass balance experiments showing >99% Pt recovery is presented adding confidence in the results. Studies of the interaction of oxaliplatin with cytoplasmic ligands found in abundance in human cells are presented including the assignments of all species observed as well as the CID fragments observed. DFT calculations were used to obtain structural information and relative free energies of different isomers of the observed species both in the gas phase and in solution as well as to probe the fragmentation products, highlighting mechanisms that account for all the experimental results. Data are presented to show several binding modes between electron rich sites such as S, N, O centers of the ligands and the Pt metal of oxaliplatin.

References
GALLIUM PHOSPHINOARYLBISTHIOLATO COMPLEXES COUNTERACT DRUG RESISTANCE OF CANCER CELLS

Eva Fischer-Fodor¹, Ana-Maria Valean², Piroska Virag¹, Petru Ilea², Corina Tatominr¹, Maria Perde Schrepler¹, Florica Imre-Lucaci², Lucian Barbu Tudoran², Evamarie Hey-Hawkins³, Luminita Silaghi-Dumitrescu²

¹ Oncology Institute "I.Chiricuta", Cluj Napoca, RO-400015, Romania
² Babes-Bolyai University, Cluj-Napoca, RO-400084, Romania
³ Institut für Anorganische Chemie der Universität Leipzig, D-04103, Leipzig, Germany
Email: fischer.eva@iocn.ro

Multiple drug resistance is a serious obstacle to chemotherapeutic drugs efficiency and by default an obstacle to the anticancer treatment success rate. Since standard platinum drug-based therapeutic regimens failure is often a consequence of tumor chemoresistance in ovary carcinoma, the investigation of alternative metal-based compounds is required. An emerging element is gallium, being the central metal in important new complexes with anticancer potential. We tested two gallium phosphinarylbisthiolato complexes: \([\text{NEt}_3\text{H}][\text{Ga}\{\text{PPh}(2-\text{SC}_6\text{H}_4)_{2-}\kappa^3\text{S,S',P}}\{\text{PPh}(2-\text{SC}_6\text{H}_4)_{2-}\kappa^2\text{S,S'}}\}](1) and its analogue obtained by cation exchange with PPh_4Cl (2). The compounds in vitro effect was tested on A2780 platinum sensitive and A2780 cis platinum resistant human ovary malignant cell lines; both exhibit cell growth inhibitory effect, compound 2 is selective, being much more effective as platinum-based standard drugs. This behavior is based on gallium incorporation into ovary carcinoma cells and DNA damages occurrence, leading to early apoptosis of a significant percent of treated cells. Compounds generate ultrastructural changes, and interact with the cell signalling pathways being able to modulate TGF-beta1, bcl-xL and FasL expression. Complexes 1 and 2 are not substrates of Pgp-1 efflux pump, overexpression of Pgp-1 does not occur in the treated cells and this point toward counteracting the of multildrug resistance mechanisms in cancer cells which are defiant to standard metal drug action, and we can conclude that compound 2 has a great anticancer potential and can be further investigated as an alternative to platinum-based drugs especially in the situation of standard treatment failure.

Acknowledgements
The present work was supported by Romanian UEFISCDI Complex Exploratory Research Idea Grant No 2/2010.
THE INFLUENCE OF BUFFER COMPONENTS ON THE BINDING OF CISPLATIN TOWARD 5′-dGMP STUDIED BY MEANS OF CZE-UV AND CZE-ESI-MS

Gerlinde Grabmann¹, Bernhard K. Keppler¹, Christian G. Hartinger¹,²,³

¹ University of Vienna, Institute of Inorganic Chemistry, Waehringer Str. 42, 1090, Vienna, Austria
² Translational Cancer Therapy Research, Waehringer Str. 42, 1090 Vienna, Austria
³ The University of Auckland, School of Chemical Sciences, Private Bag 92019, Auckland 1142, New Zealand
Email: gerlinde.grabmann@univie.ac.at

The great success of cisplatin against various malignancies but also diminishing its side effects are the driving force for improving metallodrugs [1]. One of the first steps of activity profiling related to novel Pt-based anticancer agents is their reactivity toward the DNA model compound 5′-dGMP. However, interlaboratory comparability is often problematic when examining the diversity of experimental setups applied. Especially the incubation solution is one of the crucial parameters on the formation of adducts and consequently binding kinetics, as some components of the buffer might coordinate to the metal center [2]. We investigated the impact of buffer components/salts in aqueous solutions on the binding of cisplatin toward 5′-dGMP. This comprehensive study by capillary zone electrophoresis (CZE) using UV detection showed significant deviations in binding kinetics when incubated in different solutions. Phosphate coordination to the Pt center was associated with a reduced half-life of 5′-dGMP compared to the incubation in water. Carbonate further reduced the binding ability of 5′-dGMP to cisplatin even though no carbonate complexes were detected. The hyphenation of CZE to ESI-MS allowed the characterization of the formed adducts revealing co-migrating species.

References
ZINC-COMPLEX RESCUES BIOMETAL TRAFFICKING DEFECTS IN CHILDHOOD NEURODEGENERATIVE DISEASE

Alexandra Grubman¹, Katja Kanninen², Clare Duncan¹, Grace Lidgerwood¹, Xin Yi Choo¹, Aphrodite Caragounis¹, Laura Bica¹, Anthony White¹

¹ University of Melbourne, Parkville, Australia
² University of Eastern Finland, Kuopio, Finland
Email: alexandra.grubman@unimelb.edu.au

Aberrant biometal metabolism is a key feature of neurodegenerative disorders. Metal modulating compounds have shown promise as therapeutics for neurodegeneration, but their mechanism of action remains poorly understood. Neuronal ceroid lipofuscinoses (NCLs), caused by mutations in CLN genes, are fatal childhood neurodegenerative lysosomal storage diseases for which there is no cure. This study investigated the novel concept that alteration of biometal functions also drives pathology in NCLs. Regional CLN6 transcript loss occurred concomitantly with presymptomatic biometal accumulation in two natural animal models of CLN6 NCL. Furthermore, we detected loss of the ER-localized metal transporter protein Zip7 in affected brain regions of CLN6 and CLN5 sheep. Consistent with a role for Zip7 in NCL pathogenesis, biometals accumulate specifically within ER and lysosome fractions purified from CLN6 mouse brains, and CLN6 directly interacts with Zip7 in neuronal cells, as well as sheep and mouse tissues. This study was the first to demonstrate impaired metal homeostasis in NCLs, implicating Zip7 as a key contributor to this process. Treatment of primary CLN6 cells with the metal complex, Zn(atsm) induced Zip7 upregulation, and exerted neuroprotective and anti-inflammatory effects. Moreover, a single oral dose of Zn(atsm) reduced lysosomal metal content in the brains of CLN6 mice. These results suggest that Zn(atsm) may be an ideal candidate for NCL therapeutic trials.

References

ENHANCED SUSCEPTIBILITY TO SPONTANEOUS SEIZURES OF NODA EPILEPTIC RATS BY LOSS OF SYNAPTIC Zn\(^{2+}\)

Atsushi Takeda¹, Masashi Iida¹, Masaki Ando¹, Masatoshi Nakamura¹, Haruna Tamano¹, Naoto Oku¹

¹ School of Pharmaceutical Sciences, University of Shizuoka, Yada 52-1, Suruga-ku, Shizuoka, Japan
Email: takedaa@u-shizuoka-ken.ac.jp

Zinc homeostasis in the brain is associated with the etiology and manifestation of epileptic seizures [1]. Adult Noda epileptic rats (NER) exhibit spontaneously generalized tonic-clonic convulsion about once a day. To pursue the involvement of synaptic Zn\(^{2+}\) signal in susceptibility to spontaneous seizures, in the present study, the effect of zinc chelators on epileptogenesis was examined using adult NER. Clioquinol (CQ) and TPEN are lipophilic zinc chelotors, transported into the brain and reduce the levels of synaptic Zn\(^{2+}\). The incidence of tonic-clonic convulsion was markedly increased after i.p. injection of CQ (30-100 mg/kg) and TPEN (1 mg/kg). The basal levels of extracellular Zn\(^{2+}\) were decreased before tonic-clonic convulsion was induced with zinc chelators. Exocytosis of hippocampal mossy fibers was significantly increased in hippocampal slices from CQ-injected NER that did not show tonic-clonic convulsion yet. These results indicate that the abnormal excitability of mossy fibers is induced prior to epileptic seizures by injection of zinc chelators into NER. The incidence of tonic-clonic convulsion induced with CQ was significantly reduced by co-injection with aminooxyacetic acid, an anticonvulsant drug enhancing GABAergic activity, which did not affect locomotor activity. It is likely that the insufficient GABAergic neuron activity rather than abnormal glutamatergic neuron activity is involved in the enhanced seizure susceptibility in NER induced with zinc chelators.

References
SEC-ICP-AES AS A TOOL TO CONDUCT APPLIED HEALTH RESEARCH USING BLOOD PLASMA

Jürgen Gailer¹, Thomas Morris¹, Elham Zeini Jahromi¹, Melani Sooriyaarachchi¹

¹ University of Calgary
Email: jgailer@ucalgary.ca

The exposure of humans to a variety of toxic metals (e.g. As, Cd, Hg) is associated with adverse health effects, including cancer. Relatedly, the injection of cancer patients with metal-based anticancer drugs (e.g. cis-platin) often results in severe and often dose limiting side-effects, such as hearing loss and/or neurotoxicity. In order to develop strategies to mitigate the adverse human health effects that are associated with these metal species, it is critical to unravel the underlying molecular mechanisms of their toxicity. Since both toxic metals and metal-based drugs enter the bloodstream, a better understanding of their biochemical fate therein can provide new insight. To this end, we apply a metallomics approach that is based on the analysis of blood plasma by SEC-ICP-AES. This methodology allows one to simultaneously observe the size distribution of multiple endogenous metal species (e.g. Fe, Cu, Zn, Ca) and exogenous metal species (e.g. As, Cd, Pt, Ru). Thus, the analysis of plasma to which either a toxic metal or a metal-based drug is added allows to simultaneously observe its metabolism (e.g. hydrolysis), its plasma protein binding and a toxic metal-species induced perturbation of the 'metalloproteome' (defined as the entirety of all endogenous plasma Fe, Cu and Zn-metalloproteins). Some recent examples which illustrate the potential of this metallomics approach to conduct applied health research will be presented [1, 2, 3].

References
STABLE IRON ISOTOPE TRACING REVEALS SIGNIFICANT BRAIN IRON UPTAKE IN ADULT RATS

Jiehua Chen¹, Shahreena Shahnawas¹, Nadia Singh¹, Wei Yi Ong¹, Thomas Walczyk¹

¹ National University of Singapore, Singapore, 117543
Email: walczyk@nus.edu.sg

Neurodegenerative disorders are on the rise. Iron deposits are common in brain of Alzheimer and Parkinson patients. They may result in oxidative stress and free radical damage. It remains unknown if an excessive dietary iron intake can lead to brain iron accumulation. Early radiotracer studies have led to the conclusion that iron uptake of adult brain is very low. Using stable iron isotopes we determined iron transfer from diet to brain directly. We used for the first time ever a continuous feeding approach. A constant amount of a stable iron isotope ($^{57}$Fe) was given daily with the drinking water over 4 months to adult male rats (n=7). Iron isotopic analysis of tissues was conducted using Negative Thermal Ionization Mass Spectrometry (NTI-MS). Isotopic enrichment for the brain was 6.94 ± 0.57 % with very small inter-individual variation (8% RSD). Brain iron uptake was low but substantial. Only 0.000537 ± 0.000076 % of total dietary iron was detected in brain at 4 months ( = 7-10 human years), which appears to be negligible, but this amount constitutes ca. 9% of total tissue iron with figures being similar for other organs (11.5 - 36.3%). Whereas it remains unclear if high systemic iron correlates to iron deposits in brain, our study suggests that uptake of dietary iron is much higher than found in short-term tracer studies. This finding challenges current beliefs and points to a possible role of iron nutrition in the pathogenesis of neurodegenerative disorders.
METALLOPROTEASES AND METAL IONS IN ALZHEIMER’S DISEASE:
RECENT FINDINGS

Giuseppe Grasso¹, Enrico Rizzarelli¹, Francesco Bellia², Antonio Magri², Diego La Mendola³

¹ University of Catania, Viale A. Doria 6, 95125 Catania
² Istituto di Biostrutture e Bioimmagini, C.N.R.,95125, Catania
³ Department of Pharmaceutical Sciences-University of Pisa, 56126 Pisa

Email: grassog@unict.it

In recent years, many metalloproteases have drawn attention because of their capability to degrade β-amyloid peptides and they are currently studied as pharmacological targets for Alzheimer’s disease (AD). We have recently shown that the activity of insulin-degrading enzyme, but not of neprilysin, could be differently modulated by metal ions, proposing that the binding of the latter to the catalytic site could be responsible for such peculiar behavior [1-3]. Here, we have synthesized peptides that mimic the different amino acidic sequences of the various catalytic sites. The binding of such peptides with zinc (II) and copper(II) has been investigated by thermodynamic and spectroscopic techniques. At physiological pH, results show that the thermodynamic stability of complexes with copper(II) are different for the different consensus sequences investigated. Moreover, novel metalloproteases which are able to degrade Aβ have been also identified and the results will be discussed.

Acknowledgements

Financial support from FIRB MERIT Prot. RBNE08HWLZ and MIUR FIRB-ItalNanoNet RBPR05JH2P_021 is acknowledged.

References

SELENIUM BIOMINERALIZATION BY VARIOVORAX PARADOXUS

Lucian Staicu¹, Christopher Ackerson¹, Jennifer Cappa¹, Elizabeth Pilon-Smits¹

¹ Colorado State University
Email: staiculucian@yahoo.com

Selenium (Se) is an essential trace nutrient for mammals but deleterious at slightly higher levels. Several bacteria can reduce the toxic oxyanion selenite, SeO$_3^{2-}$, to red elemental selenium, Se$_0$, deriving energy. Selenium-reducing bacteria have been successfully employed for cleaning up Se-laden wastewaters, but the mechanism of Se reduction and transport are still poorly understood. We isolated novel Se-reducing bacteria that dwell inside Se hyperaccumulator plant Stanleya pinnata. Using universal primers, 8f and 926r, we identified the isolates by 16S rRNA PCR. Among them, Variovorax paradoxus, a metal tolerant species, was selected for the investigation of the reduction mechanism and of the proteins involved in Se$_0$ formation and transport. When grown in the presence of 10 mM SeO$_3^{2-}$, V. paradoxus excreted 5 times more proteins into its growth medium than the control and showed additional bands on an SDS protein gel, suggesting that the Se$_0$ formed is coated with a protein layer and then expelled from the cell. The Se-coating protein candidates will be further sequenced and identified.

We hypothesize the mechanism of elemental Se formation involves proteins that act as transporters and stabilizers. Further identification and characterization of these proteins will shed light on the biomineralization process of Se in V. paradoxus. Additionally, the new data will provide better understanding of the environmental fate of toxic Se and of nanoSe synthesis and application.

References


LC-ICP-MS AND LA-ICP-MS AS ANALYTICAL TOOLS FOR ANALYSING THE EFFECTS OF Zn FERTILIZATION ON THE MOLECULAR SPECIATION OF Zn IN CEREAL GRAINS

Daniel Pergament Persson¹, Thomas Hesselhøj Hansen¹, Jan Kofoed Schjoerring¹, Søren Husted¹

¹ University of Copenhagen
Email: dap@life.ku.dk

Zinc (Zn) is an essential trace element to plants and humans. From a human perspective both the Zn concentration and the Zn bioavailability in plant based foods are low, leading to massive deficiency problems in regions where the main calorie intake comes from e.g. cereals. The low concentration of Zn in cereal grains can be alleviated by repeated foliar applications of Zn-solutions (Kutman et. al 2011). Since there is strong evidence that Zn is primarily bound to small peptides and proteins in the endosperm of cereal grains (Persson et al. 2009), the Zn concentration in cereal grains is in most cases correlated to the protein and nitrogen content. The efficiency of foliar Zn fertilization strategies relies on: 1) How much of the applied Zn is transported and assimilated into the edible parts of the grain, and 2) How this fertilization affects the Zn speciation and protein composition; factors which influence the bioavailability of the Zn.

At University of Copenhagen, we combine state-of-the art plant molecular biology approaches with elemental bio-imaging and speciation analyses, using LA-ICP-MS, LC-ICP-MS, ESI-TOF-MS and MALDI-TOF-TOF-MS/MS. By adding oxygen to the octopole reaction cell of the ICP-MS we have lowered the LOD for sulphur significantly, enabling simultaneous analysis of Zn, S, Fe and P with high sensitivity, both in combination with LA-ICP-MS and multi-dimensional LC-ICP-MS. Along with a continuous development of these analytical methods, the elucidation of Zn speciation and localization in cereal grains is progressing.

Our most recent results indicate that foliar application of Zn in combination with high or low nitrogen fertilization of plants, respectively, has a major influence on both tissue localization and speciation of Zn. Up until now we have identified several dynamic Zn-species, i.e. Zn species which respond strongly to Zn fertilization, using multi-dimensional chromatography for Zn metalloprotein isolation, followed by trypsin digestion and MALDI-TOF-TOF-MS/MS analysis. Also, using LA-ICP-MS for elemental bio-imaging of grain tissue we have fine-mapped the localization of Zn in the cereal grain and identified a marked uncoupling of Zn from other essential trace elements, including iron (Fe). These results will be presented at the conference.

References

BIOSPECIFIC ELEMENT-TAGGING STRATEGY FOR ICPMS-BASED QUANTIFICATION OF PROTEINS AND CELLS

Qiuquan Wang¹, Xiaowen Yan¹, Zhubao Zhang¹, Limin Yang¹

¹ Key Lab of Analytical Science & Department of Chemistry and State Key Lab of Marine Environmental Science, Xiamen University
Email: qqwang@xmu.edu.cn

Quantitative information regarding proteins and cells in a biological system is significant for understanding biological process and early diagnosis and prognosis of a disease. I will talk about strategies of biospecific element-tagging towards a protein (or an enzyme) including the design and synthesis of element-tags and characterization of them by spectroscopic methods, and then quantification of the protein (or the enzyme) via the element-tag determination using ICPMS.1,2 Moreover, these strategies were extended to cells quantification and imaging using ICPMS and CLSM through the design and synthesis of a trifunctional probe, which contains an element-tag moiety and fluorescent moiety and a homing group.3

References
[1] Yan, Xiaowen; Luo, Yacui; Zhang, Zhubao; Li, Zhaoxin; Luo, Qiang; Yang, Limin; Zhang, Bo; Chen, Haifeng; Bai, Peiming; Wang, Qiuquan. Europium-Labeled Activity-Based Probe through Click Chemistry: Absolute Serine Protease Quantification Using 153Eu Isotope Dilution ICPMS. Angew. Chem. Int. Ed. 2012, 51: 3358-3363
[3] Zhang, Zhubao; Luo, Qiang; Yan, Xiaowen; Li, Zhaoxin; Luo, Yacui; Yang, Limin; Zhang, Bo; Chen, Haifeng; Wang, Qiuquan. Integrin-Targeted Trifunctional Probe for Cancer Cells: A “Seeing and Counting” Approach. Anal. Chem. 2012, 84: 8946-8951
ELEMENTAL AND MOLECULAR STRATEGIES FOR QUANTUM DOTS SYNTHESIS, DERIVATIZATION AND BIOCONJUGATION TO DEVELOP ICP-MS BASED BIOANALYTICAL APPLICATIONS

Jorge Ruiz Encinar¹, Antonio R. Montoro Bustos¹, Laura Trapiella-Alfonso¹, José Manuel Costa-Fernández¹, Rosario Pereiro, Alfredo Sanz-Medel¹

¹ Department of Physical and Analytical Chemistry, University of Oviedo. Julian Clavería 8, 33006 Oviedo, Spain.
Email: ruizjorge@uniovi.es

Photoluminiscnet semiconductor nanocrystals, also known as Quantum Dots (QDs), can be bioconjugated to specific biomolecules making such nanoparticles very attractive as novel fluorophores for improved bioanalytical applications. Particularly, in the last decade, QDs have been often used in the development of immunosensors and immunoassays in different formats (e.g. microarrays). However, such methods based on QDs are still not very reliable for quantitative measurements. In fact, the analytical potential of QDs to be used as labels for the quantitative analysis of biomolecules will require for the control of the synthesis process, shelling and coating by solubilization reactions. Finally, the stoichiometry of the QD to the single recognition moiety of the analyte in real samples after the bioconjugation reaction should be assessed.

Previous research carried out in our laboratory demonstrated that inductively coupled plasma mass spectrometry (ICP-MS) can play a pivotal role in the assessment of CdSe QDs synthesis processes, helping to control the conditions to synthesize well-defined and reproducible nanoparticles. By using elemental and molecular information together the exact number of Cd and Se atoms existing per QD nucleus (core) was worked out. The precision of the measurements was limited by the spectrophotometric molecular measurements [1]. Furthermore, ICP-MS was proved very useful, in conjunction with chromatographic and molecular fluorescence techniques, as diagnostic tools to characterize and evaluate the quality of water soluble QDs. The effectiveness of QD bioconjugation to antibodies can also be determined, showing that the integration between elemental and molecular information is invaluable for a complete characterization of the bioconjugated QDs [2].

Also, a critical assessment between elemental mass spectrometry (ICP-MS) and molecular fluorescence, as detection principles in immunoassays formats, has been carried out for sensitive detection and quantification of progesterone in cow’s milk as a “model” analyte. Although fluorescence detection is simpler, less expensive and less time consuming, ICP-MS detection affords an important improvement in terms of sensitivity and unattainable information using fluorescence [3]. We will also show the generic “platform” for protein determinations consisting of polymerized QDs with streptavidin and biotinylated antibodies. Results obtained show the great potential of such QD-antibodies bioconjugates for the absolute quantification of target proteins in complex samples will be discussed in detail.
Abstracts

References


META
L CODED TAGS FOR DEVELOPMENT OF MULTIPLEXING DIAGNOSIS METHODS

Larissa Waentig¹, Sandra Techritz¹, Simone Hardt¹, Norbert Jakubowski¹, Christian Scheler², Peter H. Roos³

¹ BAM Federal Institute for Materials Research and Testing, Richard-Willstaetter-Str. 11, D-12489 Berlin, Germany
² Proteome Factory, Magnusstraße 11, D-12489 Berlin, Germany
³ Institute of Physiological Chemistry, Ruhr-University Bochum, Universitäetsstr. 150, D-44801 Bochum, Germany
Email: larissa.waentig@bam.de

A large set of biochemical, toxicological and in particular medical questions like biomarker development, chemical-induced protein expression or early recognition of cancer demand the development of new robust multiplexing methods for analysis. For instance, in cancer diagnosis many disease-related proteins (biomarker signatures) have to be identified and if possible quantified. But the quantification of multiple proteins is presently difficult or not possible and most often only one protein after the other can be analyzed by an immunoassay. Therefore, the analytical tool of choice should be able to detect many proteins simultaneously (multiplexing) with high sensitivity and accuracy. Inductively coupled plasma mass spectrometry (ICP-MS) might be such a powerful analytical tool. ICP-MS excels by high accuracy, high dynamic range and extremely low limits of detection for most metals down to attogram level. Furthermore ICP-MS offers a very high multi-element coverage so that many elements of the periodic table can be detected simultaneously. The idea to develop multiplexing immunoassays for ICP-MS detection is based also on the simplicity of the “mass fingerprints” in comparison to the data evaluation of multiple fluorescence immunoassays, which are limited by spectral overlap and saturation effects [1]. The combination of immunoassays with ICP-MS is based on modifying antibodies with artificial element tags acting as an indicator for the target protein (antigen) in the biological sample. Laser ablation (LA-) is used as sample introduction system. A laser ablates the whole biological sample (e.g. tissue sections, microarray slides) line by line. The ICP-MS data measured for the multiple line scans is converted into two dimensional intensity profiles presenting the local distribution of the target proteins in the biological sample. Different applications for MeCAT (metal coded tag) modified antibodies [2] were designed by the authors, in particular a multiplex immunohistochemical approach for cancer tissue sections and for Cytochrome P450 expression profiling using a novel multi-parametric protein microarray. Cytochromes P450 enzymes play an important role in the metabolism of xenobiotics and drugs. For the LA-ICP-MS based multi-parametric protein microarray multiple different proteomes were spotted onto a nitrocellulose slide and were incubated with eight different antibodies at the same time. The methodology shows excellent detection limits in the lower attomol range and a very good linearity of $R^2 = 0.9996$ which is a prerequisite for further quantification strategies.
Abstracts

References
ICP-MS-BASED DETERMINATION OF OXIDISED CYSTEINES IN PEPTIDES/PROTEINS

Ahmed H. El-Khatib¹, Diego Esteban-Fernández¹, Michael W. Linscheid¹

¹ Humboldt Universität zu Berlin, Institute of chemistry, Brook-Taylor-Str 2, 12489 Berlin
Email: Ahmed.elkhatib@chemie.hu-berlin.de

In the last years, interesting approaches [1] have used ICP-MS for quantitative proteomics taking advantage of the sensitivity, wide dynamic linear range, structure-independent response, and multiplexing capabilities of this technique. Cysteines’ thiols are well-known to undergo intracellular oxidation through reactive oxygen species (ROS) such as \( \text{H}_2\text{O}_2 \). This oxidation process is involved in oxidative stress, post-translational modifications, disease states and cell damage. The stabilization of the transient oxidised cysteine product would allow better understanding of many redox-mediated intracellular events [2]. Whereas most of the few available methods rely on isotope labeling combined with molecular MS [3] we present in this work an ICP-MS-based method that allows quantitative determination of oxidised cysteines. The development of a metal-containing oxidised-cysteine-reactive ligand will allow using ICP-MS as detector. The specificity of the proposed method and the superb sensitivity of ICP-MS compared to molecular MS could lead to unprecedented qualitative and quantitative information pertaining to the cysteine oxidation. In vitro oxidation of cysteines using \( \text{H}_2\text{O}_2 \) was studied and the developed ligand was tested with peptides/proteins and proved to be reactive and specific. Thus, this DOTA-based ligand allows the development of a simple, sensitive and straightforward ICP-MS-based method that enables the determination of oxidised thiol content of peptides/proteins.

References
SELENIUM METABOLISM AND EXCRETION IN MICE AFTER INJECTION OF $^{82}\text{Se}$-ENRICHED SELENOMETHIONINE

Naoki Furuta$^1$, Yoshinari Suzuki$^1$, Yoshiteru Hashiura$^1$, Tatsuya Sakai$^1$, Takao Yamamoto$^2$, Takahisa Matsukawa$^2$, Atsuko Shinohara$^2$

$^1$ Faculty of Science and Engineering, Department of Applied Chemistry, Chuo University, 1-13-27, Kasuga, Bunkyo-ku, Tokyo 112-8551, Japan

$^2$ Department of Epidemiology and Environmental Health, Department of Internal Medicine, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Email: nfuruta@chem.chuo-u.ac.jp

The organic Se compounds (particularly selenomethionine [SeMet]) in plants and yeasts are very effective chemoprotectants for mammalian cancer. To characterize the dynamics of selenomethionine utilization pathways, we intravenously injected $^{82}\text{Se}$-enriched SeMet into mice under different nutritional states (Se-adequate and Se-deficient mice) and then measured their endogenous and exogenous $^{82}\text{Se}$ levels. Furthermore, we quantified Se compounds and selenoproteins in liver, kidneys, plasma, and urine. The average recovery of exogenous $^{82}\text{Se}$ from solid tissues, urine, and feces was 81% for Se-adequate mice and 84% for Se-deficient mice. Exogenous $^{82}\text{Se}$ was distributed in the hepatic and renal cytosols as cellular glutathione peroxidase (cGPx), selenosugar, and SeMet within 1 h after injection. Synthesis of cGPx was maintained until 72 h after injection, regardless of the Se nutritional status. Whereas plasma levels of exogenous $^{82}\text{Se}$ as selenoprotein P (Sel-P) peaked at 6 h after injection, those of Se-containing albumin (SeAlb), extracellular GPx, and SeMet peaked at 1 h after injection. These results suggest three Se transport pathways in mice injected with SeMet: SeAlb (within 1 h after injection); SeMet (from 1 to 72 h after injection); and Sel-P (from 6 to 72 h after injection). The amount of Sel-P in Se-deficient mice was 1.5 times that of Se-adequate mice, and this increase was much larger than Se-containing compounds other than Sel-P. Our results indicate that Sel-P has an important role in Se transport when the nutritional supply of Se is insufficient.
APPLICATION OF METALLOMICS AND METABOLOMICS IN EXPOSURE EXPERIMENTS OF MICE FOR METAL TOXICITY ASSESSMENT. POTENTIAL OF ISOTOPIC DILUTION ANALYSIS

José Luis Gómez-Ariza¹,²,³, Tamara García-Barrera¹,²,³, Miguel García-Sevillano¹,²,³

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
³ Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
Email: ariza@uhu.es

Metals have a central role in biological systems. Hence, the study of metal-induced changes in cellular metabolic pathways is crucial for understanding the biological response in environmental issues. In this field, the study of biomarkers has a great interest but -omics techniques, such as metallomics [1] and metabolomics [2], represent a more powerful alternative [3]. In addition, experiments exposure of model organisms to enriched stable isotopes allow the evaluation of element metabolism under experimental conditions. In these approaches is fundamental the use of inorganic and organic mass-spectrometry [4].

The aim of the present work is to evaluate the exposure of laboratory mouse Mus musculus to toxic (As, Cd and Hg) and non-toxic (Se and Zn) metals and the changes induced in both the metallome and metabolome. For this purpose a metallomic approach based on size exclusion chromatography (SEC) in combination with other complementary orthogonal separation techniques and heteroelements monitoring by ICP-MS was performed, followed by identification of metallobiomolecules by organic mass spectrometry. In addition, simultaneous speciation of selenoproteins and selenometabolites in mouse plasma was performed by tandem (double) SEC- (dual) affinity chromatography (AFC)-HPLC and online isotope dilution analysis (IDA)-ICP-qMS. Finally, the simultaneous changes of metabolic expression in mice caused metals exposure (metabolome) was considered, using direct infusion mass spectrometry (DI-ESI-QqQ-TOF-MS) to extracts from liver, plasma and kidney of exposed animals. Subsequently altered metabolites were identified using MS/MS experiments. Conclusions on effects of these metals and their interactions on toxicity have been drawn.

References
RAPID TRANSPORT OF DIPHENYLARSINIC ACID INTO BRAIN THROUGH BLOOD-BRAIN BARRIER AS REVEALED BY MICRODIALYSIS COMBINED WITH LCMSMS

Yasuyuki Shibata¹, Toyoshi Umezu¹, Tomoko Hosoya¹, Mai Takagi¹, Kunichika Nakamiya¹

¹ National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan
Email: yshibata@nies.go.jp

Groundwater pollution by an organoarsenic compound, diphenylarsinic acid (DPAA), was uncovered in 2003 in Kamisu, Ibaraki pref., Japan. Neurological symptoms, such as ataxia, tremors, myoclonus and sleep disturbance, were observed among the people who consumed DPAA-contaminated well water (Ishii, et al., 2004), and DPAA was found to accumulate in brain of animals to which DPAA was orally administered (Ishii, Shibata et al., unpublished). To better understand its neurotoxicity mechanism, we investigated time-course of the appearance of DPAA in brain of mice by in vivo microdialysis sampling method combined with LCMSMS analysis. After ingestion of DPAA-containing water, DPAA started to appear within the first 15 min fraction into mouse brain dialysate, reached maximum after about 1.5 hrs and gradually decreased with a half life of c.a. 2 hrs. While DPAA was converted to various chemical forms, including methylated and/or thiol-containing forms, in terrestrial environment (Nakamiya et al., 2013), it was absorbed into mice body and transported to the brain through BBB rapidly, apparently as free form, after oral administration. Results of analysis under different DPAA concentration will be presented together with survey results of other components in the dialysates.

References
Gd-BASED CONTRAST AGENTS AND NEPHROGENIC SYSTEMIC FIBROSIS: A RESULT OF METABOLIC REACTIONS?

Michael Sperling¹, Lena Telgmann¹, Helene Faber¹, Daniel Melles¹, Hanna Simon¹, Christoph Wehe¹, Uwe Karst¹

¹ University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstrasse 30, D-48149 Münster, Germany
Email: Michael.Sperling@uni-muenster.de

In 2006, a disease called nephrogenic systemic fibrosis (NSF) was found to occur only under rare specific conditions in renal failure patients examined with Gd-based contrast agents. While the relation between NSF and Gd-based contrast agents is clear the mechanism for the development of the disease is not really understood. In order to investigate the reactivity of those Gd-based compounds under physiological conditions, we performed in vitro experiments in human blood. We studied the hypothesis that the Gd might be released from the complex by a transmetallation reaction. Both elemental and molecular mass spectrometry coupled to HPLC was used to investigate reaction products. Using these techniques we were able to show that transmetallation is possible for Gd complexes with linear ligands if free iron is accessible over a prolonged period of time. We also investigated the hypothesis that the stability of the Gd-complex is compromised by metabolic degradation of the complexing ligands. An electrochemical approach was used to model the metabolic reactions starting with oxidation of gadopentetate (Gd-DTPA). Mass voltammograms generated with online electrochemistry/electrospray ionization mass spectrometry (EC/ESI-MS) gave a first overview of oxidation products. Two potential metabolites could be detected, with the major metabolite originating from an N-dealkylation. Our results indicate that a metabolic transformation should not be disregarded as a possible trigger of NSF.

References
DETERMINATION OF THE TRYPANOCIDAL DRUG MELARSOPROL IN BIOLOGICAL FLUIDS WITH HPLC-ICPMS/ESMS: SHEDDING LIGHT ON ITS HUMAN METABOLIC FATE?

Georg Raber¹, Manuela Murko¹, Kevin A. Francesconi¹, Thomas Raber², Reingard Raml³, Christoph Magnes³

¹ Institute of Chemistry-Analytical Chemistry, University of Graz, Universitaetsplatz 1, 8010 Graz, Austria
² Medical University Graz; Auenbruggerplatz 4, 8010 Graz, Austria
³ Institute for Biomedicine and Health Sciences, Joanneum Research, Auenbruggerplatz 20, 8010 Graz, Austria
Email: georg.raber@uni-graz.at

Although melarsoprol, an organoarsenic compound, is widely used for the treatment of trypanosomiasis (human African sleeping sickness), very little is known about its fate in the human body, its active metabolites passing the blood brain barrier and the mode of action. Previous pharmacological studies based on the determination of melarsoprol by HPLC-UV or a bioassay method produced disparate results. We report a HPLC-ICPMS method suitable for determining melarsoprol and its metabolites in biological fluids. The arsenic-selective capability, robustness and sensitivity of the method allowed the quantitative measurement of melarsoprol and three arsenic-containing conversion products formed when melarsoprol was incubated with human serum and blood at therapeutically realistic concentration levels. Further investigations with HPLC-electrospray MS allowed identification of the major product from melarsoprol. Consequences of this new analysis method to the interpretation of pharmacological data are briefly discussed.
MONITORING THE INTERACTION BETWEEN TRANSFERRIN AND GADOLINIUM
BY MEANS OF PAGE AND LA-ICP-MS

Kristina Wentker¹, Olga Reifschneider¹, Christoph A. Wehe¹, Michael Sperling¹, Uwe Karst¹

¹ University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr. 30, 48149 Münster, Germany
Email: kristina.wentker@uni-muenster.de

Unraveling the interaction between proteins and metals has become more important during the last decade. When dealing with metalloproteins from an analytical point of view, investigation and optimization of suitable analytical techniques are required. The preservation of the native metal-protein complex is thereby of special importance.

In this work, we study the interaction between the iron-transport protein transferrin (Tf) and gadolinium (Gd). Because free Gd³⁺ is toxic, it is chelated by polyaminocarboxylic acids when it is administered during magnetic resonance imaging. Nevertheless, transmetallation could cause the release of Gd³⁺ from the chelate into the blood stream, resulting in a possible coordination and distribution by Tf. Monitoring the interaction between Tf and Gd was performed by means of polyacrylamide gel electrophoresis (PAGE). The use of laser ablation (LA) hyphenated to inductively coupled plasma-mass spectrometry (ICP-MS) represents a sensitive and selective method for the detection of protein-bound metals inside the focused protein bands in the dried gel. The obtained time-resolved line spectra for the monitored element were processed with a home-made software for the elucidation of the elemental distribution in the ablated area of the gel. Based on the intensity distribution of $^{158}$Gd in the generated image, the protein band of Tf was visualized in the unstained gel. This way, the coordination of Gd³⁺ by iron-free Tf was demonstrated.
GENETIC MODULATION IN FEMALE MICE AFTER INTERNAL CONTAMINATION WITH SOLUBLE PLUTONIUM

Rebecca J. Abergel¹, Erin E. Jarvis¹, Cindy Wu¹, Dahlia D. An¹

¹ Chemical Sciences Division, Glenn T. Seaborg Center, Lawrence Berkeley National Laboratory, Berkeley, CA 94720
Email: rjabergel@lbl.gov

The use of actinides in the civilian and defense industries over the past 60 years has resulted in persistent environmental and health issues, since a large inventory of radionuclides, including actinides such as plutonium (Pu) are generated and released during these activities. Since its beginning in the early 1940’s, the main objective of biological research with actinides has been protection of workers. It therefore focused on quantifying the uptake and tissue retention of actinides, and defining the dose-dependent relationships between toxicity and uptake. However, with the exception of the uranyl ion, little attention was given to the understanding of bio-molecular pathways triggered by actinide exposure in mammalian systems. A set of functional genomic experiments was performed to determine whether specific experimental conditions could lead to the identification of genes modulated after Pu exposure. Mice were contaminated with selected doses of soluble Pu-238 and euthanized at different time points. Gene expression analysis was performed through microarrays and qRT-PCR, using liver, kidney and lung samples. These first-of-a-kind experiments led to the identification of several genes for which expression was significantly altered after a single exposure to Pu. Similar changes have been observed after exposure to external ionizing radiation. These findings may therefore permit the discrimination between effects of radiation damage and metal toxicity.
DECIPHERING THE ACCUMULATION OF URANIUM IN THE BONES

Vidaud Claude¹, Basset Christian¹, Averseng Olivier¹, Pible Olivier¹, Hagege Agnes², Qi Lei³

¹ CEA/iBEB/SBTN, BP 11 171, 30207 Bagnols Sur Cèze Cedex
² CNRS-UMR 6191, 13108 Saint Paul lez Durance
³ State Key Laboratory of Agro-biotechnology and College of Biological Sciences, China
Email: claude.vidaud@cea.fr

Comprehension of the mechanisms of metal toxicity at the molecular level necessitates the identification of biomolecules such as proteins able to bind, transport and promote accumulation in the target organs. Uranium is widespread in our environment, and is found in the form of uranyl (UO₂²⁺, UVI) in aqueous media such as biological fluids [1]. Despite its abundance, it has never been reported as integral part in any biochemical system. Whatever the route of contamination, UVI is found in the blood, mainly associated with proteins and carbonates. The non-excreted fraction of UVI then reaches its target organs which are the kidneys and the bones, where it accumulates and remains for years [2]. Searching for target proteins by biochemical methods is a very challenging task and until now, the biochemical mechanisms leading to this UVI accumulation have been poorly described. Their elucidation requires the identification of UVI target proteins and precise quantification of their affinity for the metal. We used our sensitive SPR-based detection method (Biacore® T100, GE Healthcare) [3] to measure the apparent affinity of various proteins involved in bone turn-over. Some of them, displaying a nano-molar affinity for UVI, particularly attracted our interest. Through varied biophysical approaches we investigated the impact of uranyl-binding on protein structures and evaluated UVI: protein ratios following Size Exclusion Chromatography. These results will expand the scope of investigation of UVI accumulation mechanisms.

References
SCREENING AND IDENTIFICATION OF IN VIVO URANIUM-PROTEIN COMPLEXES IN CRAYFISH BY GEL ELECTROPHORESIS, LA-ICP MS AND µRPC-ESI MS/MS

Ming Xu¹, Sandrine Frelon², Olivier Simon², Ryszard Lobinski¹, Sandra Mounicou¹

¹ LCABIE - UMR5254, Technopôle Hélioparc Pau Pyrénées, 2 avenue du Président Angot, 64053 Pau Cedex 09 - FRANCE
² IRSN/PRP-ENV/SERIS - Laboratoire de Biogéochimie, Biodisponibilité et Transferts des radionucléides - BP3 - 13115 St Paul lez Dura
Email: ming.xu@univ-pau.fr

Uranium (U), naturally occurring or anthropically (nuclear and military applications) released in the environment, is a key element to study because of its chemical and radiological toxicity leading to deleterious biological effects. As the most stable U species, uranyl ion (UO$_{2}^{2+}$) in aerobic media, is able to link oxygen and nitrogen atoms of biomolecules to form non-covalent complexes. The understanding of cellular processes involved in uranium trafficking in living cells requires the identification of U molecular targets among which proteins play an important role. In our previous work, cytosolic U-protein complexes were extracted from a waterborne U exposed crayfish (Procambarus clarkii, a biological model) and quantitatively analyzed in one dimension by native isoelectric focusing (ND-IEF) gel electrophoresis coupled to LA-ICP MS before identification of potential molecular targets by µRPC-ESI MS/MS. To confirm these results and go into depth, the crayfish cytosols were subjected to two-dimension gel electrophoresis (2-DE) before LA-ICP MS analysis for U detection and imaging in 1D- and 2D-gel electrophoresis. Both denaturing and non-denaturing 2-DE protocols were used to screen for the presence of in vivo U-protein targets. Finally, the corresponding protein targets detected in the gels were identified by µRPC-ESI MS/MS. These advances will make an input to the understanding of U trafficking and detoxification mechanisms.

References
"A TROJAN HORSE EFFECT" IN THE DELIVERY OF RUTHENIUM(III)-BASED METALLODRUGS - MYTH OR REALITY?

Magdalena Matczuk¹, Monika Prządka¹, Svietlana S. Aleksenko², Andrei R. Timerbaev², Katarzyna Pawlak¹, Maciej Jarosz¹

¹ Chair of Analytical Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland
² Vernadsky Institute of Geochemistry and Analytical Chemistry, Russian Academy of Sciences, Kosygin 19, 119991 Moscow, Russia

Email: mmatczuk@ch.pw.edu.pl

Indazolium trans-[tetrachloridobis(1H-indazole)ruthenate(III)] (I) is one of the most promising non-platinum anticancer drug candidates. However, despite many studies carried out in the past (e.g., on albumin and transferrin binding [1,2]), an exact drug transport mechanism is still the matter of speculations. One of the most often asked questions is whether ruthenium is able to enter the cell by the same uptake mechanism as iron, i.e. via transferrin receptors (occasionally named “a Trojan horse effect”), and thus impair the iron homeostasis. Getting the answer is the main purpose of our on-going research.

In this presentation, a scheme of speciation changes of I, after intravenous administration till transformation (activation) inside cancer cells, will be proposed. To follow these changes, separation and spectrometric techniques were employed, namely, capillary electrophoresis coupled to inductively coupled plasma mass spectrometry (to explore the Ru speciation in time) and electrospray ionization mass spectrometry (to identify new species formed as hydrolysis products, adducts with plasma transport proteins, or due to interactions in cytosol). Special emphasis will be put on a possible exchange of iron(III) by ruthenium when I binds to holo-transferrin. Also, the inclination of I to transformations and liberation of possibly active Ru(II) species in simulated cancer cytosol will be explored.

References

COPPER-IONOPHORES AS THERAPY FOR PROSTATE CANCER

Michael A. Cater¹²³, Helen B. Pearson¹, Kamil Wolyniec¹, Paul Klaver¹, Patrick O. Humbert¹², Brett M. Paterson², Ashley I. Bush², Paul S. Donnelly², Maree Bilandzic³, Sharon La Fontaine³, Ygal Haupt¹²

¹ Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia.
² The University of Melbourne, Parkville, Victoria 3010, Australia.
³ Deakin University, Burwood, Victoria 3125, Australia.

Email: mcater@deaki.edu.au

Patients with prostate cancer accumulate high levels of copper in their cancerous tissue [1]. Elevated intracellular copper levels somehow predispose cancerous cells to ionophoric-copper sensitivity and several classes of copper-coordinating lipophilic compounds are being investigated as potential anticancer therapeutics. We have undertaken comparative in vitro toxicity studies of candidate copper-ionophores and short-listed a handful for preclinical analysis. Prime examples are two bis-thiosemicarbazonato compounds, CuII(gtsm) and CuII(atsm), which have emerged as promising therapeutics and have additionally provided invaluable research tools to investigate mechanism(s) of action. CuII(gtsm) and CuII(atsm) effectively kill a spectrum of human hyperplasic and carcinoma prostate lines with differences in p53 status, androgen receptor status and metastatic potential. In contrast, human primary prostate epithelial cells (PrECs) remained refractory to CuII(gtsm) and CuII(atsm) treatment. TRAMP prostate cancer mice 20 weeks of age were treated (oral gavage) daily with CuII(gtsm) (2.5mg/kg/day), or vehicle alone (control), for 4 weeks. TRAMP mice treated with CuII(gtsm) displayed a significant reduction (68.8%) in the weight of their genitourinary tract (GUT) in comparison to vehicle controls; an accepted indicator for prostate tumour burden [2]. Together, these results reveal the exciting potential for bis-thiosemicarbazanato compounds to be developed as therapeutics for prostate cancer.

References

COORDINATION OF RUTHENIUM COMPLEXES TO SULFUR LIGANDS EMBEDDED IN A LIPID BILAYER: A TWO-STEP MECHANISM

Sylvestre Bonnet¹, Azadeh Bahreman¹, Martin Rabe¹, Alexander Kros¹, Gilles Bruylants², Kirstin Bartik²

¹ Leiden University, Einsteinweg 55, 2300RA Leiden, The Netherlands
² Université Libre de Bruxelles, 50 avenue F.D. Roosevelt, 1050 Brussels, Belgium

Email: bonnet@chem.leidenuniv.nl

The coordination chemistry of metal complexes with ligands embedded in a lipid bilayer may be of relevance in metal-based chemotherapy. In this presentation we will show that the coordination of an aqua ruthenium complex to a membrane-embedded thioether ligand must take place in two steps: first, adsorption of the complex to the surface of the bilayer, and only then, coordination of the ligand to the metal center. When ruthenium(II) polypyridyl complexes and unilamellar vesicles functionalized with thioether-cholesterol conjugates are mixed together, a Ru-S coordination bond forms at the water-bilayer interface only when the lipids composing the membrane are negatively charged. With neutral liposomes, coordination does not take place even at long reaction times, which we interpret as being due to the absence of adsorption of the complex to the membrane. Isothermal Titration Calorimetry experiments (ITC), monolayer experiments at the water-air interface, and spectroscopic studies at different ionic strengths, were undertaken to study these phenomena. Our data clearly show that with negatively charged membranes the positively charged aqua metal complex adsorbs at the bilayer interface at shorter time scales compared to that of the formation of the sulfur-metal bond. Unexpectedly, the adsorption observed at short time scales is not driven by hydrophobic interactions but by entropy.

References

Utilization and Evaluation of a Dedicated Low Volume Autosampler for Biological Applications in Inorganic Mass Spectrometry

Christoph Alexander Wehe¹, Michael Sperling¹, Uwe Karst¹, David Clarke², Michael Sgroi², Damon Green²

¹ University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr. 28/30, 48149 Münster, Germany
² CETAC Technologies, 14306 Industrial Rd., Omaha, NE 68144 USA
Email: christoph.wehe@uni-muenster.de

Major challenges in modern inorganic mass spectrometry for biological and medical applications include concentrations in the sub ng/L-range and limited available sample volume. While lowest detection limits can be achieved by means of inductively coupled plasma mass spectrometers (ICP-MS), treatment of small sample volumes is often difficult due to a lack of reproducibility and extensive time consumption. Hence, automation utilizing dedicated sampling devices is worthwhile. Here, an inert autosampler system for low volumes of biological matrices is presented. Typical benefits like handling of different sizes of tempered well plates, automated addition of reagents, diverse injection modes, appropriate repeatability or low wash-out times and sufficient carry-over effects are demonstrated. Furthermore, its applicability towards different types of samples, e.g. diluted whole blood, cell lyses solutions or culture media, was evaluated by hyphenation to an ICP-MS. This combination not only enabled the fast and accurate quantification of elements like platinum or titanium through generation of transient signals (flow injection), but could also be further expanded. Application of an external, high precision syringe pump for the ICP-MS compatible carrier solution and usage of a total consumption nebulizer permitted the recording of steady-state signals for improved isotope dilution analysis.
**Abstracts**

**Poster Session**  Analytical Tools & Bioimaging & Nanometallomics

**Poster**  P 002  

**Tuesday, 9th July 2013, 11:00 - 12:00  Room “Exhibition Hall”**

**Tuesday, 9th July 2013, 18:00 - 20:00**

---

**EXPLORING THE LIMITS OF ELEMENTAL BIOIMAGING: FAST SURVEY ACQUISITION, MULTIPLE DETECTOR UTILIZATION AND LINE-DEPENDING CELL MODE SWITCHING**

Christoph Alexander Wehe¹, Olga Reifschneider¹, Ann-Christin Bülter¹, Georgina M. Thyssen¹, Michael Kieshauer¹, Michael Sperling¹, Uwe Karst¹

¹ University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr. 28/30, 48149 Münster, Germany  
Email: christoph.wehe@uni-muenster.de

Coupling of laser ablation (LA) to a selective detector such as the inductively coupled plasma mass spectrometer (ICP-MS) is nowadays a commonly used technique to obtain information about the distribution and concentration of numerous elements in biological tissue sections. While main advantages include a high spatial resolution in combination with ultimate sensitivity, this technique suffers from isobaric interferences, the destruction of the sample during the ablation process and the time needed to analyze larger samples in a multiline scan. Here, novel approaches to overcome these issues are presented. As the laser scanned across the sample, the quadrupole of the ICP-MS was set to dwell times as low as 500 µs and therefore scanning the whole mass range of interest within one second. The hereby acquired raw data was subsequently imported into a dedicated software for reduction and visualization purposes. In a second step, an additional ICP-MS was coupled to the mentioned system, further expanding the capabilities by utilizing helium/oxygen as cell gas, in order to improve the limits of detection for elements like e.g. sulfur and phosphorus. In addition, the gained results were compared to those obtained by using one ICP-MS, but switching the mode of the collision/reaction cell at the end of each line. By means of linear interpolation, an excellent correlation was achieved and samples including dried droplets, cerebellum and implant thin sections were successfully analyzed.
ISOTOPIC LABELED STANDARD FOR THE ROUTINE ANALYSIS OF ARSENOBETAINE

Christian Piechotta¹, Susanne Lischka¹

¹ BAM Federal Institute for Materials Research and Test, Richard-Willstaetter-Str. 11, D-12489 Berlin, Germany
Email: christian.piechotta@bam.de

The hyphenated technique HPLC-ICP-MS is currently the method of choice for the analysis of most of the arsenic species. Unfortunately, arsenic does not provide the opportunity to enhance the validity and robustness of the method by using isotopic dilution, since arsenic is a monoisotopic element. On the other hand, the HPLC-ESI-MS/MS is applicable as a sensitive and selective detection method for arsenobetaine. Moreover the system is in an economical point of view the more favourable choice concerning the costs of operation and purchase. Generally, it has the benefit to prevent the user to misinterpret peaks in a resulting chromatogram and to make an error in quantification due to the coelution of organoarsenicals. Another advantage is based on the possibility to identify arsenic species by specific fragmentation experiments like MS/MS-measurements. To avoid common ion suppression effects, caused by a complex matrix of food products or biota, isotopic labeled internal standards play an important role for a valid quantitative analysis. The synthesis, purity assessment and application possibilities of a twofold 13C labeled arsenobetaine as a calibration standard for the analysis of a wide range of samples types will be a topic of this poster (Lischka et al. 2011).

References
DETERMINATION OF FIVE ARSENIC SPECIES IN BIOLOGICAL SAMPLES
BY ICP-MS IN HIGH SENSITIVITY MODE

Peio Riss¹, Rene Chemnitzer², Meike Hamester²

¹ Bruker Daltonique, Champs Sur Marne, France
² Bruker Daltonic GmbH, Fahrenheitstrasse 4, 28359 Bremen, Germany
Email: Pierre-Emmanuel.riss@bruker.com

As toxicity, mobility, and bioavailability can differ greatly between the various chemical species in which an element occurs, reporting only the total concentrations can often be misleading. Arsenic is one such example where the various species differ; the inorganic trivalent form (AsIII) is the most toxic, followed by the inorganic form (AsV). Other common forms of arsenic include monomethyl arsenic (MMA), dimethyl arsenic (DMA) and arsenobetaine (AsB), which have significantly reduced toxicities. Therefore, separation and detection of these species can greatly assist risk-based toxicity assessments.

When Liquid Chromatography (LC) is interfaced with Inductively Coupled Plasma Spectrometry (ICP-MS), species elute one by one from the LC column directly to the ICP-MS for detection by elemental speciation. Coupling an LC to a Bruker aurora Elite has the added advantage of offering high sensitivity, with over 95% of the analyte ions passing through the skimmer cone being transferred to the quadrupole.

The following procedure outlines a simple yet sensitive method for the analysis of 5 Arsenic species in biological samples using the Bruker aurora Elite in high sensitivity mode.
IDENTIFICATION AND FRAGMENTATION STUDIES OF PHYTOCHELATINS AND THEIR ARSENIC COMPLEXES

Jürgen Mattusch¹, Maria Tobies¹, Uriel Arroyo-Abad¹, Thorsten Reemtsma¹

¹ Helmholtz Centre for Environmental Research - UFZ, Department of Analytical Chemistry
Email: juergen.mattusch@ufz.de

Phytochelatins (PC) are synthesized enzymatically in plants to reduce the toxic stress of metals and metalloids. Their composition follows the formula (γ-Glu-Cys)n-Gly (PCₙ, n = 2-6). The thiol groups originating from the cysteine moiety can form covalently bonds to arsenious acid as a detoxification mechanism in plant cells. Synthetic PC’s and their arsenic complexes used in this study were investigated with respect to their accurate masses and fragmentation behavior. The elemental and molecular analysis was carried out simultaneously using UHPLC-ICP-MS/ESI-Q-TOF-MS. The fast separation of the free and complexed PC’s could be performed with an analytical column Zorbax Eclipse Plus C18, RRHD (2.1x50mm, 1.8µm) within less than 10 minutes applying a elution gradient with 1 to 20% acetonitrile. Since no data about MS/MS of As-PC’s are available in the literature, in this work the structural composition of the fragments are proposed for the first time. Fragmentation parameters of the Q-TOF-MS were optimized to get the best accurate mass spectra of the product ions in MS/MS mode. The MS/MS fragmentation pattern for the arsenic complexes with PC₃ to PC₆ was established and showed characteristic arsenic containing product ions. These results can be used for the reliable prediction and identification of As-PC’s in plants without elemental selective detection like ICP-MS.
**SPECIATION OF TRACE ELEMENTS IN BIOLOGICAL MATRICES USING ION CHROMATOGRAPHY (IC) COUPLED TO INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY (ICP-MS)**

Daniel Kutscher¹, Shona McSheehy-Ducos¹, Lothar Rottmann¹

¹ Thermo Fisher Scientific, Hannah-Kunath-Straße 11, Bremen, Germany
Email: daniel.kutscher@thermofisher.com

Many trace elements like As or Se appear in more than one chemical forms, that exhibit different properties, such as toxicity or bioavailability. Especially for these two elements, the total element concentration found in a sample is not sufficient to indicate potential hazards. Species may also undergo conversion processes depending on their surrounding so that the species distribution may change. Ion chromatography is a powerful tool for the separation of such species, as many of them naturally contain charges or can be converted into charged species depending on the pH. In this presentation, different applications of IC coupled to ICP-MS are shown in biological or clinical sample types like urine or milk to show the potential of this combination. Due to the completely metal-free solvent path of the Thermo Scientific Dionex Ion Chromatography systems, instrumental background can be reduced, and also aggressive mobile phases such as acids can be used. Also, the use of Reagent-free ion chromatography (RFIC) additionally simplifies the daily work. The new iCAP Q ICP-Q-MS with its QCell can effectively remove all kinds of spectral interferences that might affect the detection of trace elements, and the Qtgra ISDS software platform enables fully integrated control of both the IC- and the ICP-MS systems as well as dedicated chromatographic integration and data processing.
ARSENOLIPID PATTERN IN FRESH COD LIVER: APPLICATION OF RP-HPLC WITH SIMULTANEOUS ICP-MS AND ESI-Q-TOF-MS DETECTION

Uriel Arroyo-Abad¹, Jürgen Mattusch¹, Thorsten Reemtsma¹, Susanne Lischka², Christian Piechotta²

¹ Helmholtz Centre for Environmental Research - UFZ, Department Analytical Chemistry, Permoserstr. 15, 04318 Leipzig, Germany
² BAM - Federal Institute for Materials Research and Testing, Department Analytical Chemistry, R-Willstaetter-Str. 11, 12489 Berlin
Email: uriel.arroyo-abad@ufz.de

Due to the ubiquitous presence especially in the marine environment arsenic species belong to a significant part of a worldwide risk. Arsenate as predominant arsenic species in seawater is biologically available like phosphate and can be accumulated in aquatic biota, seafood like crustaceans and fish, to attain humans by their staple diet. Therefore EFSA (2009) and JECFA (2011) recommended guidelines for dietary arsenic exposure under the seafood safety aspect caused by arsenic and demanded the development of new analytical methods for arsenic speciation in marine organisms. The present study was focused on the determination and identification of arsenolipids in methanolic extracts of cod liver. Arsenic species were fractionated and the fractions analyzed by RP-HPLC-ICP-MS/ESI-Q-TOF-MS. The total concentration of arsenic in the fresh cod liver was analyzed by ICP-MS to be 1.53 ± 0.02 mg As kg⁻¹ w.w. and the extraction recovery was ca. 100% and the column recovery >93%. Besides polar inorganic and methylated arsenic species (>70%) more hydrophobic arsenic-containing fatty acids and hydrocarbons occurred. Based on the element-selective detection of As, retention times and mass spectrometric data, like accurate masses and product ions, proposals for molecular structures were elaborated for 20 of the organic As species included 10 arsenic-containing fatty acids (AsFA) and an arsenic-containing hydrocarbon (AsHC) mentioned for the first time in fresh cod liver. Arsenobetaine was found as main water-soluble arsenic compound in cod liver followed by higher molecular mass arsenic-containing fatty acids and hydrocarbons.
ACCURATE QUANTITATION OF SELENIUM SPECIES IN KOREAN SEAFOOD DIET

Pak, Yong Nam¹, Kim, Eun Ju¹, Lee, Jong Hae²

¹ Department of Chemistry, Korea National University of Education, Cheong-won, Korea 363-791
² Division of Metrology for quality of Life, Korea Research Institute of Standards and Science, Daejeon, Korea
Email: pakyn@knue.ac.kr

Selenium exists in a variety of chemical species. Its chemical activity or bioavailability is strongly dependent on chemical form and concentration. Therefore, the information on each selenium species as well as the concentration in our daily uptake food should be precisely known. In this study, selenium species in various Korean seafood have been quantified by high performance liquid chromatography (HPLC) coupled with inductively coupled plasma mass spectrometry (ICP-MS). Accurate quantitation has been established using post-column isotope dilution. Selenium standards mixture (SeIV, SeVI, SeCys, SeMCys, SeMet) could be fully separated by Reversed Phase HPLC-ICP/MS. Microwave-assisted enzymatic extraction could provide fast and efficient extraction. 80BrH⁺ interference can be effectively removed either by using the solid phase extraction or the mathematical correction. Selenocystine(SeCys) and Selenomethionine(SeMet) are the major species detected in most of sea food such as codfish, sole, anchovy, belt fish, squid, herring, and whale meat. The concentrations found in Korean sea food for SeCys and SeMet are in the range of 0 - 661.6 mg/kg and 137.3 - 462.7 mg/kg, respectively.
STUDIES ON QUANTIFICATION OF PEPTIDES BY INSERTION OF SELENO-AMINO ACIDS

Laura Hyrup Møller¹, Charlotte Gabel-Jensen¹, Bente Gammelgaard¹

¹ University of Copenhagen
Email: Laura.Hyrup@sund.ku.dk

Cell penetrating peptides are an important part of several new drug delivery systems. The efficiency of the cellular uptake of these peptides and their cargos are often characterized by coupling of a fluorophore to the peptide and monitoring the fluorescence of the isolated cells [1]. These measurements, however, do not reveal if the peptide has actually penetrated the cell wall or if the fluorophore has been released during the transport. Another possibility is that the peptide and/or the fluorophore is only attached to the cell surface. Furthermore, attachment of the comparable large fluorophore may change the cell penetrating properties of the peptides. In the present study, seleno-amino acids were synthesized into different peptides. Introduction of the hetero-element selenium opens the possibility of selective and sensitive ICP-MS detection of the intact peptide and by hyphenation of the ICP-MS detector to chromatographic separation systems, biotransformation of the peptide can be revealed. Complementary ES-MS analysis may establish the identity of the degradation products of the peptides. Results from different cellular uptake studies on different peptides will be given.

References

LOW LEVEL DETERMINATION OF SELENOMETHIONINE IN HUMAN SERUM BY DOUBLE SPECIES-SPECIFIC ID HPLC-ICP-MS: THE IMPORTANCE OF BLANK QUANTIFICATION

Maria Estela del Castillo¹, Caroline Oster¹, Guillaume Labarraque¹, Paola Fisicaro¹, Petru Jitaru², Heidi Goenaga-Infante³

¹ Laboratoire National de Métrologie et d’Essais (LNE)
² HydrISE, Institut Polytechnique LaSalle Beauvais
³ LGC Limited
Email: Maria-Estela.Del-Castillo@lne.fr

Selenium (Se) is an essential element for human health due to its antioxidant properties. The biological activity of Se is mediated by selenoproteins and/or Se-containing proteins (selenoprotein P, SelP; glutathione peroxidase, GPx and selenoalbumin, SeAlb), however the Se status in humans so far has been estimated via the determination of its total content and not taking into account its speciation. Nowadays the accurate determination of selenoproteins is still a challenge due to the lack of certified reference materials (CRMs), pure primary standards and reference methods. In this regard, the quantification of Se by species specific isotope dilution mass spectrometry (SSIDMS) in human serum has only been conducted at the selenoaminoacids level (selenomethionine, SeMet and selenocysteine, SeCys) [1]. Here a metrological procedure has been developed for the quantification of SeMet (as released from SeAlb) at low ng/g levels in commercially available human sera by double SSIDMS using the $^{76}$Se isotopically enriched SeMet. In order to assure the correct quantification of SeAlb via SeMet, the possible free SeMet present in the sera was removed by ultrafiltration using a cut-off of 3kDa. After the enzymatic hydrolysis of the serum with protease and lipase, the released SeMet was analysed by ion-pairing reverse phase HPLC-inductively coupled plasma mass spectrometry (ICP-MS). The extraction procedure was carefully optimized in order to assure the complete hydrolysis of SeAlb. The presence of significant SeMet levels in the blank in addition to the low ng/g content in the serum has made necessary the quantification of the blanks by SSIDMS. A complete uncertainty budget is described for the first time by combining the uncertainties associated with each parameter of the analytical procedure (e.g. purity of the primary standard, operator, etc.), finding the blank contribution the main parameter to be considered. This work derives from the JRP (Joint Research Projects) Tracebioactivity (Traceable measurements for biospecies and ion activity in clinical chemistry) and Metallomics (Metrology for metalloproteins) funded by the European Metrology Research Programme (EMRP). The challenge of SSIDMS at the selenoprotein level is nowadays one of the tasks of the JRP Metallomics.
References

INNOVATIVE ANALYTICAL STRATEGY USING ION-MOBILITY FOR STRUCTURAL OR FUNCTIONAL SELENIUM ISOMERS IDENTIFICATION BY ION MOBILITY SPECTROMETRY

Johann Far¹, Christopher Kune¹, Edwin de Pauw¹, Gauthier Eppe¹, Ryszard Lobinski²

¹ University of Liège Belgium
² CNRS/UPPA, Laboratoire de Chimie Analytique Bio-inorganique et Environnement (LCABIE)

Email: johann.far@hotmail.fr

Selenium (Se) is a trace element which is both essential and toxic depending on its concentration and its chemical form. Se-rich yeast is one of the most popular Se source for supplementation. The classical method of speciation is related to multidimensional liquid chromatography (LC) hyphenated to mass spectrometry (MS) Recent advances in Se speciation led to greatly improve the Se speciation in these samples but isomers identification and quantification remain challenging. This work focuses on the elaboration of an innovative analytical strategy for the detection and the structural elucidation of isobaric selenium compounds present in Se-rich yeast. A specific complex formation agent acts as a chemical probe for the detection of chemical function. The addition of a complexing agent can improve the discrimination between structural or functional Se isomers using ion mobility techniques as Ion Mobility Spectrometry (IMS) by increasing the molecular weight (i.e. the m/z ratio in MS) and the collision cross section of a target ion after selective complexation. This Ion Mobility orthogonal separation improves the structural elucidation. Crown ethers used as shifting agents can specifically form complexes with primary amines. The addition of crown ether to different low molecular weight fractions obtained by multidimensional LC of a water extract from Se-rich yeast permitted to detect Se isomers and confirmed their structure using IMS.
### DEVELOPMENT OF ANALYTICAL STRATEGIES TO STUDY SELENOPROTEIN EXPRESSION IN HUMAN CELL LINES

Juliusz BIANGA¹, Zahia TOUAT-HAMICI¹, Anne-Laure BULTEAU¹, Sandra MOUNICOU¹, Ryszard LOBINSKI¹, Joanna SZPUNAR¹, Laurent CHAVATTE¹²  

¹ CNRS/UPPA, LCABIE, UMR5254, Helioparc, Pau, France  
² CNRS, UPR 3404, Gif sur Yvette, France  
Email: laurent.chavatte@univ-pau.fr

Selenoprotein family is one of the most important bioactive forms of selenium in human health. Selenium, which is an essential trace element, is incorporated as a rare aminoacid, selenocysteine, in twenty five selenoproteins. Chronic selenium deficiency is hypothesized to decrease antioxidant defenses and redox regulatory pathways through a dysregulation of selenoprotein expression. We are interested in understanding the synthesis and regulation of human selenoproteins, which is critically dependent on the availability of adequate analytical methodology. The instrumental developments will be carried out on selenoproteomes expressed in well defined human cell lines in culture which are known to express detectable selenoproteins levels: Hek293, HepG2, HaCat, and LNCap cell lines. In the present work protein extracts were separated by isoelectric focusing gel electrophoresis using immobilized pH gradient (range 3-10 not linear) strips. Selenoproteins were detected by laser ablation - ICP MS and the gel pieces of interest were excised, digested with trypsin and analyzed by capillary RP HPLC with parallel ICP MS and ESI MS/MS. The high resolution of the ESI LTQ Orbitrap mass spectrometer allowed identification of selenoproteins in complex high abundant protein matrix. The identified selenoproteins included glutathione peroxidases (GPx1, GPx2, GPx4), thioredoxin reductases (TR1 and TR2), selenoprotein P (SelP) and selenoprotein 15 (Sel15).
HIGHLY SENSITIVE LC-MS-MS ANALYSIS OF SELENOAMINO ACIDS IN BIOLOGICAL MATRICES

Charlotte Gabel-Jensen¹, Bente Gammelgaard¹

¹ University of Copenhagen, Faculty of Health and Medical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark
Email: charlotte.gabeljensen@sund.ku.dk

Selenoamino acids are ubiquitously involved in selenium metabolism and both the cancer protective effect and the recently suggested obesity related methylation mechanisms involve the selenoamino acids. LC-ICP MS is widely used in speciation analysis and is very well suited for species detection and quantification. However, the inherent loss of molecular information in LC-ICP MS analysis demands for alternative detection methods, such as molecular MS, to confirm the identity of the detected species. Generally, LC-MS analysis of amino acids is challenging because of their hydrophilic nature. The analytical challenges are reflected in the literature on in vivo as well as in vitro selenium metabolism studies. In a vast part of the publications, identification of selenium species is performed by LC-ICP MS and retention time matching or by LC-MS in which samples are subjected to extensive pre-cleaning and pre-concentration procedures. The purpose of the presented study was to develop a simple LC-MS based method for identifying seleno-amino acids by derivatisation with (5-N-succinimidoxy-5-oxopentyl)triphenylphosphonium bromide which is selective for amines and amino acids [1]. The developed method does not require extensive sample preparation, except removal of proteins prior to derivatisation. This simple procedure provides detection limits similar to LC-ICP MS and is suitable for routine verification of the presence of the seleno-amino acids in small amounts of biological matrices.

References
HIGH SENSITIVE ELEMENTAL ANALYSIS OF SINGLE MICROBIAL CELLS
BY TIME-RESOLVED ICP-MS

Kazumi Inagaki¹, Shin-ich Miyashita¹, Alexander S. Groombridge¹, Shin-ichiro Fujii¹, Akiko Takatsu¹, Koichi Chiba¹

¹ NMIJ/AIST, Umezono1-1-1, AIST Tukuba center 3-9,Tsukuba,Japan

Email: k-inagaki@aist.go.jp

High sensitive elemental analysis of single microbial cells with time-resolved Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) was successfully carried out, where a high efficiency cell introduction system (HECIS) consisting of the High Performance Concentric Nebulizer (HPCN) and a low-volume (15 ml) on-axis spray chamber utilising a sheath gas flow were used. Cell adsorption to the flow injector and sample tubing was reduced through addition of simple 0.1 %wt/wt of NaCl solution to the cell suspension and cell flowing liquid, allowing consecutive measurements without fear of significant contamination from previous measurement. Using ICP-QMS at lowest integration time (50 µs), current transient signals corresponding to separate cell events were detected for several elements on introduction of the cell suspension (Mg, Al, P, S, K, Cr, Mn, Cu and Zn). On comparing the number of peaks in the spectrum for phosphorous with the cell count using a haemocytometer, a reproducible cell transport efficiency of above 70 % was achieved. We will show the analytical results for different sized unicellular microbes, yeast, cyanobacteria, red alga, and green algae.

References
IMPROVED DETECTION CAPABILITY FOR ELEMENTAL SPECIES

Meike Hamester¹, René Chemnitzer¹, Pierre-Emmanuel Riss¹

¹ Bruker Daltonic, Fahrenheitstrasse 4, 28359 Bremen, Germany
Email: meike.hamester@bdal.de

ICP-MS is a powerful technique for the determination of trace elements in various matrices. Beyond that when coupled to separation devices ICP-MS is able to determine elemental species. The work will describe the achievements for speciation analysis by a new quadrupole based ICP-MS (Bruker aurora Elite). Key characteristic is a very high ion transmission achieved by an optimized interface, and a unique ion optical system with low chromatic and spherical aberrations, which focuses ions into one focal point. In result sensitivities of up to 4 Mio cps / ppb can be obtained, which even exceeds sensitivities of magnetic sector field ICP-MS significantly. The high sensitivity attainable allows detection of elemental species at lowest levels and utilization of the fast scanspeed and short integration times (0.1 msec) of the instrument. The presentation will discuss all relevant instrumental characteristics of ICP-MS when coupled to chromatographic devices such as:
- scan speed
- plasma robustness for organic solvents
- sensitivity
- interference management.
IMPROVING LIMITS OF DETECTION FOR SPECIATION ANALYSIS OF Gd-BASED MRI CONTRAST AGENTS BY HILIC-ICP-MS WITH SAMPLE INTRODUCTION AS DRY AEROSOL

Marvin Birka¹, Christoph Alexander Wehe¹, Lena Telgmann¹, Michael Sperling¹, Uwe Karst¹

¹ University of Münster
Email: marvin.birka@uni-muenster.de

Gadolinium(Gd)-based contrast agents are widely applied since 1988 to support medical examinations using magnetic resonance imaging (MRI). For that purpose, the toxic Gd³⁺ is complexed by polyaminocarboxylic acid chelating agents [1]. The polar and thermodynamically stable contrast agents are known for a fast and unmetabolized renal excretion. Hence, there is a large input of Gd species into the environment, which leads to an enrichment of Gd compared to the other lanthanide elements. The ecotoxicological effects of this phenomenon are unknown. Furthermore, the administration of Gd-based MRI contrast agents is related to a fatal disease known as nephrogenic systemic fibrosis (NSF) for patients with kidney failure [2]. The identification and quantification of contrast agents in biological and environmental samples requires the use of speciation analysis methods. For that purpose, the hyphenation of hydrophilic interaction liquid chromatography (HILIC) and inductively coupled plasma mass spectrometry (ICP-MS) was employed. In order to further improve the limits of detection (LOD), a method of sample introduction into the ICP as dry aerosol generated by a desolvation system was developed. This resulted in improved transport efficiency in comparison to conventional methods of sample introduction. The achieved LODs for this method were found to be below 0.1 nmol/L for commonly applied contrast agents. The development, optimization and application of these methods are presented.

References
ANALYTICAL SPECIATION OF PHOSPHITE AND PHOSPHATE IN TRANSGENIC PLANTS BY HPLC-ICP-MS WITH PHOSPHORUS DETECTION AT M/Z 31 AND M/Z 47

Julio Torres Elguera¹, Katarzyna Wrobel¹, Eunice Yañez Barrientos¹, Kazimierz Wrobel¹

¹ University of Guanajuato, Department of Chemistry, L. de Retana 5, 36000 Guanajuato, Mexico
Email: jc.torreselguera@ugto.mx

Many biotechnological studies focus today on phosphite [P(III)] as an alternative source of phosphorus for crops. In this regard, transgenic plants capable to carry out oxidation of phosphite to phosphate [P(V)], are engineered. As a support for such biotechnological task, analytical speciation of P(III)/P(V) is required [1]. Using polymeric-based anion exchange column and potassium phthalate mobile phase (pH 2.5 adjusted with nitric acid), the two species were baseline resolved with retention times 6.95 ± 0.03 min and 7.90 ± 0.03 min and with total chromatographic run time 10 min. Milimolar concentration of nitric acid in the mobile phase enabled for stable rate of PO₄³⁻ formation in plasma. The detection limits were 1.58 μgP L⁻¹ and 1.74 μgP L⁻¹ at m/z 47, as compared to 2.18 μgP L⁻¹ and 2.04 μgP L⁻¹ at m/z 31, respectively. The results obtained in the analysis of real-world samples using the two detection modes were in good agreement, yet signal acquisition at m/z 47 enabled for better precision (RSD below 2% versus around 4% at m/z 31). Most importantly, in contrast to other procedures based on PO₄³⁻ monitoring, cool plasma conditions or the collision/reaction cell were not needed and the proposed here procedure can be accomplished on any ICP-MS instrument [2,3].

References
ABSOLUTE AND SITE-SPECIFIC QUANTIFICATION OF PHOSPHORYLATION USING THE NEW ICP-QQQ-MS

Silvia Diez Fernández¹, Jorge Ruiz Encinar¹, Alfredo Sanz-Medel¹

¹ University of Oviedo, Faculty of Chemistry, Julián Clavería 8, Oviedo, 33006, Spain
Email: silvia.diez1986@gmail.com

Protein phosphorylation is one of the most important post-translational modification as it is involved in many significant biological processes. Traditionally, most of the studies have focused on phosphorylation sites determination rather than quantification or dynamic studies. Previous efforts in determination of the phosphorylation stoichiometry/protein concentration levels were limited by the absolute quantification of the protein [1]. In this context, ICP-QQQ-MS has been recently proposed as a very sensitive detector for P and S species [2]. Here we propose the use of chromatography hyphenated to the new ICP-QQQ-MS for the simultaneous determination P/S ratios on transient signals in phosphoproteins and phosphopeptides. Bovine milk β-casein was chosen as protein model as it is a commercially available and well characterized phosphoprotein containing 6 methionines (6 sulfur atoms) and 4-5 phosphates moieties per protein molecule. The analysis of the intact protein was carried out with SEC-ICP-MS in order to obtain the global phosphorylation degree using P and S species (bis-nitrophenyl phosphate and methionine) as unspecific calibrants. In addition, tryptic digestion of β-casein followed by capLC coupled to the ICP-QQQ-MS analysis showed the phosphorylation degree along the individual phosphorylation sites (individual phosphopeptides).

References


Cancer biomarkers discovery is one of the most active scientific fields within the Clinic Analytic Chemistry investigation. However, there are few cancer biomarkers in use on a routine basis in the clinical practice. The analytical techniques that are often used for these types of studies include Matrix Assisted Laser Desorption Ionization (SELDI), Mass Spectrometry coupled with Bidimensional Chromatography (2D-LC-MS) and the Bidimensional Gel Electrophoresis (2D-GE). SELDI are the most promising techniques in this field but there is still a long way to go until they can be implement on the clinical laboratories. We propose the combination of HPLC-ICP-MS with post-column isotope dilution analysis using 33S and the oral administration of 34S-labelled yeast as the base for in vivo protein synthesis kinetic studies. For this purpose, a single dose of 34S-labelled yeast, containing methionine and other 34S-enriched compounds, is given to laboratory mice. Then the concentration and the molar fraction ratio 34S/natS in the different sulfur-containing metabolites in urine is monitored, at different times, through the coupling of liquid chromatography with a multicollector ICP-MS instrument. Quantitative data is obtained by post-column isotope dilution with 33S. It can be expected that isotopic equilibration between natural abundance sulfur and the enriched sulfur will not be achieved and that certain urinary metabolites will get a higher 34S isotopic enrichment than other due to their particular synthesis and degradation kinetics. Ideally, isotope enrichment curves of the different urinary metabolites will be different in healthy and diseased individuals with could serve as the base for biomarkers identification. In this communication we will present the results obtained in the optimization of the chromatographic separation of the principal sulfur metabolites in urine of Wistar rats and C-57 mice. For this purpose, a Discovery BIO Wide Pore C18 column and a mobile phase of ammonium acetate 75 mM, pH 7,4 with a methanol gradient (2% to 30% in 60 minutes) was employed. In order to avoid sulfur signal suppression in the ICP-MS due to the methanol content in the mobile phase, a concentric nebulizer with a membrane desolvator system (Aridus II, CETAC) was used. On the other hand, the suitability of two double focusing ICP-MS instruments for on-line sulfur isotope ratio measurements were compared: the Element 2, with a sequential detection system and the Neptune Plus with simultaneous detection. The main analytical characteristics for isotope ratio measurements in both instruments were compared. Finally, we will present the first results on the comparison between urine of healthy and prostate cancer mice. The data processing to measure the isotope enrichment in HPLC-ICP-MS was based on isotope pattern deconvolution (IPD).


References


The methylation of the C-5 position of cytosine in genomic DNA resulting in the formation of 5-methylcytosine (5-MeC) is an epigenetic event that controls gene expression, genome stability and cellular differentiation. In addition, alterations in DNA methylation play an important role in the development and pathogenesis of tumors and their resistance to cytotoxic drugs such as cisplatin [1]. Therefore, it has become clinically important to know the methylation status of genomic DNA in diagnosis, in adapting cancer therapies or for prognostic purposes. Here we describe the development of a high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) method for the determination of 5-MeC content in DNA monitoring the $^{31}$P present in the nucleotides. The method has been applied to the analysis of global DNA methylation in cisplatin sensitive and resistant cells. Alternatively, strategies based on performing polymerase reaction chain (PCR) to amplify a particular region of interest in DNA are used for different purposes in the field of genomics [2]. In the work, we also show the potential of elemental mass spectrometry coupled to on-column gel electrophoresis (GE-ICP-MS) using $^{31}$P detection for quantitative analysis of PCR products using a phosphate standard. This methodology permits to obtain information about the size of the obtained amplified DNA fragments by using a DNA ladder as well as the concentration of the PCR products by normalizing the signal to the phosphate peak area.

References
A NEW METAL TAG FOR HIGHLY SENSITIVE ANALYSES OF METABOLITES BY RP-HPLC/ICP-MS

Daigo Iwahata¹, Kazuki Nakamura¹, Rie Yamada¹, Hiroshi Miyano¹, Naoyuki Yamada¹

¹ 1-1, Suzuki-cho, Kawasaki-ku, Kawasaki-shi, Japan
Email: daigo_iwahata@ajinomoto.com

We have developed a novel metal tag, bis (ethylenediamine)-4'-methyl-4-carboxybipyridine-ruthenium N-succinimidyl ester (ECRS) for sensitive analysis of amino acids and dipeptides using reversed phase high performance liquid chromatography/ inductively coupled plasma mass spectrometry (RP-HPLC/ICP-MS). ECRS is a functional reagent, containing an ester group at one end that can be activated to bind to amino group and a chelated ruthenium at the other. The activated ester was reacted briefly with amino groups under weakly alkaline conditions. The ruthenium was detected sensitively by ICP-MS. ECRS was reacted with the proteinogenic amino acids in borate buffer. The derivatives were separated by reversed phase HPLC and identified by quadrupole-based ICP-MS. ECRS was suitable for speciation; low molecular weight compounds containing amino groups. We will present the application of ECRS and another metal tags for the low molecular weight metabolites.

References
NEW DOTA-BASED METAL COMPLEXES FOR BIOPOLYMER LABELING USING CLICK CHEMISTRY REACTIONS

Yide He¹, Diego Esteban-Fernández¹, Michael W. Linscheid¹

¹ Department of Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany
Email: heyide@cms.hu-berlin.de

In the last years, our group has contributed to the challenge of quantitative analysis of biomolecules by developing metal-coded affinity tag (MeCAT) labels which target cysteine-thiol groups [1]. In this regard, a new labeling strategy-based on click chemistry is being studied to reduce the steric hindrance and improve the labeling efficiency. First, the thiol group of the cysteine is modified with a small terminal alkyne residue. Then, the metal-harboring DOTA-azide complexes are directly introduced via click chemistry. The labeling efficiency of the methodology has been proved with standard peptides and digested standard proteins. At least 88% of the cysteinyl sequence of bovine serum albumin (25 cysteine-containing peptides) was fully labeled. DVCK and CCAADDK cannot be detected, neither unlabeled nor labeled. Peptide ECCHGDLLECADDR was found partially labeled. It is worth to mention that six peptides with two adjacent cysteine residues were totally labeled. Therefore, the lower steric hindrance and the implementation of novel assisted-procedures for the click chemistry reaction, enhance the labeling efficiency. Fragmentation experiments led to satisfactory peptide identifications. Interestingly, a specific reporter ion from the label was also detected.

In conclusion, a novel click chemistry-based strategy for labeling biopolymers using DOTA complexes is presented for first time. High labeling efficiency and robustness are the main advantages of this approach.

References

COMPARISON OF COPPER LABELING FOLLOWED BY HPLC-ICP-MS AND IMMUNOCHEMICAL ASSAYS FOR SERUM HEPcidIN-25 DETERMINATION

Tobias F. Konz¹, F. Javier Alonso-García¹, María Montes-Bayón¹, Alfredo Sanz-Medel¹

¹ University of Oviedo, Faculty of Chemistry, Oviedo, 33006, Spain
Email: fjavieralonsogarcia@gmail.com

Hepcidin-25 has been defined as the key biomarker in iron metabolism. This peptide binds to the iron transporter ferroportin to cause its degradation. Therefore, the need for specific, accurate and precise methods for the quantification of hepcidin-25 in biological fluids is dramatically increasing [1]. In this regard, the use of rapid immunochemical methods that provide low limit of quantification is desired for routine clinical use. However, these methodologies should be first analytically evaluated and compared with other strategies to check for their advantages and limitations. Here we compare the use of a commercial immunochemical assay for hepcidin determination with a novel analytical approach based on Cu-labeling of the peptide followed by Cu determination using liquid chromatography (HPLC) and plasma mass spectrometry (ICP-MS) [2]. The figures of merit of both systems reveal similar characteristics and both seem to be adequate for the determination of the peptide at biologically relevant concentrations in serum samples. The analysis of a larger number of samples (n=50) by both techniques showed a good agreement in the concentrations found. Such finding permits to address the recovery of the sample preparation procedure for hepcidin-25 from human serum necessary for the HPLC-ICP-MS. Additionally, the existing limitations due to cross-reactivity issues of the ELISA method will be addressed in some of the samples.

References

ABSOLUTE AND RELATIVE QUANTIFICATION OF MULTIPLEX DNA ASSAYS BASED ON ELEMENTAL LABELING STRATEGY[1]

Guojun Han¹, Sichun Zhang¹, Zhi Xing¹, Xinrong Zhang¹

¹ Tsinghua University, Beijing, China, 100084
Email: gjhanchem@gmail.com

Rapid, multiplex, and quantitative detection of sequence-specific or mutated genes associated with human diseases has played a central role in modern clinical treatments of molecular diagnostics and genomics research. There are ever-increasing requirements for improving analytical capabilities, in particular for signal multiplexing and precise quantification. Elemental labeling strategy for bioassay has been regarded as an emerging methodology, in which large biomolecules are labelled with elemental tags and subsequently detected by elemental mass spectrometry such as inductively coupled plasma mass spectrometry (ICP-MS) [2-3].

Herein, we report the multiplex nucleic acid assays based on the elemental labeling strategy, which take advantages of DNA hybridization reactions for specific recognition, rare earth elements for multiplex labeling, magnetic microparticles for fast separation, and ICP-MS for ultrasensitive detection. In addition, both absolute and relative quantification could be performed for multiplex analysis of DNA targets, respectively. As a proof-of-concept study of high-level multiplexing, 15 kinds of DNA targets associated with clinical diseases were simultaneously detected via elemental labeling tags of Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu. The relative quantification was carried out by using internal calibration curves. Subsequently, the absolute quantification with chromatography-free hydridization isotope dilution analysis (HIDA) strategy was developed.

References

ELEMENTAL LABELED ANTIBODY FRAGMENTS FOR LC-ICP-MS BASED IMMUNOASSAYS

Teresa Mairinger¹, Daniela Kretschy¹, Gunda Koellensperger¹, Stephan Hann¹

¹ Department of Chemistry, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria
Email: teresa.mairinger@boku.ac.at

The derivatisation of antibodies with elemental labels and their ICP-MS based detection offers the possibility for indirect quantification of various targets (e.g. cancer markers or cellular metabolites). This straightforward technique is applicable in the clinical field for imaging via LA-ICP-MS [1] or for immunoassays via ICP-MS based absolute quantification of the targets in biological samples. The objective of our work is the design of an immunoassay with LC-ICP-MS based detection. As the assessed samples are characterized by a complex matrix, limited sample volume and low concentrations of the analyte (e.g. a biomarker), quantification requires highly sensitive, selective and robust strategies. We are currently optimizing the labeling procedure of a genetically modified ThioFab with rare earth elements using macrocycles as chelators. The employment of the genetically modified ThioFab allows straightforward labeling without a previous reduction step. Optimization is performed in terms of coordination of selected lanthanides by the complexing moiety, the linking reaction of the complex to the ThioFab and the formation of the final antibody-antigen conjugate. Within our presentation the state of our work regarding an immunoassay based on LC-ICP-MS-based separation and quantification of ThioFab-Antigen conjugates employing AntiHer2/Her2 is shown.

References

Yong Liang¹, Xiaowen Yan¹, Limin Yang¹, Qiuquan Wang¹
¹ Key Lab of Analytical Science & Department of Chemistry and State Key Lab of Marine Environmental Science, Xiamen University
Email: yongliang@stu.xmu.edu.cn

P450 3A4 isoform is a member of cytochrome P450 family playing a key role during the metabolism of drugs and xenobiotics. In liver, P450 3A4 accounts for approximately 50% of total expressed P450 content and participates greater than 60% of toxicological process. In addition to its importance, lack of more sensitive and liable means to quantify this vital enzyme drive us to develop a robust and sensitive method to determine P450 3A4 isoform in vitro and/or in vivo. Here we designed and synthesized an alkyne-modified ethinylestradiol as a probe to selectively label P450 3A4 isoform, in which the reactive intermediate (ketene) resulted from the bio-oxidation of the native alkyne group of ethinylestradiol by the 3A4 and, in turn, the ketene can covalently conjugate to the 3A4; finally, the other alkyne group modified on the ethinylestradiol can perform the so-called click chemistry of Copper-Catalyzed Azide-Alkyne 1,3-Dipolar Cycloaddition with a azido-DOTA-europium synthesized previously to achieve europium-tagging towards targeted 3A4. The 3A4 could thus be quantified via europium determination using SEC/ICP-MS. To the best of our knowledge, this is the first report using an alkyne-modified activity-based inhibitor and ICP-MS based on europium-tagged strategy via click chemistry for highly bioselective and sensitive quantification of P450 3A4. It is expected to apply to absolute quantification of other p450 isoforms in living system in near future.

References
METAL LABELLING WITH SUBSEQUENT ICP-MS DETECTION AS A NOVEL TOOL FOR THE RECOGNITION OF SPECIFIC DNA SEQUENCES

L. López Fernández¹, E. Blanco González¹, M. Montes Bayón¹, J. Bettmer¹

¹ University of Oviedo, Faculty of Chemistry, C/ Julián Clavería 8, Oviedo, 33006, Spain.
Email: lucia.lopezfdez@gmail.com

Following by the genome sequencing and looking for diagnostic information, new sensitive and multiplexed DNA technologies have appeared. The most commonly approaches make use of optical or electrochemical detection [1], but the potential of elemental mass spectrometry has only been rarely explored in this field [2]. Inductively coupled plasma-mass spectrometry (ICP-MS) is well known for its high sensitivity and simultaneous multi-isotope detection. In this work, the concept and initial results will be presented on its potential as detection system for the sensitive and multiplexed analysis of specific DNA targets. The method is based the labelling of modified oligonucleotides (with complementary sequence to the target sequence) applying maleimide-DOTA (2,2',2''-(10-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl) triacetic acid) loaded with lanthanide ions. This type of labelling is well-known for protein or antibody modification [3] and was optimised in order to obtain a metal-labelled DNA probe. After being incubated with the complementary oligonucleotide (DNA target) the DNA probes were analysed by size-exclusion chromatography (SEC) coupled to ICP-MS. This set-up allowed the monitoring of the hybridisation process and the recognition of specific DNA sequences. Simultaneous detection of several DNA targets was feasible. Initial data on analytical figures of merit will be given.

References
OLIGONUCLEOTIDE TARGETING STRATEGIES FOR THE DETECTION OF DNA-PROTEIN CROSS-LINKS

L. López Fernández¹, E. Blanco González¹, J. Bettmer¹, M.J. Solivio², J. Landero², E.J. Merino², J. Caruso²

¹ University of Oviedo, Faculty of Chemistry, C/Julián Clavería 8, Oviedo, 33006, Spain.
² University of Cincinnati, Department of Chemistry, Cincinnati, USA
Email: lucia.lopezfdez@gmail.com

Oxidative stress causes different damages in biomolecules. DNA lesions are the best known but there are others which have not been studied in great detail. Here DNA-protein-cross link, generated by the link between DNA and proteins, should be pointed out. Despite of the presence of this damage, there is a lack of biochemical characterization and methodology to evaluate it [1]. Recently, a model system using ribonuclease A and a 27-mer oligonucleotide was used to determine the propensity of oxidative cross-linking [2]. According to this experiment and our previous experience with DNA labeling, a new HPLC-ICP-MS methodology for the identification and quantification of DNA-protein cross-links is shown. The combination of elemental tags and inductively coupled plasma-mass spectrometry (ICP-MS) offers high sensitivity, selectivity and simultaneous multi-isotope detection. Based on the sequence of the DNA which forms part of the cross-link product, a DNA probe was synthesized and hybridized with the DNA-protein complex. This DNA probe was previously labeled with the maleimide-DOTA-lanthanide complex. After the hybridization process, the mixture was analyzed by size exclusion chromatography (SEC) coupled to ICP-MS. Due to the metal-labeled oligonucleotide the crosslink product is detectable with high sensitivity. Additionally, structural information was elucidated by the combination of tryptic digestion and peptide identification by complementary mass spectrometric techniques, including ESI-MS.

References
GOLD AS AN ACTIVITY-BASED PROBE TO ADDRESS THE ACTIVE THIOREDOXIN REDUCTASE CONCENTRATION IN CELLS AND BIOLOGICAL FLUIDS BY HPLC-ICP-MS

Juan Gómez Espina¹, María Montes Bayón¹, Elisa Blanco González¹, Alfredo Sanz Medel¹

¹ University of Oviedo, Faculty of Chemistry, Julián Clavería 8, Oviedo, 33006, Spain
Email: juangomesp@hotmail.com

Thioredoxin Reductase 1 (TrxR1) is a cytosolic enzyme containing a selenenylsulfide/selenolthiol redox active site which catalyzes the reduction of disulfide containing substrates. Therefore, the enzyme plays a critical role in regulating cellular redox homeostasis and also signalling pathways which are involved in cell survival and proliferation. In fact, TrxR1 overexpression have been associated with enhanced tumour proliferation, decreased apoptosis, increased angiogenesis, increased resistance to chemotherapeutic drugs, and reduced survival [1]. Hence, accurate and sensitive measurement of the thioredoxin level in biological fluids, cells and tissues is of paramount importance in the field of anticancer research studies. Most analytical methods for TrxR1 determination are based on immunoassays using polyclonal antibodies or relative activity measurements using spectrophotometric assays, which are not specific for TrxR [2]. Here we propose the absolute quantification of this protein in biological samples through the measurement of the Se present in their structure using isotope dilution-ICP-MS after anion exchange HPLC separation. Moreover, the determination of the active form of the enzyme was also conducted by using auranofin as an activity-based probe. This compound reacts with the selenolthiol group of the active enzyme. Once optimized this reaction, the derivatized active form of the enzyme was quantified by HPLC with ICP-MS detection of both Au and Se.

References
QUANTIFICATION OF IRON CONTAINING BLOOD PROTEINS BY MEANS OF ISOTOPIC DILUTION ICP-MS APPLYING $^{57}$Fe ENRICHED PROTEIN SPIKE MATERIAL

Christine Brauckmann¹, Claudia Frank¹, Claudia Swart¹, Detlef Schiel¹

¹ Physikalisch-Technische Bundesanstalt, Bundesalle 100, 38116 Braunschweig
Email: christine.brauckmann@ptb.de

Although, iron is an essential metal for human life, free iron ions are toxic to cells and might damage tissues. Therefore, different proteins are involved in storage, transportation and use of iron in the human body. The following investigation focuses on two iron containing proteins: haemoglobin (HGB) and transferrin (TRF). TRF is one of the major proteins that control the iron transport whereas HGB is responsible for the oxygen transport. The blood concentrations of these two proteins are important indicators in clinical diagnostics. The acute phase protein TRF is a biomarker for congenital disorders of glycosylation, cerebrospinal fluid leakage as well as for certain cancers. In case of HGB, changes in concentration might induce diseases like heart attacks, strokes or fatigue. Up to now, most metalloproteins are quantified using immunoassays, immunoturbidimetry or fluorometry; however, no primary reference measurement procedure with results traceable to the SI is available.

One aim of the EMRP project HLT05 is to develop primary reference measurement procedure for the quantification of these proteins. Species specific isotopic dilution (SS-ID) by means of LC/ICP-MS was chosen for the quantification, as ICP-MS offers low detection limits and a selective detection of iron. Due to the fact that all molecular information is lost in the plasma source, a LC separation was developed. SS-ID-MS is the most precise method to achieve a reliable and matrix independent quantification.

References
Towards SI Traceable Clinical Measurements and Materials of Iron Biomarkers

Yoana Nuevo Ordonez¹, Clay Davis¹

¹ National Institute of Standards and Technology, 331 Fort Johnson Rd, Charleston, SC, 29412
Email: yoana.nuevoordonez@nist.gov

Iron is an essential nutrient for all organisms and acts as a cofactor for many key enzymes of major metabolic pathways. Measurements of iron status are needed for clinical diagnosis of diseases such as anemia, autoimmune diseases, hemochromatosis or cancer. Clinical measurements of iron status include serum iron, transferrin (Tf), percentage of transferrin saturation (% TS), total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), ferritin and soluble human transferrin receptor (sTfR). Generally, clinical measurements are determined using immunoassays, immunoturbidimetry, immunonephelometry or fluorometry, which are not primary reference measurement procedures as they may be biased by cross-reactions or require external calibration. Consequently, the established “normal” ranges of iron biomarkers vary greatly when used for clinical diagnosis (i.e. “normal” ferritin levels in men range from 20-300 µg/L). Inter-laboratory comparisons of different clinical diagnostic tests typically yield only a method specific consensus value or show large variations (20-40%) in measurements of pooled serum samples. Consequently, reliable and traceable results for clinical parameters are crucial in clinical studies to assess the effects of new or improved treatments. In this regard, NIST is currently working to compile all of the aforementioned measurements in one reference material yielding a SI traceable measurement vehicle for clinical iron biomarkers and iron status.
A NOVEL TECHNIQUE TO DETERMINE NON-TRANSFERRIN-BOUND IRON (NTBI) IN HUMAN BLOOD BY SPECIES-SPECIFIC ISOTOPE DILUTION MASS SPECTROMETRY (IDMS)

Yao Ren¹, Thomas Walczyk¹

¹ Department of Chemistry, 3 Science Drive 3, National University of Singapore, Singapore, 117543
Email: g0900908@nus.edu.sg

Non-transferrin-bound iron (NTBI) is a known source of oxidative stress and considered to play a central role in the development of many disorders including cardiovascular disease and type 2 diabetes. NTBI is currently quantified by chelation, fluorescence-quenching or biochemically. An international round robin yielded highly inconsistent results for the different techniques. Needs for sensitive and reliable NTBI quantification were stressed.

Here we present a novel technique based on species-specific isotope dilution mass spectrometry (IDMS). Isotopically labeled Fe-nitrilotriacetic acid is spiked directly after blood sampling to allow instant NTBI capture. Iron species are separated by ultrafiltration followed by isotopic analysis of each species and total serum iron using Negative Thermal Ionization Mass Spectrometry. NTBI concentration is calculated following IDMS principles. Our IDMS approach was found to be accurate and robust against species conversion which is a major source of bias of conventional techniques. Repeatability is of the order of <2 % at an absolute NTBI detection limit of 60 ng.

First applications of our technique show that current techniques have underestimated NTBI burdens significantly, especially in individuals of normal iron status. We found that NTBI concentration drops rapidly post sampling following a power law. In contrast to current techniques, our method conserves NTBI concentration immediately at the point of sampling without delay.

References

DEVELOPMENT OF NON-DENATURING OFF-GEL FRACTIONATION TECHNIQUES FOR THE SEPARATION OF INTACT URANIUM-PROTEIN COMPLEXES IN BIOLOGICAL SAMPLES

Guillaume Bucher¹², Sandrine Frelon², Olivier Simon², Ryszard Lobinski¹, Sandra Mounicou¹

¹ LCABIE - UMR5254, Technopôle Hélioparc Pau Pyrénées, 2 avenue du Président Angot, 64053 Pau Cedex 09 - FRANCE
² IRSN/PRP-ENV/SERIS - Laboratoire de Biogéochimie, Biodisponibilité et Transferts des radionucléides - BP3 - 13115 Cadarache
Email: guillaume.bucher@etud.univ-pau.fr

Uranium is a naturally occurring radioelement which is both radio- and chemo-toxic. Generally found as hard uranyl cation (UO$_2$$^{2+}$) in aerobic media, uranium can bind proteins to form mainly non-covalent complexes [1]. Because of the electrostatic nature and the low abundance (ca. few ng per mg of tissue) of uranium-protein complexes in biological media (e.g. cytosol), their analysis requires therefore non-denaturing separation protocols and ultrasensitive detection techniques. In this regard, a liquid-phase isoelectric focusing (i.e. off-gel IEF) method was developed as a first dimension for the separation of intact U-protein complexes from the gills cytosolic fraction of U-exposed zebrafish. The liquid fractions were subsequently fractionated by size exclusion chromatography in a second dimension. U focusing and elution were monitored respectively, by off-line and on-line coupling to highly sensitive ICP-SF-MS. This alternative to conventional time-consuming gel-based methods enabled us to establish a 2D mapping of U-protein in less than 24h and overlapping of U, P and Fe signals was observed at trace level. Off-gel and on-gel IEF are compared in terms of resolution, protein and uranium recoveries. Although, off-gel IEF exhibits a lower resolving power, this technique offers several advantages among which its speed (e.g. less than 3h), the use of low voltage and the convenience of liquid fractions handling for downstream analysis in view of U protein targets identification.

References
COMPLEMENTARY USE OF MOLECULAR AND ELEMENTAL MASS SPECTROMETRY FOR THE INVESTIGATION OF THE ADDUCT FORMATION OF MERCURY SPECIES WITH BLOOD PROTEINS

Jens Hogeback¹, Miriam Schwarzer¹, Christoph A. Wehe¹, Michael Sperling¹, Uwe Karst¹

¹ University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr. 28/30, 48149 Münster/Germany
Email: jens.hogeback@uni-muenster.de

Mercury (Hg) and its organometallic species are known to be highly toxic. Due to consumption of marine food, administration of thimerosal containing vaccines and dental amalgam, humans are exposed to different Hg species in different ways. Since Hg toxicity depends massively on its species, an individual view on their toxicokinetics is important and therefore, a suitable speciation analysis is essential. In biological matrices, mercury species do not occur freely in solution. Because of the high affinity to sulfur, Hg species are usually bound to thiol containing peptides and proteins [1]. This species information is eliminated during sample preparation for the common Hg speciation analysis by means of GC/ICP MS, where Hg is alkylated completely. Therefore, analytical methods are required, in which the original species are conserved. Earlier studies indicated that the majority of blood mercury is accumulated in red blood cells [2]. In this work the adduct formation of Hg species with proteins of human erythrocytes was investigated. Cell hemolysates and native proteins were incubated with different Hg species and analyzed by means of LC/ESI-ToF-MS and LC/ICP-MS. The complementary use of molecular and elemental mass spectrometry provides both information for identification of the Hg protein adducts (ESI-MS) and species independent elemental response for quantification (ICP-MS). These results may lead to a better understanding of the distribution and toxicity of mercury species.

References
TWO-DIMENSIONAL ISOELECTRIC FOCUSING OFFGEL AND MICROFLUIDIC LAB-ON-CHIP ELECTROPHORESIS FOR ASSESSING MARINE PROTEINS IN SEAWATER

Natalia García-Otero¹, María del Carmen Barciela¹, Pilar Bermejo-Barrera¹, Antonio Moreda-Piñeiro¹

¹ University of Santiago de Compostela, Department of Analytical Chemistry, Nutrition and Bromatology
Email: antonio.moreda@usc.es

Dissolved marine proteins were assessed in surface and deep seawater by two-dimensional isoelectric focusing (IEF) OFFGEL - lab-on-chip (LOC) electrophoresis after tangential flow ultrafiltration followed by centrifugal ultrafiltration (both pre-concentration systems use 10 kDa cut off membranes). The sample pre-treatment involves a pre-concentration factor of 3000 (60 L of seawater up to 20 mL of ultrafiltered retentate by centrifugation). Different methods for marine proteins isolation from the ultrafiltrated retentate were then tested, and the best electrophoretic behaviour of the isolated proteins was obtained after protein precipitation by adding a chloroform/methanol/water mixture. Operating parameters affecting the LOC electrophoresis, such as the temperature and the heating time, were optimised, and repeatability and accuracy of the whole two-dimensional IEF OFFGEL - LOC electrophoresis were further evaluated. Metals bound to proteins in the different OFFGEL fractions were assessed by inductively coupled plasma - optical emission spectrometry (ICP-OES) and electrothermal atomic absorption spectrometry (ETAAS) under optimised operating conditions. Experiments regarding stability of the metal-binding proteins were performed by subjecting two proteins (superoxide dismutase and alcohol dehydrogenase, as protein models) to the IEF OFFGEL electrophoresis method. Results showed that the electrophoretic conditions used appear to be less drastic than those required in conventional polyacrylamide gel electrophoresis (PAGE). The first electrophoretic dimension (IEF OFFGEL) showed that marine proteins exhibit mainly alkaline isoelectric points (pis), while LOC showed that the isolated proteins exhibit a spread molecular weight range; although, high molecular weights (ranging from 61.8 to 66.2 kDa) were the most commonly found. Elements such as Cd, Cu, Mn and Ni are mainly associated to marine proteins of alkaline pIs, while Zn is mainly associated to proteins of acid pIs.
Abstracts

Poster Session  Analytical Tools & Bioimaging & Nanometallomics

Poster  P 036  Tuesday, 9th July 2013, 11:00 - 12:00  Room “Exhibition Hall”

Tuesday, 9th July 2013, 18:00 - 20:00

QUANTIFICATION WITH INTERNAL STANDARDIZATION IN LA-ICP-MS - THE EGG OF COLUMBUS?

Sarah Theiner¹, Alexander Egger¹, Wolfgang Kandioller¹, Bernhard Keppler¹, Petra Heffeter², Christoph Kornauth³

¹ Institute of Inorganic Chemistry, University of Vienna, Waehringerstrasse 42, 1090 Vienna, Austria
² Institute of Cancer Research, Medical University of Vienna, Borschkegasse 8a, 1090 Vienna, Austria
³ Institute of Clincial Pathology, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria
Email: sarah.theiner@univie.ac.at

Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has emerged as promising imaging technique in the medicinal sector to study e.g. the distribution of metal-based chemotherapeutics in biological tissues or to monitor disease-induced alterations of metals in inner organs [1]. Quantification with LA-ICP-MS still remains challenging due to the broad range of multifaceted applications of the method and the requirement of appropriate calibration standards and internal standardization. In this study, homogenized liver standards spiked with different amounts of trace elements are used for calibration. The concentrations of the standards are validated via solution-based ICP-MS after microwave assisted acid digestion. The mostly employed internal standard is the isotope $^{13}$C due to its presence in biological samples but poses problems concerning its significantly different atomic mass and first ionization potential compared to many analytes [2]. Therefore, the suitability of different alternative methods for internal standardization is tested and compared. In a final step, a quantification method is applied to different tissues (e.g. tumor) originating from mice treated with metal-based anticancer agents. The correlation of the metal intratissue accumulation pattern with the histology provides important information on the distribution of the drug on the microscopical scale.

References

A NOVEL CRYOGENICALLY COOLED ABLATION CELL FOR SOFT TISSUE ANALYSIS BY LASER ABLATION ICP-MS

I. Konz¹, B. Fernández¹, M. L. Fernández¹, R. Pereiro¹, A. Sanz-Medel¹

¹ University of Oviedo, Faculty of Chemistry, c/ Julián Clavería 6, Oviedo, 33006, Spain
Email: konzioana@uniovi.es

The analysis of essential, toxic, and therapeutic metals as well as metalloids in biological materials is a key task in life sciences, and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has proven to be an excellent tool to study their distribution in biomedical tissues. The analysis of soft tissues by LA-ICP-MS is generally carried out using either paraffin-embedded or native frozen sections. Nevertheless, recent studies have shown that during paraffin-embedding metal losses could occur leading to a distorted interpretation of the results [1]. In this vein, the application of cooled laser ablation cells has proved to be advantageous not only for the analysis of cryo-preserved materials but also for dried biological samples [2]. In this work we present a new cooled ablation cell developed for LA-ICP-MS analysis. The proposed design is based on the use of several internal Peltier elements which are controlled by a sensitive sensor directly on the sample surface and maintain the desired temperature of the sample (in the range between -20°C and +20°C). Moreover, to guarantee an adequate design for imaging applications where high resolution images from small sample areas are required, the internal volume of the ablation cell as well as the shape of the gas inlet and outlet have been investigated in detail. The successful analytical performance of the proposed cell will be demonstrated for the analysis of different type of samples.

References
QUANTITATIVE ELEMENTAL IMAGING OF TRACE ELEMENTS IN HUMAN EYE SECTIONS BY LA-ICP-MS

I. Konz¹, B. Fernández¹, M. L. Fernández¹, R. Pereiro¹, A. Sanz-Medel¹, H. González², M. Coca-Prados²

¹ University of Oviedo, Faculty of Chemistry, c/ Julián Clavería 6, Oviedo, 33006, Spain
² Fundación de Investigación Oftalmológica, Instituto Oftalmológico Fernández-Vega, Oviedo, 33012, Spain

Email: konzioana@uniovi.es

Bioimaging techniques with adequate spatial resolution are today of crucial interest in life science to achieve a deeper understanding of the role of metals in biological systems. In this vein, the use of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has demonstrated great potential for spatially resolved analysis of metals in different types of tissues. However, due to the complexity of the sample matrix, tissue thickness, water content and density of different tissue regions could affect the analytical performance. In order to achieve precise and accurate measurements the selection of an appropriate internal standard becomes crucial.

In a previous study, a new internal standard correction strategy was developed for the qualitative bioimaging of Mg, Fe and Cu in eye microstructures. The proposed normalization approach is based on the simple deposition of a thin and homogeneous gold film on the surface of the tissue section and the use of the \(^{197}\text{Au}^+\) signal as internal standard [1].

In this work, the proposed internal standardization approach was applied to quantitative elemental imaging of native human eye lens sections using matrix-matched laboratory standards. Experimental results obtained by LA-ICP-MS showed an inhomogeneous distribution of Fe, Cu and Zn in the human lens sections with elevated concentration in the lens capsule, being these results in agreement with those obtained by ICP-MS after the microwave-assisted digestion of the samples.

References

Abstracts

Poster Session  Analytical Tools & Bioimaging & Nanometallomics

Poster  P 39  Tuesday, 9th July 2013, 11:00 - 12:00  Room “Exhibition Hall”

Tuesday, 9th July 2013, 18:00 - 20:00

QUANTITATIVE BIOIMAGING OF NOBLE METALS IN THIN TISSUE SECTIONS

Olga Reifschneider¹, Christoph A. Wehe¹, Michael Sperling¹, Uwe Karst¹, Indra Raj², Giuliano Ciarimboli², Jens Ehmcke², Martin Wiemann³

¹ University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstrasse 30, 48149 Münster, Germany
² University of Münster, Experimental Nephrology, Albert-Schweizer-Campus 1-A14, 48149 Münster, Germany
³ EBI R&D gGmbH, Mendelstrasse 11, 48149 Münster
Email: o.reifschneider@gmail.com

To understand the side effects and underlying transport mechanisms of silver nanoparticles and platinum-based cytostatics in the living organism, the study of distribution and retention behavior in affected organs is of strong interest. Bioimaging by means of laser ablation coupled to inductively coupled plasma mass spectrometry (LA-ICP-MS) is a powerful approach for the investigation of the lateral distribution in thin tissue sections in the low µm range. Quantitative imaging methods by means of LA-ICP-MS were established to investigate the distribution and retention behavior of selected platinum-based pharmaceuticals and silver nanoparticles in biological samples. Hence, the distribution at different time intervals after treatment was determined in diverse types of tissue such as kidney, cochlea, testicles and lung prepared as in paraformaldehyde fixed or polymer embedded sections. The quantification was performed by external calibration using homemade matrix-matched standards. This method proved to yield precise and reproducible quantification results for embedding medium and biological matrix based standards with respect to the sample type. High spatial resolution of 10 µm and limits of detection in the very low ppb range were achieved using this simple and efficient sample preparation. The hyphenation of LA to ICP-MS proved to be a valuable tool for studying the pathways and retention mechanisms of widely used pharmaceuticals and nanoparticles in tissues. The quantitative distribution of noble metals in biological tissue was comprehensively visualized using the new calibration methods.
NEW INSIGHTS INTO LA-ICP-MS IN BIOCHEMISTRY USING A HIGH SENSITIVE MASS SPECTROMETER

Rene Chemnitzer¹, Meike Hamester¹, Pierre-Emmanuel Riss²

¹ Bruker Daltonic GmbH, Fahrenheitstrasse 4, 28359 Bremen, Germany
² Bruker Daltonique, Champs Sur Marne, France
Email: Rene.Chemnitzer@bruker.com

The complete characterization of biological samples like tissues samples includes the elemental distribution but also the isotopic composition for a broad range of elements. Laser ablation coupled to an ICP-MS has become an indispensable method. New instrumental developments allow resolutions to single-digit µm spots and new ablation cells show improved transport characteristics. With a high sensitive ICP-MS (Bruker auroraElite) these developments lead to a tremendous increase in information. The work presents results from a LA-ICP-MS setup that provides the highest sensitivity currently available and shows results of the quantitative and isotope ratio analysis of various samples like rice grains or plant leaves. Different parameters including Laser spot size and energy density as well as the sample introduction in the ICP-MS, 3D-ion focusing and scan speed were investigated. The results verify that the considerable higher sensitivity leads to new alternative approaches in LA-ICP-MS, that will be discussed in detail.
CADMIUM LOCALIZATION AND SPECIATION IN THE SYMBIOTIC ASSOCIATION

ANTHyllIS VULNERARIA - MEzORHIZOBiUM METALLIDURANS

Stéphanie Huguet¹, Souhir Soussou², Jean-Claude Cleyet-Marel², Hiram Castillo-Michel³, Nicolas Trcera⁴, Marie-Pierre Isaure¹

¹ LCABIE/IPREM-UMR 5254, Université de Pau et des Pays de l’Adour, Technopôle Hélioparc Pau-Pyrénées, Pau - France
² LSTM - UMR 113 : IRD/CIRAD/SupAgro/UM2, USC 1242, INRA, Campus International de Baillarguet, Montpellier - France
³ Beamline ID21, ESRF, Polygone Scientifique Louis Néel, Grenoble - France.
⁴ Beamline LUCIA, Synchrotron SOLEIL, Gif-sur-Yvette Cedex, France.

Email: stephanie.huguet@univ-pau.fr

The legume plant *Anthyllis vulneraria* and its associated microorganism *Mezorhizobium metallidurans* occur naturally on mine tailings from South of France (les Avinières, 161 000 ppm Zn, 1382 ppm Cd [1]). Able to grow in high toxicity conditions, *A. vulneraria* was studied using original combination of chemical and physical techniques to obtain insights on Cd tolerance mechanisms. Metal sequestration mechanisms can be different in each plant compartment, involving specific ligands in each organs/tissues of plant. This work aims to determine Cd distribution and the ligands binding Cd in the leaves and roots of *A. vulneraria* as well as in its rhizobium nodules in various conditions of rhizobium inoculation. Micro X-ray absorption spectroscopy (µXAS) is a synchrotron based technique that has demonstrated great potential for the study of the chemical form of trace metals in biological samples [2, 3]. In this work, XANES (X-ray Absorption Near Edge Structure) and µXANES were applied to probe Cd chemical environment at the bulk and micrometer scales and µX-ray Fluorescence (µXRF) was used to determine elemental distributions and co-localizations. In leaves of *A. vulneraria*, Cd is mainly in vascular bundles, cells of epidermis and in trichomes. Cd seemed to be stored as Cd-O ligands (bulk analyses), whereas Cd seemed to be transported as Cd-S ligands (micro-analyses). In roots, Cd is mainly bound to S ligands and found in vascular bundles, cortex and more concentrated in endodermis.

References

VISUALIZING THE BIODISTRIBUTION OF ZINC COMPLEXES AS PROMISING METAL DRUGS BY GAMMA-RAY EMISSION IMAGING (GREI)

Masayuki Munekane¹, Shinji Motomura², Shinichiro Kamino², Hiromitsu Haba³, Yutaka Yoshikawa⁴, Hiroyuki Yasui⁵, Makoto Hiromura²,⁶, Shuichi Enomoto¹,²

¹ Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University
² Next-generation Imaging Team, RIKEN Center for Life Science Technologies
³ RIKEN Nishina Center for Accelerator-Based Science, RIKEN
⁴ Faculty of Health and Welfare, Kobe Women’s University
⁵ Kyoto Pharmaceutical University
⁶ Daiichi University of Pharmacy

Email: ph20140@s.okayama-u.ac.jp

The medicinal uses and applications of metal complexes are of increasing clinical and commercial importance, and various types of promising metal drugs have been developed. Zinc (Zn) complexes are candidate for treatment of type 2 diabetes. However, the drug metabolism of complexes is poorly understood. In addition, no methodology is established well for analyzing noninvasively. Here, a novel nuclear medicine modality of GREI was applied to the noninvasive analyses of metal drug metabolism. Di(1-oxy-2-pyridinethiolato)Zn complex (Zn(opt)₂), di(L-histidino)Zn complex (Zn(His)₂) and ZnCl₂ were used. The compounds were radiolabeled with ⁶⁵Zn, and injected to C57BL/6J mice. GREI experiments were conducted under isoflurane anesthesia for 8 hours. In addition, the radioactivity in each tissue removed from sacrificed mice was determined by Ge detector. In the 2D images obtained by GREI experiments, the accumulations of ⁶⁵Zn were commonly high in the liver for all compounds. Moreover, the accumulation of ⁶⁵Zn was increased in the heart specifically for Zn(opt)₂. These results were consistent with the destructive distribution analyses. In addition, temporal changes of ⁶⁵Zn distribution were observed for all compounds. The present study indicated that GREI would be a novel noninvasive method to visualize the temporal distribution of zinc complexes.
INSIGHTS INTO THE NANOWORLD -
ANALYSIS OF NANOPARTICLES WITH ICP-MS

Daniel Kutscher¹, Jörg Bettmer², Torsten Lindemann¹, Shona McSheehy-Ducos¹, Lothar Rottmann¹

¹ Thermo Fisher Scientific, Hannah-Kunath-Straße 11, Bremen, Germany
² University of Oviedo, Department of Physical and Analytical Chemistry, C/ Julián Clavería 8, Oviedo, Spain
Email: daniel.kutscher@thermofisher.com

The analysis of Nanoparticles (NPs) has become one of the hot topics in analytical chemistry. Although many everyday products contain such material, detailed knowledge about potential risks or hazards is still unavailable. In order to leverage the potential of ICP-MS for the analysis of NPs, two approaches have been developed in recent years: 1. Hyphenation of an appropriate separation technique like Field-Flow-Fractionation (FFF), or, 2. Direct analysis of nanoparticles using spICP-MS. FFF has a separation principle based on the differing mobilities of different particle sizes in a laminar liquid flow. FFF is compatible for particle sizes in the low nm to low µm range and is thus perfectly suited for the separation of different NPs. In comparison, spICP-MS is able to analyze NPs directly based on the signal intensity of single particle events in the plasma which are directly proportional to the size of the NP. This direct approach greatly simplifies the experimental set-up. ICP-MS instrumentation with outstandingly high detection sensitivity extends the particle size limit of detection into the low nm range. In this presentation, the theory and typical requirements of both techniques are going to be presented. The key benefits and drawbacks of each technique are going to be illustrated with samples that contain nanoparticles of different structure and size.
IN VITRO STUDIES WITH GOLD NANOPARTICLES MONITORED BY HPLC-ICP-MS AND TEM

Juan Soto-Alvaredo¹, María Montes-Bayón¹, Jörg Bettmer¹, Carlos López-Chávez², Cristina Sánchez-González², Juan Llopis-González²

¹ Department of Physical and Analytical Chemistry, University of Oviedo, c/ Julian Claveria 8, 33006, Oviedo, Spain.
² Department of Physiology, University of Granada, Campus Cartuja, 18071, Granada, Spain.
Email: juanst@gmail.com

Gold nanoparticles (GNPs) are supposed to be extensively used in many biomedical applications like bio-imaging, gene delivery, drug delivery and other diagnostic applications, thanks to their small size-to-volume ratio, ease of synthesis, chemical stability, and optical properties [1]. Due to its increasing use, the knowledge about their effect on environmental and biological systems has gained great interest in the last years. Based on a recent work [2], a hyphenated technique, namely HPLC-ICP-MS, will be presented for the analysis of GNPs. The method allows the discrimination between particle-bound Au and low-molecular species of Au³⁺. Moreover, GNPs of different size can be separated, so that this study aims the application of the developed method to the analysis of GNPs after incubation of cell lines. For this purpose, in-vitro studies with HT-29 cells are carried out with citrate-protected GNPs of 10 and 30nm diameter (NIST 8011 and 8012). Information on viability, oxidative stress, and proliferation of the cells are accompanied by transmission electron microscopy (TEM) imaging in order to locate the GNPs within the cells. The detection of potential degradation of the GNPs (e.g. due to release of low-molecular Au³⁺ species) will be monitored by HPLC-ICP-MS after subsequent extraction of the different Au species from the biological matrix [3].

References

BIOTRANSFORMATION OF CERIA NANOPARTICLES IN CUCUMBER PLANTS

Peng Zhang¹, Yuhui Ma¹, Xiao He¹, Yuliang Zhao¹, Zhiyong Zhang¹

¹ Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Science
Email: zhangzhy@ihep.ac.cn

Biotransformation is a critical factor that may modify the toxicity, behavior and fate of engineered nanoparticles in the environment. Ceria NPs are generally recognized as stable under environmental and biological conditions. The present study aims to investigate the biotransformation of ceria NPs in plant systems. TEM images show needle-like clusters on the epidermis and in the intracellular spaces of cucumber roots after a treatment with 2000 mg/L ceria NPs for 21 days. By using soft X-ray scanning transmission microscopy technique, the needle-like clusters were verified to be cerium phosphate. Near edge X-ray absorption fine structure spectra show that Ce presented in the roots as ceria and cerium phosphate while in the shoots as ceria and cerium carboxylates. Simulated studies indicate that reducing substances (e.g. ascorbic acids) played a key role in the transformation process and organic acids (e.g. citric acids) can promote particle dissolution. We speculate that ceria NPs were firstly absorbed on the root surfaces and partially dissolved with the assistance of the organic acids and reducing substances excreted by the roots. The released Ce(III) ions were precipitated on the root surfaces and in intercellular spaces with phosphate, or form complexes with carboxyl compounds during translocation to the shoots. To the best of our knowledge, this is the first report confirming the biotransformation and in-depth exploring the process of ceria NPs in plants.
INTERACTIONS OF FETUIN WITH URANYL IONS AND URANYL HYDROGEN PHOSPHATE NANOPARTICLES

Łukasz Szyrwiel¹, Katarzyna Bierła², Ryszard Łobinski²

¹ Department of Chemistry of Drugs, Wrocław Medical University, ul. Tamka 1, 50-137 Wrocław, Poland
² CNRS/UPPA, LCABIE, UMR5254, Hélioparc, 2 av. Pr. Angot, F-64053 Pau, France
Email: lukasz.szyrwiel@umed.wroc.pl

Uranium is known for radio- and chemical toxicity [1] and has been found to accumulate in various organs of living organisms leading to pathological calcification and nephrotoxicity. In blood, UO₂²⁺ is transported in low molecular complexes and various proteins. Fetuin, known well as calcium-biomineral chaperone [2] was very recently demonstrated to be the principal target protein for uranyl ions in serum [3]. Here, we put forward the hypothesis that fetuin can complex uranium in the form of mineral (uranyl hydrogen phosphate) nanoparticles, thus limiting the formation of uranium deposits. The experiments were carried in system containing UO₂(NO₃)₂ [2,5×10⁻⁴M] and Na₂HPO₄ [2,5×10⁻⁴M] in tris buffer system at pH 7,4, T= 37ºC, time of incubation 12h. After the incubation the reaction mixture was centrifuged with 12000×g and the supernatants were analysed for uranium concentration and speciation. Fetuin was found to increase the amount of mobile uranyl to 700% in investigated systems. The presence of aggregates of uranyl hydrogen phosphate and fetuin was demonstrated by size-exclusion LC-ICP MS analysis.

References
BSA AS TEMPLATE FOR SILVER AND GOLD NANOCLUSTERS: SYNTHESIS AND CHARACTERIZATION BY COMPLEMENTARY ANALYTICAL TOOLS

Nerea Fernández Iglesias¹, Jörg Bettmer¹

¹ University of Oviedo, Faculty of Chemistry, C/Julián Clavería 8, Oviedo, 33006, Spain.
Email: nereastur@gmail.com

The special properties of gold and silver nanoparticles (NPs) are rapidly revolutionizing many different areas of science, such as chemistry, engineering and medicine, e.g. in diagnostics, treatment and novel functional materials [1,2]. The crucial role of the interaction between NPs and biomolecules (e.g. proteins) results in the formation of a biological corona on the NP ’ s surface, the so-called “protein corona”. The identification and understanding of the “biological identity” of metal NPs are of major interest. Therefore, the first step to understand the complicated molecular aspects of bio-interactions is to synthesize and characterize NPs. In this sense, gold and silver nanoclusters (NCs) were synthesized using a modified procedure of that used by Xie et al. [3]. Herein, bovine serum albumin (BSA) was used as template in the synthesis procedure, due to its many advantages as a protecting agent in biological applications. In this work, hyphenated techniques, mainly size-exclusion chromatography (SEC-HPLC) coupled to UV-Vis and ICP-MS detection, were used to evaluate the synthesis procedure and separate/purify the selected NCs. In addition, electrospray ionisation-mass spectrometry (ESI-MS), matrix-assisted laser desorption and ionisation-mass spectrometry (MALDI-MS) and high-resolution transmission electron microscopy (HR-TEM) were employed for the characterisation of these NCs. The results reveal that the synthesis of Ag and Au NCs can result in defined species, which might have great potential as probes in bioanalytical applications [2].

References
ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION FOR THE CONTROL OF THE SYNTHESIS AND PHOTOSTABILITY OF SILVER NANOCLUSTERS

Laura Trapiella-Alfonso¹, Mario Menéndez-Miranda¹, José M. Costa-Fernández¹, Rosario Pereiro¹, Alfredo Sanz-Medel¹

¹ Department of Physical and Analytical Chemistry, University of Oviedo
Email: trapiellalaura@uniovi.es

To date it exits a great demand of homogeneous and monodisperse nanoprobes, with optimum optical features for the development of quantitative bio applications. Therefore, the search for highest quality luminescent labels and nanoprobes is a present challenge. Advances in nanomaterial research has produced a new class of nanostructures known as Metal Nanoclusters (NCs) which are composed of a few to roughly a hundred atoms; these sizes place them between isolated atoms and larger nanoparticles. Due to their reduced size, NCs present discrete electronic energy levels conferring them molecule-like behaviour and leading to the observation of dramatically different optoelectronic properties as compared to larger nanoparticles or bulk material. Some of their attractive features include tunable photoluminescence, large Stokes shifts, lack of intermittency, low photobleaching and low toxicity. In this context, the use of Asymmetric Flow Field-Flow Fractionation (AF4) coupled on-line to several detectors (elemental and molecular) is a good strategy to both characterize the synthesis and other processes that can affect NCs properties (e.g. effect of UV irradiation), as well as to elucidate the mechanisms involved in such processes. Therefore, in this communication new insights will be pointed out about the mechanism and structural transformations involved in the growth of highly fluorescent silver nanoclusters and in the UV irradiation process, based on the use of AF4.
NOVEL PHOTOLUMINESCENT NANOPROBES: SYNTHESIS AND CHARACTERIZATION OF METAL NANOCUBKUSTERS

Laura Trapiella-Alfonso¹, Mónica Fernández-Ujados¹, José M. Costa-Fernández¹, Rosario Pereiro¹, Alfredo Sanz-Medel¹

¹ Department of Physical and Analytical Chemistry, University of Oviedo
Email: trapiellalaura@uniovi.es

Metal nanoclusters (NCs) are a new class of nanomaterial composed of several to tens of atoms that present distinct optical, electrical, and chemical properties as compared to other large-scale nanomaterials or bulk materials with the same chemical composition. NCs exhibit luminescence from ultraviolet to the near infra-red regions, large Stokes shifts, two-photon absorption, lack of intermittency and low photobleaching. Thus, NCs can be treated as alternatives to quantum dots and organic dyes, being highly attractive for bio-imaging and bio-labeling applications due to their smaller size and because they do not present the stigma of potential toxicity. As this is a new nanomaterial, they offer a challenging fundamental problem to understand cluster growth, stability, and functionality. Herein we present a simple and efficient synthetic route approach for the preparation of Ag and Cu nanoclusters in aqueous media. The synthesis is based on the direct reduction of the metal in the presence of lipoic acid-appended-poly(ethylene glycol) functionalized ligands. These ligands promote aqueous compatibility over a broad range of conditions (pH, ionic strength media, cell culture media) and reduce significantly the nonspecific interaction in biological systems. We will describe in detail the proposed synthetic route along with the structural, optical and spectroscopic characterization of these materials, pointing out the differences found between the metals that composed the core.
SELENIUM SPECIATION

Eeva-Maria Rintala¹, Aino Känsälä¹, Pertti Koivisto¹, Kimmo Peltonen¹, Eija-Riitta Venäläinen¹, Päivi Ekholm²

¹ Finnish Food Safety Authority Evira, Chemistry and Toxicology Research Unit, Mustialankatu 3, 00790 Helsinki, Finland
² University of Helsinki, Faculty of Agriculture and Forestry, P.O. Box 66, 00014 University of Helsinki, Finland
Email: eeva-maria.rintala@evira.fi

In this exercise we developed and validated a high performance liquid chromatographic - inductively coupled plasma - mass spectrometric (HPLC-ICP-MS) method for the identification and quantification of selenium species in vegetables and nuts. Beneficial and toxic effects of selenium in human and in animals depend on the amount of selenium intake and the chemical form of selenium. The concentrations of selenium species in unenriched foodstuffs tend to be low. Six commercially available selenium species were used as standards: SeCyst, SeMet, SeIV, SeVI, Se-(methyl)selenocysteine and γ-glutamyl-SeMC. A selenium enriched yeast Selm-1 was used as a reference material. It has a certified value for SeMet and total selenium. The samples were different garlcs and nuts. Also leaves of plants cultivated in selenium fertilized soil were used in method development because of their high selenium content.

Grounded samples were incubated in a laboratory microwave oven in the presence of TRIS-HCl-buffer and Proteinase K -enzyme. The samples were centrifuged and subsequently filtered. In this study we used ammonium citrate buffer (pH 5.2, 30 - 50 mM) as an eluent. The HPLC column was Hamilton PRP-X100 anion exchange column (250 × 4.6 mm, 5 µm). The injection volume was 100 µl, the column temperature was ambient and the eluent flow rate was 1 mL/min. The results were quantitated with an external standard method. The results and discussion will be presented in Metallomics 2013.

References
ACCUMULATION, ENZYMATIC AND MORPHOLOGIC PARAMETERS IN CHICKPEA PLANTS EXPOSED TO SELENIUM

Lyudmila Lyubenova¹, Xenia Sabodash¹, Peter Schröder¹

¹ Helmholtz Zentrum München GmbH, German Research Center for Environmental Health, Ingolstäder Landstr. 1, 85764 Neuherberg Germany
Email: lyudmila.lyubenova@helmholtz-muenchen.de

Selenium (Se) is a micronutrient with multiple antioxidative capacities. The suitable Se concentrations are of high importance for the functions of some enzymes, hormones and for metabolic processes. Se parties an essential factor for the enzymes glutathione peroxidase (GPOX) and thioredoxin reductase (TrxR) in humans. It participates also the conversion of L-thyroxin (T4) to triiodothyronine (T3) and its concentration in human serum is between 100 and 160 µg/l (µmol/l) (Gröber, 2011). Se deficiency in humans leads to interference of some organ and tissue functions. The aim of the present study is to investigate the accumulation of Se, the enzymatic and the morphologic factors to which it may lead in chickpea plants after short and long time of exposure. Se exposure experiments were carried in semi-hydroponic conditions in a greenhouse. Se concentrations were in the range 10, 25, 50 and 100 µM for 14 days (short term) and 71 days (long term) exposure. The results of our study show that Se accumulates in higher range in chickpea’ s roots compared to the shoots. The 25 µM Se exposure support and 100 µM Se inhibit the plant’s development. The investigated enzymes let us to assume that the Se detoxification in chickpea proceeds without the participation of the glutathione cycle. The applicable aspect of the study is to use Se fortified chickpea plants as a functional food in order to affect its role in the global health problems.

References
STUDY ON ACCUMULATION MECHANISM OF ARSENIC, CHROMIUM, AND SELENIUM IN *PTERIS VITTATA* L. USING SYNCHROTRON RADIATION X-RAY FLUORESCENCE ANALYSIS

Akiko HOKURA¹, Hiroki HANASHIMA¹, Mao HONDA¹, Nobuyuki KITAJIMA², Tomoko ABE³

¹ Department of Green and Sustainable Chemistry, Tokyo Denki University, Senju-Asahicho, Adachi, Tokyo 120-8551 Japan
² Fujita Co. Ltd., 2025-1 Ono, Atsugi, Kanagawa 243-0125 Japan
³ Nishina Center, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198 Japan

Email: hokura@mail.dendai.ac.jp

*Pteris vittata* L. is known as an arsenic hyper-accumulator [1-3]. We investigated the accumulation mechanism of arsenic, chromium, and selenium in the fern cultivated with the culture solution containing As(V), Cr(VI), or Se(VI) by utilizing synchrotron radiation X-ray fluorescence analysis. The chemical speciations of arsenic, chromium, selenium, and sulfur in the fern were carried out by X-ray absorption near edge structure (XANES) analyses and the elemental distributions in their tissues were visualized by micro-XRF imaging.

Arsenic was highly accumulated in pinna especially in the base of sporangium as As(III). Chromium was also reduced to Cr(III) and accumulated in leaves. Selenium could be taken in the root and less accumulated in the aerial parts. About 60% of Se in the fern was present as Se(-II) and this indicated the selenium was reduced from Se(VI) during uptake process. The chemical speciation of sulfur revealed that the ratio of S(-II) and S(V) compounds such as glutathione (GSH) and cysteic acid, respectively, increased when the fern was cultivated with As(V), Cr(VI) and Se(VI). This result demonstrated that sulfur had a certain function in detoxification process of *P. vittata* L.

References

Effect of Nickel on Plant Membrane Bioelectrical Parameters

Vilma Ksnieriene¹, Olga Sevriukova¹, Kristina Panaciova¹, Indre Lapeikaite¹, Vidmantas Sakalauskas¹

¹ Vilnius University, Lithuania, Ciurlionio 21, Vilnius, LT- 03101
Email: vilma.ksnieriene@gf.vu.lt

Although Nickel (Ni) is essential for plants at low concentrations, our knowledge of its toxic effect is incomplete. It is important to find markers of early Ni toxicity. Some responses of plant cells to Ni are related to the alteration of plasma membrane (PM) properties. The PM of plant cells constitutes not only the first barrier for the entry of heavy metals but also a target of their toxic action mediated by changes in the flow of ions. The electrophysiological response pattern could be used to evaluate the short term effect of Ni on the activity of ion transport systems in PM. The algae Nitellopsis obtusa provides an ideal model system for study effects of Ni on plant bioelectrical signals generation. The experiments were performed using intracellular electrophysiological methods in current and voltage clamp modes. Although, the depolarizing effect of Ni (0.1- 5 mM) on resting potential after 30 min application was slight, the increment of membrane conductance was observed. Concentration-dependent effect on action potential (AP) was found. Prolongation of AP and amplitude reduction due to decrement of both membrane potential and AP peak were observed. While Ca²⁺ activated Cl⁻ current initiates the generation of AP in plant cells, we have investigated changes in Cl⁻ current – voltage relations after Ni treatment. Concentration-dependent reduction of Cl⁻ current was found. We conclude that electrical signaling pathway in plant cells could reflect early Ni toxicity.
EFFECT OF EXOGENOUS REAGENTS ON METAL BINDING PROPERTIES OF TWO PLANT METALLOTHIONEIN ISOFORMS

Hasan Tanvir Imam¹, Claudia Andrea Blindauer¹

¹ The University of Warwick, Department of Chemistry, Gibbet Hill Road, Coventry, CV4 7AL, UK
Email: H.T.Imam@warwick.ac.uk

Arabidopsis thaliana has two seed specific type 4 metallothionein isoforms - MT4a and MT4b. The proteins have been expressed in E. coli, and purified by gel filtration chromatography. Electrospray ionisation mass spectrometry (ESI-MS) and elemental analysis by Inductively coupled plasma-optical emission spectroscopy (ICP-OES) reveal that both MT4a and MT4b can bind six zinc ions, similar to their homolog EC from wheat.¹,² Like EC, MT4a and MT4b are found exclusively in reproductive tissue, and it is believed that they release zinc during seed germination. Hence, using UV-Vis and NMR spectroscopies and native ESI-MS, we have studied how exogenous reagents - low pH, 4-(2-Pyridylazo)resorcinol (PAR) and Ethylenediaminetetraacetic acid (EDTA) - affect their metal binding dynamics. In addition, metal exchange with cadmium was studied; as it has been hypothesised that type 4 MTs may be able to prevent the accumulation of cadmium in plant embryos. The experimental results suggest that all these exogenous reagents promote zinc release from MT4a and MT4b.

Cadmium binds non-isostructurally to both type 4 MTs.

Acknowledgement

Thanks to Professor Peter Goldsbrough (Purdue University, USA) for providing expression plasmid of MT4a and MT4b, Esther M. Martin for help with mass spectrometry, the University of Warwick and Department of Chemistry (Scholarship to HTI), and Advantage West Midlands and the European Regional Development Fund (Birmingham Science City) for support.

References


CADMIUM EFFECTS ON THE COPPER TRANSPORTER PROTEIN COPT5 MUTANT IN ARABIDOPSIS

Àngela Carrió¹, Amparo Sanz¹, Lola Peñarrubia¹

¹ Universitat de València (UVEG), Faculty of Biology, Av. Doctor Moliner 50, Burjassot, 46100, Spain
Email: angela.carrio@uv.es

Copper (Cu) is an essential plant micronutrient whose uptake into the cytosol takes place through a high affinity family of transporters named COPT in *Arabidopsis thaliana*. COPT5 is expressed in the tonoplast and is involved in Cu recycling from the vacuole under severe deficient conditions. In addition to fulfil the requirements of copper homeostasis, Cu transport towards the cytosol, either from internal compartments or the external medium initiates a signalling pathway involving reactive oxygen species, calcium and hormones, such as abscisic acid and ethylene. One of the most studied processes under Cu deficiency is the substitution of the antioxidant activities of the copper/zinc superoxide dismutase (Cu/Zn SOD) by the iron (Fe-SOD) counterpart. We have analyzed the effect of different metals on the replacement of SODs in response to Cu deficiency in both wild-type and copt5 mutants. Cd shows a complex effect on the responses to Cu deficiency in Arabidopsis seedlings, since it reduces the expression of FSD1 and increases the expression of the CSD1 in the copt5 mutant. In hydroponic cultures the FSD1 response to Cu deficiency is much more exacerbated on the root than in the aerial part. However, CSD1 expression is mostly exacerbated in the aerial part in the presence of Cd. Cu deficiency affects ethylene biosynthesis, which is further inhibited in the mutant and in the presence of Cd.
CIRCADIAN REGULATION OF CTR/COPT TRANSPORTER PROTEINS IN ARABIDOPSIS

Ana Perea García¹, Amparo Sanz¹, Lola Peñarrubia¹

¹ Avda Dr Moliner, 50 46100 Burjassot (Valencia)
Email: ana.perea@uv.es

Since copper down-regulates the expression of members of the high affinity copper transporters family COPT in Arabidopsis, it is plausible that the cytosolic copper content could oscillate with a daily rhythm as a result of this feedback loop. This biochemical oscillator could be a part of the endogenous processes controlled by the circadian clock. We have reported that deregulated overexpression of COPT1 and COPT3 affects plant performance in the absence of environmental cycles and now we show that rhythmic patterns in these plants are altered. We have also checked the expression of COPT2 and FSD1 (iron superoxide dismutase 1), markers of Cu deficiency responses, under different either continuous or cycling environmental conditions. To that end, both promoters have been fused to the luciferase reporter, and its activity measured into free running conditions. As a conclusion, while the oscillation in COPT2 expression shows a circadian rhythmic pattern, the FSD1 oscillating expression pattern seems to be mostly regulated by light/dark cycles. Moreover, a time-course expression analysis reveals the induction by copper deficiency around 6 hours after treatment, and the establishment of a rhythmic expression pattern around 24 hours. Taken together our data point to copper homeostasis as an integrated part of biochemical processes, controlled by the circadian clock, that allow cells to better adapt to the environmental and internal metabolic cycles.
Abstracts

Poster Session  Metalloproteins & Metallodrugs & Plant Metallomics

Poster  P 057  Tuesday, 9th July 2013, 18:00 - 20:00  Room “Exhibition Hall”
Wednesday, 10th July 2013, 11:10 - 12:10

COPPER BINDING TO ZINC-METALLOTHIONEINS: FUNCTIONAL PROPERTY OR STRUCTURAL DISASTER?

Maria Tareen¹, Claudia Blindauer¹

¹ University of Warwick, Gibbet Hill Road, Coventry CV4 7AL UK
Email: M.Tareen@warwick.ac.uk

Metallothioneins are a group of small proteins with low molecular weight and high content of cysteinyl residues. Since their discovery, the physiochemical properties of Zn-, Cd-, and Cu-containing MTs have been studied. However, copper metallothioneins (Cu-MTs) have been examined less intensively, with only two MTs containing Cu(I) having been structurally characterized to date i.e., yeast Cup1 [1] and Neurospora crassa MT [2]. The present study focuses on the stoichiometry and structural effects of binding of Cu(I) to the natively zinc binding MT, SmtA from the cyanobacterium Synechococcus elongatus sp. PCC7942 [3]. For this purpose, a Cu(I) titration study of Zn₄SmtA was anaerobically performed utilizing the collective detection of ESI-MS, UV-Vis and ¹H-¹⁵N NMR spectroscopies. Fully exchanged Cu(I)-SmtA was also generated from the apo protein which formed relatively stable Cu₇SmtA, which was further tested for metal release by reaction with bathocuproine and pH titrations. Titrations of folded Zn₄SmtA with Cu(I) followed by UV-Vis spectroscopy and ESI-MS revealed that Cu(I) is able to displace Zn(II) partially, resulting in predominantly monomeric Cu₆Zn₁SmtA as end product. Structural effects of Zn(II) displacement by Cu(I) were studied by ¹H,¹⁵N NMR spectroscopy.

References
2D DIGE COUPLED WITH DUAL THIOL-STAINING AND METALLOMICS REVEALS PROTEINS TARGETED BY Cd INDUCED OXIDATIVE STRESS IN ARABIDOPSIS THALIANA

Sacha Bohler¹, Jaco Vangronsveld¹, Ann Cuypers¹

¹ Centre for Environmental Sciences, Hasselt University, Agoralaan, Building D, 3590 Diepenbeek, Belgium
Email: sacha.bohler@uhasselt.be

Reactive oxygen species (ROS) play a major role in signaling and regulation. Enzyme activity can be controlled by oxidation or reduction of cysteine thiols. Furthermore, the natural formation of ROS during photosynthesis renders mechanisms of protection against excess ROS vitally important. The accumulation of ROS during cadmium (Cd) exposure can exhaust protective measures and create an oxidative stress by inducing a shift in redox homeostasis. The increase of the oxidative state in the cell can lead to the oxidation of regulatory cysteines and have a major impact on enzyme activity in plant cell metabolism. Differential in Gel Electrophoresis (DiGE) allows the quantification of protein abundance on 2D gels. With a new approach, the dual staining of thiols, the redox state of protein thiols can be measured as well. The reduced and oxidized thiols are labeled in a same sample with different dyes. After separation by 2D electrophoresis the ratio between oxidized and reduced forms of proteins is calculated. With this technique it is possible to detect key proteins that undergo redox changes during Cd induced oxidative stress. The Cd ion readily binds to protein thiols and disrupts protein integrity and activity. Using a metallomics approach can lead to the identification of proteins directly influenced by Cd, opposed to proteins targeted by oxidative damage. The technique is highly complementary to the redox-proteomics approach.
IDENTIFICATION OF METALS BOUND TO MARINE PROTEIN BY TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS AND LA-ICP-MS

N. García-Otero¹, M.C. Barciela-Alonso¹, A. Moreda-Piñeiro¹, P. Bermejo-Barrera¹, M.S. Jiménez², M.T. Gómez², J.R. Castillo²

¹ University of Santiago de Compostela, Spain
² University of Zaragoza, Zaragoza, Spain
Email: pilar.bermejo@usc.es

Different studies based on laser ablation - inductively coupled plasma - mass spectrometry have been performed to assess metals bound to dissolved marine proteins and proteins from marine plankton fractionated by two-dimensional polyacrylamide gel electrophoresis. Dissolved marine proteins were pre-concentrated from seawater (60 L) by tangential ultrafiltration with 10 kDa molecular weight cut-off membranes until obtaining a retentate fraction of 0.5 L. After further centrifugal ultrafiltration (10 kDa molecular weight cut-off), proteins pellet was obtained by methanol/chloroform/water precipitation. Proteins isolation from plankton was assessed after different TCA/acetone and methanol washing stages, and further proteins extraction with a phenol/SDS buffer solution. Proteins were first separated on the basis of their (pH-dependent) net charges by isoelectric focusing, IEF, and then separated on the basis of their molecular masses in the presence of SDS. LA-ICP-MS analysis of the electrophoretic profiles obtained for marine dissolved proteins shows the presence of cadmium, copper, iron, zinc and chromium in five spots analyzed. Regarding proteins isolated from plankton, the analysis of several proteins spots has shown the presence of small amounts of cadmium and chromium.
A MODULAR MODEL FOR FUNGAL COPPER-THIONEINS:
BECOMING VIRULENCE FACTORS

Anna Espart¹, Silvia Atrian¹, Òscar Palacios², Mercé Capdevila², Chen Ding³, Dennis J. Thiele³

¹ Universitat de Barcelona, Facultat de Biologia, Av. Diagonal 643, Barcelona, 08028, Spain
² Universitat Autònoma de Barcelona, Facultad de Ciències, Cerdanyola del Valles, 08193, Spain
³ Duke University, School of Medicine, Durham, North Carolina, 27710, USA
Email: satrian@ub.edu

Copper plays a critical role in the mammalian immune system and e.g. macrophages accumulate Cu to be used as an antimicrobial agent, so that diverse microbial pathogens developed Cu detoxification systems to elope its toxic effects. This is the case that we recently described for Cryptococcus neoformans, a fungus causing respiratory infections that may end up in lethal meningitis if disseminated to brain. The molecular study of Cu resistance in C. neoformans led to the identification of two metallothioneins (CnMT1 & CnMT2), which are essential for its virulence. Their synthesis is triggered by high copper and they show an exceptionally high and specific copper binding capacity. The features of the Cu-CnMT1 and Cu-CnMT2 complexes revealed that, under this selection pressure, they probably evolved by tandem amplification of a basic Cu-binding subunit (similar to N. crassa or A. bisporus Cu-thioneins) encompassing seven Cys and some variable intercalating residues. This “Cys-box” appears triplicated in CnMT1 and five times in CnMT2, and it would form unusual 5 Cu(I)-thiolate clusters. An also conserved “spacer region” separates adjacent Cys-boxes. To lay experimental grounds on this model, we designed and recombinantly-expressed in Cu-enriched bacteria, a series of CnMT1-derived peptides consisting of a different number of repetitions of the basic Cys-box, with and without flanking spacers, N- or C-terminally located. Our results fully corroborate the aforementioned hypothesis.

References
INVESTIGATING CUPROPROTEINS EXPRESSION IN OYSTER (CRASSOSTREA GIGAS) AFTER COPPER STRESS BY METALLOMIC AND PROTEOMIC APPROACHES

Ming Xu¹, Hugues Bijoux², Patrice Gonzalez², Sandra Mounicou¹

¹ LCABIE - UMR5254, Technopôle Hélioparc Pau Pyrénées, 2 avenue du Président Angot, 64053 Pau Cedex 09 - FRANCE
² EA, Université Bordeaux 1, UMR EPOC 5805, Place du Docteur Bertrand Peyneau, 33120 Arcachon
Email: sandra.mounicou@univ-pau.fr

Living organisms exposed to contaminants can display several responses, such as the induction of enzymes or proteins, as a response to the oxidative stress. These inducible biomolecules can repair some of the molecular damages occurring when toxic chemicals exceed pollutant-elicited defences, notably by complexing metal ions or pumping them out of the cytoplasm. In our previous proteomic study, copper (Cu), one of the contaminants of the Arcachon bay (France), was found to correlate with the expression of several protein biomarkers (e.g. extracellular superoxide dismutase [Cu-Zn] (EC Cu/Zn-SOD)) in the pacific oyster (Crassostrea gigas) grown in field. In this work, to validate these findings and access the mechanism of Cu accumulation in oyster, a laboratory Cu-exposed oyster experiment was carried out and cuproproteins involved in the protection against Cu toxicity have been investigated based on metallomic and proteomic approaches. The Cu distribution in oyster organs (i.e. gills and digestive gland) was firstly determined by ICP MS. Then, the cuproproteins in cytosols were probed under non-denaturating conditions by SEC-ICP MS and ND-PAGE-LA-ICP MS, and identified by μRPC-ESI MS/MS. Finally, the expression of cuproproteins was evaluated by SDS 2-DE.

Acknowledgements

ANR (RIPOST project 09-CESA-005), Region of Aquitaine and the FEDER funds via CPER A2E (31486/08011464) project are acknowledged for financial support.
INFLUENCE OF LONG-TERM CERULOPLASMIN-ASSOCIATED COPPER DEFICIENCY ON COPPER METABOLISM IN RATS

Ekaterina Ilyechova¹, Alexey Skvortsov¹, Nadezhda Tsymbalenko¹, Ludmila Puchkova¹²

¹ Department of Molecular Genetics, Institute of Experimental Medicine of the NorthWest Branch of the Russian Academy of Medical Sciences (IEM NWB RAMS), St.-Petersburg, Russia.
² Department of Biophysics, St-Petersburg State Polytechnical University (SPbSPU), St.-Petersburg, Russia.

Email: ilichevaey@gmail.com

At present the extensive use of silver as mild germicidal agent is dramatically increasing. Yet there are insufficient biochemical studies that could explain dubiously low toxicity of silver in mammals. Ag(I) is isoelectronic to Cu(I), thus it can be captured by Cu-transporting proteins and incorporated into cuproenzymes as it was shown recently. So during chronic exposures silver can disturb copper metabolism (CM) and may lead to disorders due to the loss of cuproenzyme functions.

In the present study we assessed the changes of CM in rats that received Ag with food for long time (50 mg AgCl/kg/day from birth for 6 months-Ag rats). They were compared to control rats and adult rats that received AgCl for 4 weeks. Silver accumulated in liver, blood serum, and brain of Ag-rats. In Ag-rats the oxidase activities of ceruloplasmin (Cp; the major cuproenzyme of blood and copper donor to non-hepatic cells) were ~50% of physiological level. This was different from rats fed with AgCl in adult age (~0% oxidase Cp). Serum copper content was proportional to oxidase activity, immunoreactive Cp protein content did not change. Cp samples from the blood of Ag-rats were isolated and characterized. The levels of expression of CM-related genes in the liver were analyzed by RT-PCR. In Ag-rats, expression of CTR1, CTR2, Mt1a, Commd1, Ccs decreased; the transcription of the other examined genes did not change.

The data suggest that the effect of Ag-ions depends on the age of mammals. Disturbance of CM in early ontogenesis, apparently invokes alternative, previously unknown Cu-transport pathways. This leads to reprogramming of CM and thus reduces copper deficiency. The study of this mechanism is important both for understanding the environmental consequences of the extensive use of silver and the mechanisms of copper homeostasis in mammals. Moreover Ag-rat may be used as valuable model of low copper status for biological and medical studies.

Acknowledgements

The work was supported by RFBR grant 12-04-31518.
EXTREME HIGH STABILITY OF INTERPROTEIN ZINC HOOK MOTIF UNCOVERS CRITICAL CORRELATION BETWEEN STRUCTURE AND STABILITY IN RAD50 PROTEIN

Tomasz Kochanczyk¹, Artur Krezel¹

¹ University of Wrocław, Faculty of Biotechnology, Laboratory of Chemical Biology, Tamka 2, 50-137 Wrocław, Poland
Email: krezel@biotech.uni.wroc.pl

In comparison to the vast number of well characterized intramolecular catalytic and structural zinc sites, little is known about zinc sites located at protein interfaces [1]. An example of such Zn(II) site is the highly conserved zinc hook motif present in Rad50 protein, which is a part of complex that form cellular DNA double-strand break repair machinery [2]. In Rad50 zinc hook two pairs of conserved Cys-X-X-Cys form an unique interlocking metal binding site with one tetrahedrally coordinated Zn(II) which bridges protein dimer and enables long-range (~10nm) tethering of two DNA molecules. Question that we try to answer is how two ligands can assemble to form Zn(II) binding site with sufficient stability to occur at very low free zinc ion concentrations present in the cell. Our studies performed on model peptides showed that they form complexes similarly to Rad50 with 1:2 stoichiometry and remarkably high stability (-logKd = 19.19) [3]. Alanine scanning of relevant amino acids in the sequence uncovered a network of electrostatic and hydrophobic interactions that are the structural basis of observed high stability. Optimized sequence have been subsequently used as a very efficient tool for reversible Zn(II) dependent protein dimerization. We discuss the biological consequences and application in protein engineering and metallomics.

Acknowledgements

We thank MNiSW (Grant No. DI2011 031341 and IP2011 026971) and FNP (Grant F1/2010) for support.

References

AFFINITY OF METAL IONS TO ZINC PROTEINS - FACTORS AFFECTING THE DETERMINATION OF STABILITY CONSTANTS

Anna Miloch¹, Artur Krezel¹

¹ University of Wrocław, Faculty of Biotechnology, Laboratory of Chemical Biology, Tamka 2, 50-137 Wrocław, Poland
Email: krezel@biotech.uni.wroc.pl

Zn(II) is one of the most widespread metal ion in biology. Between 4-10% of the genes encode zinc proteins which in humans amount to at least 3000 proteins [1]. Speciation of zinc proteins and zinc dynamics are the fundamental questions addressed in metallomics. Free zinc concentration at hundreds of pM indicates that some of the zinc proteins might be present in the cell in unbound form. However, this conclusion is based on known stability constants. Recent studies performed on zinc finger peptides suggested that metal ion affinities to proteins might be underestimated [2]. Here, we discuss factors affecting the determination of affinities by different experimental approaches using as an example six ββα zinc finger peptides. The dissociation constants were determined by spectrophotometric titrations, using chelator competition or direct pH titrations. We show that widely practiced determination of stability constants of zinc proteins with Co(II) titration and subsequent Zn(II) reverse titration is strictly limited only for low and medium affinities and its usage results in significant experimental errors in many cases. In our opinion determination of higher affinities, which are typical for most structural sites requires either strong competitors or spectrophotometric titrations using presented here three-step titration method with Ni(II), Co(II) and Zn(II).

Acknowledgements
We thank FNP (Focus F1/2010), MNiSW (Iuventus Plus IP2012 018272) for support.

References
**Abstracts**

**Poster Session**  Metalloproteins & Metallodrugs & Plant Metallomics

**Poster**  P 065  **Tuesday, 9th July 2013, 18:00 - 20:00**  Room “Exhibition Hall”  
**Wednesday, 10th July 2013, 11:10 - 12:10**

---

**METALLOPROTEINS IN DEINOCOCCUS RADIODURANS**

Cecília S. Miranda¹, Patrícia T. Borges¹, Sandra P. Santos¹, João Carita¹, Célia V. Romão¹

¹ Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República - EAN, 2780-157 Oeiras, Portugal  
Email: cmromao@itqb.unl.pt

*Deinococcus radiodurans* is considered an extremophile bacterium, having the capacity to survive to different extreme conditions such as high doses of ionic irradiation, desiccation and other stress conditions [1]. This organism has low cellular iron and high manganese contents when compared to other radiation sensitive bacteria [2]. Studies on the manganese cellular localization suggested that this element concentrates near DNA; recently it was proposed to be associated with low-molecular mass molecules [3]. A search on the Dr genome for proteins that have been annotated as having iron centers, reveals several proteins, for instance cytochromes and iron-sulfur proteins, while for manganese the only known example is the superoxide dismutase. However, since around 50% of the total genes have an unknown function, and as there is a limitation on the bioinformatics tools to predict metal binding sites, the current project aims to identify, characterize and to determine the protein X-Ray structure of metalloproteins purified directly from Dr. We have undergoing in our lab a Small Scale Structural Metallomics Project in Dr, in which the pipeline consisted on the protein purification directly from the native source followed by metal centers characterization and protein crystallization. The results obtained concerns the identification of several soluble and membrane bound metalloproteins some of them are hypothetical proteins.

**References**


CRYSTAL STRUCTURE DETERMINATION OF THE HEME B CATALASE DR1998 FROM DEINOCOCUS RADIODURANS

Patrícia T. Borges¹, Cecília S. Miranda¹, Sandra P. Santos¹, Carlos Frazão¹, Célia V. Romão¹

¹ Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Avenida da República (EAN), 2780-157 Oeiras, Portugal
Email: pborges@itqb.unl.pt

Deinococcus radiodurans (Dr) is considered an extremophile bacterium, having the capacity to survive to different extreme conditions that damage DNA, including high doses of ionizing and ultraviolet radiation and well as other stress conditions [1]. Dr exhibits significant resistance to oxidative stress that is incurred by an increase of reactive oxygen species (ROS) formation, which are produced metabolically or can be formed upon exposure, for instance, to radiation [2]. The protective mechanism of Dr in response to oxidative damage involves a powerful enzymatic antioxidant system including catalases, peroxidases and superoxide dismutases, against primary ROS: superoxide anion and hydrogen peroxide (H₂O₂). Catalase enzymes catalyze the conversion of H₂O₂ to O₂ and H₂O and thus protect the organisms from the oxidative effect of H₂O₂. It is described that Dr genome encodes for three catalases (katE catalases DR1998 and DRA0259 and an eukaryotic-type DRA0146) [3]. Under this study, we are studying the catalase DR1998, a heme b containing protein purified from the native source as part of the Dr Metalomic Project. This protein was submitted to crystallization trials, the crystal structure was determined by X-Ray diffraction to a resolution of 2.6 Å from data collected at ID29 from ESRF. An overview to this crystal structure will be present.

Acknowledgments
Project funding by FCT (PTDC/BIA-PRO/100365/2008)

References
ACIDIANUS AMBIVALENS ERYTHRIN: BIOCHEMICAL AND STRUCTURAL STUDIES

Joana Carrilho¹, Pedro M. Matias¹, Liliana Pinto¹, Miguel Ribeiro¹, Miguel Teixeira¹, Célia V. Romão¹

¹ Instituto de tecnologia química e biológica, Oeiras, Portugal
Email: Joanacarrilho@itqb.unl.pt

The erythrins are the simplest examples of the ruberythrins family, composed only by a four-helix bundle domain containing a diiron center [1]. Since the identification of the first ruberythrin in the sulfate reducing bacterium Desulfovibrio vulgaris, they have been found in the three life domains. Several in vitro enzymatic activities have been assigned to ruberythrins, such as pyrophosphatase, ferroxidase and superoxide dismutase, but so far the most consensual activity is that of H$_2$O$_2$ reductase, linked to NADH oxidation by a redox partner enzyme [2]. Structurally, these proteins are composed by a four-helix-bundle domain harboring a non-sulfur μ-oxo diiron center which is the catalytic center, and a rubredoxin-like domain at C-terminal. The protein under study is the erythrin from the hyperthermophile Acidianus ambivalens, characterized by the ability to grow by oxidation or reduction of sulfur with O$_2$ or H$_2$. It was overexpressed in E. coli, yielding a dimeric protein harboring a diiron center, confirmed by UV-Visible and EPR spectroscopies. The absence of additional domains in this protein made it possible the study in detail of the molecular mechanism of hydrogen peroxide reduction. Protein crystals were also obtained, which diffracted to a resolution of 1.5 Å in our in-house diffractometer, and the structure was then solved by Molecular Replacement. A detailed description of this structure will be presented.

References


SUPEROXIDE REDUCTASE MOLECULAR MECHANISM KEY RESIDUES, EXPLORING ARCHAEoglobus FULGIDUS SOR STRUCTURE

Tiago M. Bandeiras¹, Cristiana M. Sousa¹, João V. Rodrigues², Ana F. Pinto², Miguel Teixeira², Pedro M. Matias², Célia V. Romão²

¹ Instituto de Biologia Experimental e Tecnológica
² Instituto de Tecnologia Química e Biológica
Email: tiagob@itqb.unl.pt

Superoxide radical $\text{O}_2^-$ is the univalent reduction product of molecular oxygen, known to be involved in a variety of cell toxicity mechanisms, like DNA damage. While aerobes contain several antioxidant defence systems, such as superoxide dismutases and catalases/peroxidases, anaerobes and microaerophiles may depend only on superoxide reductases to keep oxygen toxic species below poisonous thresholds. *Archaeoglobus fulgidus*, a hyperthermophilic sulfate-reducing archaeon, was shown to have superoxide reductase as a toxic oxygen species scavenger. SOR catalyses the one-electron reduction of superoxide to hydrogen peroxide, and in its ferrous form, have a catalytic non-heme iron centre coordinated in a square-pyramidal geometry to four histidines in the equatorial plane, and a fifth axial position occupied by a cysteine-sulfur. In the SOR oxidized state a glutamate is present as sixth ligand, completing an octahedral geometry. Our research focuses on the SORs molecular mechanism, aiming to understand the role of the conserved key glutamate and lysine residues, proposed to return the enzyme to its oxidized resting state, and to direct superoxide to the active site, respectively. Herein, with the wild-type, E12Q and E12V *A. fulgidus* SORs structures, we demonstrate how the glutamate is not necessary for the reaction to occur, while the lysine plays a second role in stabilizing reaction intermediate species and promoting fundamental protonation steps, during the reaction cycle.

References


HIGHLY PURIFIED TYROSINASE FROM AGARICUS BISPORUS ALLOWS UNAMBIGUOUS POLYPEPTIDE COMBINATION DETERMINATION OF LATENT ISOFORM PPO4

St. G. Mauracher¹, C. Molitor¹, C. Michael², A. Rizzi², A. Rompel¹

¹ University of Vienna, Institute of Biophysical Chemistry, Althanstrasse 14, 1090 Vienna, Austria
² University of Vienna, Institute of Analytical Chemistry, Währinger Straße 38, 1090 Vienna, Austria
Email: stephan.mauracher@univie.ac.at

Tyrosinases are type-3 copper enzymes which catalyze the ortho-hydroxylation of phenols and their subsequent oxidation to ortho-quinones. These compounds are capable of polymerizing to larger pigments called melanins. Isolation and purification of tyrosinases had always been a process accompanied with various problems. Primarily, these difficulties are related to enzymatic browning processes and their eventuated reactions. In this poster an approach for the purification of the enzyme from white edible mushrooms (Agaricus bisporus) is presented which averts and removes interfering polyphenols and their subsequent products (e.g. pigments) in advance to the extraction process. With this approach the latency of the enzyme can widely be conserved. The enzyme was identified by peptide sequencing via nanoLC-ESI-MSMS as a polyphenol oxidase corresponding to the known PPO4 sequence of Agaricus bisporus [1]. With sequence coverage of over 90 %, peptides starting from Ser² and reaching to Thr⁵⁶⁵ were identified. The isolated intact protein showed a molecular mass of 64,247.7 Da as determined by high resolution mass spectrometry. Based on these data, the polypeptide backbone is concluded to start with Ser² and ending with Thr⁵⁶⁵ including several distinct post translational modifications, missing a part of the C-terminal tail. The data prove the existence of a latent precursor form of tyrosinase in mushrooms and document that the reported purification procedure is able to maintain the protein in its latent form.

Acknowledgements

Financial support by the Fonds zur “Förderung der wissenschaftlichen Forschung” (FWF) under P25217-N28 is gratefully acknowledged. Stephan Mauracher is grateful to the University of Vienna for financial support of the graduate training program entitled “Functional Molecules (doctoral program, Initiativkolleg Functional Molecules IK I041-N).

References

UNVEILING THE SUPEROXIDE REDUCTASE MOLECULAR MECHANISM - EXPLORING *ARCHAEOGLOBUS FULGIDUS* AND *IGNICOCCUS HOSPITALIS* SOR STRUCTURES

C.M. Sousa¹, T.M. Bandeiras¹, A.F. Pinto², J.V. Rodrigues², M. Teixeira², P.M. Matias², C.V. Romão²

¹ Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2701-901 Oeiras, Portugal
² Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Apartado 127, 2781-901, Oeiras, Portugal
Email: csousa@itqb.unl.pt

Superoxide radical $O_2^-$ is the univalent reduction product of molecular oxygen, known to be involved in a variety of cell toxicity mechanisms. While aerobes contain several antioxidant defence systems, such as superoxide dismutases, anaerobes and microaerophiles may depend only on superoxide reductases to keep oxygen toxic species below poisonous thresholds. SORs are 14 kDa mononuclear iron proteins, classified as 1Fe-SOR (neelaredoxins) or 2Fe-SOR (desulfoferrodoxins). SORs, in ferrous form, have a catalytic non-heme iron centre coordinated in a square-pyramidal geometry to four histidines in the equatorial plane, and a fifth axial position occupied by a cysteine-sulfur. In the SOR resting state (oxidized form) a glutamate is present as sixth ligand, completing an octahedral geometry. The active center of SORs is present in a domain common to both 1Fe- and 2Fe-SORs, organized in a seven-stranded β-barrel with an immunoglobulin-like fold. Our research focuses on the molecular mechanism of SORs, and aims to understand the role of the conserved key glutamate and lysine residues, proposed to return the enzyme to its oxidized resting state, and to direct superoxide to the active site, respectively. Herein we present the crystal structures of two wild-type SORs from *A. fulgidus* and *I. hospitalis*, in its reduced and oxidized-form, unveiling the roles of these key residues in superoxide reduction.

References


CUPINS, A VERSATILE FAMILY OF METALLOENZYMES

Kerstin Steiner¹, Ivan Hajnal¹, Helmut Schwab¹

¹ ACIB, Austrian Centre of Industrial Biotechnology, TU Graz, 8010 Graz, Austria
Email: kerstin.steiner@acib.at

Cupins are a versatile yet quite unexplored superfamily of proteins with many structures deposited in the protein database (pdb), which have no annotated function. All proteins that belong to this family adopt a barrel-like structure. The cupin superfamily comprises 53 families with members performing diverse functions ranging from enzymatic activities such as dioxygenases, hydrolases, and isomerases, and non-enzymatic functions [1]. Most cupins are metalloproteins. The metal binding site is located at the base of a large cavity in the centre of the cupin. The residues involved in metal binding are located within two conserved sequence motifs. In most cupins the metal cofactor, bivalent metal ions like Fe²⁺ and Mn²⁺, plays an important role in the function, either directly in the reaction mechanism or via interaction with and activation of the substrate. The metal cofactor can influence the chemistry of the catalytic reaction and examples of promiscuous activities depending on the metal ion in the active site have been observed. We recently discovered an additional enzyme class in the cupin superfamily, namely hydroxynitrile lyases (HNL), which catalyse the reversible lysis of cyanohydrins yielding HCN and aldehydes or ketones [2]. These HNLs were subsequently characterised in detail by our group including site-directed mutagenesis and metal analysis. We showed that the enzyme is metal-dependent in both, cyanolysis and cyanohydrin synthesis reaction [3].

References
DNA CLEAVAGE, SOD MIMICS ACTIVITY OF MIXED LIGAND HETEROBIMETALLIC COMPLEXES

Ahmad Asim¹, Sartaj Tabassum¹

¹ Department of Chemistry, Aligarh Muslim University, Aligarh, INDIA
Email: ahmad.has.asim@gmail.com

Metal complexes have been extensively explored for anticancer activities after the protocol success of cisplatin for treating most aggressive solid tumors. Many of these chemotherapeutic agents act by inhibition of the synthesis of deoxyribonucleic acid DNA, a natural target due to its predominant role in cellular replication. However, the chemical entities which exhibit different mechanisms of action are gaining more importance. Among the non-platinum complexes for metal based chemotherapy, copper and zinc complexes have been much explored due to the fact that both copper and zinc are bio-essential elements responsible for numerous bioactivities in living organism. In this study, monometallic viz, Cu, Zn complexes and heterobimetallic complexes viz, Cu-Sn, Zn-Sn complexes, were synthesized by reacting with Schiff base (L-valine + o-vanillin) as primary ligand, and pyrazole as secondary ligand. These complexes were characterized by using various spectroscopic techniques viz, IR, ¹H, ¹³C, ¹¹⁹Sn, ESR, ESI-MS and elemental analysis. In vitro DNA binding studies of all the complexes with CT DNA were carried out by employing different optical methods viz, UV-vis, fluorescence, viscosity measurements and compared with classical anticancer drug cisplatin. SOD-like or SOD mimcs activity of all the complexes have been evaluated by means of modified (NBT Assay) by the O₂⁻ generated by the xanthine/xanthine oxidase system. These results suggested that the heterobimetallic complexes possess significantly higher potential in comparison to monometallic complexes to act as superoxide dismutase (SOD) mimics.

References
IDENTIFICATION AND ACCURATE DETERMINATION OF Mn-SOD IN HUMAN LIVER

Yoana Nuevo Ordóñez¹, Clay Davis¹

¹ National Institute of Standards and Technology, 331 Fort Johnson Road, Charleston, SC 29412
Email: yoana.nuevoordonez@nist.gov

Manganese superoxide dismutase (MnSOD) is the primary antioxidant enzyme that protects cells from oxidative stress by catalyzing the dismutation of superoxide (O$_2^-$) to hydrogen peroxide and oxygen in the mitochondria of eukaryotic cells. MnSOD is a homotetramer with a manganese in its active site, which carries out a one electron transfer between two O$_2^-$ radicals. Alterations in MnSOD levels have been associated with a number of neurodegenerative diseases, including Parkinson’s disease, Duchenne muscular dystrophy, Charcot-Marie-Tooth disease, and Jennedy-Alter-Sung syndrome [1]. While many different types of tumors express low levels of MnSOD, overexpression of MnSOD has demonstrated suppression of the tumorigenicity of human melanoma cells, breast cancer cells and glioma cells, suggesting that MnSOD can serve tumor suppressor gene in a wide variety of cancers [2,3]. Therefore, MnSOD could be used as an effective tumor suppressor and applied to cancer therapy in the near future. Taking into account that the MnSOD is located in mitochondria and the liver cells have maximum number of mitochondria (1000-2000 per cell), a human liver sample was screened for MnSOD by ICP-MS and Mn containing fractions identified by LC/MS/MS. Proteotypic peptides were determined in order to select isotopically labeled heavy peptides used for quantification of MnSOD in human liver by AQUA.

References


CHARACTERIZATION OF THE INTERACTION OF SlyD WITH HypB IN HELICOBACTER PYLORI: IMPLICATIONS IN HYDROGENASE MATURATION

Hongyan Li¹, Tianfan Cheng¹, Xinming Yang¹, Wei Xia¹, Hongzhe Sun¹

¹ The University of Hong Kong, Pokfulam Road, Hong Kong
Email: hylichem@hku.hk

Helicobacter pylori infects nearly half of the population worldwide, which causes of chronic gastritis, peptic and duodenal ulcers and even stomach cancer. The survival and successful pathogenesis of the bacterium is heavily relied on two nickel-containing enzymes i.e. urease and [NiFe] hydrogenase [1]. A number of proteins including HypA/HypB, HspA, SlyD [2] has been demonstrated to be involved in the nickel ion acquisition and maturation of the hydrogenase. It was shown that SlyD interacts with HypB [3], however, detailed study in H. pylori is lacking.

In the present study, we characterized the interaction of SlyD with HypB from H. pylori. Using cross-linking assay combined with MALDI-TOF MS/MS indicated the formation of heterodimeric complex of SlyD with HypB and such an interaction was also confirmed in vivo by GFP-fragment reassembly technique. The binding residues of SlyD examined by NMR chemical shift perturbation are located mainly in the IF domain. The binding of SlyD to HypB led to either enhance or diminish the GTPase activity of HypB in the presence of Ni²⁺ or Zn²⁺ respectively, in consistence with the effect of metal ions on the GTPase activity of HypB alone, indicating that metal ions were transferred from SlyD to HypB. Subsequently, our chromatography study confirmed that the translocation of Ni²⁺ from SlyD to HypB is regulated by the protein-protein interaction. Our study provides an evidence of participation of SlyD in hydrogenase maturation process.

References

Evolving Metal Binding Site of Methyl Parathion Hydrolase

Nur Hafizah Azizan¹, David Ollis¹

¹ Australian National University
Email: u4808655@anu.edu.au

In light of the current environmental problem which severely affects other living organisms, organophosphate degrading enzymes has become the focus of recent attentions due to their potential utility for detoxification of chemical waste. Methyl parathion hydrolase (MPH) for instance, a bacterial enzyme has drawn our considerable interest due to the capability of catalysing the degradation of methyl parathion. This bacterium uses methyl parathion as a sole C/N source and can completely degrade p-nitrophenol, the product of methyl parathion. Here, we studied the changes and enhancements to the catalytic bonding of MPH through site-directed evolution to the substrate-binding cavities. In an effort to better understand the role of metal in catalysis and to understand how the protein binds the metal, we adopted isothermal titration calorimetry (ITC) method to measure the metal binding affinity of MPH variants.
VANADIUM-BINDING PROTEIN IN MARINE PLANKTON FROM CABO FRIO - BRAZIL

Vinicius T. Kütter¹, Maria Montes-Bayón², Emmanoel V. Silva-Filho¹, Silvia M. Sella³, Monique C. Souza¹, Alfredo Sanz-Medel²

¹ Department of Geochemistry - University Federal Fluminense, Outeiro São João Batista s/n, 24020141, Niterói, Brazil
² Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, Julian Clavería 8, 33006 Oviedo, Spain
³ Department of Analytical Chemistry - University Federal Fluminense, Outeiro São Joao Batista s/n, 24020141, Niterói, Brazil
Email: viniciuskutter@yahoo.com.br

Vanadium (V) is an essential element to some species of marine phytoplankton, macroalgae and invertebrates. Many enzymes such as haloperoxidases, nitrate reductase and nitrogenase contain this element. The Cabo Frio region has great abundance and diversity of ascidians which can accumulate high levels of vanadium-binding proteins, so called Vanabins [1]. The family of Vanabins consists of at least five closely related small proteins (Vanabin 1 to 4, and Vanabin P) which are composed of approximately 90 amino acids including 18 cysteine residues. Vanabin 1, 2 and P were found recombinent ligand more than 20 ions in oxidative state V(IV) with a dissociation constant of 2 \(10^{-5}\) M [2]. In order to investigate the role of plankton in the cycling of V, samples of different plankton fractions (20, 64 and 150 μm) were collected in the region to determine the total content of V by ICPMS. The concentrations of V in total plankton found were 45.2 ng g\(^{-1}\) (20 μm), 28.4 ng g\(^{-1}\) (64 μm) and 2.81 ng g\(^{-1}\) (150 μm). The best extraction procedure for the analysis of biomolecules associated to V was obtained using 50 mM ammonium acetate (pH 5) with stirring for 12 hours. The V speciation in biomolecules was performed by two strategies: i) coupling of size exclusion chromatography (SEC) for the fractionation of species with ICP-MS and ii) with SEC-AE-UVVIS-ICPMS. The results showed a single fraction containing V associated to a biomolecule in the range of 8 to 16 kDa, with isoeletric points above 8, which is very similar to that found in vanabins. The preliminary analysis using MALDI-TOF indicates that the biomolecule can be linked to one V type vanabin, requiring further studies for confirmation.

References
A STUDY ON MERCURY-BINDING PROTEIN IN FISH BY MASS SPECTROMETRY

Zurahanim Anual¹, Simon Foster¹, William Maher¹

¹ University of Canberra, Australia
Email: zurahanim.anual@canberra.edu.au

Balancing the risk and benefits of fish consumption has always been a public health agenda. Although eating fish has always been associated with health benefits due to high amounts of omega-3 fatty acids (EPA and DHA), consumption of fish is regarded as the major pathway of mercury accumulation in humans. Through biomethylation and biomagnification processes, mercury concentrations in fish at the top predatory level are often higher than fish at the bottom of the food chain. Speciation analysis of methylmercury (MeHg) is important due to its elevated toxicity and potential for bioaccumulation in food chain. Numerous studies report only on the speciation of MeHg⁺ and Hg²⁺ but more often than not, the real chemical form of MeHgX is neglected (X = low-molecular ions, peptides, proteins or other potential binding ligands). As mercury has a high affinity for sulphur, the most likely binding ligand of mercury is free sulfhydryl groups in protein cysteine residues. However, there is limited information on the binding sites of mercury in fish proteins. In this study, the binding behaviour of MeHg is investigated in different species of fish namely John’s Snapper (Lutjanus johnii), Doublewhip Threadfin Bream (Nemipterus nematophorus) and Torpedo Scad (Megalaspis cordyla). Fish samples were first extracted before size exclusion chromatography as well as SDS-PAGE was used to determine the molecular weights of protein bound mercury. Ion chromatography and reverse phase chromatography were used to determine the chemical associations of mercury. The implications for the metabolism and toxicity of mercury in fish were further discussed.
USE OF INORGANIC ICP MS AND MOLECULAR MASS SPECTROMETRY TO STUDY THE METABOLISATION OF PLATINUM ANTICANCER DRUGS DURING PERITONEAL CARCINOMATOSIS HIPE

Carine Arnaudguilhem¹, Brice Bouyssiere¹, Nicoel Bec², François Quenet², Amina Bouslimani², Christian Laroque²

¹ LCABIE-IPREM UMR 5254, Hélioparc, 2 Av. Pr Angot, 64053 Pau
² IRCM/INSERM U896, ICM Val d’Aurelle, 208 rue des Apothicaires, 34296 Montpellier
Email: carine.arnaudguilhem@univ-pau.fr

Peritoneal carcinomatosis (PC) is a frequent terminal evolution from digestive or ovarian cancers. About 20% of the patients affected by a colorectal cancer develop this disease, resulting into widespread peritoneal dissemination. Systemic treatment based on intravenous injection of platinum drugs, are few efficient for PC. Survival prognosis rarely exceeds 6 months and until recently, PC was considered to be the final stage. During the last decade, a new treatment based on hyperthermic intraperitoneal chemotherapy (HIPEC) has been developed. It consists in bathing the open peritoneum after cytoreductive surgery with a heated chemotherapy solution, allowing the use of higher drugs concentrations while minimizing secondary effects. This local treatment has considerably extended the five-year survival rate to 51%. Oxaliplatin, a 1.2-diamino-cyclohexane (dach)-Pt complex, and cisplatin are used in HIPEC depending on the tumor origin. Oxaliplatin is less toxic and can be used at higher concentration. Recently, it has been shown that the patient outcome to the HIPEC treatment depends on the tumor histologic type. Then, the understanding of the degradation, metabolisation, and penetration of the drug in tumors is a crucial point to improve treatment efficiency. To date, few studies have been reported and only total Pt has been considered. Here, we present the complementary of ICP MS and ESI-MS to investigate oxaliplatin metabolisation and evolution along the HIPEC procedure.
SPECIATION ANALYSIS OF PLATINUM CYTOSTATICS AND ITS METABOLITES IN BIOLOGICAL SAMPLES BY HILIC/ICP-MS AND HILIC/ESI-MS

Christina Herdering¹, Michael Sperling¹, Uwe Karst¹

¹ University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr. 30, 48149 Münster, Germany
Email: c.h@wwu.de

Platinum cytostatics are highly effective against cancer but suffer from toxic side effects. However, there are some approaches to reduce these toxic effects, but they fail in many cases as the mechanism of action is still not completely understood. Examination of interactions of these drugs within the organism represents a great challenge for analytical methods especially when biological samples have to be analyzed. The identification of platinum drugs and its reaction products in biological samples requires a sensitive analytical method that is able to handle complex biological matrices like blood or urine. Furthermore, sufficient information to identify the platinum drug and its partly unknown reaction products has to be received.

An analytical method to identify Cisplatin, Carboplatin and its reaction products with small molecules like creatinine in biological samples like urine samples of pediatric cancer patients is presented. Hydrophilic interaction liquid chromatography (HILIC) is coupled with inductively coupled plasma (ICP) mass spectrometry (MS) and electrospray ionization (ESI) high resolution MS. Accordingly, platinum species are identified by their retention times in HILIC/ICP-MS as well as the exact masses and isotopic patterns of the intact complexes in HILIC/ESI-MS.
MALDI IMAGING MASS SPECTROMETRY FOR UNRAVELING THE MOLECULAR MECHANISMS OF CISPLATIN-INDUCED RENAL DAMAGE AND NEPHROPROTECTIVE STRATEGIES

Estefanía Moreno-Gordaliza¹, Diego Esteban-Fernández², Alberto Lázaro³, Blanca Humanes³, Alberto Tejedor³, M. Milagros Gómez-Gómez¹, Michael W. Linscheid²

¹ Universidad Complutense de Madrid, Faculty of Chemistry, Dept. Analytical Chemistry, Avda. Complutense s/n 28040 Madrid (Spain)
² Humboldt Universität zu Berlin, Department of Chemistry, Brook-Taylor Str. 2, 12489 Berlin, Germany
³ Hospital General Universitario Gregorio Marañón, Renal Physiopathology Laboratory, C/Doctor Esquerdo 46, 28007, Madrid (Spain)

Email: estefania.moreno@quim.ucm.es

Nephrotoxicity is the major side effect of cisplatin-based antitumor therapies. Previous elemental bioimaging studies in rat kidney sections revealed that cisplatin accumulates preferentially in the renal cortex and corticomedullary junction [1]. This is in agreement with the fact that the induced damage is mainly located in proximal tubule cells. This accumulation is significantly reduced by cilastatin, which has been recently proposed as a nephroprotector for these therapies [2]. However, the molecular basis for cisplatin renal damage and protection are still not completely clear.

In the last years, MALDI imaging mass spectrometry (IMS) has proved to be a valuable tool for studying molecular species distributions in tissue slices with μm-scale spatial resolution and minimal sample preparation [3]. A methodology has been developed for MALDI-LTQ-Orbitrap-IMS in kidney sections from rats under cisplatin treatments, in an attempt to study the effect of the drug on the renal lipidome and proteome. This approach offers the possibility to get a whole picture of both their distribution and alterations within renal substructures. Sample preparation, which is a critical step, was optimized including aspects such as matrix deposition, sample delipidification or tryptic in-tissue digestions. In addition, the effect of the co-administration of the drug with cilastatin on the renal molecular distributions found to be altered by cisplatin was also evaluated.

References

## Abstracts

**Poster Session**  
*Metalloproteins & Metallodrugs & Plant Metallomics*

<table>
<thead>
<tr>
<th>Poster</th>
<th>P 081</th>
<th>Tuesday, 9th July 2013, 18:00 - 20:00</th>
<th>Room “Exhibition Hall”</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wednesday, 10th July 2013, 11:10 - 12:10</td>
<td></td>
</tr>
</tbody>
</table>

### CISPLATIN TREATMENT: INDUCED DNA ADDUCTS AND DETECTED DNA STRAND BREAKS IN HUMAN CELL LINES, UNDER DIFFERENT RESISTANCE CONDITIONS

Espina, Marta¹, Corte, Mario¹, Montes, María¹, Sierra, Marta¹, Blanco, Elisa¹, Sierra, L. María¹

¹ University of Oviedo, Faculty of Medicine and Health Sciences, Julián Clavería s/n, Oviedo, 33006  
Email: espinamarta@uniovi.es

Cisplatin, a chemotherapeutic drug widely used in cancer treatment, presents important resistance problems. Several processes, like the cell intake, chemical metabolism or the DNA repair activity, can contribute to cisplatin response. To study the contribution of repair activity in this response, the levels of cisplatin induced adducts are related to the induced DNA damage in terms of DNA strand breaks, in different conditions. Two pairs of human cell lines are chosen for this study: the first one is formed by a nucleotide excision repair (NER) deficient cell line (GM04321) and a NER efficient cell line (A549); the second one is formed by a cisplatin sensitive cell line (A2780) and a resistant one, obtained from this by cisplatin treatment, (A2780cis). Two different treatments are performed with both pairs: treatment of 3 hours with different cisplatin concentrations, and treatment of 3 hours plus 1 hour recovery without cisplatin. The DNA strand breaks are measured with the Comet assay. The cisplatin induced adducts are quantified using HPLC, followed by ICP-MS, as described before [1]. Preliminary Comet assay results, with the first pair of cell lines, show that 10 µM cisplatin concentration induces significant DNA strand breaks in GM04321 but not in A549 cell line, in the three hours treatments. These results will be correlated with the levels of induced adducts to check for a possible relationship in human cells, as that described recently in Drosophila *in vivo* [2].

### References


EVALUATION OF DRUG UPTAKE AND EFFLUX FOR Pt-DRUGS ALTERNATIVE TO CISPLATIN IN SENSITIVE AND CISPLATIN-RESISTANT CELL LINES

Mario Corte Rodríguez¹, María Montes Bayón¹, Elisa Blanco González¹, Alfredo Sanz Medel¹, Marta Espina Fernández², Luisa María Sierra Zapico², Matthew Price³

¹ University of Oviedo, Faculty of Chemistry, Julián Clavería 8, Oviedo, 33006, Spain
² University of Oviedo, Faculty of Medicine, Julián Clavería s/n, 33006, Oviedo
³ Phosplatin Therapeutics, 1350 Avenue of the Americas, 3rd Fl., NY 10019-4703, New York
Email: macortero@gmail.com

Cisplatin (CP) is one of the most effective antineoplastic drugs used in chemotherapy that exerts its action by binding to DNA. However, the therapeutic outcome of Cisplatin-based chemotherapy can be impaired by intrinsic or acquired resistance that is the consequence of multifactorial events. An increasingly recognized factor affecting DNA platination is the change of intracellular platinum concentrations. Reduced drug accumulation is frequently observed in CP-resistant cell lines but the mechanism has remained uncertain [1]. In order to overcome such limitations, alternative Pt-based treatments have been developed over the last years and continue nowadays. In this regard, some Pt pyrophosphate compounds have shown excellent properties as cytotoxic agents in CP-resistant cell lines and are currently undergoing preclinical studies [2]. Therefore, in this work we present some comparative studies on the effect of different Pt drugs (cisplatin, oxaliplatin and a phosphaplatin) in terms of cellular accumulation and DNA interactions in cell cultures. Two cell lines of ovarian carcinoma A2780 and A2780cis (cisplatin-resistant) and one of lung cancer (A549) have been exposed to different drugs concentrations (0, 5, 10 and 20 µM). Analytical methodologies for the determination of Pt in whole cells and in DNA samples have been developed and will be illustrated. Furthermore, accurate quantification of DNA concentration based on $^{31}$P detection using ICP-MS will also be shown.

References
INVESTIGATING Pt-DNA ADDUCT FORMATION IN SALIVA AND LEUKOCYTES IN PATIENTS RECEIVING PLATINUM-BASED CHEMOTHERAPY

Sarah Taylor¹, Barry Sharp¹, Helen Reid¹, George Don Jones², Anne Thomas³, Joanna Wood³

¹ Centre for Analytical Science, Department of Chemistry, Loughborough University, Loughborough, LE11 3TU, UK
² Department of Cancer Studies and Molecular Medicine, University of Leicester, University Road, Leicester, LE1 7RH, UK
³ Department of Cancer Studies and Molecular Medicine, University Hospitals of Leicester NHS Trust, Osborne Building, Royal Infirmary, Leicester, UK.

Email: S.E.Taylor@lboro.ac.uk

A comparison of the formation of Pt-DNA adducts as determined from the leukocytes and saliva taken from patients undergoing Pt-based chemotherapy is presented. Samples were taken pre- and post-treatment and were analysed via sector-field, inductively-coupled plasma mass spectrometry (SF-ICP-MS) to determine the level of Pt-DNA adducts formed. Approximately 65% of the patients undergoing chemotherapy receive a platinum-based drug (such as Oxaliplatin, Cisplatin or Carboplatin usually in combination treatments), but at the point-of-care clinicians have no indication how individuals will respond and therefore patients receive a standardized dose based on body surface area and kidney function. Recent work in this laboratory and elsewhere has shown considerable patient variability in adduct formation and whilst it is accepted that formation and repair correlate with efficacy, there is indicative evidence that toxicity and side effects may also be predicted from adduct yields [1, 2]. In order to make it easier to obtain DNA from patients over extended time scales following day clinic drug infusion, the use of commercially available saliva-DNA extraction kits, which can be used by patients at home, was investigated. Here we report our experiences with this approach, and report our findings regarding the equivalence of data derived from DNA obtained from saliva and leukocytes.

References

QUANTIFICATION OF GOLD AND PLATINUM METALLODRUGS IN CELL SUSPENSIONS

Armin Gross¹, Andreas Meyer²

¹ Bruker Nano GmbH, Am Studio 2D, 12489 Berlin, Germany
² Institute for Pharmaceutical Chemistry, University of Braunschweig, Beethovenstrasse. 55, 38106 Braunschweig, Germany
Email: armin.gross@bruker-nano.de

Total reflection X-ray spectrometry (TXRF) is an instrumental technique, which offers detection limits low enough to quantify trace element concentrations with negligible interference from matrix components [1]. The objective of this pilot study was the applicability of TXRF for the quantification of low concentrations in the μg/L range of gold and platinum based drugs in biological matrices (cell suspensions). For this purpose cisplatin, a common chemotherapeutic, and auranofin, a gold complex classified as antirheumatic agent, were chosen as relevant metal based drugs.

This paper shows that gold and platinum from metal based drugs can be quantified by TXRF in the ppb range with acceptable precision and recovery rates in aqueous samples as well as in cell suspensions. The easy preparation and handling of samples make TXRF very useful and recommend it as an alternative method for clinical use. TXRF does not require any media or consumables and supports a cost-efficient laboratory practice.

References

STUDIES ON PROTEIN METALATION BY SELECTED GOLD COMPOUNDS AS PROSPECTIVE ANTICANCER AGENTS

Lara Massai¹, Federica Scaletti¹, Tiziano Marzo¹, Luigi Messori¹, Chiara Gabbiani², Elena Michelucci³

¹ Department of Chemistry, University of Florence, via della Lastruccia 3 Sesto Fiorentino, (Italy).
² Department of Chemistry and Industrial Chemistry, University of Pisa, Pisa (Italy).
³ Mass Spectrometry Centre (CISM), University of Florence, Via U. Schiff 6, 50019 Sesto Fiorentino (Italy).
Email: lara.massai@unifi.it

Recent studies highlighted the importance of gold compounds as a new family of cytotoxic agents with the potential of becoming anticancer drugs candidates. Gold has a rich coordination chemistry and very interesting redox properties. The goals of our researches are: to model the binding interactions of gold with proteins, to understand the mode of action of cytotoxic gold compounds at the cellular level, to identify their primary molecular targets. Our gold compounds (AubipyC, Auoxo6, Au2Phen and AuNHC) manifest an acceptable stability in aqueous solutions at physiological pH that makes them amenable for biological testing. These compounds were assayed in vitro in a variety of cancer cell lines and have revealed remarkable anti proliferative properties. We have analysed, through ESI MS, the interactions of gold compounds, with two model proteins - i.e. lysozyme and cyt c - in order to disclose molecular details of the inherent metalation processes, and with the copper Chaperone Atox1 in order to understand the intracellular metabolism of medicinal gold species. Atox-1 contains a conserved CXXC motif for copper(I) binding, located in a solvent-exposed loop, in the vicinity of the N-terminus. The latter CXXC motif confers to Atox-1 a high reactivity toward soft metal ions. Adducts contain gold in the oxidation state +1 as a consequence of gold(III) reduction. Gold(I) ions are soft Lewis acids and, as such, should react readily with the copper(I) binding site of Atox-1.

References
IMAGING LA-ICP-MS FOR THE INVESTIGATION OF Pd-TAGGED PHOTOSENSITIZERS IN TUMOR SPHEROIDS

Ann-Christin Bültet, Christoph A. Wehe¹, Franziska Blaske¹, Olga Reifschneider¹, Uwe Karst¹

¹ Institute of Inorganic and Analytical Chemistry, University of Münster
Email: a.buelter@uni-muenster.de

Photosensitizers are frequently used as drugs in photodynamic therapy. Due to accumulation of the photosensitizer within malignant tissue and subsequent illumination with light of a specific wavelength, highly reactive oxygen species arise, which are able to induce apoptosis of the tumor cells.[1] Laser ablation coupled to inductively coupled plasma mass spectrometry offers high sensitivity enabling the investigation of the fate of the photosensitizers. Tagging of the drugs with Pd allows the detection by means of ICP-MS. The distribution of Pd in biological matrices was determined. Spheroidal cell cultures were used as a model system for malignant tissues and were incubated with different concentrations of Pd-tagged photosensitizers. Additionally, effects of the dosage form of the photosensitizer were elucidated by incubation of the tumor spheroids with the pure substance and poly(lactic-co-glycolic acid) (PLGA) nanoparticles. The incubated cells were embedded and sliced into 5 µm thin cryosections. The cellular uptake and intracellular accumulation of the drugs was investigated by visualization of the distribution of Pd. Thus, images with a lateral resolution of 10 µm were generated. The enrichment of the drug occurs within the first cell layers of the spheroid. In case of incubation with the pure substance, an accumulation of the drug in specific areas can be shown, while the nanoparticles are distributed more homogeneously.

References

RUTHENIUM METALLATION OF PROTEINS: RAMAN-ASSISTED CRYSTALLOGRAPHIC STUDIES

Alessandro Vergara¹,², Daniela Montesarchio¹, Luigi Paduano¹, Antonello Merlino¹,²

¹ Department of Chemical Sciences, University of Naples Federico II, Napoli, Italy.
² CNR Institute of Biostructures and Bioimages, Napoli, Italy.
Email: antonello.merlino@unina.it

Raman assisted-crystallographic studies on the formation of adducts between AziRu (trans-RuCl4(pyridine)(dmso-S), a Ru(III) complex with high anti-proliferative activity [1], and two model proteins (hen egg white lysozyme [2] and bovine pancreatic ribonuclease [3]) are presented. The results of these analyses show that protein structures are not perturbed significantly by the ruthenium label. The metal coordinates to ND atoms of His residues losing all its original ligands, but retaining octahedral, although highly distorted, coordination geometry. The data provide clear evidences on the mechanism of AziRu-protein adduct formation and of ligand exchange in the crystal state.

References
ANTI-DIABETIC ACTION OF Zn COMPLEX Zn(OPT)$_2$ ON THE EXPRESSION AND ACTIVATION OF PDX-1 IN PANCREAS OF MICE

Hiroyuki Yasui$^1$, Yutaro Natsume$^1$, Miki Kobayashi$^1$, Yuki Naito$^1$, Yutaka Yoshikawa$^1$

$^1$Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan
Email: yasui@mb.kyoto-phu.ac.jp

Zinc (Zn) is one of the most important trace elements in the human and associated with various physiological processes. The relationship between Zn and diabetes has been recognized and discussed. We have synthesized and evaluated 200 kinds above Zn complexes, the bioavailability of which was improved by means of both in vitro insulinomimetic activity and in vivo anti-diabetic action. Then, we focus on a potent Zn complex, di(l-oxy-2-pyridinethiolato)Zn(II) (Zn(opt)$_2$), action of which is suggested to be related with the expression and activation of pancreatic duodenal homeobox-1 (Pdx-1) in pancreas. Pdx-1 is also known as IDX-1, IPF-1 and STF-1, which are key factors of β-cell differentiation and function. We tried to investigate the protein expression and activation of Pdx-1 in pancreas of normal and STZ-induced diabetic mice treated with Zn(opt)$_2$. STZ-induced diabetic mice received daily oral administrations of Zn(opt)$_2$. After 14 days, pancreases of mice were homogenized and the protein expression and intranuclear transition of Pdx-1 was measured by Western blotting method. The total protein expression level of Pdx-1 in pancreas was more increased in the Zn(opt)$_2$-treated group than control group of STZ-mice, and the translocation of Pdx-1 into nuclear was enhanced by Zn(opt)$_2$-treatment. We conclude that Zn(opt)$_2$ would act on conserving β-cell differentiation and function in a diabetic state through the expression and activation of Pdx-1.

References
Changes in circulating leptin levels after treatment with vanadium in STZ-induced diabetic rats

Carlos Lopez-Chaves¹, Cristina Sánchez-Gonzalez¹, Juan Llopis¹, Carmen Bermudez-Peña², Maria Montes-Bayón³, Alfredo Sanz-Medel³

¹ University of Granada. Faculty of Pharmacy
² Mexican Social Security Institute. Biomedical Research Unit
³ University of Oviedo, Faculty of Chemistry

Email: crissg@ugr.es

Purpose: The adipokine leptin is primarily synthesized by adipose tissue. Leptin promotes body weight loss, participates in the regulation of appetite and improves glucose tolerance. Vanadium reduces food and water intake and glycaemia. The present study was undertaken to investigate the effect of V treatment on food and water intake and serum leptin levels of type I diabetic rats.

Methods: Four study groups were examined: Control; Diabetic; Diabetic-treated with 1mgV/day, and Diabetic-treated with 3mgV/day. In all cases diabetes was induced by streptozotocin. Vanadium was supplied in drinking water as bis(maltolatooxovanadium(IV))(BMOV). The experiment had a duration of five weeks. Body weight and food and water intake were monitored. Glucose, insulin and leptin were determined in blood.

Results: The untreated-diabetic rats shown polydipsia, hyperphagia, hyperglycaemia, body weight loss, and a decrease in serum levels of leptin. The treatment with 1mgV/day decreased the food and water intake, without showing effects on body weight, glycaemia and serum leptin levels vs untreated diabetic group. The treatment with 3mgV/day had no effect on body weight, but decreased food and water intake and glycaemia, moreover, this dose of V restored the circulating levels of leptin to those found in the control group.

Conclusion: Our results shown that BMOV, orally administered at the dose of 3mgV/d to diabetic rats, normalized the feeding behaviour and the serum levels of leptin and glucose.
**Abstracts**

**Poster Session** Metalloproteins & Metallodrugs & Plant Metalomics

**Poster** P 090  
**Tuesday, 9th July 2013, 18:00 - 20:00**  **Room “Exhibition Hall”**  
**Wednesday, 10th July 2013, 11:10 - 12:10**

---

**DEVELOPMENT OF A $^{64}$Cu-LABLED ANTIBODY PROBE FOR VISUALIZING CTLA-4 IN THE TUMOR**

Kei Higashikawa¹, Masayuki Munekane¹, Keiko Watanabe², Shinichiro Kamino², Masashi Ueda¹, Makoto Hiromura² ³, Shuichi Enomoto¹ ²

¹ Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University  
² Next-generation Imaging Team, RIKEN Center for Life Science Technologies  
³ Daiichi University of Pharmacy  
Email: gph422011@s.okayama-u.ac.jp

**Objective:** Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) has attracted the attention as a target molecule of cancer immunotherapy, and anti-human CTLA-4 monoclonal antibodies, such as ipilimumab and tremelimunab, are remarkably effective for patients with cancer. However, these pharmaceutical agents are expensive and may cause side effects, such as autoimmune diseases. Therefore, we thought the development of a molecular imaging probe visualizing CTLA-4 in the tumor would lead to cost-efficient medical care and avoidance of the side effects by ineffective therapy. In the present study, we developed a probe targeting CTLA-4.

**Experiments:** We prepared tumor bearing mice by subcutaneous administration of CT26, a colon tumor cell line, and analyzed the expression of CTLA-4 in the tumor tissue by RT-PCR. Next, we developed $^{64}$Cu-DOTA-anti-CTLA-4 antibody and $^{64}$Cu-DOTA-IgG2A as a control, and compared the amount of accumulation in the tumor between these probes by PET imaging. Results and Discussions: The result of RT-PCR analysis showed the expression of full-length CTLA-4, which is a representative immunosuppressive form of CTLA-4, was increased in the tumor. PET imaging indicated that $^{64}$Cu-DOTA-anti-CTLA-4 antibody accumulated in the CT26 tumor with high-contrast, as compared with $^{64}$Cu-DOTA-IgG2A. These results suggested tumor imaging using $^{64}$Cu-DOTA-anti-CTLA-4 antibody leads to valuable diagnosis for evaluation of CTLA-4 expression as well as the tumor detection.
IN VIVO STUDY OF THE INTERACTION OF BISMUTH-BASED DRUGS WITH METALLOCHAPERONE HSPA IN HELICOBACTER PYLORI

Yuchuan Wang¹, Ligang Hu¹, Yau-Tsz Angel Lai¹, Xinming Yang¹, Yuen-Yan Candice Chang¹, Hongyan Li¹, Hongzhe Sun¹

¹ Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong
Email: yuchwang@hku.hk

Bi-based antiulcer drugs have long been used for the treatment of H. pylori infection. Interference with biologically essential metal homeostasis in the pathogen, subsequently disrupting the function of important metalloproteins was suggested to be a key molecular mechanism of drug action. HspA is a potential drug target in H. pylori, which will undergo structural and functional perturbation upon Bi binding. In spite of extensive in vitro studies on the interactions of bismuth drugs with metalloproteins, there appear limited studies in vivo. In this report, we examined metal-binding specificity of HspA as well as the effects of bismuth drugs on the binding. HspA selectively associated with Co, Ni, Cu and Zn from an essential metal pool when overexpressed in E. coli, with Zn taking up the largest proportion. In the presence of Bi, binding of Zn and Ni to HspA was significantly suppressed, while the binding of Co and Cu was unaffected, indicating that under the increased cellular Bi stress, HspA functionality may be abolished due to lack of essential metal ions such as Zn and Ni in H. pylori. Interestingly, no structural changes of HspA were observed upon Bi binding as previously reported, suggesting that the in vivo mechanisms of drug action may differ from that observed in vitro. We provide the first in vivo evidence of a metallodrug competing with essential metals of the key metalloproteins in the bacterium.

Acknowledgements
This work was supported by the RGC of HK (N_HKU75209 and 7046/12P).

References
CAN PHYSICIANS REALLY RELY ON LABORATORY DIAGNOSTICS?

Claudia Swart¹, Sabine Zakel¹, Paola Fisicaro², Heidi Goenaga-Infante³

¹ PTB, Bundesallee 100, 38116 Braunschweig, Germany
² LNE, rue Gaston Boissier, FR-75724 Paris Cedex 15, France
³ LGC, Queens Road, Teddington, Middlesex, TW11 0LY, UK
Email: claudia.swart@ptb.de

About 30 % of all proteins contain metals in one form or another, either as cofactor or covalently bound as part of the protein. Some of these metalloproteins such as transferrin (TRF) and haemoglobin (HGB) are regularly analyzed in clinical laboratories all over the world. However, for most of them, no primary reference measurement procedures exist. Only such measurement procedures enable the establishment of a traceability chain and thus ensure the comparability of the results for these proteins in different clinical laboratories. Directives such as the EC-directive covering in vitro diagnostic medical devices ((IVD-)directive 98/79/EC) and standards such as EN ISO 17511:2003 demand the traceability of the results received for analytes in samples of human origin. Therefore, it will be important to establish a traceability chain for metalloproteins as is already in place for inorganic and small organic molecules in biological samples. Only in this way, it can be ensured that results obtained in different laboratories and different countries are comparable and thus provide physicians with the same reliable information. The project HLT 05 in the framework of the European Metrology Research Programme (EMRP) is presented here. It aims at developing reference measurement procedures for the iron containing proteins TRF and HGB, the Cu containing proteins superoxide dismutase and ceruloplasmin (CRP) as well as for selenoproteins.

References

Abstracts

Poster Session  Toxicological, Essential & Medical Aspectes of Metals

Poster  P 093  Tuesday, 9th July 2013, 18:00 - 20:00  Room “Exhibition Hall”
Thursday, 11th July 2013, 11:20 - 12:20

TITANIUM DEBRIS FROM METALLIC IMPLANTS: FROM IONS TO NANOPARTICLES

Juan Soto-Alvaredo¹, Jörg Bettmer¹, María Montes-Bayón¹, Alfredo Sanz-Medel¹, Carlos López-Chávez², Cristina Sánchez-González², Juan Llopis-González²

¹ Department of Physical and Analytical Chemistry. University of Oviedo. C/ Julian Claveria 8, 33006, Oviedo, Spain.
² Department of Physiology. Facultad de Farmacia. Campus Cartuja. Universidad de Granada. 18071 Granada. Spain
Email: juanst@gmail.com

The increasingly use of titanium implants in orthopedical and odontological surgery is producing great concern about the possible metal delivery from them into the biological fluids and the subsequent effect that this metal might cause in short and long term in the human body. Some works have already pointed out that the titanium is delivered in form of Ti ions and titanium dioxide nanoparticles coming from the wear of the metal surface [1] and that these nanoparticles may produce physiological problems. For this reason, based on previous experience [2], a quantitative strategy for the determination of total Ti concentration in human serum samples is implemented for two kind of patients having dental implants and spinal fusion implants respectively, by isotope dilution analysis using a double-focussing inductively coupled plasma mass spectrometer (DF-ICP-MS). The aim is to minimize sample handling and therefore contamination issues. Obtained detection limits of about 0.05 μg L⁻¹ permit the determination of Ti debris in biological fluids of exposed patients and as well as control individuals. On the other hand, in vitro studies have been approach for the evaluation of cytotoxicity and proliferation of cells after incubation with different concentration of titanium dioxide nanoparticles and Ti ions. TEM images of the cells were also taken to get an insight into the uptake, fate, accumulation and size of the nanoparticles after incubation.

References

INCREASE OF HIPPOCAMPAL ALUMINUM IS LINKED TO THE PROGRESSION OF AMYLOID PATHOLOGY IN A TRIPLE TRANSGENIC MOUSE MODEL OF ALZHEIMER’S DISEASE

Consalvo Ada¹

¹ University "G. d'Annunzio" of Chieti-Pescara
Email: ada.consalvo@libero.it

Brain deregulation of endogenous (Fe, Cu, Zn) and exogenous metals (Al) contributes to several neurodegenerative diseases. Al is a known neurotoxin that has been implicated in Alzheimer’s disease (AD). Previously we found that, Al is significantly increased (at 14 months of age, m.o.a.) in the cerebral cortex of female triple transgenic AD mice (3xTg-AD), an AD model overexpressing human mutant APP, PS1 and phosphorylated tau. In this study, we analyzed Al levels in the hippocampus of 3xTg-AD mice at 14 m.o.a. and found the metal increased when compared with age-matched wild-type (WT) mice. Suggesting the age dependency of the phenomenon, Al was not detected in the hippocampi of mice of both strains at 7 m.o.a.. Interestingly, hippocampal Al concentrations showed a significant positive correlation with the increased levels of human Aβ 1-40 and Aβ1-42 present in the 3xTg-AD mice, suggesting a functional link between changes in Al uptake and the development of the amyloid pathology. In order to assess the molecular basis of this age-dependent increase of hippocampal Al uptake, we evaluated, using microarray analysis, changes in expression levels of genes known to be involved in brain Al influx such as the transferrin receptor (TFR), the monocarboxylate transporters (MCTs), and the cystineglutamate-transporter (Xc-). When analyzing hippocampi of WT and 3xTg-AD mice at 3 and 12 m.o.a., we found a significant up-regulation of MCT1 and Xc- in 3 months old 3xTg-AD mice while this up-regulation was lost in AD mice at 12 m.o.a.. In contrast, TFR and MCT2 were up-regulated in young and old AD mice. We found that TRF and MCT2 mRNA levels increased with aging, matching the age-related increase in hippocampal Al. Our findings suggest that Al homeostasis is linked to the progression of the amyloid pathology, likely through an altered expression of TFR and MCT2.

References
THE EXPRESSION OF PROSAP/SHANK PROTEINS IN DEVELOPMENT AND AGING IN HEALTHY AND ALZHEIMER’S DISEASE BRAIN

Resham Chhabra¹, Jürgen Bockmann¹, Tobias M. Böckers¹, Andreas M Grabrucker¹

¹ Ulm University
Email: resham.chhabra@uni-ulm.de

Synaptic homeostasis is an essential phenomenon for normal functioning of the central nervous system (CNS) and alterations in synapse formation, maturation and plasticity are tightly controlled during development and aging. It is thus not surprising that an imbalance of the establishment and maintenance of synapses is an underlying factor for many synaptopathies including Alzheimer’s disease (AD), the most common cause of dementia. AD is clinically characterized by gradual and global cognitive decline and an increased synaptic loss during aging can be considered as an example for an imbalance in synapse maintenance. Various recent studies revealed that the proteins of the ProSAP/Shank family act as major scaffolding elements in the postsynaptic density (PSD) of excitatory synapses and the expression level of ProSAP2/Shank3 is able to influence synapse formation. ProSAP/Shank assembly within the PSD is Zn²⁺-dependent and Zn²⁺ might be a major factor in controlling synaptic homeostasis. Intriguingly, an imbalance in brain Zn²⁺ levels as well as ProSAP/Shank protein levels has been associated with a variety of neuropsychological and neurodegenerative disorders. For example, Zn²⁺-binding by Aβ leads to synaptic loss via dysregulation of ProSAP2/Shank3 scaffold in AD. Thus, the expression of ProSAP/Shank proteins during development and aging in a brain region- and isoform specific manner may be a major indicator of the condition of a brain under investigation. Therefore, here, we performed in vivo studies on mouse models investigating the expression levels of ProSAP/Shank family members during development and aging in a brain region specific manner analyzing all ProSAP/Shank family members and their known isoforms. Moreover, since the expression of ProSAP/Shank proteins is tightly regulated by Zn²⁺, we analyzed the levels of zinc during aging. To this end, we performed protein biochemistry and immunohistochemistry as well as zinc staining using wildtype mice. Next, we investigate how this expression pattern is altered in various mouse models for brain disorders such as AD. Further based on our results, we currently develop novel strategies to influence observed alterations and induce the expression levels of ProSAP/Shank proteins as seen in healthy animals. To that end, we evaluate the use of nanoparticles targeting the CNS. Taken together, this study will provide new insights into many synaptopathies such as AD and hopefully provide a basis for the evaluation and screening of substances to rescue observed pathologies.
ZINC DEFICIENCY DYSREGULATES A SYNAPTIC PATHWAY ASSOCIATED WITH AUTISM SPECTRUM DISORDERS

Andreas M. Grabrucker¹, Stefanie Grabrucker¹, Matti Eckert¹, Linda Jannetti¹, Stefanie Pfaender¹, Tobias Boeckers¹

¹ University Ulm, WG molecular Analysis of Synaptopathies, Albert Einstein Allee 11, 89081 Ulm, Germany
Email: andreas.grabrucker@uni-ulm.de

The idea that many genes implicated in Autism might converge on a single pathway present at excitatory glutamatergic synapses has recently been raised by multiple studies. For instance, our recent data provide evidence that a common synaptic pathway (NRXN-NLGN-ProSAP/SHANK) found at excitatory synapses is disrupted in ASD. However, although genetic factors might be largely responsible for the occurrence of autism they cannot fully account for all cases and it is likely that in addition to a certain combination of autism-related genes, specific environmental factors might act as risk factors triggering the development of autism. Our recent work also focuses on the influence of zinc ions on the synapse. Intriguingly, the concerted action of Shank3 and zinc ions is essential for the structural integrity of the PSD regulating postsynaptic receptor composition. It might be possible that both, environmental factors like nutritional Zn^{2+} status or metal ion homeostasis in general have a crossing point with this distinct autism-associated pathway in excitatory synapses and the deregulation of any of these two factors may lead to ASDs. Thus, here, we investigate, if zinc ions regulating Shank3 provide a crossing point between genetic forms of ASD and zinc deficiency as an environmental risk factor.

References

CHANGES IN IL-1β, IL-6 AND TNF-α LEVELS AFTER TREATMENT WITH VANADIUM IN STZ-DIABETIC RATS

C. Sanchez-Gonzalez¹, C.E. Trenzado¹, C. López-Cháves¹, P. Aranda¹, J. Llopis¹, L. Vera-Ramirez², M. Montes-Bayón³, A. Sanz-Medel³

¹ University of Granada, Granada, Spain.
² Pfizer-University of Granada and Andalusian Government Centre for Genomics and Oncology, GENyO Center, Granada, Spain.
³ University of Oviedo, Faculty of Chemistry, Department of Physical and Analytical Chemistry, Oviedo, Spain.
Email: crissg@ugr.es

Purpose: Vanadium is an element whose essentiality, distribution and toxicology effects, as well as its biological and pharmacological activity, are still not fully understood. The present study was undertaken to investigate the inflammatory status of diabetic streptozotocin rats following treatment with vanadium.

Methods: Four study groups were examined: Control; Diabetic; Diabetic treated with 1 mg V/day (DMV); and Diabetic treated with 3 mg V/day (DMVH). The vanadium was supplied in drinking water as bis(maltolato) oxovanadium (IV) (BMOV). The experiment had a duration of five weeks. Transferrin, alanine aminotransferase (ALT), C-reactive protein (CRP), interleukin-1β, interleukin-6, TNF-α, red blood cells, white blood cells, lymphocytes, platelets, haemoglobin and haematocrit levels were determined in blood.

Results: In DMVH group, there was a significant decrease in fasting glycaemia, transferrin and haemoglobin, and increased serum CRP, IL-6 and ALT levels in comparison with the DM group.

Conclusion: Our results shown that BMOV is a good hypoglycaemic agent and its effect is dose dependent. However, treatment with 3 mg V/day causes a moderate anaemic state associated with chronic inflammatory disorders.
DETERMINATION OF GLUTATHIONE PEROXIDASE AND SELENIUM LEVELS IN OCULAR FLUIDS AND TISSUES

Raquel Gonzalez de Vega¹, M. L. Fernandez Sanchez¹, Alfredo Sanz-Medel¹, Hector Gonzalez Iglesias², Miguel Coca-Prados²

¹ Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, Spain
² Ophthalmologic research Foundation Fernández-Vega, Oviedo, Spain

Email: devega.raquel@gmail.com

Selenium is an essential trace element for human present in numerous selenoproteins, including several enzymes with physiological key roles, such as the glutathione peroxidases (GPx). GPx is the general name of an enzyme family with peroxidase activity. This enzyme, together with catalase, SOD and vitamin E are an essential part of the cellular defense mechanisms against oxidative damage [1]. Oxidative stress can be defined as an increase over physiological values of the intracellular concentrations of reactive oxygen species (ROS). Also, oxidative stress has been implicated in the possible pathophysiology of some ocular diseases, such as retinopathy of prematurity, glaucoma, macular degeneration, and uveitis [2]. The glaucoma is an optical neuropathy characterized by specific structure alteration of the optic nerve accompanied by progressive damage to the visual field. Untreated glaucoma can lead to permanent damage of the optic nerve and resultant visual field loss, which over time can progress to blindness. The aim of this particular study is to investigate the possible role of the GPx in the pathogenesis of glaucoma. So speciation of Se in ocular fluids and tissues (vitreous humor, lens, cornea, retina, RPE) has been performed by HPLC-ICP-MS. Quantification of Se and of GPx levels in the fluids under study was carried out by isotope dilution analysis. Also GPx activity in each tissue and ocular fluid will be determined and their relation with GPx levels will be discussed.

References


METABOLIC SIGNATURES ASSOCIATED WITH NON-ALCOHOLIC STEATOHEPATITIS PATIENTS USING A METABOLIC FINGERPRINTING APPROACH AND DIRECT INFUSION ESI-MS

María Alvarez-Martinez¹², Tamara García-Barrera¹², José Luis Gómez-Ariza¹², Manuel Romero-Gomez³, Isidora Ranchal³, Rocio Gallego-Duran³

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
² Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
³ UG MQ Enf. Digestivas, Hospital Universitario Nuestra Señora de Valme, Universidad de Sevilla, Spain
Email: tamara@dqcm.uhu.es

Non-alcoholic steatohepatitis (NASH) is hallmark by lipid accumulation in the liver (steatosis) along with inflammation (hepatitis) which can lead to fibrosis and finally, irreversible cirrhosis. There is a corresponding increase of body mass index (BMI) with steatohepatitis, and due to obesity is a growing international epidemic, steatohepatitis is about to become the most common cause of liver cirrhosis and end-stage liver diseases [1]. Nowadays, the pathogenesis behind hepatic inflammation is not well understood but, until now, several mechanisms have been proposed mainly related with the abnormal visceral adipose tissue (VAT) function, primarily due to obesity, and its secretory products. Recent evidences point towards another tissue, the gastrointestinal tract, as the source for liver inflammation since studies showed increased intestinal permeability during NASH, which could lead to elevated levels of plasma lipopolysaccharide (LPS) [2]. Recently, increasing amounts of data show the involvement of oxLDL in hepatic inflammation emerging as a new factor.

The aim of the current study was to characterize the serum NADH metabolic profiles through metabolic fingerprinting, that means the high throughput, rapid, global analysis of samples to provide sample classification, usually without quantification and metabolic identification. For this purpose, a method based on direct infusion coupled to triple quadrupole mass spectrometry has been developed and applied to serum samples of NASH patients. The approach gave comprehensive metabolic profiles followed by discriminant analysis (PLS-DA) to compare control and NASH patients, that allow establishing metabolites altered during this disease.

References

THE ROLE OF SELENIUM AND LIPID METABOLISM DURING PREGNANCY PERIOD IN HUMAN

Belén Callejón-Leblic¹³, Tamara García-Barrera¹³, José Luis Gómez-Ariza¹³, Inés Velasco Lopez²

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
² Obstetrics and Gynecology Service. General Hospital of Riotinto-Huelva. Spain
³ Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
Email: tamara@dqcm.uhu.es

Pregnancy is associated to significant changes in liver function and understanding these changes is essential in clinical evaluation of liver abnormalities during this period. Selenium is an essential micronutrient related to thyroid hormone metabolism [1,2]. Selenium intake is critical during pregnancy and lactation, and its supplementation has beneficial effects on thyroid function, oxidative balance, immunomodulation and improvements in lipid profile [2,3]. It is also important long chain polyunsaturated fatty acid intake that is related to neurogenesis, neurotransmission and oxidative stress protection. Therefore, this study provides further contributions on the nutritional status of selenium, fatty acids and phospholipids in pregnant women.

In this work, a method for the simultaneous speciation of selenoproteins and selenometabolites in human serum has been developed based on in series double size exclusion (SE) and dual affinity (AF) HPLC (1+1SE-1+1-AF-HPLC) hyphenated to ICP-QMS, using species-unspecific isotope dilution (SUID). In addition, a metabolomic approach based on direct infusion electrospray mass spectrometry (DI-ESI-QTOF-MS) was used, combining a two-step extraction of polar and lipophilic metabolites and further analysis by ESI(+)/ESI(-) ionization modes. The approach gave comprehensive metabolic profiles followed by PLS-DA to compare control and pregnant women at perinatal status, which allows establish metabolites altered during perinatal and lactation period. Finally, these altered metabolites were quantified by GC-MS.

References
CHARACTERIZATION OF METALS PROFILE IN SERUM SAMPLES DURING THE PROGRESSION OF ALZHEIMER’S DISEASE

Raúl González-Domínguez¹²³, Tamara García-Barrera¹²³, José Luis Gómez-Ariza¹²³

¹ Department of Chemistry and CC.MM. Faculty of Experimental Sciences. University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
² Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
Email: ariza@uhu.es

Metal dyshomeostasis is closely related to Alzheimer’s disease (AD), so the characterization of the elemental profile is a suitable tool for studying neurodegenerative processes associated and to discover potential markers of disease [1]. In this context, the study of metal containing biomolecules [2] gives more valuable information than characterization of total concentration of these elements. Thus, elements can be mainly present as free ions, complexed with low molecular mass ligands, or in form of metalloproteins. The distinction between high molecular mass (HMM) and low molecular mass (LMM) species is very important, since finally affects to biological activity or toxicological potential of the element, as well as its mobility across different biological compartments. An analytical approach based on non-denaturing precipitation of proteins has been optimized for the fractionation of HMM and LMM species from human serum, which were subjected to multielemental analysis by inductively coupled plasma mass spectrometry. The methodology was applied to healthy controls, Alzheimer’s disease and mild cognitive impairment patients (pre-clinical stage of AD), in order to study the progression of dementia. In this way, we demonstrated the involvement of relevant metals such as iron, copper, zinc, selenium, aluminium and manganese in the development of Alzheimer’s disease.

References

INTEGRATED ENVIRONMENTAL METABOLOMICS AND METALLOMICS TO ASSESS THE EFFECT OF POLLUTION ON PROCAMBARUS CLARKII IN DOÑANA NATIONAL PARK

A. Gago-Tinoco¹²³, R. González-Domínguez¹²³, T. García-Barrera¹²³, J.L. Gómez-Ariza¹²³

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
² Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
Email: ariza@uhu.es

Aquatic ecosystems of Doñana National Park (DNP) were monitored using the crab Procambarus clarkii as bioindicator to measure environmental pollution. This organism has been previously studied in this area through the use of conventional biomarkers [1]. In this work, a metabolomic approach was carried out by direct infusion mass spectrometry and multivariate statistical analyses were used to differentiate crabs groups from heavy metal-polluted and clean areas. Additionally, to evaluate heavy metal contamination, total metal content was determined. Thus, the integrated analysis of metabolomic and metalloomic data enabled to study the metabolic response of the organism against pollution. Several metabolites were discovered as potential biomarkers, such as decreased levels of carnosine, alanine, niacinamide, acetoacetate, pantothenic acid, ascorbate, glucose-6-phosphate, arginine, glucose, lactate, phospholipids and triglycerides, as well as over-expression of acetyl carnitine, phosphocholine, choline and uric acid. In this way, metal-induced toxicity could be related to metabolic impairments, principally oxidative stress, metabolic dysfunction and dyslipidemia. Therefore, the results obtained demonstrate that tandem mass spectrometry can be used as an efficient method for characterizing heavy metal contamination derived from polluted area compared to clean area and to identify metabolites related to environments that are contaminated with heavy metals.

References
DISTRIBUTION OF METAL COMPLEXES OF METALLOTHIONEIN ISOFORMS IN MUS MUSCULUS LIVER AFTER CADMIUM EXPOSURE

R. Jara-Biedma¹²³, R. González-Domínguez¹²³, T. García-Barrera¹²³, J.L. Gómez-Ariza¹²³

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
³ Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
Email: ariza@uhu.es

Many heavy metals are major environmental hazards due to toxicity that derives from their affinity for metal sensitive groups, such as thiols. In order to elucidate the mechanisms that control the uptake of metals by organisms exposed to environmental metal pollution, as well as metal transport, metabolism and detoxification processes, the laboratory mouse Mus musculus has been used as laboratory model [1]. Cellular metal overload has a great impact on organisms, causing upregulation of different metal-containing biomolecules, in organs of high metabolic activity such us liver. One of them are metallothioneins (MT), a family of low molecular weight proteins that easily complex metals with apparent element specificity. For these reasons, MTs have been used as conventional biomarkers in environmental studies. In this work, ICP-ORS-MS and nESI-qTOF-MS were used in parallel in combination with two-dimensional chromatography for the characterization of metal complexes with metallothionein isoforms, which are produced at different exposure days in hepatic cytosols of Mus musculus exposed to increasing Cd dose. Different complexes of MT isoforms with cadmium, copper and zinc were found in the two most exposed groups, which were purified by size exclusion chromatography (SEC) [2] and separated by reversed-phase chromatography (RPC). Further characterization by nESI-qTOF-MS was performed in order to gain further insight into the mechanisms involved in metal detoxification by MTs.

References
CADMIUM TOXICITY IN MICE ASSESSED BY METALLOMIC AND METABOLOMIC APPROACHES. ANTAGONISTIC INTERACTION BETWEEN Cd/Se UNDER CONTROLLED EXPOSURE

Miguel García-Sevillano¹²³, Tamara García-Barrera¹²³, José Luis Gómez-Arizá¹²³

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
² Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
Email: ariza@uhu.es

Cadmium (Cd) is an important toxic metal, which is the origin of numerous environmental contamination episodes and health problems, such as cancer. In addition, it is well known that selenium presents numerous antagonistic interactions with Cd like the prevention of Cd-induced oxidative stress [1], protection of Cd-induced nephrotoxicity and hepatotoxicity [2] and antagonist action against Cd-induced inhibition of hepatic drug metabolism [3]. In contrast, the toxicological effects on metabolism, trafficking and alterations of metabolic cycles are still unclear. To this end, metallomic and metabolomic approaches have been applied to evaluate the effects of mouse exposure to Cd and Se.

In this work, a metallomic approach based on SEC coupled to ICP-MS is combined with anion or cation exchange chromatography to achieve better understanding of the function, detoxification processes and regulation of metals in biological systems under controlled exposure. In addition, isotopic dilution analysis (IDA) was performed for quantifying the selenium containing proteins in plasma using ICP-qMS as a multielemental detector. On the other hand, to get suitable metabolic information, several organs and biological fluids taken from the laboratory mouse Mus musculus at different exposure times have been studied. The analysis was carried out by direct infusion high-resolution mass spectrometry (DI-ESI-QqQ-TOF-MS) and statistical analysis of results allowed us to compare the different metabolic profiles, establishing those metabolites altered by the presence of these contaminants. Finally, altered metabolites were quantified by gas chromatography-mass spectrometry (GC-MS).

References

Abstracts

TOXICOLOGICAL EFFECTS OF ARSENIC/CADMIUM INTERACTIONS IN MICE BASED ON -OMICS TECHNOLOGIES

Dolores Silvera Garrido¹,²,³, Tamara García Barrera¹,²,³, José Luis Gómez Ariza¹,²,³

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
² Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
Email: tamara@dqcm.uhu.es

Arsenic (As) and Cadmium (Cd) are important inorganic toxicants in the environment, which are the origin of numerous environmental issues. Arsenic toxicity is due to its reaction with sulphhydryl groups in cells [1], although recent studies suggest the generation of reactive oxygen species (ROS) during arsenic compounds metabolism [2]. Arsenic exposure has shown to depress the functions of antioxidant defense system leading to oxidative damage of cellular macromolecules including DNA, proteins and lipids. Interactions between As and Cd in acute liver injury have been reported [3], but little is known about their potential interaction in testicular damage and neurotoxicity, especially during chronic exposure. It has been issued that the exposure of both elements in humans produces more pronounced renal toxicity than each of the agents alone, and in rats they induce lipid peroxidation, glutathione and metallothionein, as well as the redistribution of essential elements.

For this purpose, a metallomic approach based on size characterization of metal biomolecules by SEC-ICP-MS has been applied and combined with organic mass spectrometry for the identification of altered biomolecules. The metallomic approach has been complemented with an environmental toxicometabolomic study based on direct infusion to triple quadrupole time of flight mass spectrometer (DI-ESI-QTOF-MS) followed by discriminant analysis (PLS-DA). Finally, these altered metabolites were quantified by GC-MS.

References
MERCURY/SELENIUM INTERACTIONS IN LABORATORY MICE
COMBINING -OMICS METHODOLOGIES

Gema Rodríguez-Moro¹-²-³, Tamara García-Barrera¹-²-³, José Luis Gómez-Ariza¹-²-³

¹ Department of Chemistry and CC.MM. Faculty of Experimental Sciences. University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
³ Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
Email: tamara@dqcm.uhu.es

Mercury (Hg) is a very toxic element for human and other organisms with great relevance in the environment [1]. It has been found that selenium has a beneficial effect on the toxicity of Hg [2] and other metals due to antagonist interactions involving a variety of biochemical and toxicological processes. Since Parizek and Ostadalova (1967) [3] found that inorganic mercury toxicity simultaneously decreased with selenite administration, many studies have been performed to examine the role of Se in this process.

In this work, a column switching method coupling two columns (reversed phase and chiral stationary phase) and two mobile phases was used for the simultaneous speciation of mercury (inorganic and methylmercury) and selenium (selenomethylselenocysteine, selenite, selenate, L-selenomethionine, D-selenomethionine, methylmercury and inorganic mercury). In addition, high molecular mass biomolecules have been determined by size exclusion (SE) coupled to inductively coupled plasma-(quadrupole) mass spectrometry (ICP-QMS). In addition, a metabolomic approach based on direct infusion electrospray mass spectrometry (DI-ESI-QTOF-MS), together with suitable two-steps metabolite extraction (for polar and lipophilic compounds) and analysis by ESI(+) / ESI(-) ionization modes, provide comprehensive metabolic profiles that can be followed by discriminant analysis (PLS-DA). This metabolomic approach allows the comparison between control mice (Mus musculus) against others exposed to mercury and mercury/selenium mixtures. In addition, altered metabolites were quantified by gas chromatography-mass spectrometry (GC-MS).

References
STUDY OF METABOLITES OF SELENIUM BIOMOLECLES PRODUCED BY THE MICROALGAE CHLORELLA SOROKINIANA CULTURED IN MEDIA ENRICHED WITH THIS ELEMENTS

Verónica Gómez-Jacinto¹², Tamara García-Barrera¹², Inés Garbayo-Nores¹³, Carlos Vílchez-Lobato¹³, José Luis Gómez Ariza¹²

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
² Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
³ Algal Biotechnology Group, International Centre for Environmental Research (CIECEM), Parque Dunar s/n, Matalascañas, Almonte, 2176
Email: tamara@dqcm.uhu.es

The need for food supplemented with essential elements has becoming an important issue. Because of the high content of proteins and other nutritional elements in Chlorella sorokiniana, this microalgae is widely cultivated for the production of health food products. Chlorella grows very fast and it is easier to follow the selenium intake process. Some elements such as selenium has been biotechnologically incorporated to the microalgae Chlorella sorokiniana as source of elemental species for human consumption. Selenium is an essential trace element of fundamental importance to human health. Chlorella has been exposed to Se in the form of selenate which is rapidly absorbed within first few minutes at the cell surfaces where it is irreversibly fixed. In the After 24-48 hours, about 40% of the total fixed Se was inside the cells in organic-bound Se in Chlorella biomass, in the form of selenomethionine¹. Investigation of the cellular response showed that Chlorella cells can tolerate sodium selenite up to 100 mg l⁻¹ [2]. In this work, the microalgae Chlorella sorokiniana has been used for selenium enrichment with a biotechnologic production platform. Therefore, a simultaneous study of selenium intake and bioavailability can contribute to a better understanding of the toxicity or benefits associated to the consumption of food containing levels of selenium. Selenium containing metabolites were extracted from the biomass and characterized with by RP-HPLC-ICPMS.

References
STUDY OF THE ANTAGONISTIC INTERACTION OF SELENOMETHIONINE ON METHYLMERCURY TOXICITY IN THE MICROALGAE CHLORELLA SOROKINIANA

Verónica Gómez-Jacinto¹², Fernando Moreno-Roldán¹², Tamara García Barrera¹², Inés Garbayo-Nores¹³, Carlos Vilchez-Lobato¹³, José Luis Gómez-Ariza¹²

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
² Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
³ Algal Biotechnology Group, International Centre for Environmental Research (CIECEM), Parque Dunar s/n, Matalascañas, Almonte, 2176
Email: veronica.gomez@dqcm.uhu.es

The protective effect of selenium against mercury toxicity is well known especially between selenomethionine and methylmercury and it has been studied in several living organisms. Moreover, the investigation about which chiral form of selenomethionine effectively acts against the toxic effects of methylmercury has not been carried out previously. Chlorella sorokiniana is an alga that is known for its ability to accumulate metals and this work explores the ability of Chlorella to bioconcentrate and to detoxify different species of selenium and mercury. In the present study, control cultures and cultures of C. sorokiniana grown in standard medium with D,L- SeMet, L-SeMet and D-SeMet. At a certain time after experiment started up MeHg+ was added to the cultures with D,L-SeMet, L-SeMet and D-SeMet and to the controls.

The use of ICP-MS is essential for multispeciation purposes as it allows obtaining multielement profiling in real samples in a single chromatographic run and for this reason, it constitutes a reliable technique with high throughput. None mercury-selenium complex was detected. Present paper reports new data about the relationship between ability of detoxification of methylmercury and selenomethionine enantiomers through the study of the metabolic intermediates by means of speciation analysis.
MERCURY SPECIATION BY HPLC-IDA-ICP-MS. APPLICATION TO THE
DETERMINATION OF MERCURY SPECIES IN CUBAN COMMERCIAL EDIBLE FISH

M.R. Fernández de la Campa¹, A. Montero Alvarez², A. Sanz-Medel¹

¹ Department of Physical and Analytical Chemistry, University of Oviedo, Oviedo, Spain.
² Analytical Chemistry Lab. Center of Applied Technologies and Nuclear Development(CEADEN) La Habana, Cuba.
Email: mrfcampa@uniovi.es

A sensitive and accurate quantitative method for the speciation for inorganic \( \text{Hg}^{2+} \) and methylmercury by species specific isotope dilution analysis-HPLC-ICP-MS has been optimized and implemented for marine fish samples. Quantitative extraction of Hg species was achieved with 0.1 % (v/v) 2-mercaptoethanol, 0.05 % (m/v) L-cysteine and 0.10 % (v/v) HCl solution using ultrasounds for 30 min. Chromatographic separation of mercury species was carried out on a C8 reverse phase column with 0.05 % (v/v) 2-mercaptoethanol, 0.075 % (m/v) L-cysteine and 0.06 mol·L⁻¹ ammonium acetate as the mobile phase. A species specific isotope dilution analysis approach, using \( ^{201}\text{CH}_3\text{Hg}^+ \) and \( ^{200}\text{Hg}^{2+} \) was employed for quantification of both species [1-3]. Two biological Certified Reference Materials (DOLT-2, DORM-2) were analyzed to assess the analytical performance of total Hg and its species. No significant differences were found between the obtained concentration and the certified reference values for total Hg and \( \text{CH}_3\text{Hg}^+ \). The results indicate that no species interconversion reactions occurred during the used extraction and chromatographic separation procedures. The detection limits for \( \text{CH}_3\text{Hg}^+ \) and \( \text{Hg}^{2+} \) species were 7.7 and 5.2 ng·g⁻¹, respectively. The method recovery (expressed as the sum of both species contents in relation to total Hg concentration analyzed by ICP-MS) was about 97±5 %. The procedure was applied to speciation of mercury in 12 species of the most consumed and commercial fish in Cuba.

References
EVIDENCE OF DIRECT ADSORPTION OF Hg\textsuperscript{0} IN HUMAN HAIR DURING OCCUPATIONAL EXPOSURE TO MERCURY VAPOUR ESTABLISHED BY LA-ICP-MS

José Ignacio García Alonso\textsuperscript{1}, Cristina Sariego Muñiz\textsuperscript{1}

\textsuperscript{1} Mass Spectrometry Service, University of Oviedo
Email: jiga@uniovi.es

The concentration of mercury in human hair has been traditionally employed to assess environmental exposure as the level of mercury in hair is about 250 times that in blood. However, we have found evidence that the determination of mercury in human hair can provide misleading data because of the direct adsorption of mercury onto the hair in case of occupational exposure to elemental mercury vapour. We have performed longitudinal analysis of human hair by Laser Ablation ICP-MS of hair of 5 individuals which were exposed to high levels of mercury vapour. Sulfur was used as internal standard. The ratio \(^{202}\text{Hg}/^{34}\text{S}\) showed a distinct pattern of mercury concentration with much lower levels of mercury near the root of the hair and high levels of mercury near the end of the hair. In all cases a big jump in mercury concentration in hair occurred at a given distance from the root indicating approximately the dates when the contamination occurred. In some cases the concentration of mercury at the tip of the hair was ca. 1000 times higher that that near the root. In this communication we will also discuss the consequences of these findings on mercury toxicology assessment in cases of occupational or environmental exposure to elemental mercury vapour.
A QUANTITATIVE PROTEOMIC APPROACH IDENTIFIES KEY ENZYMES OF THE METHYLATION CYCLE AS SPECIFIC TARGETS FOR MeHg NEUROTOXICITY

Pablo Cabezas Sánchez¹, Carmen Camara Rica¹, Jose L. Luque Garcia¹

¹ University Complutense of Madrid, Faculty of Chemistry, Av Complutense S/N, 28040, Madrid, Spain
Email: pablo.cabezas@ucm.es

Methylmercury (MeHg) is a persistent environmental toxicant and it represents a risk to human health, particularly causing brain and neural damage. Many studies have shown that MeHg induces apoptosis in neuronal cells at low doses, whereas higher concentrations provoke necrosis. In order to elucidate the biomolecular mechanisms involved in the MeHg-induced neurotoxicity, we compared the differential protein expression of neuroblastoma cells (N2a) exposed to 2 mg/L of MeHg for 8 h. The use of a quantitative proteomic approach based on stable isotopic labeling (SILAC) and mass spectrometry allowed us the identification of a set of de-regulated proteins. 55 proteins were found up-regulated and 71 down-regulated. Clustering of these de-regulated proteins showed that the main biological functions affected by MeHg included RNA post-transcriptional modifications, cellular assembly and organization, proteins synthesis, cell cycle and cell death. Among the de-regulated proteins, key enzymes of the methylation cycle were inhibited after MeHg exposure. Validation of the proteomic results was carried out by immunobloting. A comparison of the effect of MeHg on N2a cells with inorganic Hg and additional heavy metals (Cu and Pb) was also performed showing a specific mechanism associated to MeHg-induced toxicity.
STUDY OF THE MERCURY-SELENIUM INTERACTION IN TERRESTRIAL MAMMAL KIDNEYS

María José Patiño Ropero¹, Nuria Rodríguez Fariñas¹, Fco. Javier Guzmán Bernardo¹, Rosa C. Rodriguez ¹, Juan J. Berzas Nevado¹

¹ University of Castilla-La Mancha, Avda. Carlos III s/n, E45071, Toledo, Spain
Email: fcojavier.guzman@uclm.es

Little is known about the mechanism that enables toxic metals uptake, bioaccumulation and toxicity. Mercury binds to thiols and these are likely to be bound to sulphur-containing proteins/biomolecules. On the other hand, selenium is essential but toxic at high concentrations, and its biological functions are believed to be carried out by selenoproteins in which Se is specifically incorporated as the amino acid selenocysteine. Several authors reported a protective function of Se against toxic effects caused by Hg, but the way it happens has not yet been fully elucidated [1-3]. In this study, we investigated biomolecules containing Hg and Se in kidney tissues from red deer and wild boar exposed to Hg pollution under real conditions in the Almadén mercury mining area (Spain). The extraction of the biomolecules was carried out by homogenization of the tissues with Tris-HCl buffer. The extracts were analysed by size exclusion chromatography (300-1 kDa) coupled to ICP-MS. Similar profiles were observed in red deer and wild boar kidney tissues. Mercury and selenium were mostly bound to proteins of high molecular weight. On the other hand, kidney tissues were digested enzymatically (using a non-specific protease) and analysed by anionic exchange chromatography coupled to ICP-MS detection. In this case, a number of different organic selenium species were detected. In order to go further in this study, the fractions containing Se-biomolecules are currently being studied by HPLC-MS-QTOF.

References

MERCURY BIOACCUMULATION AND DEPURATION BY THE SEA ANEMONE 

**BUNODOSOMA CAISSARUM**

Nafisa Rizzini Ansari¹, Renato Campello Cordeiro¹, Raquel R. S. Correia², Marcos A. S. Fernandez³, Jean Remy Davée Guimarães²

¹ Geochemistry Department, Fluminense Federal University, Niterói, RJ, 24020-150
² Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, 21941-902
³ Faculty of Oceanography, University of Rio de Janeiro State, Rio de Janeiro, RJ, 20550-013
Email: nafisarizzini@yahoo.com

Aiming the evaluation of mercury (Hg) incorporation in the sea anemone Bunodosoma caissarum specimens were sampled at the Geribá Beach, Brazil. In this experiment there were two types of microcosms. The first with seawater marked with the radiotracer $^{203}\text{Hg}$, in beakers, containing one specimen each. The other microcosm had only seawater marked with $^{203}\text{Hg}$, as controls. The beakers were placed inside sealed desiccators with continuous air renovation and sampling of the Hg volatile forms. The $^{203}\text{Hg}$ was determined by gamma spectrometry. A single initial spike of inorganic $^{203}\text{Hg}$ was added to the seawater of each microcosm. In the bioaccumulation experiment the $^{203}\text{Hg}$ activity was determined periodically in the seawater and in the specimens of the different systems during six days. Then, the methylmercury (MeHg) was quantified in the seawater by liquid scintillation. In the dissolved fraction from 4.32 to 39.30% and in the particulate from 0 to 9.5% of the total Hg (THg) in the seawater was of MeHg. Afterwards, the specimens were submitted to a depuration process of 48 days. The $^{203}\text{Hg}$ activity was determined just before each renewal with unspiked seawater. After the depuration the organisms maintained from 35 to 70% of the bioaccumulated Hg. In addition, the percentages of MeHg relative to the THg in the tissues ranged from 0.18 to 2.36%. The microcosms containing only seawater had approximately 17% of Hg volatilization. While in the systems with seawater and *B. caissarum* it was 33%.

References

EFFECT OF SELENIUM ON TRANSFER OF MERCURY TO EGGS IN JAPANESE QUAILS

Yuta Tani¹, Tomohito Kaito¹, Yasumi Anan¹, Yasumitsu Ogra¹

¹ Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan
Email: ogra@ac.shoyaku.ac.jp

Selenium (Se), one of the essential micronutrient for animals, is known well to play a role in detoxification of mercury (Hg). Although it has been reported that Se detoxifies inorganic Hg by direct interaction in mammals, certain mechanisms of Se to the Hg detoxification in other animals are limited. Several avian species are important for human health because they are bred as poultry for the consumption of their meat and eggs. In this study, we administrated inorganic Hg (iHg) to the female Japanese quails ingesting selenite, and analyzed the transfer to eggs and body distribution of mercury. The egg-laying quails were fed water containing sodium selenite for 16 days. iHg was administered orally 9 days after beginning of the Se ingestion. The laid eggs and excreta of mother birds were collected every 24 h after the iHg administration. The quails were sacrificed 7 days after the iHg administration, and then tissues/organs and blood were collected. The Hg concentration in the yolk of the Hg-administered quails was 14-fold higher than that of control quails on day 3. On the other hand, the increase in the Hg concentration in the yolk was diminished in the quails ingesting selenite. In addition, the Hg concentration in the egg white did not change significantly after the administration. These results suggested that iHg was more preferably transferred to the yolk than the white from the mother quails, and Se protected the transfer of iHg from the mother to the eggs in avian.
CHANGES IN COPPER CONCENTRATION AND EXPRESSION OF COPPER-REGULATING GENES DURING DIFFERENTIATION OF PHEOCHROMOCYTOMA, PC12, INTO NEURAL CELLS

Aya Tejima¹, Maki Tokumoto¹, Yudai Ishizuka¹, Yasumi Anan¹, Yasumitsu Ogra¹

¹ Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan
Email: ogra@ac.shoyaku.ac.jp

Copper (Cu) is an essential metal and its homeostasis is strictly regulated by several Cu-regulating factors, such as Cu transporters, Cu chaperons, and Cu-binding proteins. It is suggested that there is a relationship between some neurodegenerative disorders, e.g., Alzheimer’s disease and amyotrophic lateral sclerosis, and the disruption of Cu homeostasis. This implies that the disruption of Cu homeostasis specifically affects neural cells. Neural cells have a specific pathway for Cu metabolism that differs from other types of cells. To reveal the specific pathway for Cu metabolism in neural cells, we investigated changes in the copper concentration and the expression of copper-regulating genes in PC12 cells. PC12 is a cell line derived from a pheochromocytoma established from rat adrenal medulla, and can differentiate into a neuron when induced by nerve growth factor (NGF). The mRNA expression of Ctr1, a Cu influx transporter, was reduced after inducing the differentiation in a time-dependent fashion. Contrary to the Ctr1 expression, the mRNA expression of Atp7a, a Cu efflux transporter, was increased. Although the expression of metallothionein (MT)-1 and MT-2, which are ubiquitously expressed in all cell types, was decreased after the differentiation, MT-3 which is mainly expressed in the brain was increased with the differentiation. The concentration of Cu was specifically increased but those of iron, manganese, and cobalt were not altered after the differentiation. Cu imaging by the cuprous-ion-specific fluorescent probe, CS-1, also showed that Cu increase in the differentiated PC12 was localized in the cytoplasm. These results indicate that the differentiation into neural cells initially induces the expression of MT-3, which results in the increase in cellular Cu concentration because MT-3 preferably binds Cu. Then, the expression of Ctr1 and Atp7a is reduced and induced, respectively, to ameliorate the increase in Cu concentration in the cells. Hence, MT-3 seems to play a key role in specific Cu homeostasis in neural cells.
IDENTIFICATION OF SELENIUM METABOLITE IN CULTURED CELLS AND ELUCIDATION OF ITS BIOLOGICAL ROLES

Momoko Kimura¹, Yasumi Anan¹, Marina Hayashi¹, Maki Tokumoto¹, Yasumitsu Ogra¹

¹ Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan
Email: ogra@ac.shoyaku.ac.jp

Selenium (Se) is an essential micronutrient for animals. Animals can metabolize both naturally occurring inorganic and organic selenium compounds such as selenite, selenate and selenoamino acids to produce selenoproteins. Se is then excreted into urine as selenosugar and trimethylselenonium. However, the metabolic pathway of Se in animal cells is not fully understood. In this study, we exposed sodium selenite to mammalian cell lines. An unknown Se metabolite was detected in the supernatant of the cells by an HPLC-inductively coupled plasma mass spectrometry (ICP-MS). Namely, the retention time of the Se metabolite did not matched with those of any standard Se compounds which we had. The unknown Se peak was also detected in the supernatant added with GSSeH in vitro. To obtain molecular information of the Se metabolite, it was applied to HPLC-electrospray tandem mass spectrometry (ESI-MS-MS) and electrospray quadrupole and time-of-flight mass spectrometry (ESI-Q-TOF). The results obtained by the molecular mass spectrometry, i.e., the molecular mass of the Se metabolite (106) and the fragments ions suggest that the unknown Se metabolite was selenocyanate (SeCN⁻). The spike of authentic SeCN⁻ increased the peak height of the unknown Se metabolite, and then, simultaneous exposure of GSSeH and cyanide (CN⁻) increased the peak corresponding to the unknown Se metabolite. These results suggest that SeCN⁻ is a novel Se metabolite in cultured cells. The biological significance of SeCN⁻ is now being evaluated.
COMPREHENSIVE STUDY ON SPECIATION OF LOW-MOLECULAR WEIGHT SELENIUM METABOLITES IN BLACK MUSTARD SEED BY CHROMATOGRAPHIC AND MS-BASED TECHNIQUES

Laurent Ouerdane¹, Paulina Flis¹, Katarzyna Bierla¹, Hugues Preud'honne¹, Joanna Szpunar¹, Federica Aureli², Francesco Cubadda²

¹ Laboratoire de Chimie Analytique Bio-Inorganique et Environnement, CNRS/UPPA UMR 5254 (University of Pau), 64053 Pau, France
² Istituto Superiore di Sanità, Department of Food Safety and Veterinary Public Health, Rome, Italy
Email: laurent.ouerdane@univ-pau.fr

The beneficial effect of selenium, an essential micronutrients for humans and animals, on health is strongly dependent on its concentration and speciation hence the huge interest in studying Se speciation especially in plants as they are the main source of Se in diet [1]. Mustard belongs to the family called *Brassicaceae* which are primary or secondary Se-accumulators. The black mustard (*Brassica nigra*) seeds are commonly used as spice or for medical purposes but also in oil production and the residual fraction of this process (the meal) is an important protein source in animal diet and component of feed concentrates [2]. Despite of its wide use, the information of Se speciation in these seeds is very limited. A complete speciation of low-molecular weight Se metabolites in mustard seeds is essential to evaluate their beneficial effects on human health (anti-inflammatory activity or cancer prevention). Therefore, an analytical methodology based on high-resolution ESI MS assisted by Se-specific detection by inductively coupled plasma mass spectrometry (ICP MS) [3] has been improved for Se speciation in seeds of black mustard grown on Se-rich soil. The HILIC and cation-exchange chromatography combined with ESI Orbitrap MS allowed detection and characterization of over 30 low-molecular weight Se metabolites belonging to different families such as Se-sinapine, Se-amino acid, Se-sugar, Se-choline, Se-carbohydrate, Se-glucosinate and their metabolites Se-urea and Se-cyanate.

References
SYSTEMATIC STUDY OF THE SELENIUM FRACTIONATION IN HUMAN PLASMA SAMPLES FROM A CANCER PREVENTION TRIAL: THE POWER OF HYPHENATED MASS SPECTROMETRY FOR CLINICAL SPECIATION

Deitrich, C.L.¹, Rayman, M.P.², Moesgaard, S.³, Goenaga-Infante, H.¹

¹ LGC Limited, Queens Road, Teddington Middlesex TW11 0LY, United Kingdom
² University of Surrey, Guildford GU2 7XH, United Kingdom
³ Pharma Nord ApS, Sadelmagervej 30-32, Dk-7100 Vejle, Denmark
Email: christian.deitrich@lgcgroup.com

Selenium (Se) is an essential element of high relevance to human nutrition and cancer. Increasing evidence suggests that Se plays an important role in cancer prevention by reducing the risk of certain types of cancer such as prostate, lung, colorectal and bladder cancers [1]. Several mechanisms for the anti-cancer effects of selenium have been proposed, and research efforts have focused on low molecular weight Se compounds and selenoproteins as cancer preventive agents. However, there is still lack of clinical speciation (profiling) data to help to understand the metabolism and anti-cancer effects of dietary Se compounds in the human body. Moreover, recent literature has highlighted the fact that supplementation with selenium will only confer benefits to people with low Se status. Unfortunately, this fact has not been considered in some previous cancer prevention trials with selenium, leading to mixed findings.

This work represents the first systematic study of the selenium fractionation in human plasma of patients from the PRECISE (Prevention of Cancer with Se) pilot study. This study involved individuals from UK and Denmark with low Se status (baseline plasma Se). Supplementation of the individuals with selenised yeast at levels of 0, 100, 200 and 300 μg Se per day was undertaken and samples were collected after 0 months, 6 months and 5 years. Since selenoprotein polymorphism vary between individuals of different sex, the speciation measurements were only performed in plasma samples from male volunteers. The determination of total Se in baseline plasma and plasma from supplemented individuals was performed using collision-reaction cell ICP-MS after 10-fold sample dilution. Determination of the total Se in the high molecular weight pool (most likely associated with proteins) and low molecular weight pool (free Se metabolites) was performed by ICP-MS in fractions of plasma obtained after filtration using 10kDa cellulose membranes. Determination of selenomethionine (mainly incorporated non-specifically into Se-albumin) was achieved by HPLC-ICP-MS after enzymatic hydrolysis. The Se content of SeMet in 10 kDa filtered plasma was considered as an approximate estimate of the fraction of Se associated to Se albumin. By subtracting this Se value from the total Se in the high molecular weight pool, the approximate amount of Se associated with selenoproteins (SEPP1 and GPX-3) could be determined. Finally, the speciation of Se in the low molecular weight pool is of interest for nutritional and clinical studies.
Abstracts

molecular weight pool was performed by using complementary ion pairing reversed phase and anion-exchange HPLC coupled to ICP-MS. Using this approach and retention time standards, preliminary identification of known Se-metabolites that may have anti-cancer effects such as Methyl-2-acetamido-2deoxy1-seleno-β-D-galactopyranoside (Selenosugar-1), selenomethionine and Se-methyl-Se-cysteine in plasma without the need for further sample preparation was achieved. Verification of the presence of selenosugar 1 in plasma was also achieved using ESI MS/MS in SRM mode. The relevance of the Se speciation data obtained in this work to future trial design and the improvement of the existing understanding of the cancer preventative effect of Se in humans will be discussed.

References

Abstracts

**Poster Session**  Toxicological, Essential & Medical Aspects of Metals

**Poster P 119**  
*Tuesday, 9th July 2013, 18:00 - 20:00  Room “Exhibition Hall”*  
*Thursday, 11th July 2013, 11:20 - 12:20*

**DEVELOPMENT OF AN ANALYTICAL STRATEGY TO MEASURE MAJOR SELENIUM-CONTAINING SPECIES IN JUVENILE TURTLES (TRACHEMYS SCRIPTA SCRIPTA) BY SAX-HPLC-ICP MS**

Johann Far¹, Christelle Dyc¹, Krishna Das¹, Gauthier Eppe¹

¹ University of Liège Belgium  
Email: johann.far@hotmail.fr

Sea turtles are exposed to many environmental elements such as selenium (Se). Sea turtles are listed under the Red List of threatened species by the International Union for Conservation of Nature. It is thus mandatory to use low-invasive tissue collection (skin, carapace, blood…) for estimating Se exposure in these highly protected turtles. For this purpose, a biological modal *Trachemys scripta scripta* (or slider turtle) was selected. For two months, juvenile turtles were dietary exposed to Se by spiking the food with Selenomethionine (SeMet) or Methionine as control groups. Individuals were sacrificed after different time of exposure and tissues (skin, liver, muscle, carapace and blood) collected to perform Se speciation and determine some biological endpoints. An analytical strategy was developed to cope with the very low amount of available sample. It is briefly consisting by reduction, alkylation and proteolysis of the entire freeze-dried tissues followed by sample clean-up using ultra-filtration membrane. Then anion exchange HPLC using salt and pH gradient was developed to prevent the introduction of organic solvents, which cause severe fouling of ICP MS and avoid ultra-trace analyses of sea water in routine analysis. This method successfully achieved the detection and quantification at ppm level of expected species (i.e SeMet, selenocysteine, inorganic Se) and also unknown species but their relative amounts were time and tissues dependent.
SPECIATION OF SELENIUM COMPOUNDS IN Se-ENRICHED MUSHROOMS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

L. Soares de Assunção¹,³, M. Cássia Soares da Silva¹, M. C. Megumi Kazuya¹, M. González Fernández², T. García-Barrera², J.L. Gómez-Arizá², J.D. Bautista Palomas³

¹ Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, 36570-000, Brazil
² Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen.21007 Huelva. SPAIN
³ Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Sevilla, Sevilla, 41012, Spain

Email: macarena.gonzalez@dqcm.uhu.es

In this study Pleurotus ostreatus and Lentinula edodes mushrooms have been enriched with Se to be used as food source with high content of bio-available Se species, nutritionally effective and less toxic to humans. To this end, P. ostreatus mushrooms have been cultivated on coffee husk substrates exposed to 25 mg/kg Se, and L. edodes mushrooms have been produced on wood sawdust exposed to 50 mg/kg Se. Samples of freeze-dried mushrooms were extracted with protease Type XIV and later analyzed by high performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS). The concentrations of total 80Se of enriched P. ostreatus and L. edodes are 267.6 and 174.4 mg/kg Se, respectively, showing the potential of these mushrooms in accumulating high concentrations of Se in its fruiting body. In P. ostreatus mushrooms, more than 82 % of the accumulated Se is bio-transformed into organic Se (selenocystine, selenomethylselenocysteine and selenomethionine) and 25 % is inorganic (SeIV and SeVI). L. edodes mushrooms have more than 68 % as inorganic Se (Se IV) and only 26 % of organic Se (selenocystine and selenomethionine). Thus, P. ostreatus mushrooms become a better alternative as food supplement of Se against enriched L. edodes mushrooms because they have higher concentration of species of Se that are considered more bio-available and less toxic.
SURVEY OF TOTAL, INORGANIC AND BIOAVAILABLE ARSENIC IN CEREAL PRODUCTS (RICE, WHEAT, OATS AND CORN) FROM SPANISH MARKET

J.A. Gómez-Martín¹, M. González Fernández¹²³, T. García-Barrera¹²³, J.L. Gómez-Ariza¹²³

¹ Department of Chemistry and CC.MM. Faculty of Experimental Science. University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN
² Research Center of Health and Environment (CYSMA). University of Huelva. Campus de El Carmen. 21007 Huelva. SPAIN

Email: macarena.gonzalez@dqcm.uhu.es

Arsenic (As), especially inorganic arsenic (i-As), is an environmental and food-chain contaminant that is carcinogenic to humans after chronic oral exposure. The intake of this element in human (by water and food) has been estimated to be in the range 12 to 20 µg As per day [1], contributing in a greater extent a diet rich in seafood with typical levels ranging from 5 to 50 µg kg⁻¹. However, As in seafood is not considered as a health hazard because this element is mainly present as non-toxic organoarsenicals (arsenobetaine and arsenosugars, this later predominantly in algae). Other foods such as milk, meat, cereals and vegetables also contribute to arsenic intake in the diet although in a lower magnitude [2]. More recently, however, rice and rice-based products have been identified as significant dietary sources of i-As [3], increasing the concern about potential exposure of specific groups of population to these toxic species. In the present work, a procedure based on microwave-assisted extraction with HNO₃ and ICP-MS has been developed for determination of total As in different cereals products, as breakfast cereals, rice crackers, pasta, flours, brans, infants foods rice-based rice, wheat, oats and corn from different market of Huelva (Spain). For As speciation in rice products, an anion exchange chromatographic method with inductively coupled plasma mass spectrometry detection (ICP-MS) has been developed using a PRP-X100 column with 10 mM malonic acid at pH 5.6 mobile phase which gave sharp well-resolved HPLC peaks and short retention times [4]. Finally, in vitro bioavailability of As has been assessed in different rice products samples by using simulated saliva, gastric and intestinal fluids.

References
SPECIATION OF URINARY ARSENIC METABOLITES IN CAMBODIAN RESIDENTS LIVING IN ARSENIC-POLLUTED AREAS

Seiichiro Himeno¹, Hideki Miyataka¹, Harue Morita¹, Suthipong Sthiannopkao²

¹ Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Yamashiro, Tokushima, Japan
² Dong A University, Department of Environmental Engineering, Busan, Korea
Email: himenos@ph.bunri-u.ac.jp

Consumption of arsenic (As)-polluted well water causes a variety of health hazards in Asian countries. Human samples including hair, nail, and urine have been utilized to assess the exposure levels of As. Speciation of urinary As metabolites can provide information on the capacity of methylation of inorganic As in the body as well as the consumption of fish products. In the present study, we examined As concentrations in hair, nail, urine, and well water collected from the residents living in four villages (3 in As-polluted area and 1 in non-polluted area) in Cambodia. The average concentrations of As in well water in As-polluted villages exceeded 500 ppb, which is 50-fold of the WHO recommendation (10 ppb). The concentrations of As in well water showed significant correlations with those in hair and nail. On the other hand, urinary As concentrations among four villages were almost similar. Speciation analysis of urinary As metabolites by HPLC-ICP-MS showed that the concentrations of arsenobetaine in non-polluted village were much higher than those in other villages. A nutritional survey showed that the residents in As-polluted areas eat freshwater fish exclusively, while those in a non-polluted area eat both marine fish and freshwater fish. These data suggest that urinary arsenic concentration may not be a good indicator for arsenic exposure, especially in Cambodia where people eat both freshwater and marine fish as a source of animal protein.
EVALUATION OF RECOMBINANT HUMAN ARSENIC (+3 OXIDATION STATE) METHYLTRANSFERASE FOR THE METHYLATION OF TELLURITE IN VITRO

Maki Tokumoto¹, Natsuko Kutsukake¹, Yasumi Anan¹, Yasumitsu Ogra¹

¹ Showa Pharmaceutical University, 3-3165, Higashi-Tamagawagakuen, Machida, Tokyo 194-8543, Japan
Email: maki@ac.shoyaku.ac.jp

Inorganic arsenic (iAs) is metabolized by arsenic (+3 oxidation state) methyltransferase (As3MT) to methylarsonous acid (MMAsIII) and dimethylarsinous acid (DMAsIII). It is known that inorganic tellurium (iTe) is also methylated to monomethyltellurol (MMTe), dimethyltelluride (DMTe) and trimethyltelluronium ion (TMTe) in vivo. However, the enzyme(s) being responsible to the iTe methylation is unclear. In this study, we prepared recombinant human As3MT (hAs3MT) and evaluated the activity of methylation for iAs and iTe. cDNA of hAs3MT was insert to pBAD vector to express histidine-tagged hAs3MT. Then, the recombinant protein was purified by a nickel-chelate column. The reaction mixture containing 20 µg recombinant hAs3MT, 1 mM S-adenosyl-L-methionine, 7 mM glutathione and 1 µM arsenite (iAs) or tellurite (iTe) in 20 mM phosphate buffer were incubated at 37°C for 4 h. The mixture was treated with 3% H₂O₂ to oxidize methylated metabolites of metalloids, and the oxidized metabolites were determined by an HPLC coupled with an inductively coupled plasma-mass spectrometer (HPLC-ICP-MS). Although hAs3MT was able to transform iAs to MMAs and DMAs, MMTe, DMTe and TMTe were not detected in the reaction mixture under the reaction condition. These suggest that As3MT more preferably metabolize iAs than iTe, and iTe seems to be methylated by another methyltransferase rather than As3MT.
ALTERATIONS IN NEUROBEHAVIOR AND NEUROLOGICAL ENZYME ACTIVITIES IN CADMIUM-TREATED FRESHWATER PLANARIAN, DUGESIA JAPONICA

Jui-Pin Wu¹, Mei-Hui Li¹

¹ Environ. Tox. Lab., Department of Geography, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei 106, Taiwan
Email: rb5_wu@yahoo.com.tw

Planarian represents the most primitive example of centralization and cephalization of the nervous system in Bilateria. Many studies demonstrated that planarian show specific behavioral patterns in response to drugs acting on neural system. Thus, this animal was often used as a practical model for investigation on drug action and abuse. With the similar concept, we attempted to develop planarian as a practical animal model for neurotoxicological research on environmental neurotoxins and to establish the methodology for useful biomarkers. In this study, freshwater planarians, Dugesia japonica, were exposed to artificial freshwater (ISO water) containing cadmium (Cd) at different concentrations for 60 min. During the exposure period, neurobehavior of Cd-treated planarians was continuously observed and recorded. Neurobehavioral responses observed in treated planarians were divided into three different categories, morphology, neurology, and morbidity. In control groups, planarians were in either movement or rest position, and did not show any abnormal neurobehavior. However, in planarians treated with Cd at high concentration (20 mM), neurobehavioral changes in morphology and neurology were immediately observed after the beginning of exposure (0-1 min after exposure), followed by morbidity responses that appeared 3 min later (3-4 min after exposure). Appearances of neurobehavioral alterations in planarians treated with Cd at lower concentrations (1.25-10 mM) were delayed, in accordance with decreasing Cd concentration. Morphological responses in Cd-treated planarians included irregular-shaped, elongated, screw-like, and bridge-like positions, which the latency time of their appearance decreased dose-dependently. Similar trend was also observed in morbidity responses of treated planarians that included labored movement, depression, and unconsciousness. Furthermore, effects of Cd on in vitro and in vivo activities of neurological enzymes in planarians were also investigated, including acetylcholinesterase (AChE), ATPase, and monoamine oxidase (MAO) A and B. For in vitro study, tissue extracts from intact untreated planarians were incubated with Cd before measuring activities of neurological enzymes immediately. For in vivo study, planarians were exposed to ISO water containing Cd, and were sampled at 1, 2, 4, and 7 days to determine neurological enzyme activities. Results showed that neurological enzyme activities in freshwater planarians after Cd treatment were significantly altered. This indicated that alterations in neurobehavior and selected neurological enzyme activities were useful biomarkers for Cd neurotoxicity.
References


Abstracts

Poster Session  Toxicological, Essential & Medical Aspectes of Metals

Poster  P 125  Tuesday, 9th July 2013, 18:00 - 20:00  Room “Exhibition Hall”
Thursday, 11th July 2013, 11:20 - 12:20

COMPARATIVE TOXICITIES OF Cd, Cu, AND Zn TO FRESHWATER PLANARIANS WITH COMPARISONS TO OTHER INVERTEBRATES

Jui-Pin Wu¹, Mei-Hui Li¹, Hui-Ling Lee²

¹ Environ. Tox. Lab., Department of Geography, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei 106, Taiwan
² Department of Chemistry, Fu Jen Catholic University, 510, Zhongzheng Road, Xinzhuang District, New Taipei City 24205, Taiwan
Email: rb5_wu@yahoo.com.tw

Due to its wide geographical distribution, low cost when being maintained in the laboratory, and responsiveness to xenobiotics, the freshwater planarian is considered a potential animal model for studying metal toxicity. In the present study, we determined the acute toxicities of cadmium, copper, and zinc to Dugesia japonica and patterns of metal bioaccumulation and metallothionein induction in treated planarians. According to the results, planarians showed different susceptibilities to different metals. To determine the possible causes for inter-metal differences in susceptibility, acute toxicities of metals to planarians and several metal ion characteristics were correlated. We found that the acute toxicities of metals to planarians were related to their softness indices. Additionally, after comparing acute toxicities of metals to freshwater invertebrates reported in the literature, an impressively higher tolerance of planarians to metal exposure was revealed. By further conducting a comparative study, we found that different susceptibilities of freshwater invertebrates with different taxonomic statuses to metals might be related to interspecific differences in their bioaccumulation patterns. Although the mechanistic causes for inter-metal and interspecific differences in the susceptibilities of aquatic organisms to metals are likely quite complicated, our results provide some critical clues and directions for future work.
DETOXIFICATION STRATEGIES OF CADMIUM IN CERATOPHYLLUM DEMERSUM

Jürgen Mattusch¹, Uriel Arroyo-Abad¹, Elisa Andresen², Hendrik Küpper², George Thomas², Gerd Wellenreuther³

¹ Helmholtz Centre of Environmental Research - UFZ, Department of Analytical Chemistry
² University of Konstanz, Department of Biology
³ HASYLAB at DESY, Notkestr. 85, D-22637 Hamburg, Germany
Email: juergen.mattusch@ufz.de

The heavy metal cadmium (Cd) is highly toxic to plants. To understand the mechanisms of tolerance and resistance to Cd, we treated the rootless, submerged macrophyte Ceratophyllum demersum L. with sub-micromolar concentrations of Cd under environmentally relevant conditions. X-ray fluorescence measurements revealed changing distribution patterns of Cd and Zn at non-toxic (0.2 nM, 2 nM), moderately toxic (20 nM) and highly toxic (200 nM) levels of Cd. Increasing Cd concentrations led to enhanced sequestration of Cd into non-photosynthetic tissues like epidermis and vein. At toxic Cd concentrations, Zn was redistributed and mainly found in the vein. Cd treatment induced the synthesis of phytochelatins (PCs) in the plants, with a threshold of induction already at 20 nM Cd for PC₃. Our results show that also non-accumulators like C. demersum store toxic metals in tissues where the heavy metal interferes least with metabolic pathways, but remaining toxicity interferes with micronutrient distribution. The induction of phytochelatins is not proportional to metal concentration, but has a distinct threshold, specific for each PC species. Finally we could show that 20 nM Cd, which was previously regarded as non-toxic to most plants, already induces detoxifying mechanisms.
STUDY ON ACCUMULATION MECHANISM OF CADMIUM IN TOBACCO BY-2 CELLS BY SR-XRF ANALYSIS

Fumihiro Masuyama¹², Akiko Hokura¹, Tomoko Abe², Tomonari Hirano², Yasuko Terada³, Toshio Sano⁴

¹Department of Green and Sustainable Chemistry, Tokyo Denki University, Senju-Asahicho, Adachi, Tokyo 120-8551 Japan
²Ion Beam Breeding Laboratory, RIKEN Innovation Center, 2-1 Hirosawa, Wako, Saitama 351-0198 Japan
³SPring-8, JASRI, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo 679-5198 Japan
⁴Faculty of Bioscience and Applied Chemistry, Hosei University, 3-7-2 Kajinocho, Koganei, Tokyo 184-8584 Japan

Email: 12kms26@ms.dendai.ac.jp

It has been known that Cd bound to thiol compounds such as phytochelatin or metallothionein in each part of plants to be detoxified. However, the accumulation mechanism of Cd at the cellular level of plants remains unclear. In the present study, micro-XRF imaging and XAFS analyses were applied to tobacco BY-2 cells to reveal the distribution and the chemical state of Cd at the cellular level. In addition, the BY-2 transformant for overexpression of metal transporter, NtNRAMP1, was compared with the wild type of BY-2 cells based on the elemental distribution and the chemical state of Cd. The tobacco BY-2 cells were transferred to the medium containing 25 µM CdCl₂ and cultivated for a certain time at the Cd treatment. The quantifications of cell death and growth rate were examined. Tobacco BY-2 cells were placed on 4 cm × 4 cm acrylic plate and subjected to micro-XRF analysis at SPring-8. The Cd K-edge XANES measurements were carried out at beam line NW10A at PF-AR using freeze-dried tobacco BY-2 cells. After 7-days and 14-days Cd treatment, the survival rates for wild type were about 60% and 10%, respectively. However, those for transformant cells were about 80% and 40%, respectively. The growth rate under Cd treatment decreased for wild type, but kept up for NtNRAMP1 overexpressing cells. These results suggested that the transformant cells have higher resistance to cadmium than the wild BY-2 cells. The results of µ-XRF imaging clearly revealed that Cd was accumulated at whole of the cells for both wild type and transformant. These results implied that Cd was accumulated at vacuole. In addition, the Cd K-edge XANES analysis indicated that Cd accumulated in the BY-2 cells bound to sulfur atoms. It was reported that Cd accumulated in tobacco leaves bound to thiol group of phytochelatin. In this study, it was revealed by non-destructive analysis that tobacco BY-2 cells accumulate Cd at vacuole as compartmentalization, and Cd in the cell was detoxified with thiol compounds such as phytochelatin.
EFFECT OF Cd(II) AND Se(IV) EXPOSURE ON CELLULAR DISTRIBUTION OF BOTH ELEMENTS AND CONCENTRATION LEVELS OF GLYOXAL AND METHYLGLYOXAL IN L. SATIVUM

Alma Rosa Corrales Escobo¹, Kazimierz Wrobel¹, Armando Gómez Ojeda¹, Eunice Yáñez Barrientos¹, Katarzyna Wrobel¹

¹ University of Guanajuato, Department of Chemistry, 36000 Guanajuato, Mexico.
Email: alma_rce@ugto.mx

In this work, two aspects of abiotic stress imposed in L. sativum by Cd(II) and Se(IV) were investigated. The determination of glyoxal (GO) and methylglyoxal (MGO) in plants under different exposure conditions provided novel, complementary data on the element- and concentration-dependent oxidative damage occurring in the presence of a single stressor ion and on the beneficial effect of simultaneous exposure to their combination. The increase of MGO and GO in plants challenged with Cd(II) was associated with previously reported enhanced lipid peroxidation and with the impairment of MGO detoxification system [1, 2, 3]. In the case of Se(IV), such phytotoxic effect was observed only for Se in medium above 1 mg L⁻¹, whereas for its lower concentrations GO and MGO were decreased as compared to control plants, which indicates that there exists a narrow concentration range of Se, favorable for garden cress.

The attenuation of oxidative stress markers observed for simultaneous plant treatment with Cd(II) + Se(IV) was accompanied by lower uptake of the two elements and with drastic change of their cellular distribution, as compared to a single stressor treatment. In particular, under exposure to Cd(II) + Se(IV), relative contribution of the two elements in fraction containing poorly soluble sample components was increased, suggesting that direct in vivo interaction between two elements might be involved in the protective effects observed. The fluorescence spectra obtained for biomass extracts corresponding to different exposure conditions suggested formation of CdSe quantum dots in L. sativum; however further studies are needed for ultimate identification and characterization of such nanoparticulate species.

References
IRON AS A KEY PIECE IN ZINC TOLERANCE IN SACCHAROMYCES CEREVISIAE

Elena Jiménez-Martí¹, María Guirola¹, Sílvia Atrian¹

¹ Dpt of Genetics, Universitat de Barcelona (Spain)

Email: elena.jimenez@ub.edu

Trace elements are essential for all organisms and Saccharomyces cerevisiae is a useful model to study metal regulation and homeostasis. There are many reports about Fe and Zn metabolisms in S. cerevisiae, and their possible interrelationships, but this is still an open question. Our group works with different yeast mutants characterized by a high-Zn tolerance phenotype (?PIF1, ?ACO1 and ACO1 point mutants). All these strains exhibit increased Fe accumulation, increased citrate accumulation and up-regulation of most of the genes related to iron metabolism. Pif1 is a DNA helicase associated to the maintenance of mitochondrial DNA (mtDNA), whose deficiency is complemented by the Aco1 moonlighting DNA-binding function. Because of the accumulating evidence of a relation between Aco1 protein/function and Zn and Fe homeostasis, we investigated the domino effect that would link all these factors. Here, we focus our attention in the role of Fe, to ascertain if iron is the key piece of this puzzle. Our hypothesis is that any molecular event leading to Aco1 loss of function (TCA cycle arrest) will suppose an accumulation of citrate that would cause the drop of labile-iron cell contents. This would switch on the Fe operon, leading to the increase of Fe levels experimentally measured, which would confer high Zn tolerance to cells. Therefore, we suggest a clear connection between metabolic pathways (TCA cycle), mtDNA stability, oxidative stress response, and iron/zinc cell status.

References

**USE ENRICHED $^{57}$Fe ISOTOPES AND IPD-ICP-MS TO STUDY THE NUTRITIONAL EFFECT OF LACTOFERRIN IN THE IRON METABOLISM IN LACTATING RAT**

Sonia Fernández-Menéndez¹, María Luisa Fernández-Sánchez¹, Alfredo Sanz-Medel¹, Belén Fernández-Colomer², Jose Lopez-Sastre²

¹ University of Oviedo, Faculty of Chemistry, Julián Clavería 8, Oviedo, 33006, Spain  
² “Hospital Central de Asturias”, Department of Neonatology, Celestino Villamil, s/n., Oviedo, 33006, Spain  
Email: soniafdez@gmail.com

Iron is an essential micronutrient, according to the World Health Organization (WHO), iron deficiency is considered the first nutritional disorder in the world. For the newborn, breast milk is the ideal food, although it contains rather low levels of iron (0.2-0.4 mg/L) are usually enough to keep baby’s hemoglobin levels within the normal range during the first six months. No breast feed children should be fed with iron-fortified formula to prevent iron deficiencies y anemia since iron absorption from formula is very low compared with maternal milk. Lactoferrin (Lf) is added recently to milk formulas to protect the newborn against gastrointestinal infections and appears to be involved in the metabolism of Fe. Since the bioavailability of iron depends on the physicochemical form, it is convenient to study other physicochemical forms of Fe, alternatives to those used today (allowed as FeSO₄), with higher bioavailability and evaluate its potential to be used for fortification of formula milks. In this work, stable isotopes in combination with IPD-ICP-MS are used to study the nutritional effect of Lf in iron bioavailability and their metabolism. So, milk formula (supplemented with $^{57}$Fe-Lf and/or $^{57}$FeSO₄ in presence of Lf) is used to feed lactating rats. The distribution and quantification of each specie in the fluids and tissues containing endogenous and supplemented iron were determined and compared with those present in rat receiving maternal feeding.

**References**


**SPECIATION ANALYSIS OF MANGANESE IN MORINDA CITRIFOLIA**

Justyna Rybak¹, Justyna Wojcieszek¹, Katarzyna Pawlak¹, Lena Ruzik¹

¹ Department of Analytical Chemistry, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, Warsaw, Poland
Email: kasiap@rpi.pl

The project was focused on speciation analysis of the manganese species and to study the fractionation of microelements such as copper, cobalt and molybdenum in Noni juice. The investigation of manganese species in Noni juice was carried out by fractionation using SEC ICP MS and next ESI MS for their identification. Also presented the fractionation analysis of copper, cobalt and molybdenum in Noni juice sample using SEC ICP MS - juice was treated with buffer and enzymatic extraction media and analyzed. For the evaluation of the amounts of the metal fractions distinguished, the ICP MS was used off-line prior to the determination of copper, cobalt, molybdenum and manganese concentrations in the juice. The accuracy of the entire fractionation scheme and sample preparation procedures involved was verified by the performance of the recovery test. For the characterization of the bioavailability of these elements, in vitro two step model digestion was carried out simulating activity of gastric (pepsin digestion) and intestinal (pancreatin digestion) juices [1]. It was found that manganese in Noni juice is bound by bioflavonoid – rutin, anthraquinone – alizarin and glycosides – asperulosidic acid (ESI MS identification). The study shows that copper and molybdenum present in Noni juice are complexed by peptides, and cobalt by organic acids (which comprise 3.6% of juice). In addition, compounds complexing manganese, iron, copper and molybdenum are hydrophobic proteins [2].

References
STUDY OF MANGANESE LEVELS IN \textit{DEINOCOCCUS RADIODURANS}

Cecilia Miranda\textsuperscript{1}, Mafalda Rodrigues\textsuperscript{1}, Sandra P. Santos\textsuperscript{1}, Ana Margarida Rosa\textsuperscript{1}, Isabel Abreu\textsuperscript{1}, Celia Romao\textsuperscript{1}

\textsuperscript{1} ITQB-UNL, Av. da República Estação Agronómica Nacional 2780-157 Oeiras Portugal
Email: cecilia.miranda@itqb.unl.pt

\textit{Deinococcus radiodurans} (Dr) is an aerobic bacterium extremely resistant to radiation, desiccation and other stressful conditions. Several studies have been published on this organism and different hypotheses have been proposed in an attempt to understand radiation resistance, such as an efficient DNA repair system, protection against protein oxidation, compacted DNA nucleoid or high intracellular manganese accumulation \cite{1}. A relationship between intracellular Mn/Fe concentration ratio and bacteria ionizing radiation (IR) was reported, in which bacteria with high Mn/Fe ratio are extremely resistant to IR-induced protein oxidation, whereas bacteria with low Mn/Fe ratio are hypersensitive to protein oxidation \cite{2}. The presence of an enhanced Mn/Fe ratio confers an increased capacity to prevent the formation of Fe-dependent reactive oxygen species (ROS) through the Fenton reaction. Intracellular Mn can also be protective by scavenging ROS, it was shown that majority of Mn in Dr is present as small complexes with orthophosphate, amino acids ant peptides, which, by scavenging superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$), protects proteins against oxidative damage \cite{3}. However on the stationary growth phase it was proposed that the major cellular contribution of Mn is due to Mn- superoxide dismutase \cite{3}. Currently in our lab, we have a Small Scale Structural Metallomics Project on Dr, and we will present our results on the study of Mn levels on Dr submitted to different stress conditions.

References

\begin{itemize}
\item \cite{1} Slade D., Radman M., (2011),Oxidative Stress Resistance in Deinococcus radiodurans, Microb and Mol Biol Rev 75, 133-191
\item \cite{2} Daly M., (2009) A new perspective on radiation resistance based on Deinococcus radiodurans, Nat Rev Microbiol 7,297-244
\item \cite{3} Tabares L., Un S. (2013), In situ Determination of Manganese (II) speciation in Deinococcus radiodurans by High Magnetic-Field EPR: detection of high levels of Mn(II) bound to proteins J Biol Chem.288, 5050-5055
\end{itemize}
PARTICLE-INDUCED X-RAY EMISSION (PIXE) ANALYSIS OF TRACE ELEMENTS IN THE MAMMALIAN RETINA AND CORNEA: MISS MATCH BETWEEN LOCALISATION OF ZINC AND MTs

Marta Ugarte¹, Geoffrey W Grime², Neville N. Osborne³

¹ Moorfields Eye Hospital NHS Foundation Trust, 162 City Road, London EC1V 2PD, UK
² Surrey Ion Beam Centre, Advanced Technology Institute, University of Surrey, Guildford, Surrey GU2 7XH, UK
³ Fundación de Investigación Oftalmológica, Instituto Oftalmológico Fernández-Vega, Avda. Dres. Fernández-Vega s/n 33012, Oviedo

Email: marta.ugarte5@gmail.com

Metals are crucial for cell homeostasis playing structural, regulatory and catalytic functions in proteins (e.g. enzymes, receptors). Zinc has been detected in the cornea and retina. Congenital and dietary zinc deficiency is associated with corneal changes and retinal degeneration. Major zinc-binding proteins, metallothioneins (MTs), are mainly associated with the retinal pigment epithelium (RPE) and ganglion cells; corneal epithelium and endothelium.

OBJECTIVES. To characterize trace element distribution, particularly zinc, in rat cornea and retina.

MATERIALS AND METHODS. Concentration of trace elements within corneal and retinal frozen sections was established with PIXE.

RESULTS. Corneal and retinal metal distribution is non-homogenous. Calcium (corneal most abundant metal followed by zinc) is in high levels in endothelial cell bodies. Zinc widely distributed across the endothelium, which also has small amounts of iron and copper. In the retina, zinc is the most common metal (followed by iron and copper) with high levels in the RPE, photoreceptor inner segments (RIS)/outer limiting membrane (OLM), inner nuclear and plexiform layers. Iron is present in the RPE/choroid and RIS/OLM. Copper is primarily in RIS/OLM and plexiform layers.

CONCLUSIONS. Zinc, iron and copper exist in different amounts and locations in the cornea and retina. In the RPE, zinc colocalised with MTs. However, there was a clear miss-match between zinc and MT localisation in the neuroretina and cornea.

References

EFFECTS OF SMOKING HABITS ON THE SERUM LEVELS OF ESSENTIAL MINERALS IN NURSE STUDENTS FROM THE ZULIA UNIVERSITY

Alfonso Bravo¹, Fred de La Hoz¹, Nataly Zerpa¹, Daniel Cárdenas¹, Dulce Perozo¹, Daniel Villalobos¹, Lorena Bolívar¹

¹ University of Zulia, Faculty of Medicine, 65 St. 19 Av., Maracaibo 4011, Venezuela.
Email: arbravo@gmail.com

The addiction to tobacco is a critical aspect that has generated much interest today in the health area staff. But the relationship between cigarette smoking and mineral content in the body is a little studied aspect and inconsistent results nationally and internationally. The objective of the research was to determine the effects of smoking on the serum levels of essential minerals in Nursing students at the University of Zulia. This study was correlational with cross-sectional design. The sample was represented by 56 students of different genders, aged 18-30 years. A questionnaire previously validated by experts was applied to assess the prevalence, knowledge and attitudes towards smoking. The iron (Fe), zinc (Zn) and copper (Cu) in blood serum was quantified by atomic absorption spectrophotometry. The Spearman correlation analysis was applied to relate smoking to levels of minerals. A high prevalence of smoking habit (50.9%) was found among nursing students, and 26.3% started smoking during the nurse studies. The 17.5% smoked in college, 14.0% of current smokers cigarettes consumed daily. The 42.1% of smokers want to leave that habit, and 66.7% supported completely suppress smoking in hospitals or health centers. Zn levels were significantly higher (p<0.05) in student smokers (0.50 ± 0.08 mg/L) compared with nonsmokers (0.45 ± 0.10 mg/L). A significant correlation (r= 0.31, p<0.05) between serum Zn and smoking was found in Nursing student from the University of Zulia.

References

TOWARDS THE IDENTIFICATION OF NOVEL ZINC COMPLEXING LIGANDS FROM THE MARINE CYANOBACTERIUM SYNECHOCOCCUS SP. WH8102

Amira Z. Ksibe¹, James P. Barnett¹, David J. Scanlan¹, Claudia A. Blindauer¹

¹ Warwick University, Faculty of Chemistry, Coventry, UK
Email: A.ksibe@warwick.ac.uk

Marine microorganisms, including cyanobacteria, have a requirement for several trace metals that are utilized in major biochemical processes such as photosynthesis and respiration. Although an absolute requirement for zinc has not been experimentally demonstrated for marine cyanobacteria, zinc is predicted to function as a cofactor in several cyanobacterial enzymes including carbonic anhydrase. The concentration of free Zn$^{2+}$ in surface ocean waters is in the low pM range, with the majority of zinc complexed to as yet undefined organic ligands [1]. The high concentration of these ligands in the upper water column suggests a biological source, and at least some of these ligands may be produced by cyanobacteria that are already known to secrete siderophores for scavenging scarce iron [2], and copper binding ligands to mitigate potential toxic effects [3]. A method has been developed to isolate and characterise biogenic zinc-binding ligands, zincophores, using Synechococcus sp. WH8102 as a model organism. Secreted compounds were isolated and purified from culture supernatants using solid-phase extraction and liquid chromatography. Further characterisation of the isolated ligands by LC-MS and ICP-MS approaches indicated a number of low molecular weight compounds that appear to be secreted by Synechococcus sp. WH8102 in response to zinc limitation.

Acknowledgements
We gratefully acknowledge the support of Warwick University, Birmingham Science City Advanced Materials 2, and Homs University.

References
MECHANISM OF CESIUM ABSORPTION IN A SUBMERGED PLANT, *EGERIA DENSAA*

Hikaru Kowata¹, Yoshiyasu Nagakawa², Noboru Sakurai², Akiko Hokura³, Yasuko Terada⁴, Hiroshi Hasegawa¹, Emiko Harada¹

¹ Department of Biological Resources Management, School of Environmental Science, The University of Shiga Prefecture, Japan
² Tokyo Metropolitan Industrial Technology Research Institute, Japan
³ Department of Green and Sustainable Chemistry, School of Engineering, Tokyo Denki University, Japan
⁴ Japan Synchrotron Radiation Research Institute (JASRI)/SPRING-8, Japan
Email: harada.e@ses.usp.ac.jp

Radioactive cesium (Cs) has been discharged into the environment in the northeast region of Japan after an accident of the nuclear power plant in March 2011. So far, the distributions of radioactive elements by terrestrial plants have been reported to discuss the prediction of the movement of radionuclide and the removal of them in the environment.

In this work, we studied the uptake and accumulation of Cs in a fresh submerged vascular plant *Egeria densa* (Brazilian waterweed, Hydrocharitaceae) to investigate the behavior of Cs in the aquatic ecosystem. We collected plants, waters, and sediments that had been contaminated by radioactive fallout in Koori, Souma and Minamisouma in prefecture Fukushima in August and September in 2012. The activities of $^{134}$Cs, $^{137}$Cs, and $^{40}$K were measured using a germanium semiconductor detector. We found that the endogenous radioactive Cs concentrations in plants were correlated with that in the sediments.

To analyze the detailed localization of Cs in plants, synchrotron radiation-based micro X-ray fluorescence (SR-µ-XRF) analysis were performed on BL37XU of the SPring-8 (Proposal No. 2012B1556). The plants were cultivated in hydroponic medium containing 20 µM of stable isotope $^{133}$Cs for 3 days under the controlled condition. The two-dimensional metal distribution of leaves was obtained by µ-XRF with approximately 1 µm X-ray beams and showed the predominant localization of Cs as well as K in the apoplastic region. To elucidate the uptake mechanism of Cs, $^{133}$Cs were then applied separately to shoots and roots in the two-compartment bath [1]. The results showed the plants take up Cs by both shoot and root parts. We concluded that *E. densa* is playing an important role for the deposition of radioactive Cs in the fresh water.

References

EVALUATION OF THE RADIOPROTECTIVE ABILITY OF THE SPICES AND DIETARY SUPPLEMENTS BY MULTIPLE NUCLIDE IMAGING

Yukiko Murakawa¹, Masayuki Munekane¹, Masanari Taniguchi¹, Shinichiro Kamino², Masashi Ueda¹, Makoto Hiromura²,³, Shinji Motomura², Shuichi Enomoto¹,²

¹ Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University
² Next-generation Imaging Team, RIKEN Center for Life Science Technologies
³ Daiichi University of Pharmacy
Email: ph421136@s.okayama-u.ac.jp

The Fukushima nuclear accident occurred, radionuclides containing $^{137}$Cs, $^{131}$I and $^{90}$Sr were released into the environment. At present, people are concerned about internal exposure via foods contaminated with radionuclides. Therefore, effective and easily obtained radioprotector is urgently needed. We focused on Curcumin (Cur), Brazilian Propolis (BP) and Royal Jelly (RJ) as a radioprotector, because they have excitometabolic or antioxidant effects. We analyzed distribution of radionuclides in order to reveal the effects of these intakes to the dynamics and excretion of radionuclides.

Cur, BP, or RJ were orally administrated to BALB/c mice for 1 week. On the 8th day, mice were injected intravenously with $^{137}$Cs, $^{131}$I and $^{85}$Sr. After 6 hours, the radioactivity of each tissue was determined by Ge detector. In addition, we evaluated distribution of radionuclides by GREI (Gamma-Ray Emission Imaging), which is a multiple nuclide imaging modality.

There was no significant difference among Cur-treated mice and control mice. Surprisingly, radioactivity in most tissues of BP- and RJ-treated mice was reduced and excretion of radionuclides was promoted. Especially, $^{131}$I accumulation in thyroid gland of BP- and RJ-treated mice was significantly decreased (BP: 4.4 %ID; Ctrl: 9.6 %ID, p<0.01) and (RJ: 0.1 %ID; Ctrl: 12 %ID, p<0.01), respectively. In conclusion, BP and RJ would be the effective radioprotector. Moreover, GREI succeeded to visualize the effect of supplements noninvasively.