Effect of oral administration of deferiprone on cadmium accumulation, intrarenal essential elements content and renal function in cadmium-exposed mice

Y. Gluhcheva[a], I. Pashkunova[b], M. Schaier[c], K. Kamenova[d*], S. Stoykova[e], E. Petrova[a], E. Pavlova[a], Th. H.Helbich[b], B. Keppler[f], J. Ivanova[g]

[a]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

[b]Department of Biomedical Imaging and Image-guidedTherapy, Division of Molecular and Structural Preclinical Imaging, MedicalUniversity of Vienna and General Hospital of Vienna, Vienna, Austria

[c]Institute of AnalyticalChemistry, University of Vienna, Vienna, Austria

[d]Faculty of Medicine, Medical University, Sofia, Bulgaria

[e]Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

[f]Institute of Inorganic Chemistry, University of Vienna, Vienna, Austria

[g]Faculty of Medicine, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

**email address of the presenting author: kvkamenova@gmail.bg*

Cadmium (Cd) is one of the most nephrotoxic agents[1,2]. Environmental and occupational exposure to Cd induces renal dysfunction followed by loss of calcium, amino acids, enzymes, and elevated proteins in the urine[2]. Deferiprone (L_1) is a lipophilic chelating agent with low toxicity approved by U.S. Food and Drug Administration (FDA) for treatment of iron (Fe) overload in patients with thalassemia syndromes. It has been reported that the intraperitoneal administration of L_1 significantly reduced Cd content in organs of Cd-exposed rats and restored Fe concentration to normal control values[3]. Presently, there is no information about the effect of oral administration of L_1 on the essential elements' homeostasis and renal function of Cd-exposed animals. In this study, we discuss for the first time the effect of oral administration of L_1 on the Cd accumulation, intrarenal essential elements content and renal function in Cd-intoxicated mice. The results demonstrate that the effect of L_1 on the essential elements' homeostasis and renal function of Cd-exposed mice was dose-dependent. Oral administration of high dose (135 mg/kg b.w.) L_1 to Cd-exposed mice for 14 days induced an elevation of the serum renal markers, a significant depletion of the renal concentrations of Fe and selenium (Se) compared to untreated controls. The histological analysis of kidneys of Cd-exposed mice which subsequently obtained high dose L_1 revealed tubules with dilated lumen. Oral administration of low dose L_1 (19 mg/b.w.) to Cd-treated mice for 14 days exerted better therapeutic effect compared to the higher dose L_1 (135 mg/kg b.w.).

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Deciphering silver nanoparticle fate and effects in the liver

Vanessa Tardillo Suárez[a], Yousr Rekik[b], Mireille Chevallet[b], Benoit Gallet[c], Peggy Charbonnier[b], Isabelle Michaud-Soret[b], Artur Krezel[d], Wojciech Bal[e], Giulia Veronesi[a,b] and Aurélien Deniaud[b]*

[a] ESRF, The European Synchrotron. 38000 Grenoble, France
[b] Univ. Grenoble Alpes, CNRS, CEA, IRIG-LCBM, 38000 Grenoble, France
[c] Univ. Grenoble Alpes, CEA, CNRS, IBS, F-38000 Grenoble, France
[d] Department of Chemical Biology, Faculty of Biotechnology University of Wroclaw, Poland
[e] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

aurelien.deniaud@cea.fr

The widespread use of silver nanoparticles (AgNP) in consumer goods raises concerns about their toxicity to humans and their impact on the environment[1]. AgNP toxicity in cells and animals has been extensively studied but the impact of chronic exposure on human physiology and health remains poorly understood. Besides, AgNP are used as biocides in medical devices as wound dressing or catheters, from which they are known to be released in the bloodstream, causing patients to be massively exposed. In spite of this, the effects of this kind of exposure have been disregarded. In this context, the metal content was analysed in liver biopsies from patients with various pathologies after a long hospital stay. Silver accumulation was detected in both healthy and sick livers, levels being statistically higher in patients with various hepatic pathologies[2,3]. These data strongly suggest that AgNP-containing medical devices lead to Ag exposure followed by its storage in the liver of the patients, which raises interest about the fate and the effects of AgNPs on this organ. We have previously shown the intracellular dissolution of AgNPs within endo-lysosomes[4] in hepatocytes, followed by Ag(I) binding to biomolecular thiols[4].

To go further, we combined various imaging approaches such as synchrotron nanoprobe X-ray fluorescence and 3D electron microscopy to follow AgNP transformation in 2D and 3D hepatic cultures. Using these techniques, we have been able to visualize the translocation of Ag(I) molecular species to the nucleus and mitochondria of hepatocytes[5,6]. Moreover, using 3D cultures of hepatocytes that possess active biliary excretion mimicking the liver, we revealed the excretion of Ag(I) molecular species into bile canaliculi thanks to active transport *via* the ATP7B copper transporter[7]. Since Ag(I) mainly recombines with thiols in cellular proteins and peptides, we analysed the activity of specific nuclear proteins with thiol-containing Zn-finger domain. We have shown that, in the nucleus, Ag(I) species impaired the nuclear receptor activity, thus disrupting critical mechanisms of liver physiology in clinically-relevant exposure scenarios[5]. Nuclear receptors are most probably inhibited by the exchange of Zn(II) by Ag(I) in their Zn-finger DNA binding domain. This hypothesis was investigated, *in vitro*, on Zn-finger peptides and provided the molecular proof of Zn to Ag exchange[8,9].

Therefore, we showed for the first time that the exposure to non-toxic concentrations of AgNPs led to an endocrine disruptor-like effect in hepatocytes. This outcome is due to the replacement of Zn(II) by Ag(I) ions in the Zn-finger DNA binding domains of specific nuclear receptors.

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As(III) and Hg(II) interaction of an oligopeptide modelling the metal binding site of the metalloregulatory protein *Af*ArsR

A. Tóth,[a] K. Sajdik,[a] B. Gyurcsik,[a] Z. Kele,[b] E. Wéber,[b] L.I. Szekeres,[a] P.W. Thulstrup,[c] L. Hemmingsen,[c] J. Schell,[d] A. Jancsó[a]*

[a] Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7. H-6720 Szeged, Hungary

[b] 8, H-6720 Szeged, Hungary

[c] Department of Chemistry, University of Copenhagen, Universitetsparken 5., 2100 Copenhagen, Denmark

[d] European Organization for Nuclear Research (CERN) 1211 Geneva, Switzerland

toth.annamaria@chem.u-szeged.hu

Plenty of bacteria have an ars operon in their chromosomes or plasmids[1] ensuring protection against the toxic semimetals, arsenic (III or V) and antimony (III or V). ArsR proteins, responsible for the transcriptional regulation of this operon, display three cysteine residues in their sequence for the binding of trivalent semimetals that initiates the derepression of the regulated genes.[2] Metalloregulatory proteins are known to operate with a remarkable metal ion selectivity.[3] While the selectivity of the semimetal regulating ArsR proteins has not been directly studied, it was indicated by bio-reporter/sensor systems incorporating these proteins as As(III) or Sb(III) sensing elements.[4] The molecular details of semimetal recognition and the origins of the semimetal selective operation of ArsR have not yet been unfolded. In the present study we approached these questions by investigating a model peptide Ac-NCCHGTRDCA-NH_2 (**L**) displaying the amino acid sequence found in the C-terminal As(III)/Sb(III) binding site (α5) of ArsR from the *Acidithiobacillus ferrooxidans* bacterium (*Af*ArsR). With our experiments we intended to compare the interaction of the ligand with arsenous acid (the solution form of As(III)) and Hg(II), a metal ion with well-known thiophilic character and with an aptitude for tricoordinate structures under certain conditions. Various spectroscopic data reflected differences in the binding of As(III) and Hg(II) to the model peptide. While As(III) forms a single mono-complex under any conditions, species with different composition and protonation state was observed with Hg(II). As the most remarkable difference, As(III) was shown to bind to the ligand via all the three cysteines in the whole studied pH range. In contrast, {HgS_2} structures dominate in the acidic pH regime and the binding of the third thiolate takes place only above pH ~ 6.5. This results in a distorted trigonal {HgS_3} species being geometrically different from the proposed trigonal pyramidal AsL complex. The observed structural differences may play key roles in how these proteins discriminate various metal ions and respond only to the cognate semimetals.

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Zinc responsive Magnetic Resonance Imaging (MRI) contrast agent, and its interaction with Human Serum Albumine (HSA)

Patrick Malikidogo[a], Manon Isaac[a], Agnès Pallier[a], Jean-François Morfin[a], Eva Toth[a], Célia Bonnet[a]

[a] Center for Molecular Biophysics, UPR 4301 CNRS, rue Charles Sadron, 45071 Orléans, France1 affiliation

manon.isaac@cnrs-orleans.fr / celia.bonnet@cnrs-orleans.fr

Magnetic Resonance Imaging (MRI) is a powerful non-invasive imaging technique giving anatomical and functional images. Contrast agents are used for a better sensitivity. They can also be tuned to get molecular information like pH, enzyme activity, or physiological cations levels, useful to detect abnormalities underlying diseases. Gadolinium-based (Gd3+) contrast agents are most common due to their chemical stability, rapid clearance, and flexible design, allowing to turn them into probes.

Zinc cations play a key role as a cofactor in gene transcription and metalloenzyme function, or in signaling pathways. Disturbance in Zn^{2+} homeostasis is involved in neurodegenerative diseases (Alzheimer, Parkinson), diabetes, and cancers (prostate, pancreas, and breast)[1]. However, the role of zinc and its distribution remain largely to elucidate.

We will present the rational development of a small molecular zinc responsive contrast agent based on a pyridine unit already used for Gd^{3+} complexation[2], to which a zinc complexing unit has been added through a linker[3]. Its physicochemical characterizations (complexation, relaxometric properties) will be described, as well as its interaction with Human Serum Albumine, the most abundant protein in blood plasma.

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Coordination properties of ligands constituting fragments of FeoB from *Staphylococcus aureus*

V. Dzyhovskyi,[a]* K. Stokowa-Sołtys,[a]

[a] Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383, Wroclaw, Poland

valentyn.dzyhovskyi@chem.uni.wroc.pl

Staphylococcus aureus is a gram-positive a commensal bacteria. However, this microorganism might become pathogenic and has the capacity to infect nearly every tissue in the human body. Therefore, it can cause various diseases such as wound infections, sepsis, endocarditis and others[1]. *Staphylococcus aureus* uses metal ions as cofactors in numerous biochemical processes, such as the synthesis of nucleic acids and proteins, DNA replication, carbon transformations, metabolism of reactive oxidative species (ROS) and others[2]. To ensure an adequate level of essential metal ions, the bacterium uses appropriate transporters, e.g. FeoB (ferrous iron transporter). This system plays an important role in bacterial virulence. Although ferrous iron transport system is very widespread among microorganisms, it is still poorly described in Gram-positive pathogens[3].

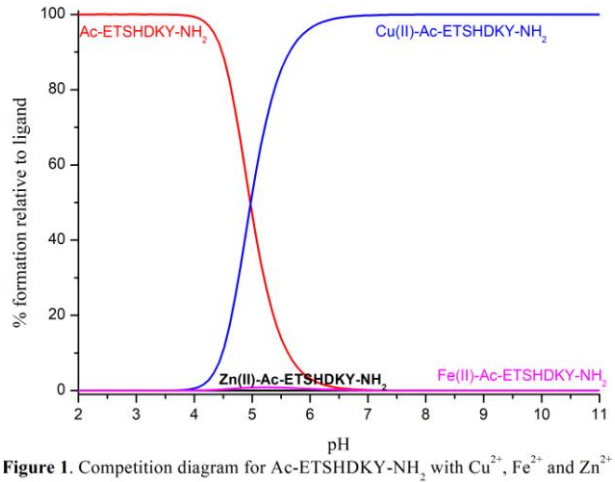

Figure 1. Competition diagram for Ac-ETSHDKY-NH_2 with Cu^{2+}, Fe^{2+} and Zn^{2+}

Herein, combined potentiometric and spectroscopic studies were carried out to determine Cu^{2+}, Fe^{2+} and Zn^{2+} binding by ligands constituting fragments of FeoB. The studied fragments are Ac-IDYHKLMK-NH_2, Ac-ETSHDKY-NH_2, Ac-SFLHMVGS-NH_2, MENYCILG-NH_2 and Ac-KNMCQIIMTE-NH_2. The calculated overall stability constants of the complex species allowed to compare the binding strength of metal ions among ligands as well as the affinity of individual ligand to different metal ions. For this purpose, previously calculated stability constants were applied to a situation, in which equimolar amounts of metal ion and each ligand are present in solution. As can be seen in the Figure 1, cupric ions are the most effectively chelated by the Ac-ETSHDKY-NH_2 fragment. Moreover, gel electrophoresis and UV-Vis spectroscopy were used to check whether the formed complexes are able to generate reactive oxygen species (ROS) in the presence of hydrogen peroxide.

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Photoactivatable zinc sensors: one step closer towards understanding zinc's role in signal transduction

A. M. Pick,[a] S. Becker [a]*

[a]*Technische Universität Kaiserslautern,*
Fachbereich Chemie,
67663 Kaiserslautern
Germany
pick@chemie.uni-kl.de

Zinc is essential for the human organism. Despite its role in protein function and Lewis catalysis, it is crucial for signal transduction.[1] Fluorescent zinc sensors are used to investigate zinc signaling at the molecular level. Building upon the work of *Lippard et al.*, who established fluorescent zinc sensors based on fluoresceine,[2] I seek to develop photoactivatable zinc sensors, whose zinc sensing property can selectively be turned on and off via an external light stimulus. This way, we want to achieve a new, spatio-temporally controllable sensor class. For this, we insert azobenzene, a chemical photoswitch, into the well-established composition of fluorescent sensors: between a zinc binding and a reporting unit. While in the *E* isomer, these units are spatially separated and thus, unable to bind zinc, the photo-isomerization to the *Z* isomer re-constitutes a fully working zinc sensor (Fig. 1.). In this presentation, the synthesis as well as first results concerning the photophysical properties of the prototype of this new sensor class is reported.

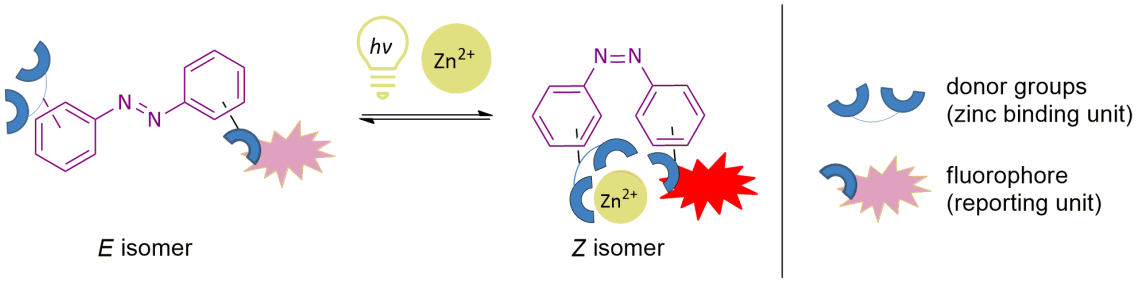

Figure 1. Photo-isomerization restores the sensor's zinc binding motif through setting off the spatial separation of the binding and reporter units.

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Towards Spatio-Temporal Control: Photoactivatable Chelators Based on Azobenzene

R. I. Petrikat[a], H. Schneider[a], L. Thibol[a], N. Dilmen[a], C. Wiedemann[a], G.-M. Sollinger[a], O. A. Müller A. M. Pick[a] and S. Becker*[a]

[a]*Technische Universität Kaiserslautern, Fachbereich Chemie, 67663 Kaiserslautern, Germany*

petrikat@chemie.uni-kl.de

Understanding the function of metal ions – and especially Zn^{2+} - in the brain and the central nervous system on the molecular level is a key for understanding the pathogenesis of neurodegenerative diseases. However, sensing zinc is challenging due to its spectroscopically silent character, thus, common tools are fluorescence sensors that indicate metal binding via a fluorescence response. Those sensors are used to observe metal ion homeostasis without interference.[1] On the other hand, metal specific chelators are used to actively disturb the metal ion homeostasis to investigate the cellular response. Common disadvantages of this approach are cytotoxicity and especially, the lack of spatio-temporal control.[2]

To yield spatio-temporal control, we are developing chelators that can be activated via an external light stimulus to change from an idle into an active state. Therefore, we employ azobenzene as photoswitchable backbone. One example is the photoswitchable chelator 3,3'-Azobenz(BPA)$_2$ (Figure 1). This ligand binds zinc in an isomer-specific fashion: while the *E* isomer does not show interaction with zinc ions, the *Z* isomer reacts with zinc. In this contribution, the systematic approach to the chelator design and spectroscopic studies concerning zinc binding are presented.

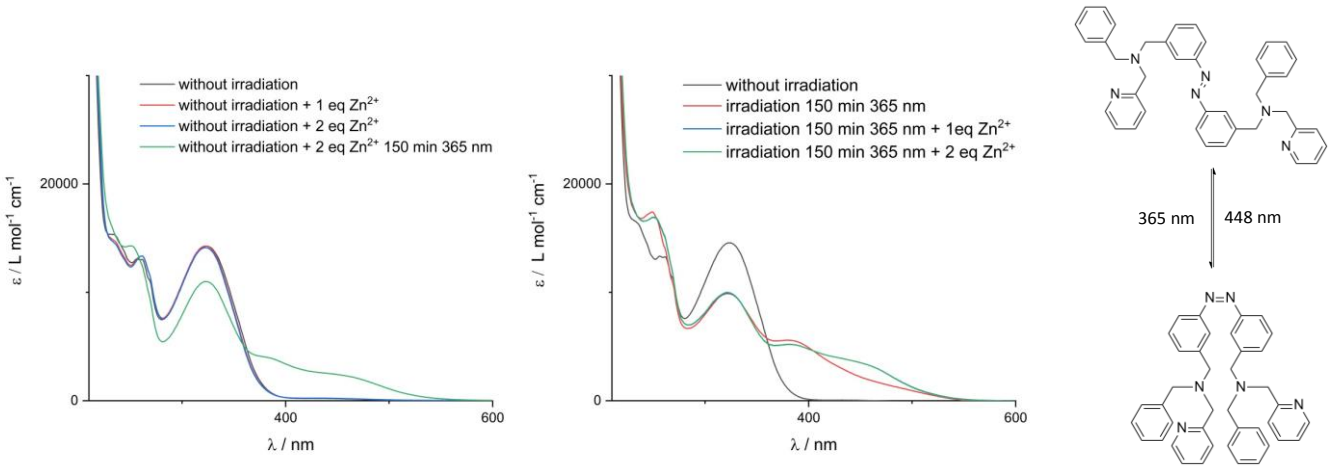

Figure 1. UV/Vis spectrum of 3,3'-Azobenz(BPA)$_2$ in MeCN after addition of one and two equivalents of Zn^{2+} ions before (*left*) and after irradiation (*right*) with light (365 nm). Isomerization from *E* to *Z* isomer of 3,3'-Azobenz(BPA)$_2$ after irradiation with light (365 nm) and re-isomerization after irradiation with light (448nm).

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Peptide-based copper(II) sensors designed for selective detection in biological samples

K. Zimmeter,[a] A. Sour,[a]* P. Faller,[a] H. Martin,[b] C. Bonnet[b]

[a] Institut de Chimie (UMR 7177), Université de Strasbourg-CNRS, Strasbourg, *France*

[b] Centre de Biophysique Moléculaire, Université d´Orléans, Orléans, France

a.sour@unistra.fr

Copper (Cu) is an essential micronutrient for humans and most other living beings. It is distributed into exchangeable (Cuexc) and inert pools, which differ by their thermodynamic and kinetic properties. The understanding of the Cu homeostasis, i.e. the distribution, uptake and trafficking of labile copper ions in vivo is essential for diagnosis and medical treatment a wide variety of diseases.[1] For example, in the case of Wilson's disease, characterized by Cu overload, it has been shown that the Cuexc quantification in blood plasma has clinical significance. Here, we propose to develop new bioinspired Cu(II) sensors and to associate one of them to a magnetic resonance imaging (MRI) contrast agent.

This imaging modality is very well suited to image the whole body and to highlight blood vessels with high spatial and temporal resolution.[2] The Cu-responsive MRI contrast agent is composed of (i) a bioinspired Cu(II)-binding unit and (ii) a gadolinium(III) complex, which is the MRI active part.[3] We will present new Cu(II) chelators with high affinity and high selectivity particularly vs Zn(II). Then, we will use the best Cu(II)-ligand to build a bioinspired Cu(II)-responsive MRI agent. The mechanism of this agent is based on the change of the number of Gd(III)-coordinated water molecules (see figure). One of the arm linked to the Gd(III) ion in absence of copper, moves to the Cu(II) ion when this ion is captured by the Cu(II) ligand, and this switch induces an increase of the image signal. The synthesis, determination of the affinity and selectivity of the different complexes as well as the first results of the MRI efficiency will be presented.

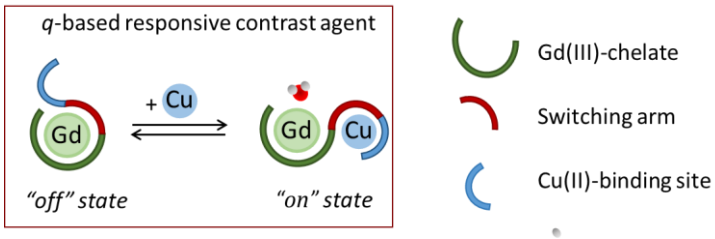

Figure: Schematic representation of the principle of a *q*-based Cu-responsive contrast agent

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