6th - 10th July 2020

BioMetals 2020

12th International BioMetals Web-Symposium

BOOK OF ABSTRACTS

https://biometals2020.sciencesconf.org/

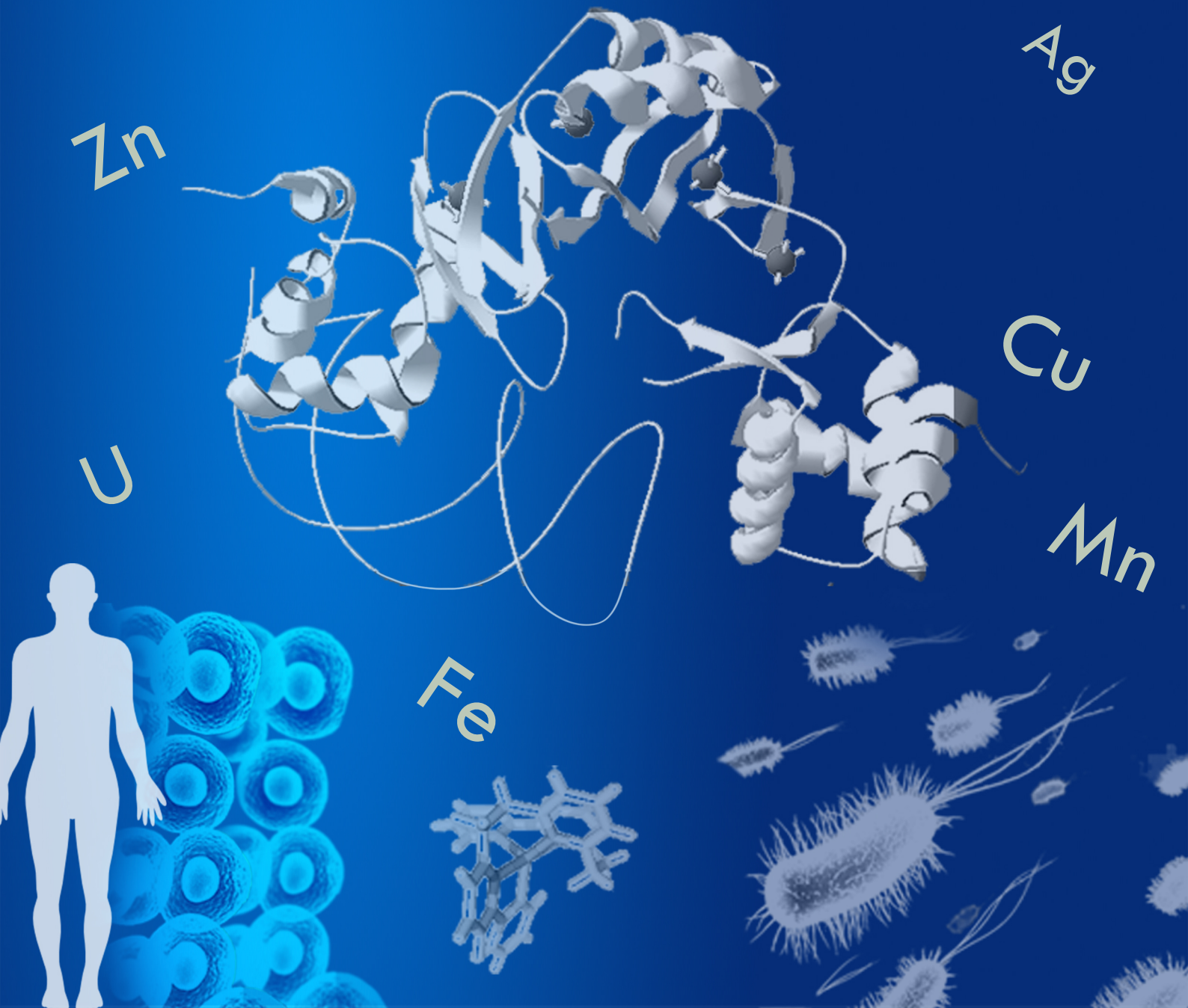


Table of contents

Please note that the titles below are not ordered exactly in the same order as the conference program, they are grouped mainly by day.

Monday

Flash presentation

Flash presentation

Tuesday

Wednesday

Thursday

Friday

Metal Sequestration by Calprotectin and Consequences on Microbial Physiology

Elizabeth M. Nolan

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Most microbial pathogens have a metabolic iron requirement, necessitating the acquisition of this nutrient in the host. In response to pathogen invasion, the human host limits iron availability to starve pathogens of this nutrient. Canonical examples of nutritional immunity are host strategies that limit pathogen access to Fe(III). In contrast, little is known about how the host restricts access to another biologically relevant oxidation state of this metal, Fe(II). This redox species is prevalent at certain infection sites and is utilized by bacteria during chronic infection. Human calprotectin (CP, S100A8/S100A9 or MRP8/MRP14 heterooligomer) is an abundant metal-sequestering innate immune protein that utilizes an unusual hexahistidine (His_6) site to coordinate multiple nutrient metal ions in the divalent oxidation state. We describe the Fe(II)-binding properties of CP, and report that CP inhibits iron uptake and induces an iron starvation response in *Pseudomonas aeruginosa* by sequestering Fe(II) at the His_6 site. We show that, under aerobic conditions in which the Fe(III) oxidation state is favored, Fe(II)-withholding by CP was enabled by (i) its ability to stabilize this redox state in solution and (ii) the production and secretion of redox-active phenazines by *P. aeruginosa* which reduce Fe(III) to Fe(II). Analyses of the interplay between *P. aeruginosa* secondary metabolites and CP indicated that Fe(II) withholding alters *P. aeruginosa* physiology and expression of virulence traits. This work implicates CP-mediated Fe(II) sequestration as a component of nutritional immunity in both aerobic and anaerobic milieus during *P. aeruginosa* infection.

KEY WORDS: Calprotectin, metal sequestration, ferrous iron, *Pseudomonas aeruginosa*
!

How cells help proteins to acquire the correct metals: Free energies of metalation

Nigel J Robinson[a]

[a] *Department of Biosciences and Department Chemistry, Durham University, UK*

nigel.robinson@durham.ac.uk

The occupancies of proteins with metals (metalation) *in vivo* is largely unknown. Metalation is enigmatic because some metals form more stable complexes with proteins than do others. Mis-metalation may involve alternative coordination geometries to the *bona fide* site; may recruit addition ligands; or exploit a sub-set of the ligands used by the cognate metal. The biological challenge is to populate each metalloprotein 'locus' with the correct ion(s), and somehow overcome competition from metals that form more stable complexes, regardless of the coordination chemistries expoited by competing ions.

In the absence of steric selection, nascent proteins bind first row essential divalent metals with an order of preference which follows the Irving-Williams series (Order of stability of metal complexes. *Nature* 1948, 162, 746-747). Cuprous ions are also highly competitive especially for binding sites containing thiols. Correct metalation can be achieved provided cells maintain the weaker binding metals at greater availabilities than the tighter binding ones (Protein-folding location can regulate manganese-binding versus copper- or zinc-binding, *Nature,* 2008, 455: 1138-1142). But it has been difficult to define and then to measure metal-availabilities inside cells.

Our research group has recently determined such intracellular metal availabilities by measuring the metal sensitivities of a complete set of DNA-binding metal-sensors (Bacterial sensors define intracellular free energies for correct enzyme metalation, *Nature Chemical Biology*, 2019, 15: 241–249; Fine control of metal concentrations is necessary for cells to discern zinc from cobalt, *Nature Communications,* 2017, 8:1884 (1-12); A tight tunable range for Ni(II) sensing and buffering in cells, *Nature Chemical Biology*, 2017, 13: 409-414). The resulting values have been used to define the intracellular free energies for metalation. Crucially, intracellular metal availabilities are maintained as the inverse of the Irving-Williams series (Bacterial sensors define intracellular free energies for correct enzyme metalation, *Nature Chemical Biology*, 2019, 15: 241–249). These values now provide a thermodynamic framework within which it becomes possible to understand and to calculate protein metalation via an associative cell biology of metals.

The development of a metalation-calculator which uses these thermodynamic values, will be described. The calculator accounts for inter-metal competition inside cells and accounts for changing metal-availabilities under different growth regimes. Use of the calculator will be exemplified by studies to understand the function and mechanism of action of proteins involved in the acquisition of cobalt for vitamin B_{12} biosynthesis, with additional implications for the bio-manufacture of this nutrient.

Specificity factors that govern zinc-dependent assembly of CD4 coreceptor and Lck tyrosine kinase

Anna Kocyla[a], Aleksander Czogalla[b], and Artur Krezel[a]

[a] *Department of Chemical Biology, Faculty of Biotechnology, University of Wroclaw, Poland*
[b] *Department of Cytobiochemistry, Faculty of Biotechnology, University of Wroclaw, Poland*

anna.kocyla@uwr.edu.pl

Since the discovery of zinc fingers the number of reports on interprotein zinc-mediated and frequently transient protein complexes has also arisen. Although they are still hard to capture one of such zinc-based complexes is Zn(CD4)(Lck), known as zinc clasp. Tetrathiolate coordination of Zn^{2+} provides structural bridge between cytoplasmic tail of CD4 (or CD8α) co-receptor and N-terminal unique domain of Lck tyrosine kinase [1]. Formation of the complex is crucial to get the Lck in the close proximity to the T cell receptor thus enabling the phosphorylation cascade to start.
Zinc-dependent interprotein complex assembly differs from intramolecular ones in terms of factors that govern its assembly, meaning that besides Zn^{2+} concentration needed to form the complex also the concentration of protein subunits matters [2]. However, this behavior well-grounded as the basic principle of the law of the mass action in chemistry, putted in biological context opens the possibility of formation e.g. $Zn(CD4)_2$ dimeric species with exclusion of zinc clasp assembly. Probing the stability of a zinc site on a model domains we showed formation of complexes with different stoichiometry using range of biophysical methods (competition studies with chromophoric zinc chelators, circular dichroism, fluorescence, chromatography) [3]. Within the CD4 cytoplasmic tail sequence the biggest influence on a composition of a formed complex had cysteine residues beyond described zinc site. Their reversible palmitoylation in cellular millieu led us to investigate and compare formation constants of a zinc clasp complex in case of the CD4 palmitoylation. To get the more reliable view we constructed model liposomal membranes and performed FLIM-FRET analysis with the embedded CD4 and Lck domains.
Altogether, we present the multiple facets of interprotein zinc clasp behavior in terms of its stoichiometry, affinity, and palmitoylation using range of biochemical and biophysical methods. Our work contribute to the not yet fully understood process of a T cell activation where the role of zinc ions is just starting to be investigated but the importance of CD4 and Lck proteins is well grounded.

[1] P. W. Kim, Z. Y. Sun, S. C. Blacklow, G. Wagner and M. J. Eck, A zinc clasp structure tethers Lck to T cell coreceptors CD4 and CD8, Science, 2003, 301, 1725-1728.
[2] A. Kocyla, J. Adamczyk and A. Krezel, Interdependence of free zinc changes and protein complex assembly – insights into zinc signal regulation, Metallomics, 2018, 10, 120–131.
[3] A. Kocyla, A. Krezel, Zinc clasp-based reversible toolset for selective metal-mediated protein heterodimerization, Chem. Commun. (Camb.), 2018, 54, 13539-13542.

Research was financially supported by the National Science Centre (NCN) under grant no. 2019/33/B/ST4/02428, 2018/28/T/NZ1/00526, and 2016/21/N/NZ1/02775.
!

The events that may contribute to subgingival dysbiosis: a focus on the interplay between iron, sulfide and oxygen

Kanchana Chathoth[1], Bénédicte Martin[1], Pierre Cornelis[2,3], Stéven Yvenou[1], Martine Bonnaure-Mallet[1,4,] and Christine Baysse[1*]

*Corresponding author: christine.baysse@univ-rennes1.fr

[1] University Rennes 1, NUMECAN INSERM U1241, CIMIAD F-35043 Rennes France

[2] Normandie Université, Université de Rouen Normandie, Laboratoire de Microbiologie Signaux et Microenvironnement, LMSM EA4312, Évreux, France.

[3.] Department of Bioengineering Sciences, Laboratory of Microbiology, Vrije Universiteit Brussel, Brussels, Belgium

[4] CHU Pontchaillou Rennes

Keywords: oral biofilm, iron, hydrogen sulfide, hydrogen peroxide, oxygen

Abstract

The establishment of a chronic infection by subversion of a commensal microbiota results from a complex and multiparametric sequence of events. This presentation narrows down to the interplay between oxygen, iron and sulfide that can result in a vicious cycle that would favour peroxigenic and glutathione producing streptococci as well as sulfidogenic anaerobic pathogens in the subgingival niche. Briefly, oxygen always being present in oral sites may promote H_2O_2 production by peroxygenic bacteria, mainly streptococci. The Fenton chemistry involving ferrous iron from the oral environment and H_2O_2 may generate ROS (hydroxyl radical) which in turn may produce more free iron via Fe-S cluster degradation. The generation of toxic hydroxyl radicals may induce the bacterial production of glutathione (GSH) as stress response, and then indirectly induce the formation of H_2S since GSH can act as a substrate in vitro, like other cysteine-containing peptides from the gingival crevicular fluid. H_2S protects anaerobes by lowering the redox potential in local niches. It may trigger more GSH production by H_2O_2 producing organisms (as described in streptococci), and can reduce ferric iron. From another point of view, H_2S can decrease the Fenton chemistry since it can react with non-heme iron, free or present in iron-sulfur cluster containing proteins, to generate insoluble precipitates (FeS). Altogether, this vicious circle starting from O_2 and ferrous iron may promote anaerobic pathogens and inflammation. We propose strategies for the therapeutic modulation of the microbiota to prevent periodontitis and promote oral health.

Title: Identification and characterization of iron transporters in *Enterococcus faecalis*

Authors: Debra N Brunson, Ling Ning Lam PhD, Jose Lemos PhD

Affiliations: College of Dentistry, University of Florida, Gainesville, Florida 32610

Keywords: Iron homeostasis, *Enterococcus faecalis,* virulence

Enterococcus faecalis is an opportunistic pathogen frequently associated with catheter associated urinary tract infections, central line associated blood stream infections, and wound infections. The virulence of *E. faecalis* largely resides in its ability to survive under harsh conditions, form biofilms on indwelling devices, and evade the innate immune system. One obstacle that must be overcome by bacterial pathogens is the restricted access to essential metal ions such as iron and manganese during infection, an active response known as nutritional immunity. To overcome host-imposed metal limitation, bacteria utilize high affinity metal transporters and may secrete metallophores that are used to scavenge metal ions from host tissues. Recently, we showed that manganese import is essential for the virulence of *E. faecalis* but the importance of iron and the mechanisms utilized by *E. faecalis* to acquire this biometal during infection are presently unknown. Genomic and global transcriptional analyses indicate that the core genome of *E. faecalis* encodes five iron transporters, the highly conserved *feoAB, fhuBCDG,* and *efaCBA,* and two putative metal ion transporters that we have tentatively named *eitABCD* and *emtABC*. Transcriptional profiling revealed temporal expression of the iron transporters with *emtB* and *efaA* strongly induced shortly after iron deprivation whereas *eitC, feoB,* and *fhuG* responses peaked around 60 minutes. With the exception of the Δ*feoB* strain, single inactivation of the other transporters reduced intracellular iron content by ~ 50%. However, this reduction did not have a significant impact on cell growth albeit the Δ*eitABCD* strain displayed a modest growth delay in iron-depleted media. Finally, we found that the virulence of the single mutants was not affected in a peritonitis mouse model. We conclude that *E. faecalis* relies on multiple and functionally redundant transporters to maintain iron homeostasis. Work is underway to isolate and characterize strains simultaneously lacking 2, 3, 4 or all 5 iron transporters.

Effect of iron on the development of oral biofilm

Kanchana Chathoth[a,b], Fabrice Mahé[c], Bénédicte Martin[a], Vincent Meuric[a,b,d], Stéven Yvenou[a], Olivier Loreal[a,b], Martine Bonnaure-Mallet[a,b,d] and Christine Baysse[a]

[a] *NUMECAN U1241 INSERM CIMIAD, Université de Rennes 1, France*
[b] *CHU Pontchaillou, 35033 Rennes, France*
[c] *IRMAR-UMR CNRS 6625, Université de Rennes 1, France*
[d] *Faculté d'Odontologie, Université de Rennes 1, France*

kanchana-nandanan.chathoth@univ-rennes1.fr

Changes in the oral environment may lead to periodontal diseases that can range from a mild gingival infection to severe periodontitis. Genetic hemochromatosis, characterized by systemic iron overload, favours the occurrence of severe periodontitis (Meuric et al., 2017 - PMID: 28586532). The mechanisms involved are not known. We **aim** to study the effect of iron excess in the pathogenic switch of the oral microbiota in the form of a new mathematical model. **Strategy:** As commonly used rich empirical growth media contain high residual iron concentration, we developed a 3-species oral biofilm model in iron-deficient medium which enables control on the concentration and source of iron. This new 'Medium for Mixed Bacterial Community' (MMBC) (Martin et al., 2018 - PMID: 30170019) shares a common core composition enabling the switch from mono- to co-cultures and efficiently promotes both planktonic and biofilm cultures of two periodontal pathogens *Porphyromonas gingivalis* (TDC60), *Treponema denticola* (ATCC35405), and a commensal, *Streptococcus gordonii* (Challis DL1). To better understand the evolution of these bacteria as three-species oral biofilm, we are developing a **mathematical model** with respect to iron concentration. This model will help to connect the different processes (mutualistic or antagonist relationship) between partners and weigh their relative contributions. To have a comprehensive view, planktonic cultures, mono-, bi- and tri-species biofilms were grown at varying iron concentrations. The biofilm development was monitored by qPCR and confocal fluorescent microscopy. **Results:** We choose three iron concentrations (0.8, 8, and 80 µM) corresponding to suboptimal, optimal and excess levels according to the growth rate of *P. gingvalis* in planktonic culture. Planktonic conditions show no effect of iron on the growth of *T. denticola* and *S. gordonii*. Interestingly, 8 µM iron is optimal for the mono-species biofilms of *P. gingivalis* (with higher thickness and biomass) and *T. denticola* (with higher biomass). The analyses of composition and physical parameters of bi- and tri-species biofilms are in progress. Finally, we will model the evolution of *in vitro* mono- or multispecies biofilms to decipher the influence of iron on the behaviour of the oral microbiota. This knowledge would help determine new treatment strategies.

The influence of iron regime on the human gut microflora – an *in vitro* batch culture approach

Amro Duhduh[a], Andrea Monteagudo[b], Arvind Salunkhe[b], Anisha Wijeyesekera[b], Dora Pereira[c], Glenn Gibson[b], Gemma Walton[b], Simon Andrews[a]

[a] *School of Biological Sciences, University of Reading UK*
[b] *Department of Food and Nutritional Sciences, University of Reading UK;*
[c] *Vifor Pharma, UK*

a.duhduh@pgr.reading.ac.uk

Iron is an essential micronutrient for nearly all living organisms, including bacteria. Iron-deficiency related anaemia (IDA) is a major public health problem that affects more than 2 billion people globally (24.8% of the world's population). IDA can be treated by oral iron supplements; however, iron supplements have been reported in different intervention studies to negatively influence the composition of the gut microbiota causing a decrease in the level of commensal bacteria and supporting the growth of pathogens. Much of iron deficiency is as a consequence of the low efficiency (~15%) of dietary-iron absorption. Dietary iron-absorption inhibition factors (iron chelators) like phytate and tannins, found in e.g. flour and tea, have been reported to act as potent iron-uptake inhibitors.

The aim of this research is to determine the influence of different iron sources as well as dietary iron inhibitors on gut microbiota composition and metabolic activity. This aim is being progressed using *in vitro* anaerobic mini-batch cultures under a range of iron regimes, inoculated with human gut microbiota. Our preliminary findings show that the absence of iron and haem is associated with a decrease in the abundance of Enterobacteriaceae whereas their presence causes a significant shift in the gut microbiota composition. Further, high doses of phytate and tannin cause a reduction in the growth of the microbiota.

Currently, other (non-dietary) iron chelators are being tested to determine their effect on gut microbiome of three healthy adults. Future works to extend this research include *In vivo* human trial to study the influence of tea on the gut microbiota (ethical application approval has been obtained). In addition, a gut culture experiment will be carried out to *in vitro* to test the same black tea and placebo extracts used in the human trial.

The role of the three lipocalins of egg-white in inhibition of *Salmonella* Enteritidis under iron restriction

Julien Louis[ab], Baron Florence[b], Bonnassie Sylvie[b], Nau Françoise[b], Kimon Andreas Karatzas[c], Andrews Simon[a] and Jan Sophie[b]

[a] *School of Biological Sciences, University of Reading, United Kingdom*
[b] *Sciences et Technologie du Lait et de l'œuf, l'institut-agro / INRAE, France*
[c] *Food and Nutritional Sciences, University of Reading, United Kingdom*

louis.julien@agrocampus-ouest.fr

Salmonella enterica serovar Enteritidis (*S*E) is a major strain associated with foodborne disease outbreaks caused by eggs and egg products. Egg white (EW) is noted for its strong antimicrobial properties which includes iron restriction resulting from the presence of the iron-binding protein, ovotransferrin. To circumvent iron restriction, *S*E synthesises two types of siderophore, enterobactin (Ent) and its di-glucosylated derivative, salmochelin (Sal). Glucosylation of Ent is considered to be a strategy employed by pathogens to prevent siderophore sequestration and removal from circulation by lipocalin-2 during infection of humans. EW also contains a siderophore-binding lipocalin, called Ex-FABP, but it is unclear whether it is present at sufficient concentration to inhibit growth. In addition, two other lipocalins are also found in EW (Cal-γ and α-1-ovoglycoprotein [α1-ovoGp]) and might also be capable of siderophore sequestration. The question addressed in this project was to determine the role that these lipocalins play in sequestering Ent and Sal in EW. This was explored by, firstly, over-expressing α1-ovoGp, Cal-γ and Ex-FABP, and then raising antibodies which were used to estimate their EW concentrations by western blotting at 233, 5.6, and 5.1 µM, respectively. This indicates that they are the 4th, 11th and 12th most abundant proteins in EW by mass (respectively). Secondly, lipocalin siderophore-binding activity was studied using isothermal titration calorimetry. This confirmed that Ex-FABP can only bind Ent, not Sal, and showed for the first time that Cal-γ and α1-ovoGp fail to bind either siderophore. The results also showed that Ex-FABP has a higher affinity for Fe^{3+}-Ent (K_d 5.3 ± 3.8 nM) than apoEnt (K_d 86.2 ± 14.6 nM), as previously reported. Thirdly, *S*E mutants knocked-out for Ent synthesis or Sal synthesis/export were used to determine whether exposure to lipocalin proteins limits *S*E growth or survival under iron-limited conditions (M9 medium). Among the three EW lipocalins, only Ex-FABP (5 µM) caused reduced growth, but this was only seen in the Sal-synthesis mutant (not the WT). This suggests that Sal secretion allows *S*E to overcome growth inhibition caused by Ex-FABP exposure. Hence, the results support the notion that Sal production acts as a mechanism to evade sequestration by Ex-FABP. The results also show that Ex-FABP is present in EW at sufficient concentrations to inhibit iron-restricted growth of *S*E producing Ent (but not Sal). This work thus provides new insight into the role that lipocalin proteins play in defence of EW against bacterial infection.

Sensing the environment and the host: Iron transport and signalling in *Pseudomonas*

S. Wettstadt, J. Otero-Asman, C. Civantos and **María A. Llamas**

Department of Environmental Protection, Estación Experimental del Zaidín-CSIC, Granada, Spain

marian.llamas@eez.csic.es

Iron is essential for bacterial survival. However, the availability of this metal both in the environment and the host is low because it is mostly present in the oxidized ferric form (Fe^{3+}) or complexed with host proteins, making it hardly accessible for bacteria. *Pseudomonas* bacteria are known for their ability to colonize many different niches, which is enhanced by their capability to scavenge iron from the environment and the host. The different strategies used by *Pseudomonas* to acquire iron include: (i) the production of siderophores (e.g. pyoverdine); (ii) the uptake of siderophores produced by other microorganisms (referred as xenosiderophores) with which *Pseudomonas* shares the niche; (iii) the utilization of host iron carriers (e.g. heme, hemoglobin); and (iv) the transport of Fe^{2+} ions. Uptake of these iron carriers through the highly impermeable outer membrane of *Pseudomonas* often occurs through specialized receptors of the TonB-dependent receptor (TBDR) family. Importantly, several *Pseudomonas* TBDRs have a dual function in both the transport of the iron carrier and signalling. This occurs through the association of the TBDR with a cytoplasmic membrane spanning anti-sigma factor and an extracytoplasmic function sigma (σ^{ECF}) factor. These three proteins form a signal transduction pathway known as cell-surface signalling (CSS) that spans from the outer membrane to the cytosol and is extensively present in *Pseudomonas*. Binding of the cognate iron carrier to the CSS receptor activates a signal transduction cascade that results in the regulated proteolysis of the anti-sigma factor and the liberation of the σ^{ECF} factor in the cytosol. The σ^{ECF} factor can subsequently bind to the RNA polymerase and promote the transcription of response genes. These always include genes involved in iron acquisition and sometimes also genes required for bacterial competition and virulence. *Pseudomonas* thus use xenosiderophores and host iron carriers not only as a source of iron but also as signalling molecules to detect competitors or the host. Therefore, blocking CSS could be a effective strategy to prevent the virulence of pathogenic *Pseudomonas* species.

Necrotrophic phytopathogens are protected from reactive oxygen species by a secreted metal-binding protein

Agnès Rodrigue [1], Lulu Liu[1,5], Virginie Gueguen-Chaignon [2,5], Isabelle R Gonçalves [1,5], Christine Rascle [1], Martine Rigault [3], Alia Dellagi [3], Elise Loisel [1], Nathalie Poussereau [1], Laurent Terradot [4*] and Guy Condemine [1*]

1 Microbiologie Adaptation et Pathogénie, UMR 5240 CNRS, Université de Lyon, INSA de Lyon, France.
2 Protein Science Facility, SFR BioSciences, UMS3444/US8, France.
3 Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech, France.
4 Molecular Microbiology and Structural Biochemistry, UMR 5086 CNRS, Institut de Biologie et Chimie des Protéines, Université de Lyon, France.
5 These authors contributed equally

agnes.rodrigue@insa-lyon.fr

To date, few secreted proteins involved in plant infection common to necrotrophic bacteria, fungi and oomycetes have been identified except for plant cell wall-degrading enzymes. Here we study a family of iron-binding proteins (named Ibp) that is present in Gram-negative and Gram-positive bacteria, fungi, oomycetes and some animals. Mutants lacking these proteins are less virulent as demonstrated in the phytopathogenic bacterium *Dickeya dadantii* or the fungal necrotroph *Botrytis cinerea*. *Dickeya dadantii* Ibp (IbpS) is secreted, can bind iron and copper, and protects the bacteria against H_2O_2- induced death. Its 1.7 Å crystal structure reveals a classical Venus Fly trap fold that forms dimers in solution and in the crystal. We propose that secreted Ibp proteins binds exogenous metals and thus limit intracellular metal accumulation and ROS formation in the microorganisms.

Effect of Iron on the Composition and Activity of the Gut Microbiota using in vitro Models

Andrea Monteagudo-Mera[a], Arvind Salunkhe[a], Gemma Walton[b], Glenn Gibson[b], Anisha Wijeyesekera[b], Dora Pereira[c] and Simon Andrews[a]

[a] [1]School of Biological Sciences, University of Reading, UK
[b] Department of Food & Nutritional Sciences, University of Reading, UK
[c] Department of Pathology, University of Cambridge, UK

a.monteagudo@reading.ac.uk

Iron is an essential nutrient for most organisms including bacteria. Under homeostatic conditions, iron availability in the intestinal lumen is limited and increases or decreases of unabsorbed iron may break the balance in the colonic microbial ecosystem. The aim of this work was to determine how different forms and concentrations of iron sources ($FeSO_4$ and heme) contained in a defined gut model medium influence the metabolic activity and composition of the faecal microbiota during growth using *in vitro* systems. First, a short term study to screen multiple iron conditions was carried out using pH-controlled anaerobic batch cultures. In the second part of the work, iron deficiency, iron supplementation and the effect of heme were studied in three-stage continuous culture human colonic models. The effect of iron on the faecal microbiota composition was determined using NGS-based community profiling (16S rRNA sequencing). Metabolic end products such as lactate and short chain fatty acids were quantified using gas chromatography (GC) and metal levels were determined by ICP-OES.
Results from batch cultures showed that high amounts of haem (77 μM) as only iron source in the medium resulted in a reduction of diversity and SCFAs production as well as a significant increase of enterobacteria. This effect was counteracted when $FeSO_4$ (18 μM) was added in the medium. Results obtained from the human colonic models revealed that iron deficiency had a greater impact on the microbiota composition and activity than iron supplementation. A significant reduction of diversity, butyrate producers, SCFAs and ammonia was detected under iron restricted conditions. On the other hand, withdrawal of heme from our heme continuous model favoured the growth of *Bifidobacterium* and *Lactobacillus* species - health promoting bacteria - although no metabolic outcomes were detected. Overall, results from this study showed that different iron regimes could impact positively or negatively in the composition and function of the gut microbial community which may have an impact on health. These findings also highlight the importance of *in vitro* models to understand the relationship between iron and gut microbiota.

Haem biosynthesis in *Campylobacter jejuni*

Jordi Zamarreño Beas[a], Marco A. M. Videira[a], Jessica F.M. Pereira[a] and Lígia M. Saraiva[a]

[a]*Instituto de Tecnologia Química é Biológica, Universidade Nova de Lisboa, Portugal*

jzamarreno@itqb.unl.pt

Campylobacter jejuni is the cause of 1/4 of diarrhoeal diseases in the world, and is considered to be the major cause of gastroenteritis worldwide. Although these infections are in general not life threatening, it can be fatal in high risk groups. The increasing number of infections due to *C. jejuni* demands a better understanding of the molecular mechanisms allowing its survival within the host that, so far, remain poorly understood. This includes haem biosynthesis that has a crucial role in the pathogen physiology, as survival within the host relies on essential haem-binding proteins. Haem cofactor is responsible for the function of several key cellular processes such as, respiration signaling, gas sensing, microRNA processing and cellular differentiation. Living organisms can obtain haem either by synthesis and acquisition from the host and environment, and in prokaryotes, most organisms are able to synthesize haem endogenously via specific Haem Synthesis Pathways (HSP).

All the currently known HSP begin with the universal tetrapyrrole precursor δ-aminolaevulinic acid and requires a cascade of enzymes to finally produce haem. The first pathway discovered, named protoporphyrin dependent pathway (PPD), requires at least eight enzymes to form haem. Recently, our group participated in the discovery of two other distinct pathways, named sirohaem dependent pathway and coproporphyrin dependent pathway, which occur in sulfate-reducing bacteria and Gram-positive pathogens, respectively (1–3). In this work, we focus on the study of *C. jejuni*'s haem biosynthesis pathway and its role in pathogenicity. We will present our currents results on the biochemical characterization of the haem biosynthesis-related enzymes and their *in vivo* function. Altogether, the data will uncover the HSP pathway active in *C. jejuni* which is essential for survival of the pathogen within the human hosts.

1. Bali S et al. *Proc. Natl. Acad. Sci. U.S.A.* 108(45):18260. (2011)
2. Lobo SAL et al. *Mol. Microbiol.* 97(3):472. (2015)
3. Videira MAM et al. *Mol. Microbiol.* 109(3):385. (2018)

Metallobiology at the host-pathogen interface: the case of tuberculosis

Olivier Neyrolles

IPBS-Toulouse, CNRS University of Toulouse, France

Transition metals are essential to all living organisms. They are also toxic in high amounts, and their intracellular concentration must be tightly regulated. For both reasons, transition metals sit at the heart of the battle between microbial pathogens and their hosts. In the early 2010's, my laboratory and another made the pioneering discovery that transition metals, namely zinc and copper, can be used by phagocytes of the immune system to intoxicate bacterial pathogens, and that metal efflux pumps are involved in bacterial resistance to immune-mediated metal intoxication. In particular, we showed that in host macrophages, zinc accumulates in phagocytosis vacuoles containing the tuberculosis (TB) bacillus, *Mycobacterium tuberculosis*, and that the putative metal efflux pump CtpC, a member of the P-ATPase superfamily, is required for *M. tuberculosis* to multiply inside these cells. To date, several studies reported convergent findings pointing to a role for zinc and copper export systems in the virulence of various fungal and bacterial pathogens, including the TB bacillus. These results opened a new chapter in the field of metallobiology of host-pathogen interactions. They also revealed metal detoxification machineries as major points of vulnerability in pathogenic microbes, which might represent promising targets for the development of novel antimicrobials. Here, I will discuss our recent advances in understanding the mechanisms of zinc detoxification in *M. tuberculosis*, with a particular focus on a novel family of proteins that we recently identified as possible zinc metallochaperones involved in the formation of P-ATPase-containing metal efflux microdomains in the bacterial plasma membrane.

A luminescent peptide probe for extracellular Cu^{2+} detection

Enrico Falcone[a], Pauliza Gonzalez[a], Lucie Lorusso[a], Olivier Seneque[b], Peter Faller[a] and Laurent Raibaut[a]

[a] *Institut de Chimie, UMR 7177, CNRS-Université de Strasbourg, Strasbourg, France*
[b] *CEA, IRIG, LCBM (UMR 5249), Univ. Grenoble Alpes, CNRS, Grenoble, France*

efalcone@unistra.fr

Copper (Cu) is an essential element for most organisms, acting mainly as a redox cofactor in enzymes, but the excess of loosely-bound copper can be also toxic, e.g. via Reactive Oxygen Species (ROS) production. Importantly, increased serum levels of the exchangeable (i.e. kinetically labile) Cu^{2+} pool have arisen as potential markers of some Cu-related pathologies, such as Wilson's disease and Alzheimer's disease, whilst total Cu levels are generally not diagnostic.[1] As elemental analysis techniques cannot directly access such exchangeable Cu pool, the development of Cu^{2+}-specific luminescent sensors is useful to circumvent sample treatments and separative steps. Despite luminescent turn-on probes are generally preferred to turn-off, the design of reversible luminescent turn-on sensors for Cu^{2+} is very challenging, as Cu^{2+} generally quenches luminescence.[2] Hence, we designed a turn-off Tb^{3+} luminescent peptide probe combining the high selectivity and suitable affinity of the N-terminal *Xxx-Zzz-His* (ATCUN) motif with the long-lifetime emission of the lanthanide.[3] This ATCUN-Tb^{3+} conjugate showed selective and reversible response towards Cu^{2+}, and time-delayed detection of Tb^{3+} luminescence allowed monitoring Cu^{2+} fluctuations even in the presence of a fluorescent biological-like background, such as Luria-Bertani (LB) cell culture medium (see Figure).

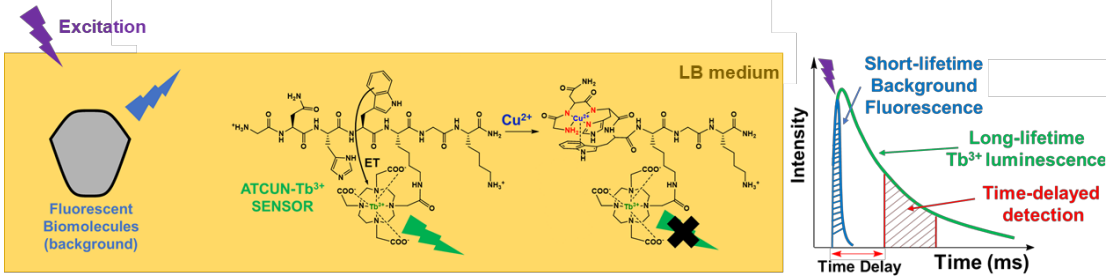


Figure. The emission of Tb^{3+} (green flash) via energy transfer (ET) from a Tryptophan residue is reversibly quenched upon selective Cu^{2+} binding to the ATCUN motif. Time-delayed detection of long-lifetime Tb^{3+} luminescence allows getting rid of the autofluorescence background (blue flash) in biological media.

References

1. R. Squitti, R. Ghidoni, I. Simonelli, I. D. Ivanova, N. A. Colabufo, M. Zuin, L. Benussi, G. Binetti, E. Cassetta, M. Rongioletti and M. Siotto, Copper dyshomeostasis in Wilson disease and Alzheimer's disease as shown by serum and urine copper indicators, *J. Trace Elem. Med. Biol.*, **2018**, 45, 181–188.
2. E. Falcone, A. Sour, V. Lebrun, G. Ulrich, L. Raibaut and P. Faller, Reversible turn-on fluorescent Cu(II) sensors: rather dream than reality? *Dalt. Trans.*, **2019**, 48, 14233–14237.
3. E. Falcone, P. Gonzalez, L. Lorusso, O. Sénèque, P. Faller and L. Raibaut, A Terbium(III) luminescent ATCUN-based peptide sensor for selective and reversible detection of Copper(II) in biological media, *Chem. Commun.*, **2020**, 56, 4797-4800.

OXIDATIVE STRESS RESPONSE ENZYMES: UNRAVELLING CATALYTIC DETERMINANTS OF SUPEROXIDE REDUCTASES THROUGH NATURAL AND SITE DIRECTED MUTANTS

Diogo Salazar Diniz, Filipe Folgosa, Bruno Salgueiro, Célia Romão and Miguel Teixeira

[a] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal*

diogodiniz@itqb.unl.pt

Candidatus *Korarchaeum cryptofilum* is a species of the proposed *phylum Korarchaeota*, or *Xenarchaeota* of the *Archaea*, which is mainly found in hydrothermal environments such as hot springs, shallow water, and deep ocean vents. *Korarcaeota cryptofilum* genome was sequenced and one of the identified proteins was a superoxide reductase (SOR). These enzymes catalyse the reduction of superoxide into hydrogen peroxide (eg. $O_2^{\bullet-} + 2H^+ + e^- \rightarrow H_2O_2$). There are two main groups of SOR's: the 1Fe-SOR containing one iron ion in the catalytic center, and the 2Fe-SOR protein containing one additional iron in a desulforedoxin-like domain[1]. The iron in the catalytic center, in the reduced, active, form, is coordinated by 4 equatorial histidineS and 1 axial cysteine. The SOR from *K.cryptofilum* is a 2Fe-SOR protein with an additional uncharacterized domain at the C-terminus. By sequence analysis it was observed that this SOR contains a natural mutation at the catalytic site, where one of the histidine ligands is substituted by a serine. After successfully growing and purification of the wild-type protein, biochemical analysis demonstrated that the protein/Fe^{2+} was 1:1 instead of 1:2, as expected. This result was confirmed by UV-Visible spectroscopy. A site directed mutant, S70H, that reestablished the fourth ligand histidine at center II revealed no spectroscopic differences from the wild-type protein, indicating that no Fe atom is present at the catalytic center. A modified growth condition by supplementation with 5 different metals (Fe, Co, Zn, Cu and Ni) is undergoing to verify the hypothesis of the presence of a different metal co-factor in this SOR. In addition, crystallization trials are in progress to evaluate not only the effect of this natural point mutation but also the spatial arrangement of the extra domain.

[1] Martins, M C, *et al,* Free Rad BiolMed, 140 (2019), 36-60

Copper (I) oxidation by a CueO-like laccase from *T. thermophilus* studied by direct electrochemistry

Vivek Pratap Hitaishi, Romain Clement, Lisa Zuily, Marianne Ilbert, Elisabeth Lojou and **Ievgen Mazurenko**

Bioenergetics and Protein Engineering (BIP), Aix Marseille Univ, CNRS, Marseille, France

imazurenko@imm.cnrs.fr

Multicopper oxidases (MCO) are a group of oxidoreductases containing a couple of Cu-centres: mononuclear T1 and trinuclear T2/T3. These enzymes are able of four-electron oxygen reduction at high potentials reaching 0.78 V (NHE) which is beneficial for different enzymatic and biohybrid fuel cells.[1] Depending on the structure, MCO can oxidise a large variety of substrates, e.g. a subgroup called laccases perform low-specificity oxidation of aromatic compounds which can also be exploited for bioremediation purposes.

In this work we present the electrochemical characterization of the laccase from a hyperthermophilic bacterium *Thermus thermophilus*[2] on various modified electrodes. We discovered an additional unusual catalytic wave upon the addition of Cu(II) into the electrochemical cell appearing only in the presence of active immobilized laccase. The onset potential and the magnitude of this wave depends on Cu(II) concentration and on the type of electrode surface. In homogeneous assays, such activation by Cu(II) is known for other MCOs, notably for copper efflux oxidase from *E.coli* (CueO), an enzyme responsible for Cu(I) detoxication of the periplasm.[3] It was suggested that it involves copper binding to the methionine-rich domain near the T1 centre.[4,5] Although the laccase from *T. thermophilus* shares only 31% of sequence identity with CueO, a similar methionine-rich domain can be identified suggesting possible copper binding. We thus propose that the observed Cu-dependent wave is related to Cu(I) generation, binding and cuprous oxidase activity displayed by laccase. We use electrochemistry to investigate the mechanism of laccase-Cu interaction and demonstrate the utility of electrochemical methods to study the metal-oxidase activity of enzymes.[6]

1. N. Mano, A. de Poulpiquet, *Chem. Rev.*, **2018**, 118, 2392–2468.
2. P. Agbo, J.R. Heath, H.B. Gray, *J. Phys. Chem. B,* **2013**, 117, 527–534.
3. S.A. Roberts, A. Weichsel, G. Grass, K. Thakali, J.T. Hazzard, G. Tollin, C. Rensing, W.R. Montfort, *Proc. Natl. Acad. Sci.*, **2002**, 99, 2766–2771.
4. K.Y. Djoko, L.X. Chong, A.G. Wedd, Z. Xiao, *J. Am. Chem. Soc.*, **2010**, 132, 2005–2015.
5. S.K. Singh, S.A. Roberts, S.F. McDevitt, A. Weichsel, G.F. Wildner, G.B. Grass, C. Rensing, W.R. Montfort, *J. Biol. Chem.*, **2011**, 286, 37849–37857.
6. V. Pratap Hitaishi, R. Clement, L. Quattrocchi, P. Parent, D. Duche, L. Zuily, M. Ilbert, E. Lojou, I. Mazurenko, *J. Am. Chem. Soc.*, **2020**, 142, 1394-1405

Biogenesis of Iron-Sulphur cluster binding proteins in human cytosol: An insight into the molecular mechanism of cluster delivery from GLRX3 to NUBP1

Francesca Camponeschi[a], **Nihar Ranjan Prusty[a]**, Sabine Annemarie Elisabeth Heider[ab], Simone Ciofi-Baffoni[ab] and Lucia Banci[ab]

[a]*Magnetic Resonance Center CERM, University of Florence, Italy*
[b]*Department of Chemistry, University of Florence, Italy*

niharp1992@gmail.com

Iron-sulfur (Fe-S) clusters are versatile cofactors found in all kingdoms of life. In eukaryotes, the assembly of Fe-S clusters is performed in different cellular compartments by two different machineries. In mitochondria, the iron-sulfur cluster (ISC) assembly machinery de novo synthesizes a [2Fe-2S] cluster, and is responsible for the maturation of mitochondrial [2Fe-2S] and [4Fe-4S] proteins. The cytosolic Fe-S protein assembly (CIA) machinery is required for the maturation of cytosolic and nuclear [4Fe-4S] cluster proteins. Fe-S cluster biogenesis in cytosol includes cluster assembly, association with chaperones, scaffold or carrier proteins and its subsequent transfer to recipient apoproteins. Human cytosolic monothiol glutaredoxin-3 (GLRX3) is a protein essential for the maturation of cytosolic [4Fe-4S] proteins. We reported that dimeric cluster-bridged GLRX3 transfers its $[2Fe\text{-}2S]^{2+}$ clusters to the human P-loop NTPase NUBP1, a scaffold for [4Fe-4S] assembly at early phase of the CIA machinery. The $[2Fe\text{-}2S]^{2+}$ clusters are transferred to monomeric apo NUBP1 by reductive coupling to form $[4Fe\text{-}4S]^{2+}$ clusters on both N-terminal CX13CX2CX5C and C-terminal CPXC motifs of NUBP1 in the presence of glutathione which acts as reductant. Our findings provide the first evidence for GLRX3 acting as a [2Fe-2S] cluster chaperone in the initial stage of the CIA machinery.

Biochemical and structural characterization of a new Flavorubredoxin from *Photobacterium swingsii*

Bruno A. Salgueiro, Filipe Folgosa, Célia V. Romão, Guillem Hermanez, Tiago Cordeiro, Carlos Frazão and Miguel Teixeira

[a] *Instituto de Tecnologia Química e Biológica, António Xavier, Universidade Nova de Lisboa*

brunosalgueiro@itqb.unl.pt

Photobacterium is one of the oldest genera established in the *Vibrionaceae* family of the Gammaproteobacteria class. *Photobacterium swingsii* was recently discovered, and is a facultative anaerobe [1]. According to its genome analysis, a putative flavodiiron (FDP) protein was found, as well as a vast set of proteins capable of performing oxygen and NO detoxification. Flavodiiron proteins have the ability to reduce oxygen to water or NO to nitrous oxide, with different specificities.

Thus far, only the FDP (flavorubredoxin) from *E.coli* was shown to be a clear NO selective FDP[2], while most of the others have a preference for oxygen[3]. This prompted us to search, among flavorubredoxins, the most divergent from the *E. coli* one. The FDP from *P. swingsii* is one of such enzymes.

The enzyme was over expressed in *E.coli*, purified and characterized biochemically and kinetically. At the structural level, Small Angle X-ray Scattering and X-ray srystallography assays were performed.

References:

1 - Gomes-Gil, B., Roque, A., Rotllant, G., Peinado, L., ... and Thompson, F. (2010) Photobacterium Swingsii Sp. Nov., Isolated From Marine Organisms, it Evol Microbiol 61(Pt 2):315-319, doi: 10.1099/ijs.0.019687-0.
2- Gomes, C. M., A. Giuffre, E. Forte, J. B. Vicente, L. M. Saraiva, M. Brunori and M. Teixeira (2002). "A novel type of nitric-oxide reductase - Escherichia coli flavorubredoxin." Journal of Biological Chemistry 277(28): 25273-25276
3- MC Martins, CV Romão, F Folgosa, PT Borges, C Frazão, M Teixeira, 2019, How superoxide reductases and flavodiiron proteins combat oxidative stress in anaerobes, Free Rad. Biol. Med.,140:36-60.

Zinc excess increases cellular demand for iron and decreases tolerance to copper in *Escherichia coli*

Z. Xu[a], P. Wang[a], H. Wang[b], Z.H. Yu[b], H.Y. Au-Yeung[b], T. Hirayama[c], H. Sun[b] and **Aixin Yan[a]**

[a] *School of Biological Sciences, The University of Hong Kong, China*
[b] *Department of Chemistry, The University of Hong Kong, China*
[c] *Laboratory of Pharmaceutical and Medicinal Chemistry, Gifu Pharmaceutical University, Japan*

ayan8@hku.hk

Transition metals serve as an important class of micronutrients that are indispensable for bacterial physiology but are cytotoxic when they are in excess. Bacteria have developed exquisite homeostatic systems to control the uptake, storage, and efflux of each of biological metals and maintain a thermodynamically balanced metal quota in the cell. However, interplay of the pathways that control the homeostasis of different biological metals and its implications for metal-based immunity and antimicrobial treatment remain largely elusive. Here, we report that zinc (Zn) excess perturbs iron (Fe) and copper (Cu) homeostasis in *Escherichia coli*, resulting in increased Fe and decreased Cu levels in the cell. Gene expression analysis revealed that Zn excess transiently up-regulates Fe-uptake genes and down-regulates Fe-storage genes and thereby increases the cellular Fe quota. *In vitro* and *in vivo* protein–DNA binding assays revealed that the elevated intracellular Fe poisons the primary Cu detoxification transcription regulator CueR, resulting in dysregulation of its downstream genes *copA* and *cueO* and activation of the secondary Cu detoxification system CusSR-*cusCFBA*. Supplementation with the Fe chelator 2,2'-dipyridyl (DIP) or with the reducing agent glutathione abolished the induction of *cusCFBA* during Zn excess. Consistent with the importance of this metal homeostatic network to cell physiology, combined metal treatment, including simultaneously overloading with both Zn (0.25 mM) and Cu (0.25 mM) and sequestering Fe with DIP (50 μM), substantially inhibited *E. coli* growth. These results advance our understanding of bacterial metallobiology and may inform the development of metal-based antimicrobial regimens to manage infectious diseases.

The three pillars of bacterial zinc homeostasis

Dietrich H. Nies[a]

[a]*Molecular Microbiology, Martin-Luther-University Halle-Wittenberg, Germany.*

d.nies@mikrobiologie.uni-halle.de

The metallophilic beta-proteobacterium *Cupriavidus metallidurans* is able to maintain its zinc homeostasis from nM to mM concentrations in the medium, and in the presence of other, chemically similar transition metals, which may also vary in concentration in a similar range. This outstanding ability is based upon three pillars: the transportome, which adjusts the cellular transition metal concentration and composition by controlled uptake and efflux reactions; the cellular zinc repository and distribution system; and a network of 11 extracytoplasmic functions (ECF) sigma factors responsible for preferences in metal homeostasis. The function of these pillars in *C. metallidurans* were investigated with state-of-the-art proteomics and transcriptomics methods. When confronted with a mixture of transition metal cations, the *C. metallidurans* cell answered with up-regulation of 63 of its predicted 3084 operons, 48 of the 63 operons encoding systems for metal efflux from the cytoplasm or periplasm, while expression of only a few metal import systems was impeded. With exception of the cobalt-nickel resistance determinant *cnr*, ECF sigma factors were not involved in this response but guaranteed that iron homeostasis was maintained over homeostasis of other metals, followed by zinc and magnesium/phosphate homeostasis as second preference. Expression of the genes for the metal efflux systems were controlled by two-component regulatory systems and MerR-type regulators. At least 10 systems were responsible for zinc uptake into the cytoplasm but only ZupT production was governed by zinc starvation via the regulator Zur. Other members of the Zur regulon were three CobW-type zinc-binding chaperones, which serve as zinc repository or post-translational regulator of transport system. Currently, the intracellular zinc distribution system is under investigation.

The *Bacillus anthracis* virulence-determinant ABC transporter MntBC-A: transport specificity and trans-membrane metal recognition

Oded Lewinson[a], I. Vigonsky[a], G. Masrati[b], N. Levanon[a], J. Rose[a], M. Grupper[a], A. Kuznetsova[a], A. Bloum[a] and N. Ben-Tal[b]

[a]*Department of Molecular Microbiology, Faculty of Medicine, The Technion-Israel Institute of Technology, Haifa, Israel*
[b]*Department of Biochemistry and Molecular Biology, Faculty of Life Sciences, Tel Aviv University*

lewinson@technion.ac.il

The pathogenicity of the potentially lethal human pathogen *Bacillus anthracis* depends on several well-characterized virulence factors such as its unique poly-D-glutamic acid capsule and its protective antigen, edema factor, and lethal factor (LF) toxins. More recently, an additional virulence factor, *mntA*, which codes a substrate binding protein of an ABC import system (*mntBCA*) was identified. Deletion of *mntA* yields a completely non-virulent strain which is currently being used for development of a more efficient vaccine.

Despite its importance, little is known of the $_{ba}$MntBC-A import system. Here, we used metal-sensitivity assays, advanced structural modelling, mutational analysis and transport experiments to study the structure-function relations of $_{ba}$MntBC-A.

We find that, despite the broad metal recognition of the substrate binding protein $_{ba}$MntA, the assembled $_{ba}$MntBC-A transporter imports only manganese, and find that zinc acts as a high-affinity competitive inhibitor.

The metal permeation pathway of the transporter is lined with a unique and highly conserved two triads of titratable residues that participate in manganese coordination. Access to the metal-coordinating residues is blocked by a ladder of hydrophobic residues which seal the access to the transmembrane metal binding site. ATP-driven conformational changes are suggested to open this hydrophobic seal to permit metal binding from the extracellular side of the membrane and metal release into the cytoplasm. The conserved positions of the titratable and hydrophobic residues among ABC transporters of manganese and other transition metals suggests that they also share this mechanism.

The molecular characterization presented here provides an advancement in our understanding of bacterial high-affinity transition metal uptake, a process which is tightly linked to bacterial virulence. The identification of a conserved and essential transmembrane metals coordination site may prove useful in the future design and development of intervention strategies.

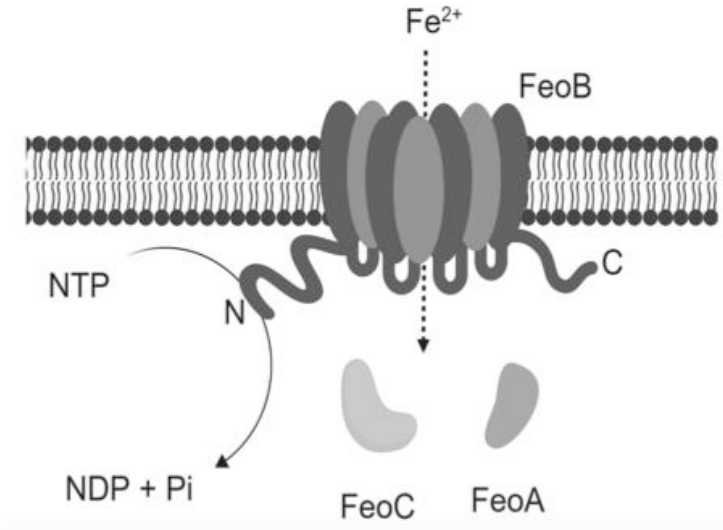
Characterization of the *Vibrio cholerae* Feo Iron Transport System

Shelley M. Payne[a], Minhye Shin[b,c] and Camilo Gomez-Garzon[a]

[a] *Department of Molecular Biosciences, The University of Texas at Austin, USA*
[b]*Department of Biotechnology, Korea University, Seoul, Republic of Korea*
[c]*Department of Agricultural Biotechnology, Seoul National University, Republic of Korea*

payne@mail.utexas.edu

The Feo ferrous iron transport system is widely distributed among prokaryotes. As shown in the figure, this transporter consists of a cytoplasmic membrane protein, FeoB, that appears to form the channel for importing ferrous iron, and two small cytoplasmic proteins, FeoA and FeoC. Our earlier work showed that the three proteins form a large membrane-associated complex, and mutations that result in loss of complex formation also eliminate ferrous iron transport. FeoB has a nucleotide triphosphatase domain in the N-terminal, cytoplasmic region of the protein, and this enzymatic activity is required for Feo-mediated iron transport. Although the NTPase activity was initially described as a GTPase in *Enterobacteriaceae*, we found that *V. cholerae* FeoB hydrolyzes both GTP and ATP, and either is sufficient for function. Mutations that eliminated the GTPase, but not ATPase, activity were identified, and these mutants retained the ability to transport ferrous iron, indicating that the ATPase activity is sufficient for ferrous iron transport. The functions of FeoA and FeoC are not clearly defined. Both FeoA and FeoC are essential for Feo-mediated transport in *V. cholerae.* FeoA, but not FeoC, is required for assembling the Feo complex, while FeoC is needed for function. In vitro assays with purified full-length FeoB showed that addition of FeoA and FeoC had no effect on NTPase activity. Our genetic and biochemical data suggest the following model for Feo function in *V. cholerae:* FeoA interacts with the membrane-associated domain of FeoB and promotes the formation of the complex, which is a trimer of FeoB trimers. FeoC interacts with FeoA, and this interaction requires FeoB. All three proteins are found in the complex, and the stoichiometry is 1:1:1. The fully assembled complex transports ferrous iron via the transmembrane domains of FeoB, and the energy for transport is likely provided by the hydrolysis of either GTP or ATP.



The importance of conserved residues and the C-terminal sub-domain in FeoB-mediated iron uptake

Al-Aidy, S.R.[1,2], Andrews, S.C.[1]
[1]School of Biological Sciences, University of Reading, Reading, RG6 6AJ, UK, [2]College of Medicine, University of Wasit, Iraq.

FeoB is common to all Feo iron transport systems and is presumed to function as the iron permease. Various issues remain to be resolved concerning its mechanism of function and its interaction with the FeoA and FeoC proteins in the case of *E. coli*. One approach that can assist such understanding involves the use of site-directed mutagenesis (SDM). This methodology can be used to target key residues, identified on the basis of conservation and/or predicted location, and determine their relevance in processes such as ferrous iron uptake assays as performed here.

Nine highly-conserved FeoB residues within the permease domain were altered by SDM. The FeoB-C403S, -C432S, -C677S and -E582Q variants failed to exhibit Feo-enhanced growth under iron restriction indicating an essential role for these residues. These four residues were found to be well positioned to act as Fe^{2+} ligands in a FeoB model structure. In contrast, the FeoB-E488Q mutant retained good Feo activity, although this was slightly reduced with FeoC aerobically, and slightly raised aerobically. This residue is predicted to be located in a cytosolic loop and thus may interact with the N-terminal G-protein domain. The FeoB-C772S/H773G and -C763S/C764S (residues in the C-terminal cytoplasmic subdomain of FeoB) variants showed greater Feo activity and showed little enhancement in the presence of FeoC, suggesting that this subdomain interferes with FeoC activation of Feo activity under aerobic conditions and that FeoC may interact with the C-terminal subdomain of FeoB.

The Cup determinant is crucial for the resistance to copper in *Cupriavidus metallidurans*

N. Hirth, M.S. Gerlach, C. Große, D.H. Nies

Martin-Luther-University, Molecular Microbiology, Halle (Saale), Germany

In its natural habitat *C. metallidurans* is often exposed to high concentrations of different heavy metals such as copper, gold or mercury. To survive in this toxic environment it has developed tools for detoxification. Here, mutants carrying multiple deletions in copper resistance genes were constructed and used to study the individual contribution of each system to copper and gold resistance. Deleted were operons encoding for the periplasmic copper oxidase *copABCD*, the P-Type-ATPase *cupCAR*, which is located in the inner membrane, the periplasmic spanning efflux pump *cusABC* as well as the *gig*-cluster whose function is yet unclear. A total of 14 multiple mutants was created in the AE104 wildtype background and their respective survival under copper and gold stress was tested. Preincubation with gold or copper led to lower gold resistance in all strains. When exposed to copper the Cup determinant was crucial for survival. So far CupA is the only known protein to detoxify the cytoplasm from copper. A triple mutant of the Cop, Cup and Cus clusters only containing the cup determinant was more resistant to copper than the other triple mutants. Copper or gold preincubation led to higher or lower resistance to copper respectively.[1] The Cus system was not able to compensate for the loss of *cup*. The periplasmic copper oxidase CopA seemed to be more important for the detoxification of the periplasm than the efflux system Cus in a single mutant background. Additional disruption of the gene *gshA* (γ-glutamylcysteinesynthetase) led to lower copper resistance which underlines the importance of glutathione in the detoxification process of copper.

[1]Wiesemann N, Bütof L, Herzberg M, Hause G, Berthold L, Etschmann B, Brugger J, Martinez-Criado G, Dobritzsch D, Baginsky S, Reith, F, Nies DH. 2017. Synergistic toxicity of copper and gold compounds in *Cupriavidus metallidurans*. Appl Environ Microbiol 83:e01679-17.

Siroheme biosynthesis in *Staphylococcus aureus*: insights of the pathway evolution in bacteria

Marco A.M. Videira[1], Susana A.L. Lobo[1,2], Filipa L. Sousa[3], and Lígia M. Saraiva[1]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República (EAN), 2780-157 Oeiras, Portugal.
[2] Instituto de Biologia Experimental e Tecnológica (iBET), Oeiras, Portugal
[4] Department of Ecogenomics and Systems Biology, University of Vienna, 1090 Vienna, Austria.

Siroheme is the cofactor of sulfite and nitrite reductases, enzymes that play a key role in sulfur and nitrogen metabolism. It is known that *Staphylococcus aureus* encodes a nitrite reductase, but so far, nothing was shown regarding the synthesis of the siroheme prosthetic group. In this work, we show for the first time that *S. aureus* has the enzymes to perform all the steps required to synthesize siroheme and we give a hint on how other bacteria evolved to produce the siroheme tetrapyrrole. We show that three enzymes are required in *S. aureus* for the synthesis of siroheme. The first gene annotated as *cysG* encodes a uroporphyrinogen III methytransferase (UroM), a second gene annotated as *cysG* encodes a precorrin-2 dehydrogenase (P2D) and a last gene that was annotated as *nirR* and previously thought to be a transcriptional regulator, encodes a protein with sirohydrochlorin ferrochelatase activity (ShfC). We further proved that the last protein acts as sirohydrochlorin ferrochelatase by successfully complementing an *Escherichia coli cysG* mutant strain and we showed that a *S. aureus* mutant in this gene is unable to perform nitrite consumption. Furthermore, we showed by homology modelling that residues H22 and H87 are located in the active site of *S. aureus* ShfC and that the site-directed mutation of these residues affects the chelatase activity of the protein. We reorganized the possible routes of siroheme biosynthesis in bacteria by naming them as Type 1 (one multifunctional enzyme), 2 (two enzymes) and 3 (three separated enzyme). We showed by phylogenetical analysis that Type 1 is the most used route and is particularly present in Gammaproteobacteria and Streptomycetales, Type 2 predominates in Fibrobacteres and Vibrionales, and Type 3 is found mainly in Firmicutes of the Bacillales order. To conclude, we show how the siroheme biosynthesis pathway is distributed in the bacterial genomes and based on our results, we suggest that these differences arose from multiple fusion/fission events since the changes occur at the genus and species level.

Email: m.videira@itqb.unl.pt

How bacteria cope with silver ions

Katharina M. Fromm[a], V. Chabert[a], B. Giese[a], L. Simond[a], Ed. Madivoli[a], M. Füeg[a], S. Vasylevskyi[a] and F. Marquenet[a]

[a] *Department of Chemistry, University of Fribourg, Switzerland)*

Katharina.Fromm@unifr.ch

Silver and its compounds are known to possess excellent antimicrobial properties that can be used to combat even multi-resistant bacteria. Yet, the question arises whether bacteria can become resistant towards silver ions. Although there is no absolute resistance, some bacteria possess the ability to cope with relatively large amounts of silver, either using a silver efflux system or the ability to reduce silver ions.

While the copper efflux pump of Gram-negative bacteria is well studied, the silver efflux system of bacteria contains a unique protein, SilE, whose role was unclear upon discovery. Initially, it was thought to bind five silver ions via 10 histidine moieties, undergoing a conformational change from an unstructured protein to an α-helical structure.

We carefully analysed the amino acid sequence of SilE, synthesized in a first step model tetra-peptides excerpt from this sequence and studied their interaction with silver ions in solution, using NMR-titrations and competition fluorescence measurements. In this way, we could identify the amino acids involved in binding to silver and determine local binding affinities. In a next step, we produced longer peptides with two or three silver binding sites and analysed the metal ion binding in order to find out whether the binding occurred in a cooperative or independent manner. In collaboration with Olivier Walker and Maggie Hologne from Lyon, the conformational changes of these model peptides upon addition of silver ions were elucidated.[1]

The second way to deal with silver ions is known in anaerobic bacteria, such as *Geobacter sulfurreducens*. Using the “mineral” respiration process, they are able to dump electrons formed within the cells into minerals outside of the cells. Hence, a long distance electron transfer needs to occur. We could kinetically study this process by following the oxidation state of the Fe-cytochromes in the bacterial periplasm and the formation of silver nanoparticles.[2]

Both results will be presented in the talk.

1 V. Chabert, M. Hologne, O. Sénèque, O. Walker, K. M. Fromm, *Chem. Comm.* **2018**, *54,* 10419-10422

2 V. Chabert, L. Babel, M. P. Füeg, M. Karamash, E. S. Madivoli, N. Herault, J. M. Dantas, C. A. Salgueiro, B. Giese, K. M. Fromm, *Angew. Chem. Int. Ed.*, **2020**, *59, https://onlinelibrary.wiley.com/doi/abs/10.1002/anie.201914873.*

Investigating components of a *Campylobacter jejuni* gene cluster upregulated for iron scavenging during human infection

Tomas Richardson-Sanchez[a], Anson C.K. Chan[a], Brendil Sabatino[a], Michael E.P. Murphy[a] and Erin C. Gaynor[a].

[a] *Department of Microbiology & Immunology, University of British Columbia, Canada*

tomas.richardson-sanchez@ubc.ca

Campylobacter jejuni is a leading cause of bacterial gastroenteritis globally and causes an enormous socioeconomic burden. *C. jejuni* is prevalent as a commensal in the gut of many domesticated animals, with transmission to humans often occurring by consumption of contaminated poultry meat or water. Ingestion of only a few hundred cells is enough to cause an acute diarrheal disease which can lead to highly debilitating secondary sequelae. The minimum iron requirement of *C. jejuni* (10^{-7} M) is well below the bioavailability within the host (10^{-24} M). *C. jejuni* overcomes this barrier by using specialized iron uptake systems to succeed as a pathogen. The *C. jejuni fetMPABCDEF* gene cluster (encoded by *cjj81176_1649-1656*) is upregulated during human infection and upon exposure to human fecal metabolites. The first two components of this gene cluster are well characterized in iron uptake: *fetM* is a homologue of an *E. coli* iron transporter and *fetP* (also known as *p19*) is a periplasmic iron binding protein. However, the six downstream genes (*fetABCDEF*) remain uncharacterized. A *C. jejuni* Δ*fetABCDEF* mutant showed reduced growth in iron-depleted medium, suggesting a role for *fetABCDEF* in iron uptake. This study aimed to determine the contribution of each gene in the *fet* cluster to iron scavenging by monitoring sensitivity of gene deletion mutants to iron availability. *C. jejuni* deletion strains were constructed for each gene of the *fet* cluster. The growth of each mutant was assessed in comparison to wild-type by OD_{600} and CFU under three levels of iron availability: standard (Mueller-Hinton broth), low iron (standard medium with 5 μM deferoxamine), and high iron (standard medium with 100 μM $FeCl_3$). As Mueller-Hinton broth iron content varies between brands and product batches, preliminary growth experiments were required to optimize the deferoxamine concentration from a test range of 0 – 20 μM and inductively coupled plasma mass spectrometry was used to measure the total ^{56}Fe content of the standard medium at 375 ± 12 ppb (6.7 ± 0.2 μM, n = 3). Compared to wild-type *C. jejuni*, major iron-dependent growth defects were observed for gene deletion strains corresponding to a predicted membrane protein (Δ*fetA*) and a predicted ABC transporter (Δ*fetB,* Δ*fetC,* Δ*fetD*). This phenotype was highly similar to that of Δ*fetABCDEF* and Δ*fetP*, indicating an important contribution by FetABCD to iron scavenging. No growth defect was observed upon deletion of *fetE* or *fetF*, although a Δ*fetEF* double deletion strain did show an iron-dependent growth defect. This demonstrated redundancy for FetE and FetF (both predicted membrane-associated thioredoxins) in a function associated with iron homeostasis. A strong growth defect which was independent of iron availability was observed for Δ*fetM*, supporting *fetM* homology to an *E. coli* iron transporter. All growth defects demonstrated by gene deletion mutants were restored to that of wild-type by ectopic chromosomal complementation. Overall, the observed phenotypes are consistent with the putative functions of FetABCDEF and these results implicate function of FetABCDEF alongside FetMP in iron uptake. Ongoing work focuses on gaining functional and structural insight into these proteins to enhance our understanding of *C. jejuni* iron uptake and pathogenesis.

EMPIRICAL EVIDENCE SUPPORTS AN AETIOLOGICAL ROLE FOR CHANGES IN BIOMETAL PATHWAYS IN PARKINSON'S DISEASE

Amr Abdeen, Benjamin Trist, Kay Lorraine Double

Brain and Mind Centre and Discipline of Pharmacology, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW 2050, Australia

Iron overload and copper deficiency are characteristic of the degenerating substantia nigra (SN) in Parkinson's disease brains, however the evidence for causality between these pathological features has not been previously assessed. We gathered empirical evidence of metal changes in the Parkinson's disease SN using a systematic review, then employed the Bradford Hill model of causation to systematically assess whether the available evidence supports a causal relationship between metal alterations and neuron death in Parkinson's disease. The systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines. An electronic search of articles published up to September 2019 was conducted in PubMed, EMBASE, Central Register of Controlled Trials and Scopus, and 8437 resultant articles were screened for pre-defined inclusion and exclusion criteria restricted to human research. The quality of the included 182 articles was assessed using our published Quality Assessment Scale and the NIH Quality Assessment tools; studies of limited quality were removed, resulting in 156 studies for final analysis. The Bradford Hill model evaluates a potential causal inference of one variable on another by assessing published evidence supporting and opposing each according to a set of nine criteria. Assignment of the final 156 studies to these nine criteria revealed that at least seven criteria supported a causal role for alterations to iron and copper levels in nigral neuron death in Parkinson's disease patients. This study supports the development and clinical testing of current and future therapeutic interventions targeting metal alterations in Parkinson's disease.

Long-lived kinetic intermediates explain Cu(II) exchange processes in ATCUN peptides

Radosław Kotuniak[a], Marc J. F. Strampraad[b], Karolina Bossak-Ahmad[a], Urszula Wawrzyniak[c], Iwona Ufnalska[c], Peter-Leon Hagedoorn[b] and Wojciech Bal[a]

rkotuniak@ibb.waw.pl

[a]Department of Biophysics, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Poland
[b]Department of Biotechnology, Delft University of Technology, The Netherlands
[c]Chair of Medical Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Poland.

The amino-terminal copper and nickel/N-terminal site (ATUN/NTS) motifs possess very high affinity to Cu^{II}. Such bioactive peptide or protein sequences participate in maintaining copper homeostasis (K. L. Haas et al. 2011) and in pathogenic processes like in Alzheimer's disease (A. Santoro et al. 2018), type II diabetes and some types of cancer. The routine steady-state experiments with UV-Vis, CD or EPR detection can deliver information about affinity and conformation of peptide-Cu^{II} complexes, but it is difficult to verify the mechanism and rate of metal binding using these techniques alone. The study of kinetics of peptide-copper complexes also provides the evaluation of timescale in which Cu^{II} should be exchanged between proteins to maintain the basic biological processes. For instance, copper transport from the bloodstream to cells is provided by a membrane protein Ctr1. Maryon et al. demonstrated that the maximal rate of copper transported is 10 ions per second per hCtr1 receptor (E. B. Maryon et al. 2013). This indicates that there is limited time in which copper needs to be delivered to the vicinity of the transporter. The comparison of equilibrium binding constants of different molecules or proteins is not sufficient to evaluate which one can deliver the ions.

Therefore we used the stopped-flow and microsecond freeze-hyperquenching (MHQ) techniques supported by steady-state spectroscopic and electrochemical data to study the kinetic properties of GGH, the simplest ATCUN/NTS peptide (R. Kotuniak et al. 2020) The kinetic methods reveal the formation of partially coordinated intermediate Cu^{II} complexes that have been unnoticed before. This long-lived ($t_{½}$ ~100 ms) species seems to be responsible for extending the whole reaction till almost 1 s although the affinity of the final complex is higher than pM (K. Bossak-Ahmad et al. 2020). Circular voltammetry proved its Cu^{II}/Cu^{I} redox activity. The reaction mechanism is completed with an early complex observed at 100-200 μs of the reaction. The presented Cu^{II}/GGH reactivity is a model for other ATCUN/NTS motifs which can be found in N-terminal domains of copper transporter (hCtr-1), many serum albumins or some Aβ-peptides. The presented methodology will help fill the gap in the understanding of extracellular copper transport.

B. cenocepacia increased antimicrobial resistance in the Cystic Fibrosis lung is associated with low pH and elevated levels of zinc and iron

L. Daniela Morales[a], Michael E. P. Murphy[a]

[a] Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada

danimora@mail.ubc.ca

Cystic fibrosis (CF) is a genetic disease caused by mutation in the Cystic Fibrosis transmembrane conductance regulator (CFTR) that mediates ion transport across the membrane of epithelial cells, leading to an accumulation of viscous and sticky mucus in the ducts and compartments of organs such as lungs. Previous analysis of sputum samples from CF patients shown elevated levels of zinc and iron (an average Fe= 800 ppm and Zn= 1300 ppm), and lower pH (2.9-6.5), compared to healthy individuals. *Burkholderia cenocepacia* is an opportunistic pathogen correlated with increased disease severity and mortality in CF patients. Additionally, *B. cenocepacia* is resistant to a wide range of disinfectants and antibiotics and no standard treatment is available to eradicate this infection. This study aimed to determine the efficacy of antibiotics in inhibiting *B. cenocepacia* growth using a medium that models iron and zinc concentrations and pH in the CF lung. A defined Synthetic Cystic Fibrosis media (SCFM) has been previously designed base on the composition of different CF sputum samples. However, ICP-MS analysis of this media shows low Fe/Zn content. Thus, for our studies we supplemented the SCFM with ferrous sulfate and zinc acetate (SCFM-FeZn) to the average values previously reported and adjusted the pH to 5.5. We assessed *B. cenocepacia* growth using optical density and a fluorescent reporter. Overall, *B. cenocepacia* growth was improved at pH 5.5 compared to pH 6.8, and supplementation with different concentrations of ferrous sulfate and zinc acetate altered growth: when supplemented with both metals the growth was improved, when just iron was supplemented the growth rate increased, while just zinc supplementation had an inhibitory effect. Additionally, a library of 591 antibiotics was screened, using SCFM-FeZn at both: pH 6.8 and 5.5. A total of 40 compounds were active against *B. cenocepacia*: 17 compounds were active at both pH values, while 12 were uniquely active at pH 6.8 and 11 at pH 5.5, surprisingly none of these compounds included antibiotics that are currently used to treat *B. cenocepacia* infections. Here we provide evidence of the impact of physiological pH, iron and zinc concentrations in *B. cenocepacia* in the growth and antimicrobial resistance during CF lung infections, and propose an improved SCFM media that can be further used for antimicrobial screening against CF infections. Furthermore, we have identified compounds that inhibit *B. cenocepacia* growth at a physiologically relevant lower pH and zinc and iron concentration.

Presenting author: Laura Daniela Morales Duran

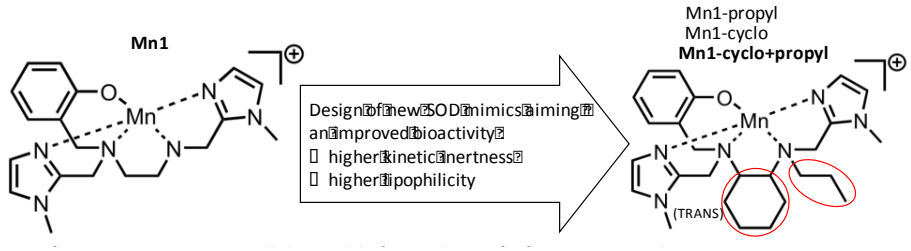
Design and study of anti-oxidant inorganic complexes mimicking the superoxide dismutase (SOD)

Gabrielle Schanne[a], Martha Zoumpoulaki[a], Nicolas Delsuc[a], Philippe Seksik[b] and Clotilde Policar[a]

[a] *Laboratory of Biomolecules, Department of chemistry, Ecole normale supérieure, Sorbonne University, Paris, France*
[b] *Metabolism Inflammation Department, Saint Antoine Research center, Paris, France.*

gabrielle.schanne@ens.psl.eu

Superoxide Dismutases (SODs) are metalloenzymes involved in the cellular antioxidant defenses. They regulate the concentration of the superoxide anion, a reactive oxygen species (ROS)[1]. It has been shown that SOD defenses are weakened in intestinal epithelial cells of patients suffering from inflammatory bowel diseases (IBDs)[2]. The resulting increase in ROS amount, leading to oxidative stress, may contribute to the pathogenesis in IBDs. Low-molecular weight complexes, mimicking SOD activity may be promising antioxidant metallodrugs for the treatment of IBDs. The research conducted in Policar's group has led to the development of the manganese complex Mn1 that has shown anti-oxidant and anti-inflammatory activities in intestinal LPS-stressed epithelial cells, an inflammation model mediated by oxidative stress[3]. However, Mn1 is very flexible compared to the native SOD and is prone to metal-assisted dissociation in cells. Indeed, metal exchanges might occur between the manganese center and metal ions present in the biological environment. Aiming at improving the bioactivity of this SOD mimic, three new MnSOD mimics derived from Mn1 have been designed. Their structure includes additional cyclohexyl and propyl groups. In one hand, by rigidifying the ligand structure, the cyclohexyl group may provide a compact and preorganized coordination cavity to encapsulate the manganese ion and thus may improve the kinetic inertness of the complexes[4]. In the other hand, the lipophilic propyl group may favor the cell penetration of the complexes and hence enhance their bioavailability.



We have assessed the potential of new SOD mimics derived from Mn1 to demonstrate higher intrinsic SOD activity, higher lipophilicity and improved kinetic inertness in the cellular environment. Very interestingly, the new Mn1 derivatives were shown to provide anti-inflammatory effects in intestinal LPS- stressed epithelial cells at lower doses than Mn1 and are hence more efficient SOD mimics.

[1] Sheng Y. et al., *Chemical Reviews* **2014,** 114 (7), 3854–3918
[2] Kruidenier L. et al., *Journal of Pathology*, **2003**, 201, 7-16
[3] Mathieu, E et al., *Inorg. Chem.* **2017,** *56*, 2545-2555
[4] Laine, S. et al., *New Journal of Chemistry.* **2018**, 42 (10)

Discovery through combinatorial chemistry of a peptidyl metal complex mimicking the antioxidant enzyme Catalase: characterization and in vitro/in vivo activity

Koudedja COULIBALY[a], Marion THAUVIN[b], Sophie VRIZ[b], Clotilde POLICAR[a] and Nicolas DELSUC[a]

[a] Laboratoire des biomolecule (Département de chimie, École normale supérieure, Sorbonne Université, France)
[b] Center for Interdisciplinary Research in Biology(College de France, CNRS, INSERM, PSL Research University, France)

koudedja.coulibaly@ens.fr

ABSTRACT

Catalase mimics (CATm) are metal complexes able to solve oxidative stress by hydrogen peroxide dismutation. Among them, peptidyl complexes are noteworthy because peptides are easily synthesized on solid support, afford a great versatility and are biocompatible. Yet, the rational design of the sequence still remains a challenge. To circumvent this difficulty, we have applied a one-bead-one-compound combinatorial approach for the discovery of peptide-based redox active complexes.[1] A screening strategy relying on an activity-based assay has been applied with an easy-to-analyze read-out for the discovery of new CATm. More than 125 peptides have been generated and we discover a first peptidyl di copper complex mimicking Catalase activity. The dinuclear copper complex was studied in details and characterized for its CAT activity out of biological context and in cells using different analytical methods. (see Fig 1).
For further investigation, the hits coordination sphere was studied using various techniques such EPR at low temperature, CD and UV-visible spectroscopy. The thermodynamic constants have been estimated with UV-visible competitive experiments and by ITC. The complex presents a very good CAT activity in vitro and more interestingly on Hyper HeLa cell, a genetically encoded ratiometric fluorescent sensor of H_2O_2., which highlights the efficiency of such combinatorial approach for the discovery of peptidyl complexes for therapeutic purposes.

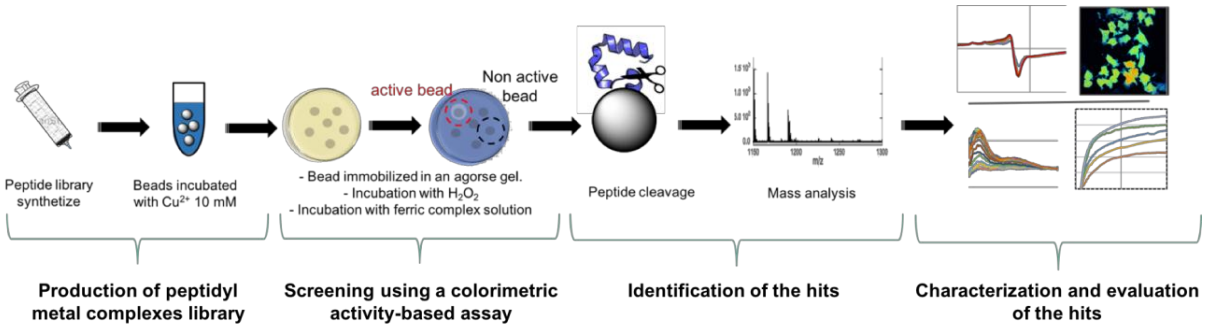


Fig. 1. *Development of novel peptide-based metal complexes mimicking CAT by a combinatorial strategy*

KEY WORDS

Peptide-Metal interaction, Screening and Identification, Characterisation methods, Catalase, Combinatorial chemistry, Oxidative stress, activity based assay, redox active complexes.

[1] Vincent, A. et al. (2019) An easy-to-implement combinatorial approach involving an activity-based assay for the discovery of a peptidyl copper complex mimicking superoxide dismutase. *Chem. Commun.* 56,399-402, https://doi.org/10.1039/c9cc07920c.

The ancient roots of nicotianamine: diversity, role, regulation and evolution of nicotianamine-like metallophores

Clémentine Laffont[a], **Pascal Arnoux[a]**

[a] *CEA Cadarache (BIAM, UMR CEA/CNRS/AMU, Saint Paul lez Durance, France)*

pascal.arnoux@cea.fr

Nicotianamine (NA) is a metabolite synthesized by all plants in which it is involved in the homeostasis of different micronutrients such as iron, nickel or zinc. In some plants it also serves as a precursor of phytosiderophores that are used for extracellular iron scavenging. Previous works have also established the presence of NA in filamentous fungi and some mosses whereas an analogue of NA was inferred in an archaea. More recently, opine-type metallophores with homology to NA were uncovered in bacteria, especially in human pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* or *Yersinia pestis*, synthesizing respectively staphylopine, pseudopaline and yersinopine.

Here, we will present the current state of knowledge regarding the discovery, biosynthesis, function and regulation of these metallophores. We will also discuss the genomic environment of the *cntL* gene, which is homologous to the plant NA synthase (NAS) gene, and plays a central role in the synthesis of NA-like metallophores. This reveals a large diversity of biosynthetic, export and import pathways. Using sequence similarity networks, we uncovered that these metallophores are widespread in numerous bacteria thriving in very different environments, such as at the host-pathogen interface but also in the soil.

We additionally established a phylogeny of the NAS/cntL gene and, as a result, we propose that this gene is an ancient gene and NA, or its derivatives, is an ancient metallophore that played a prominent role in metal acquisition or metal resistance. Indeed, our phylogenetic analysis suggests an evolutionary model where the possibility to synthesize this metallophore was present early in the apparition of life, although it was later lost by most living microorganisms, unless facing metal starvation such as at the host-pathogen interface or in some soils. According to our model, NA then emerged as a central metabolite for metal homeostasis in fungi, mosses and all known higher plants.

Simple rules govern the diversity of bacterial nicotianamine-like metallophores

Clémentine Laffont[a] and Pascal Arnoux[a]

[a] *CEA, BIAM, Aix-Marseille University, France*

clementine.laffont@cea.fr

Nicotianamine (NA) is a metabolite produced by a NA synthase (NAS) in all plants. It is involved in the homeostasis of different micronutrients such as iron, nickel, copper or zinc. In some plants, it also serves as a precursor of phytosiderophores that are implicated in extracellular iron scavenging. Previous works also established the presence of NA in filamentous fungi[1] whereas an analogue of NA was inferred from biochemical works in some archaea[2]. More recently, analogues of NA were discovered in bacteria such as *Staphylococcus aureus*[3], *Pseudomonas aeruginosa*[4], and *Yersinia pestis*[5] and were named staphylopine, pseudopaline, and yersinopine respectively.

Depending on the species, these bacterial metallophores are synthesized by two (CntLM) or three enzymes (CntKLM). When it is present, CntK, a histidine racemase, transforms L-histidine into D-histidine, such as in *S. aureus*. CntL, a NAS-like, adds an aminobutyrate moiety coming from S-adenosyl methionine (SAM) on the amino group of its second substrate (L-histidine in *P. aeruginosa* and *Y. pestis* or D-histidine in *S. aureus*) to form a pathway intermediate (noted yNA when using L-histidine, xNA when using D-histidine). Finally, CntM, an enzyme belonging to the opine dehydrogenase family, condenses the pathway intermediate with an α-ketoacid (pyruvate in *S. aureus* and *Y. pestis* or α-ketoglutarate in *P. aeruginosa*) using NAD(P)H to form an opine-type metallophore.

Focusing on the diversity of bacterial nicotianamine-like metallophores, we explored the substrate specificity of CntM by combinatorial approaches including bioinformatics, structural analysis, chemical synthesis and enzymatic studies. We unraveled that the selectivity toward α-ketoacids is largely governed by a single residue at position 150 of CntM (*S. aureus* numbering): an aspartate at this position ensures selectivity toward pyruvate, whereas an alanine leads to the consumption of both pyruvate and α-ketoglutarate. Modifying this residue in *P. aeruginosa* reverses the selectivity of the CntM enzyme. Altogether, we were able to define two simple rules governing the diversity of opine-type metallophores. First, the presence or absence of a *cntK* gene leads to the use of respectively D-histidine or L-histidine by CntL. Second, the presence of an aspartate or an alanine at position 150 on CntM (*S. aureus* numbering) results in pyruvate or α-ketoglutarate incorporation, respectively. Based on these two simple rules, the production by *Paenibacillus mucilaginosus* of a fourth metallophore called bacillopaline was discovered *in vitro*. Overall, the nature of the nicotianamine-like metallophore produced by bacteria possessing a *cnt* system could now be predicted[6].

[1] Trampczynska, A., *et al.* (2006) *FEBS Lett., 580(13), 3173–3178.*
[2] Dreyfus, C., *et al.* (2009) *Proc. Natl. Acad. Sci. USA 106(38), 16180–16184.*
[3] Ghssein, G. *et al.* (2016) *Science, 352(6289), 1105–1109.*
[4] Lhospice, S. *et al.* (2017) *Sci. Rep., 7(1), 17132.*
[5] McFarlane, J. S., *et al*. (2018) *J. Biol. Chem., 293(21), 8009–8019.*
[6] Laffont, C. *et al.* (2019) *Biochem. J., 476(15), 2221–2233.*

Phenotypic adaptation of *Pseudomonas aeruginosa* in the presence of exosiderophores.

Vincent Normant[1], Quentin Perraud[1], Sarah Fritch[1], Lauriane Kuhn[2], Philippe Hammann[2], Gaëtan LA Mislin[1] and Isabelle J Schalk[1].

[1]UMR7242, ESBS, Université de Strasbourg, CNRS, [2]Plateforme Protéomique Strasbourg-Esplanade, IBMC, CNRS,

Iron is essential for most organisms because it is involved in several biological processes such as DNA repair and replication, respiration or tricarbolxylic acid cycle. However, in aerobic condition, iron is highly insoluble and poorly bioavailable. Thus, organisms have developped many strategies to acquire this metal from the environment.

Among them is the use of siderophores. Siderophores are small molecules produced and secreted by bacteria. These compounds scavenge iron with high affinity in the bacterial environment, and transport the metal back into the bacteria via specific transporters.

P. aeruginosa, an opportunist Gram-negative multi-resistant pathogen, has developed many strategies to acquire iron from the environment: it produces two siderophores, pyoverdine and pyochelin, and has also developed many strategies to acquire iron by using siderophores (exosiderophores) produce by other bacteria and fungi.

Using proteomic and molecular biology approaches, we have investigated how *P. aeruginosa* adapts the expression of its iron acquisition pathways depending on its environment and in different growth conditions: iron restricted growth conditions, epithelial cell infection assay, in the absence or presence of several exosiderophores. In all growth conditions tested, the exosiderophores induced the expression in *P. aeruginosa* cells, of the proteins involved in iron acquisition by this chelator. Moreover, catechol-type exosiderophores were clearly more efficient in inducing the expression of their corresponding transporters than the others, showing that bacteria opt for the use of catechol siderophores to access iron when they are present in the environment. In parallel, we also observed a down-regulation of TonB-dependent transporters involved in iron acquisition by other siderophores: expression of the proteins of the pyochelin pathway was significantly repressed under most conditions tested, as well as that of proteins of the pyoverdine pathway, but to a lesser extent. In fact, *P. aeruginosa* uses preferentially exosiderophores than pyoverdine or pyochelin to get access to iron in the presence of exosiderophores. In contrast, we did not observed effect on the expression of heme and ferrous uptake pathways. All, these results were also confirmed in infection models with lung epithelial cells.

Understanding this phenotypically plasticity will allow to develop new therapeutic molecules using siderophores coupled to antibiotics in Trojan-horse strategy.

Iron acquisition by *Streptococcus pneumoniae*: PiuA specifically recognizes tetradentate Fe^{III}-catechol complexes

David P. Giedroc[a], Yifan Zhang[a], Katherine A. Edmonds[a] and Malcolm E. Winkler[b]
[a]Department of Chemistry, Indiana University, Bloomington, IN USA
[b]Department of Biology, Indiana University, Bloomington, IN USA

Streptococcus pneumoniae (Spn) is an important human pathogen that causes millions of infections worldwide with an increasing occurrence of antibiotic resistance. *Spn* is a lactic acid, hydrogen peroxide-generating bacterium that lacks a respiratory electron transport chain and a TCA cycle, and therefore lacks a significant Fe "footprint". Nonetheless, Fe acquisition is a crucial virulence determinant in *Spn*; further, *Spn* must rely on Fe^{III}-siderophore scavenging to meet nutritional Fe needs. Recent studies suggest that the human catecholamine stress hormone, norepinephrine (NE), facilitates Fe acquisition under conditions of transferrin-mediated Fe starvation. Here we show that the solute binding lipoprotein PiuA from the *piu* Fe acquisition ABC transporter PiuABCD, previously described as a Fe-hemin binding protein, binds tetradentate *bis*-catechol Fe^{III} complex using two protein-derived ligands (H238, Y321) to create a coordinately-saturated Fe^{III} complex. Tetradentate catechol Fe^{III} complexes formed by *bis*- and monomeric catechols, including the hydrolysis products of enterobactin and NE, bind PiuA with far higher affinity ($K \geq 10^9$ M^{-1}) than hemin-Fe^{III} ($K \sim 10^6 M^{-1}$). Our *in vitro* studies using ^{54}Fe LC-ICP-MS and NMR spectroscopy confirm that Fe^{III} can move from transferrin to apo-PiuA in a NE-dependent manner. Structural analysis of PiuA Fe^{III}-*bis*-catechol (by paramagnetic resonance enhancement, PRE), Ga^{III}-*bis*-catechol and Ga^{III}-$(NE)_2$ complexes by NMR spectroscopy reveals only localized structural perturbations in PiuA upon ligand binding, consistent with a recent description of other class III (cluster A) solute binding proteins. Here, we confirm tetradentate *bis*-catechol Fe^{III} complexes as an important Fe source in a Gram-positive human pathogen, and further show that SstD from the *sst* ABC transporter from *Staphylococcus aureus* likely functions in the same way. These findings parallel recent studies in the Gram-negative intestinal pathogen *Camphylobacter jejuni*. Current work is directed toward understanding the metabolic fate of nutritionally relevant catechol-Fe^{III} complexes and the transcriptional regulation of this process. Supported by NIH grant GM118157.

Deinococcus radiodurans' protection mechanisms against oxidative stress triggers the delocalization of intracellular metals

Sandra P. Santos[a,b], Yang Yang[c], Claire Bouthier De La Tour[d], Suzanne Sommer[d], Peter Cloetens[c], Isabel A. Abreu[a] and **Célia V. Romão**[a]

[a]ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa. Av. da República, 2780-157 Oeiras, Portugal.
[b]iBET - Instituto de Biologia Experimental e Tecnológica. Apartado 12, 2781-901 Oeiras, Portugal.
[c]ESRF- The European Synchrotron, CS40220, 38043 Grenoble Cedex 9, France.
[d]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91198 Gif sur Yvette, France.

cmromao@itqb.unl.pt

Deinococcus radiodurans is the most radiation resistant organism so far identified. It is a gram-positive bacterium, and besides its high resistance to different types of radiation, it is also able to endure extreme conditions of desiccation and oxidative stress [1,2]. The proposed mechanism that avoids cell death relies on the protein protection against oxidation [3], promoted by the oxidative stress generated under these extreme conditions. Therefore, the organism contains enzymatic systems, such as superoxide dismutase and catalase, which are able to detoxify reaction oxygen species. Moreover, it contains a highly efficient non-enzymatic system that involves small complexes of manganese and other small molecules, such as phosphate [4]. Currently, neither the homeostasis of Mn nor the intracellular localization and formation of these small complexes are yet fully understood. We have been addressing this question by studying two proteins, the DNA-binding proteins, Dps1 (*dr2263*) and Dps2 (*drb0092*), under starvation conditions [5-8]. Our results showed that these proteins have the ability to store Mn and Fe as well as to bind/protect DNA under *in vitro* conditions [7]. Moreover, structurally these proteins share a highly conserved dodecameric structure, although under *in vivo* conditions, Dps1 assumes different oligomeric forms [5,6,8]. In order to complement our studies, we have used synchrotron X-ray fluorescence nano-imaging [9]. This technique allows precise intracellular localization of the trace metals within their original cellular context. Our results show that cell protection against ROS is dependent on the metal distribution inside the cell. Moreover this mechanism is dependent on both Dps. In conclusion, we will present our recent results in which the use of X-ray fluorescence nano-imaging was crucial to elucidate of how *D.radiodurans* deals with a burst of oxidative stress that could be promoted by the exposure to radiation.

References

1. White et al (1999) *Science*, 286, 1571.
2. Slade & Radman (2011). *MMBR*, 75, 133.
3. Daly et al (2007) *PLoS Biol*, 5, e92.
4. Daly et al (2004) *Science*, 306, 1025.
5. Romao et al (2006) *JBIC*, 11, 891.
6. Cuypers et al., (2007) *JMB*, 371, 787.
7. Santos et al (2015) *FEBS J*, 282, 4307.
8. Santos et al (2017) *JMB*, 429, 667.
9. Santos et al (2019) *Scient Rep,* 9, 17217.

Discovery and characterization of UipA, a uranium-binding protein involved in bacterial uranium tolerance

Abbas Mohamad Ali[a], Nicolas Gallois[a], Béatrice Alpha-Bazin[b], Nicolas Bremond[a], Philippe Ortet[a], Mohamed Barakat[a], Laurie Piette[a], David Lemaire[a], Pierre Legrand[c], Christophe den Auwer[d], Pascal Arnoux[a], Catherine Berthomieu[a], Jean Armengaud[b], and Virginie Chapon[a].

[a] *DRF/BIAM, CEA, CNRS, Aix-Marseille Université, France.*
[b] *DRF/Joliot/DMTS, CEA, INRAE, Université Paris Saclay, France*
[c] *Synchrotron SOLEIL, France*
[d] *CNRS, Institut de Chimie de Nice, Université Côte d'Azur, France*

abbas.mohamadali@cea.fr

Uranium is a naturally occurring radioactive element and its redistribution in the environment is mainly due to human activities. The bioavailability and toxicity of uranium depend on its speciation. Bacteria can interact with uranium and modulate its speciation, thus playing a major role in its mobility and transfer in the environment[1]. In addition, a number of uranium-tolerant bacterial strains have been described but the molecular mechanisms involved in this process are not fully understood. Using a *Microbacterium* strain isolated from contaminated soils from Chernobyl, we have previously characterized the mechanisms of uranium-cells interactions and the impact of uranium stress on its proteome[2,3]. To get a broader view of uranium stress response and to better understand the mechanisms of tolerance to this metal, we performed a comparative proteogenomic analysis of this strain and three other *Microbacterium* strains, which differ in their ability to tolerate uranium. We identified the key proteins involved in uranium stress response, which led to the discovery of a protein of unknown function, specific to uranium-tolerant strains and highly up-produced in response to uranyl. With a combination of spectrometric and spectroscopic approaches, we showed that this protein, named UipA (Uranium induced protein A) is a UO_2^{2+}- and Fe^{3+}-binding protein. The three dimensional structure of UipA, solved in its apo and uranium bound states, revealed two PepSY domains with uranium binding sites stabilized by a set of conserved residues. Analysis of the protein topology *in vivo* showed that UipA is a single pass transmembrane protein, with a short cytoplasmic N-ter segment and its most part exposed to the extracellular side. Based on our data, we propose that UipA could be part of a system involved in uranium sensing and tolerance in *Microbacterium*.

(1) Newsome, L., K. Morris and J. R. Lloyd (2014). "The biogeochemistry and bioremediation of uranium and other priority radionuclides." Chemical Geology **363**: 164-184.
(2) Theodorakopoulos, N., V. Chapon, F. Coppin, M. Floriani, T. Vercouter, C. Sergeant, V. Camilleri, C. Berthomieu and L. Fevrier (2015). "Use of combined microscopic and spectroscopic techniques to reveal interactions between uranium and *Microbacterium* sp. A9, a strain isolated from the Chernobyl exclusion zone." J Hazard Mater **285**: 285-293.
(3) Gallois, N., B. Alpha-Bazin, P. Ortet, M. Barakat, L. Piette, J. Long, C. Berthomieu, J. Armengaud and V. Chapon (2018). "Proteogenomic insights into uranium tolerance of a Chernobyl's Microbacterium bacterial isolate." J Proteomics **177**: 148-157.

In vitro biological water-gas shift reaction: characterization and grafting of recombinant *Rhodospirillum rubrum* CO-dehydrogenase

Umberto CONTALDO[a], Bruno Guigliarelli[b], Julien Perard[a], Clara Rinaldi[a], Alan Le Goff[c] and Christine Cavazza[a]

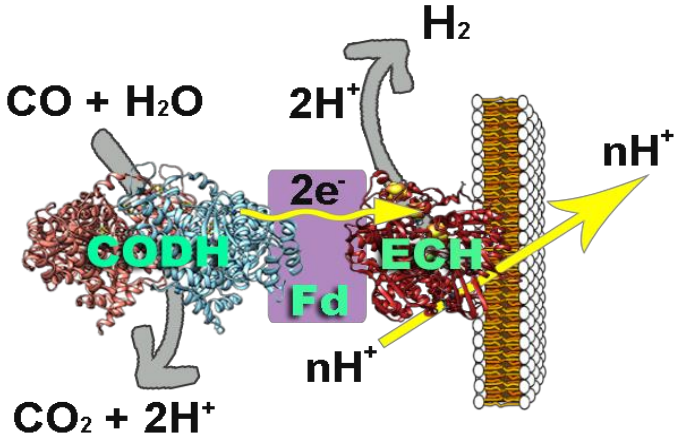
[a] *Chemistry and Biology of Metals Laboratory, CEA-Univ. Grenoble Alpes, France*
[b] *Bioenergetics and Protein Engineering Laboratory, CNRS-Aix Marseille Univ., France*
[c] *Electrochemical and Analytical Biosystems, DCM-Univ. Grenoble Alpes, France*

Umberto.CONTALDO@cea.fr

In the perspective of a green economy, one of the main challenges is the discovery of low-cost environmental friendly fuels. One interesting candidate is syngas, produced by the gasification of biomass or from waste, resulting in a variable mixture of H_2 and CO. Achieving the accurate control of this ratio is fundamental to exploit syngas at the industrial level. Currently, one of the most utilized methods is the water gas shift reaction (WGSR).

$$CO + H_2O \rightleftharpoons CO_2 + H_2$$

In spite of its potential, this reaction requires inorganic catalysts working at high temperature and pressure, with low specificity, which hinder its sustainability. As often happens in recent years, nature offers an alternative solution. Carbon monoxide dehydrogenase (CODH), an enzyme able to catalyze the oxidation of CO to CO_2, can be coupled with [NiFe]-hydrogenase to perform the biological WGSR[1].



In order to efficiently use micro-organisms, it is fundamental to deeply understand how this process occurs. Our goal is to optimize the large-scale production of fully active CODH in the easy-to-grow bacterium, *Escherichia coli.* Here we present a novel method for the production and purification of *Rhodospirillum rubrum* CODH in *E. coli*, as well as its biochemical, spectroscopical and electrochemical characterisation. The recombinant enzyme purified with a good yield shows an efficient CO oxidation activity. Moreover, the enzyme was successfully immobilized on carbon nanotubes and we observed for the first time a the direct electron transfer. The further step will be the in vitro immobilization of the recombinant *Rr*CODH with a [NiFe]-hydrogenase on carbon nanotubes to optimize the efficiency of the biological WGSR.

[1] Alfano and Cavazza, *Sustainable Energy & Fuels,* **2018**, *2*, 1653-1670.

Molecular mechanisms of Resistance to Radiation

Sara Silva[a] and Célia V. Romão[a]

[a]*ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa. Av. da República, 2780-157 Oeiras, Portugal.*

saras@itqb.unl.pt

Ionizing radiation, such as UV, X-ray and gamma radiation, has deleterious effects on molecules, and yet we are exposed to it in various situations, from sun exposure to radiation therapy and X-ray analysis. *Deinococcus radiodurans* is a gram-positive bacteria and one of the most radiation-resistant organisms known to date. We use this organism to study molecular mechanisms of protection against radiation-induced oxidative stress.

D. radiodurans employs various strategies to protect the genome, proteome and membranes from radiation. Dps (DNA-binding protein under starvation) proteins, Dps1 and Dps2, are part of a system of oxidative stress protection. These proteins bind DNA under stress conditions, and are able to store metals, such as iron and manganese.

Here we show how Dps1 and Dps2 are displaced inside the cells upon exposure to X-rays. Moreover we use X-ray crystallography to determine the structure of Dps1 loaded with Zinc and co-crystallized with DNA, which seems to induce an extended conformation of the N-terminus.

Dissecting the mechanism for cadmium toxicity in *Deinococcus radiodurans* R1

Ruchi Chaturvedi[1, 2*], and G. Archana[2]

[1]Department of Biological Sciences. P.D. Patel Institute of Applied Sciences, Charotar University of Science and Technology. Changa. Gujarat, India.

[2]Department of Microbiology and Biotechnology Centre, M. S. University of Baroda, Vadodara, Gujarat, India

*ruchichaturvedi.bio@charusat.ac.in; ruchideinos@gmail.com

ABSTRACT

Metal interactions in *D. radiodurans* R1 (DR1), a ionising radiation resistant bacteria, are of interest not only from the point of view of metal detoxification/remediation but also due to the important role that certain heavy metals play in the radiation resistance physiology of this organism. Cadmium is a highly toxic metal and a common contaminant present at the radioactive waste sites. Although it has virtually no biological functions but Cd^{2+} exerts its toxicity through the induction of oxidative stress. Cd^{2+} toxicity is also due to their strong affinity for –SH groups and ability to compete with other divalent metal ions for binding to proteins. The effect of this metal on radiation resistance in deinococci has not been well explored. The present work demonstrates that in DR1 Cd^{2+} toxicity is essentially due to induction of oxidative stress that leads to the accumulation of carbonylated proteins and lipid peroxidation. The induction of oxidative stress was demonstrated to be due to the inhibition of two key- antioxidant enzymes super-oxide dismutase (SOD) and catalase. Additionally, Cd^{2+} also negatively affected DNA repair pathway and the transcriptional factors associated in the control of expression of *rec* A and oxidative stress combative enzymes. This work is able to highlight the mechanism of Cd^{2+} toxicity despite an expansive expression of both anti-oxidative enzymes and efficient DNA repair mechanism in DR1.

The fate of uranium in plants – Physiological and cellular effects

Jacques Bourguignon[a], B. Revel[a], M. Sarthou[a], N. Serre[a], J. Martin-Laffon[a], A-M Boisson[a], S. Figuet[a], A. Favier[b], A. Vallet[b], T. Rabilloud[c], B. Brutscher[b], P. Catty[c], C. Alban[a] and S. Ravanel[a]

[a] *PCV/IRIG, Univ. Grenoble Alpes, France*
[b] *IBS/IRIG, Univ. Grenoble Alpes, France*
[c] *CBM /IRIG, Univ. Grenoble Alpes, France*

jacques.bourguignon@cea.fr

Uranium (U) is a non-essential trace metal element that is ubiquitous in the Earth crust, with average worldwide concentrations of 3 ppm in soil and 3 ppb in sea water. The radionuclide is primarily redistributed in the environment by anthropogenic activities related to U mining and milling industries, civil and military nuclear activities, and extensive enrichment of agricultural soils with phosphate fertilizers, which may be significantly contaminated with U. The accumulation of U in soil, water and air can lead to potential risks to ecosystems, agrosystems, and ultimately human health, as the radionuclide has both chemical and radiological effects. Natural U is of low radiotoxicity due to its isotopic composition (>99% ^{238}U) but the uranyl ion (UO_2^{2+}) that is prevalent in oxidizing environments is highly chemotoxic for all living organisms. Uranium is known to be taken up by plants and to have cellular toxic effects but none of the proteins involved in these processes has been identified so far. To fill this gap, we have designed a series of experiments to gain insight into the molecular mechanisms governing the fate of U in plants. Some aspects of this work will be presented.

In a previous transcriptomic analysis, we showed that *Arabidopsis thaliana* plants challenged with U displayed an important perturbation of iron uptake and signalling response. U also altered expression of genes involved in phosphate homeostasis, hormone synthesis and signalling, and cell wall metabolism (1). Recently, we showed that U might have a positive impact on plant physiology. We revealed that U is able, in a chlorotic Arabidopsis mutant, to displace iron, making it available for chloroplast biogenesis and leading to a greening of the leaves (2). More recently, we analysed the impact of U on the root system of Arabidopsis plants and dissected the sequence of cellular mechanisms that leads to the accumulation of the cell wall polymers callose and lignin to control spreading of the toxic metal (3).

We also used *Saccharomyces cerevisiae* as a model to identify yeast transporters that could be involved in the uptake of U. We performed competition experiments in the presence of U and different essential metals and used strains deleted in genes coding for metal importers.

In order to go deeper in the analysis of the molecular mechanisms of U toxicity in plants, we aim to identify the U-binding proteins (UBP) that could be either direct cellular targets of U toxicity or proteins involved in U cellular detoxification. To reach this goal, we developed metalloproteomic strategies (4 and unpublished data) and identified 38 UBP from *Arabidopsis thaliana* root, shoot and cell extracts and we studied the biochemical and structural properties of some selected UBP.

(1) Doustaly et al (2014) Uranium perturbs signaling and iron uptake response in Arabidopsis thaliana roots *Metallomics* 6: 809–821. (2) Berthet et al (2018) Arabidopsis thaliana plants challenged with uranium reveal new insights into iron and phosphate homeostasis *New Phytol*. 217:657-670. (3) Serre et al (2019) Uncovering the physiological and cellular effects of uranium on the root system of *Arabidopsis thaliana*. *Env. Exp. Bot*. 157:121-130. (4) Sarthou et al (2020) Development of a metalloproteomic approach to analyse the response of Arabidopsis cells to uranium stress. *Metallomics*. In press.

Elucidating the mechanism of uranium uptake and processing in tobacco BY-2 cells

Warren John[1,*], Nicole Matschiavelli[1], Domenika Thieme[2], Wolfgang Hoehenwarter[2], Susanne Sachs[1]

[1]Institute of Resource Ecology, Helmholtz-Zentrum Dresden – Rossendorf, Germany

[2]Leibniz Institute of Plant Biochemistry, Germany

[*]Corresponding author, Email: w.john@hzdr.de

The release of uranium from waste repositories into the ground water and surrounding soil can have adverse effects on the biomes of affected sites. The bioavailability and chemical toxicity of U(VI) species, which are the most prevalent in oxic environments of soils and water bodies, can pose serious threats as they are transferred through the food chain. Despite remediation strategies employing the cultivation of crop plants to sequester uranium, little is known of the mechanisms used by plants in processing the uranium species that they encounter. The aim of this research therefore has been to shed light on the pathways involved in the uptake and processing of uranium by plant cells, using the undifferentiated tobacco BY-2 cells as model plant cells. Former experiments showing increases in the cytoplasmic glutathione pools upon exposure of *Brassica napus* cell cultures to uranium[1] have led us to the hypothesis that tobacco cells are able to reduce U(VI) to U(IV). This research describes a novel method of exposing BY-2 cells to U(VI) in phosphate deficient medium, which maintains relatively high cell viability under phosphate deficient conditions, and reveals differentially expressed proteins in the presence of uranium. Uranium-spiked culture medium was seen to affect the uptake of trace elements and minerals as well as show changes in the profiles of polyacrylamide-resolved proteins. Proteomics is being used to identify candidate proteins involved in the processing of uranium by the cells and microscopic visualization techniques are utilized to confirm these pathways and mechanisms.

Acknowledgments: This work is funded by the German Federal Ministry of Education and Research under the contract number 02NUK051B.

[1] Viehweger, K., Geipel, G. & Bernhard, G. (2011) *Biometals* 24: 1197 - 1204

A highly sensitive magnetic whole-cell biosensor for arsenic detection

Anissa Dieudonné[a], Sandra Prévéral[a] and David Pignol[a]

[a] *Aix Marseille Univ, CEA, CNRS, BIAM, UMR 7265, Saint Paul-Lez-Durance France*

anissa.dieudonne@cea.fr

Arsenic is a metalloid naturally present in the earth crust. Because of its relative abundance and of anthropogenic activities, inorganic arsenic is released in water and is the contaminant that affects the health of the largest number of people worldwide, according to the World Health Organization. The most vulnerable populations have few resources, thus an affordable, efficient and easy-to-use way to detect arsenic is needed. A solution is whole-cell biosensors, they can meet all this criteria but they still often require optimization for the sensitivity and specific adaptations for practical use in the field. Here we use magnetotactic bacteria as cellular chassis to build magnetic bacterial biosensors for arsenic detection. Their natural magnetism allows their easy concentration and entrapment to increase the arsenic-response signal. Promoters potentially inducible by arsenic were first identified *in silico* within the genomes of two magnetotactic bacteria strains: *Magnetospirillum magneticum* AMB-1 and *Magnetospirillum gryphiswaldense* MSR-1. The ArsR-dependent regulation was confirmed by reverse transcription PCR experiments. Biosensors built by transcriptional fusion between the arsenic-inducible promoters and the bacterial luciferase *luxCDABE* operon gave an element-specific response in 30 minutes with an arsenite detection limit of 0.5 µM. After magnetic concentration, we improved the sensitivity of the biosensor by a factor of 50 to reach 10 nM, more than one order of magnitude below the recommended guidelines for arsenic in drinking water (0.13 µM). Finally, we demonstrated the successful conservation of the biosensors by freeze-drying. This paves the way for the development of sensitive and immobilized whole-cell biosensors tailored for use in the field.

In vivo speciation and uptake mechanisms of Uranium in Paracentrotus lividus sea urchin and Laminaria digitata seaweed

Maria Rosa Beccia[a], Benjamin Reeves[a,b], Romualdo Bianco[a], Pier L. Solari[c], Catherine Leblanc[d], David K. Shuh[e], Marguerite Monfort[b], Christophe Moulin[f] and Christophe Den Auwer[a]

[a] *Institut de Chimie de Nice, Université Côte d'Azur, France*
[b] *DAM/DIF/F-91297, Commissariat à l'Energie Atomique, France*
[c] *MARS beamline, Synchrotron SOLEIL, France*
[d] *Station Biologique de Roscoff, Sorbonne Université, CNRS, France*
[e] *Chemical Sciences Division, Lawrence Berkeley National Laboratory, California, USA*
[f] *Secrétariat Général de la Défense et de la Sécurité Nationale, France*

maria-rosa.beccia@univ-cotedazur.fr

Seawater is the ultimate receptacle for radioactive metals coming from contaminated waters. As a result, marine organisms may be exposed to such metals, which may affect their biological processes. Current studies on global accumulation rates are no longer sufficient because of the complexity of the impacted matrices and because metal bioavailability and toxicity strongly depend on chemical speciation and molecular mechanisms.[1] Thus, a comprehensive approach at the molecular level is necessary to assess the bio-environmental impact of radioactive metals at larger scales. We present a multi-spectroscopic investigation of uranium (U) speciation and uptake mechanisms in two model marine organisms: the sea urchin *P. lividus* (animal) and the seaweed *L. digitata* (algae). For both organisms we evaluated the amount of accumulated U in separated tissues by ICP-OES. For *P. lividus,* we determined U speciation in the gonads, the digestive tube and the test compartments, by TRLIF and XAS spectroscopy. Our results suggest that the transferrin-like protein Toposome is involved in U uptake in the first two compartments (Figure). Scanning Transmission X-ray Microscopy reveals that U is diffusely distributed over the cells. On the other hand, $CaUO_2(CO_3)_3$ is the main U species in the test.[2] For *L. digitata,* SEM microscopy reveals the formation of U nano-clusters inside the cells of blades and meristem, especially on the inside of cell walls (Figure). XAS spectroscopy shows the presence of phosphate ligands around UO_2^{2+} ion. Because alginate is one of the main constituents of algae cell walls, UO_2^{2+}-alginate interaction has also been investigated. IR spectra show an interaction between the carboxylic functions of alginate and UO_2^{2+} ion. These results provide new insights on the biochemical mechanisms of uranium uptake by marine organisms and thus on its impact on marine ecosystem.

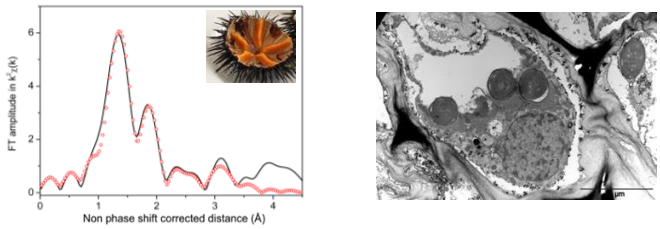


***Figure. Left**: Experimental EXAFS spectrum (circles) and best fit (line) for contaminated P. lividus gonads, at U L_{III} edge. Best fit is in agreement with a transferrin-like uranyl binding site.* ***Right:** SEM imaging of contaminated L. digitata blade. Uranium clusters visible on the inside of the cell wall.*

[1] M. R. Beccia, P. L. Solari, M. Monfort, C. Moulin, C. Den Auwer (2018). *New J. Chem*. 42: 7582-7591.
[2] B. Reeves, M. R. Beccia, P. L . Solari, D. E. Smiles, D. K. Shuh, C. Berthomieu, L. Mangialajo, S. Pagnotta, M. Monfort, C. Moulin, C. Den Auwer, (2019) *Environ. Sci. Technol*. 53, 7974-7983

Azido-Desferrioxamine Siderophores as Functional Click-Chemistry Probes Generated in Culture upon Adding a Diazo-Transfer Reagent

Michael P. Gotsbacher[a] and Rachel Codd[a]

[a] *School of Medical Sciences (Pharmacology), The University of Sydney, Australia*

michael.gotsbacher@sydney.edu.au

This work aimed to undertake the *in situ* conversion of the terminal amine group of bacterial DFO siderophores, including desferrioxamine B (DFOB, **1**), to an azide group to enable downstream click chemistry. Initial studies trialled a precursor-directed biosynthesis (PDB) approach[1]. Supplementing *Streptomyces pilosus* culture with blunt-end azido-amine non-native substrates designed to replace 1,5-diaminopentane (DP) as the native diamine substrate in the terminal amine position of DFOB did not produce azido-DFOB. Adding the diazo-transfer reagent imidazole-1-sulfonyl azide hydrogen sulfate (**17**)[2] to spent *S. pilosus* medium that was cultured in the presence of 1,4-diaminobutane (DB), as a viable native substrate to expand the suite of native DFO-type siderophores **1** and **2**–**9** (Fig. 1a), successfully generated the cognate suite of azido-DFO analogues in apo (**2** and **10**–**16**, Fig. 1b) and Fe^{III}-loaded form (Fe^{III}·**2,** Fe^{III}·**10**–Fe^{III}·**16**, Fig. 1c). Cu^{I}-mediated or strain-promoted Cu^{I}-free click chemistry reactions between this minimally processed mixture and the appropriate alkyne-biotin reagents produced the cognate suite of 1,4-disubstituted triazole-linked DFO-biotin compounds as potential molecular probes, detected as Fe^{III}-loaded species Fe^{III}·**29**–Fe^{III}·**32** and Fe^{III}·**34**–Fe^{III}·**37**, respectively (Fig. 1d–e).[3] The amine-to-azide transformation of amine-bearing natural products in a complex mixture by the direct addition of a diazo-transfer reagent to deliver functional click chemistry reagents adds to the toolbox for chemical proteomics, chemical biology and drug discovery.

References

[1] T. J. Telfer, et al., *ACS Chem. Biol.*, **2016**, *11*, 1452-1462;

[2] G. T. Potter, et al., *J. Org. Chem.*, **2016**, *81*, 3443-3446;

[3] M. P. Gotsbacher and R. Codd, *ChemBioChem*, **2020**, DOI: 10.1002/cbic.201900661.

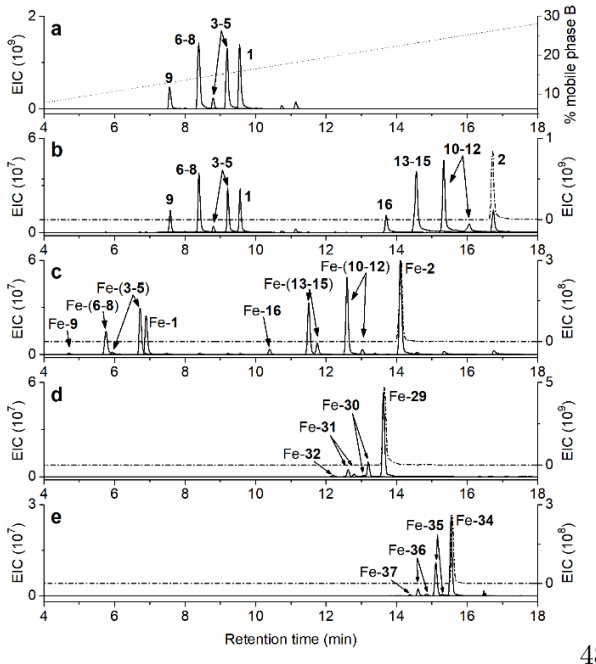


Figure 1: LC-HR-MS EIC traces of (a) **1**, **3**–**9** (DB-supplemented culture supernatant); (b) **1**–**16** upon treatment of (a) with **17**; (c) Fe-**1**–**16** upon addition of Fe^{III} to (b); (d) Fe^{III}·**29**–Fe^{III}·**32** upon treatment of (c) with a biotinylated terminal alkyne using CuAAC click chemistry; and (e) Fe^{III}·**34**–Fe^{III}·**37** upon treatment of (c) with a biotinylated strained cycloalkyne using SPAAC click chemistry. Traces from biological samples spiked with synthetic azido-DFOB (2), Fe^{III}·**2**, Fe^{III}·**29** or Fe^{III}·**34** are

shown in (b)–(e), respectively, as broken lines (scaled to the y-axis at the right).

Elucidating the mechanism for the biosynthesis of *N*-acetyl desferrioxamine siderophores

Kate P. Nolan[a], Josep S. Font[a], Athavan Sresutharsan[a], Michael P. Gotsbacher[a], Chris J.M. Brown[a,] Renae M. Ryan[a] and Rachel Codd[a].

[a] *School of Medical Sciences (Pharmacology), The University of Sydney, Australia*

knol6293@uni.sydney.edu.au

Desferrioxamine B (DFOB) is a tri-hydroxamic acid siderophore biosynthesised by *Streptomyces pilosus* and other bacteria within the Actinomycete phylum. It is used clinically to treat secondary iron overload that occurs from the treatment of transfusion-dependent blood disorders such as beta thalassaemia. DFOB biosynthesis occurs *via* a nonribosomal peptide synthetase independent pathway, involving a cascade of four enzymes, the DesABCD cluster [1,2]. Des C is an acyl transferase that catalyses the *N*-acetylation or *N*-succinylation of *N*-hydroxy-1,5-diaminopentane (HDP). This generates *N*-acetyl-*N*-hydroxy-1,5-diaminopentane (AHDP) or *N*-succinyl-*N*-hydroxy-1,5-diaminopentane (SHDP), respectively. Des D then condenses one SHDP monomer to an AHDP-SHDP dimer to produce the trimeric siderophore. [3].

$DFOD_1$ is an *N*-acetylated variant of DFOB, identified in culture supernatant of *S. pilosus* and other bacteria such as *Salinispora tropica* [4]. *N*-Acetyl fluorinated analogues of DFOB were detected in a recent study which prompted the current study on the biosynthesis of these constructs [5]. This work is focused on delineating the steps involved in the biosynthesis of *N*-acetylated $DFOD_1$ and precursors.

Des C and Des D have been recombinantly expressed from *S. tropica* and overproduced in *Escherichia coli*, with the purified protein used for functional studies with synthetic precursors of the siderophore assembly. Functional studies will focus on whether Des C is capable of iterative cycles of acylation. Studies will also address whether Des D is capable of condensing the acetylated monomers. This work will report results that provide new knowledge on the biosynthesis of this subclass of siderophore. These mechanisms could have wider implications for the *N*-acetylation pathway for other natural products.

[1] Kadi N, Barona-Gomez F, Challis GL (2007). Nat Chem Biol 3(10):652-656

[2] Challis GL (2005). ChemBioChem 6**:** 601-611.

[3] Telfer TJ, Gotsbacher MP, Soe CZ, & Codd R (2016). ACS Chem Biol 11**:** 1452-1462.

[4] Ejje N, Soe CZ, Gu JS, & Codd R (2013). Metallomics 5**:** 1519-1528.

[5] Telfer TJ, & Codd R (2018). ACS Chem Biol. 13(9):2456-2471

A Novel Total Synthetic Approach to Desferrioxamine B Analogues

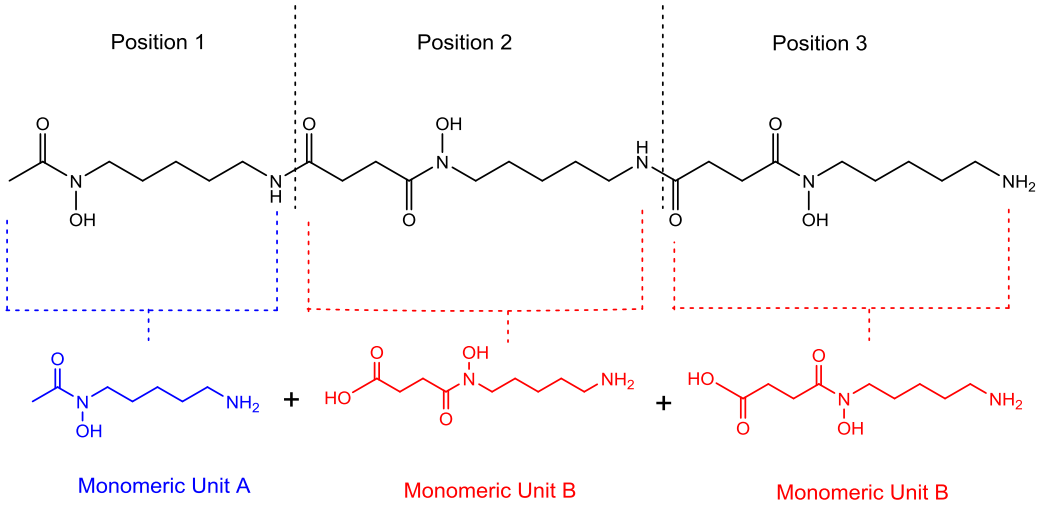
Athavan Sresutharsan[a], Christopher Brown[a], Tomas Richardson-Sanchez[a] and Rachel Codd[a]

[a]*School of Medical Sciences (Pharmacology), University of Sydney, Camperdown, NSW 2006, Australia*

athavan.sresutharsan@sydney.edu.au

Desferrioxamine B (DFOB) is a linear siderophore which forms stable hexacoordinate, octahedral complexes with Fe(III) through three bidentate hydroxamic acid functional groups. The exquisite affinity for Fe(III) indicated the clinical use of DFOB as a scavenger for excess iron in patients with transfusion-dependent haemoglobin disorders. The potential clinical utility of DFOB is under scrutiny, which warrants further study into new DFOB analogues with new properties and function.

Studies have used biological and semisynthetic approaches to produce analogues of DFOB, with other work focusing on total synthesis [1,2]. The proposed synthesis in the current work is predicated on the trimeric structure of DFOB consisting of one monomeric unit A linked to two monomeric unit Bs. Synthesis of native and ether containing monomeric units A and B allowed for the production of a suite of eight analogues of DFOB, including native DFOB, with ether subunits inserted into positions 1 and/or 2 and/or 3. These constitutional isomers are predicted to co-elute with the compounds generated in our group using biosynthetic methods. The proposed synthetic scheme is highly flexible and can be adapted to produce various structural analogues. The synthetic route provides access to trihydroxamic acid adducts as well as dihydroxamic acid and other analogues. Improved access to structural variety may reveal nuances in the relationship between DFOB structure and properties that may inspire further therapeutic use.



References

[1] T. Richardson-Sanchez, W. Tieu, M.P. Gotsbacher, T.J. Telfer, R. Codd. Org. Biomol. Chem. 27 (2017) 5719-5730.

[2] A.R. Poreddy, O.F. Schall, T.A. Osiek, J.R. Wheately, D.D. Beusen, G.R. Marshall, U. Slomczynska. J. Comb. Chem. 6 (2004) 239-254.

IRON (III) AND GALLIUM (III) COMPLEXES OF FERRIOXAMINE E BIOMIMETIC ANALOGS - COORDINATION SPECIFICITY AND POSSIBLE APPLICATIONS

Andrzej Mular M. Sc.[a], Prof. Elżbieta Gumienna-Kontecka Ph.Dsc.[a] and Prof. Henryk Kozłowski Ph.Dsc.[a]

[a]*Faculty of Chemistry, Wroclaw University, Poland*
andrzej.mular@chem.uni.wroc.pl

Multi-drug-resistant bacterial and fungal infections are emerging problem. Today 700 000 lives are lost annually because of antimicrobial resistance. This situation is more severe alongside patients with immune deficits where mild infections can become lethal despite treatment [1]. That is why we seek to develop new ways of fighting infections.

Novel treatments and ways to specifically deliver them to drug resistant bacteria and invasive mycoses are being actively sought. We aim to find pathogen-specific therapeutics that will not cause severe side-effects in patients in accordance to selective toxicity rule.

Those properties are presented by wide group of microbe-produced low molecular weight compounds – siderophores, which are used by bacteria and fungi to acquire ferric ions that are crucial for their viability. Although pathogen-selective targets are scarce, what arises from the fact that bacteria and fungi share essential metabolic pathways with humans there is at least one significant difference between the microbial and mammalian cells: the transport of transition metal ions.

We want to focus on the transport of indispensable metal ion – Fe(III). It's effective acquisition is often considered as a virulence factor. Bacteria and fungi have developed highly efficient transport systems, which rely on siderophores – metal chelating molecules which are excreted outside the pathogen in order to efficiently bind iron ion. Then, the ferric-siderophore complex is uptaken via high specificity receptors into bacterial cell [2].

To fully understand mechanisms of those processes and utilize them, we need to examine thermodynamics and coordination chemistry of siderophores towards iron ions. It is also a necessity to comprehend transportation mechanisms of siderophore complexes. To make that possible we are investigating not only natural siderophores but also analogs with structural differences.

We also incorporate gallium (III) to our research as it can successfully mimic iron (III) in its complexes and be used as radioactive probe for nuclear imaging or ferric enzymes inactivating drug [3,4].

Here we will present our current advancements in research concerning Ferrioxamine E family of siderophores alongside with its biomimetic analogs.

References:

[1] Antimicrobial Resistance in G7 Countries and Beyond: Economic Issues, Policies and Options for Action, Michele Cecchini et all, OECD report 2015

[2] Harnessing the power of fungal siderophores for the imaging and treatment of human diseases, Agnieszka Szebesczyk et all, CCR, 327–328, 84-109, 2016

[3] Repurposing of gallium-based drugs for antibacterial therapy, Carlo Bonchi et all, BioFactors, 40, 303-312, 2014

[4] Hybrid Imaging of Aspergillus fumigatus Pulmonary Infection with Fluorescent, 68Ga-Labelled Siderophores, Pfister, J, Biomolecules, 10, 16, 2020

Acknowledgements: The financial support from the Polish National Science Centre (NCN UMO-2017/26/A/ST5/00363) is gratefully acknowledged.

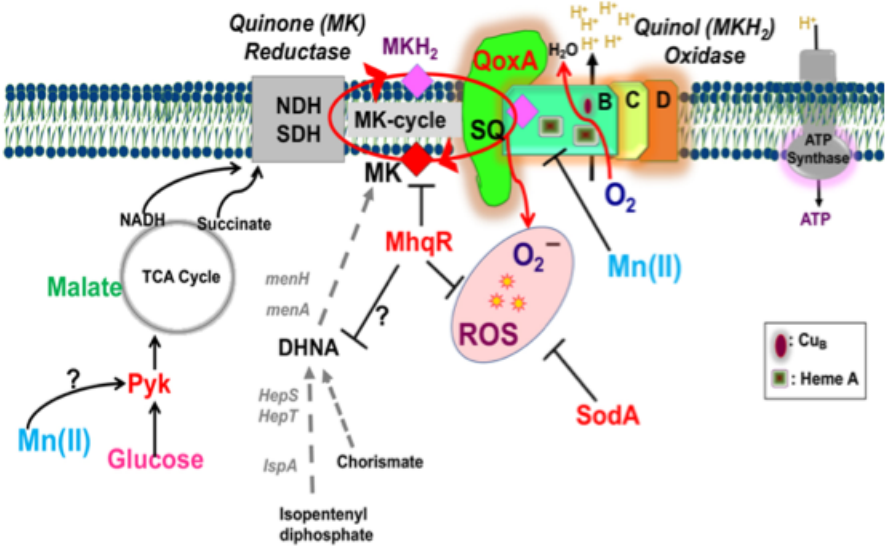
Manganese homeostasis in *Bacillus subtilis*

A. J. Sachla and **John D. Helmann**

Department of Microbiology, Cornell University, USA

jdh9@cornell.edu

Cell physiology relies on the coordinated activity of numerous metalloenzymes and can easily be disrupted by imbalances in metal ion pools. *Bacillus subtilis* requires manganese for growth and has highly regulated mechanisms for both import and efflux that help maintain homeostasis. MntR is the central regulator of Mn(II) homeostasis and acts as both a transcriptional repressor of import systems (*mntABC* and *mntH*) and an activator of two cation diffusion facilitator efflux pumps (*mneP* and *mneS*). Mutant cells (*mneP mneS*) defective for manganese efflux are highly sensitive to manganese intoxication. However, the intracellular targets of Mn(II) mismetalation are unknown, as are the physiological consequences of intoxication. Here, we employed a forward genetics approach to identify pathways affected by manganese intoxication. Our results highlight a central role for the membrane-localized electron transport chain (ETC) during intoxication. In the presence of elevated manganese there is an increased generation of reactive oxygen species (ROS) associated with dysfunction of the major terminal oxidase, the cytochrome aa_3 heme copper menaquinol oxidase (QoxABCD). Manganese intoxication is suppressed by increased expression of alternative oxidases, or by a mutation in heme A synthesis that converts QoxABCD aa_3 oxidase to a bo_3-type oxidase. Suppression is also achieved by increased expression of superoxide dismutase or by derepression of the MhqR regulon, which protects cells against reactive electrophiles. These results suggest that the cytochrome aa_3-type quinol oxidase is a target of manganese mismetalation, resulting in a diversion of electrons to generate ROS, thereby contributing to intoxication. This process may have relevance to the ability of manganese to impair mitochondrial respiration, which relies on a similar oxidase system, and thereby contribute to human disease.



Metalloregulation of cyanobacterial physiology operated by FUR (ferric uptake regulator) proteins.

María F. Fillat

Departamento de Bioquímica y Biología Molecular y Celular and Instituto de Biocomputación y Física de Sistemas Complejos, Universidad de Zaragoza, Zaragoza 50009, Spain.

fillat@unizar.es

Cyanobacteria are one of the main primary producers of the biosphere and contribute significantly to the fixation of CO_2 and atmospheric N_2. Their high metal requirements at redox-sensitive centres of the photosynthetic and respiratory electron transport chains makes metalloregulation in cyanobacteria a critical issue for their ecological success. FUR (ferric uptake regulator) proteins are the major reponsible for keeping metal homeostasis in cyanobacteria in a coordinated fashion with carbon and nitrogen metabolisms and the oxidative stress response. In *Anabaena* sp. PCC7120 (herein *Anabaena*), the FUR family consists of three members that correspond to the Fur (FurA), Zur (FurB) and PerR (FurC) paralogs. Unlike other Fur orthologues, FurA does not exhibit structural zinc and is a redox protein. Beyond the direct transcriptional control of regulated genes, FurA can bind small molecules, such as heme and 2-oxoglutarate, modulating its affinity for DNA. FurB (Zur), in addition to control zinc homeostasis, regulates a set of genes crucial for cellular defense against reactive oxygen species. FurB integrates the response to zinc availability with the redox environmental conditions through thiol-mediated redox modulation and/or heme binding. The FurC (PerR) regulon includes a set of genes involved in the response to peroxide, some components of the phycobilisome and cell division genes, including the major thylakoid membrane protease *ftsH*, among others. Interestingly, the effect of nitrogen deficiency in the transcriptome of a FurC-overexpressing *Anabaena* variant is notably higher than in the WT *Anabaena*. Direct regulation by FurC of key genes involved in nitrogen metabolism and heterocyst development has been verified, expanding the regulon of a PerR orthologue. Finally, deregulation of FUR paralogs affects the synthesis of exopolysaccharides and the formation of biofilms by *Anabaena* cultures, indicating that the FUR family modulates either directly or indirectly this process.

Characterization of the stress-induced molecular signal that activates IrrE, a metalloprotease essential for extreme radiation resistance in *Deinococcus* bacteria

Arjan de Groot, Romaric Magerand, and Laurence Blanchard

CEA-DRF-BIAM-MEM, UMR 7265 CEA-CNRS-AMU, CEA Cadarache, France

nicolaas.degroot@cea.fr

Deinococcus bacteria are extremely resistant to radiation and other DNA damage and oxidative stress-generating conditions. They are able to repair massive DNA damage, including hundreds of double-strand breaks. The extreme resistance involves many factors and several well-regulated mechanisms (1). Compared to radiation-sensitive species, proteins are much better protected against oxidative damage in *Deinococcus* and other radiation-resistant species. This limitation of protein damage has been correlated to a high intracellular concentration of manganese ions (and a high Mn/Fe ratio) and the presence of antioxidant Mn(II) complexes (2). An efficient radiation response mechanism to induce expression of several DNA repair genes is also essential for radiation resistance. This SOS-independent response mechanism is controlled by two proteins: DdrO and IrrE. DdrO is a transcriptional repressor that is cleaved and inactivated by metalloprotease IrrE (3, 4, 5). DdrO cleavage by the constitutively expressed IrrE is stimulated, by an unknown molecular mechanism, when the cells are exposed to radiation or oxidative stress. Cleavage is also induced after a short exposure of the cells to an excess of zinc ions (4), indicating that the zinc shock and the different stress conditions (radiation, desiccation, hydrogen peroxide, …) generate a common molecular signal that activates metalloprotease IrrE. Our current work aims at identifying this molecular signal.

(1) Lim et al, 2019, FEMS Microbiol Rev 43:19-52.
(2) Daly, 2012, DNA Repair 11:12-21
(3) Ludanyi et al, 2014, Mol Microbiol 94:434-449.
(4) Blanchard et al, 2017, MicrobiologyOpen 6:e477
(5) de Groot et al, 2019, Nucleic Acids Res 47:11403-11417.

Zinc and iron homeostasis adjust a core metalloproteome and is reflected by the cellular metal composition, the metallome.

Martin Herzberg[a], Lucy Bütof[a], Dirk Dobritzsch[b], Vladislava Schulz[a] and Dietrich H. Nies[a]

[a] *Institute for Biology/Microbiology, Martin-Luther University Halle-Wittenberg, Germany*
[b] *Core Facility – Proteomic Mass Spectrometry, Martin-Luther University Halle-Wittenberg, Germany*

martin.herzberg@mikrobiologie.uni-halle.de

Life depends on biometals, such as zinc and iron, as catalytically active or structurally cofactors in proteins or when they are involved in signaling and regulation processes. An adapted and controlled interplay of efflux, uptake and storage systems are required to maintenance the cellular metal homeostasis. The essential metal requirement of a cell is counterbalanced by the danger of poisoning in case of excess. As a result, the cellular metal quotas of the essential metals are strictly controlled in face of the changing environmental conditions. The β-Proteobacterium *Cupriavidus metallidurans* possess of a large number of metal efflux systems and an entire repertoire of secondary and primary systems for uptake of divalent transition metal cations such as zinc and iron.

The zinc-dependent, Zur-regulated ZIP (ZRT, IRT-like Protein) uptake system ZupT and the putative metal chaperons of the COG0523 protein family of G3E GTPases as additional members of the Zur regulon play central roles in the zinc homeostasis of *C. metallidurans*. Iron uptake systems are mainly regulated by Fur. Two paralogues are known in this organism, FurA and FurB, the former being the main iron regulator and controlling the siderophore synthesis cluster. Alternative extracytoplasmic sigma factors, such as RpoI, play a further role in the regulation of these homeostasis systems. In addition, this organism uses a zinc-dependently regulated operon consisting of zinc-independent cambialistic enzymes and putative zinc chaperones to survive under zinc limitation. Both gene clusters are coded in direct neighborhood and show a possible evolutionary driven gene arrangement. In combination with a metal depended regulated two-stage efflux consisting of CDFs, P-type ATPases and RND systems, it is able to tolerate highly toxic metal concentrations in the environment.

A quantitative proteomic approach explore that the overall number of predicted zinc-binding proteins is higher than the zinc quota, which forms in their totality the “zinc repository” as a part of the metalloproteome. There are also direct correlations between the experimentally determined and the theoretical metalloproteome, which is reflected in the quantity and the percentage distribution of the bioelements of the metallome.

This composition and regulation of a complex interplay of the involved systems makes the organism to a survivalist in metal-contaminated environments, as

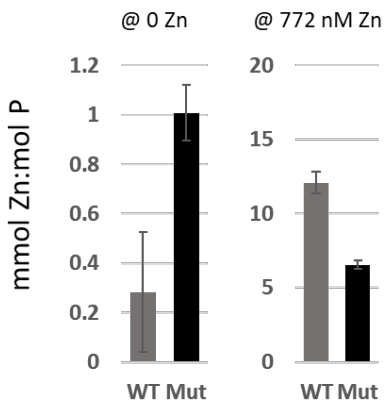
A single zinc sensor mediates the response of an oligotrophic marine cyanobacterium to zinc excess and starvation

Claudia A. Blindauer[a], Vilmos Fülöp[b], Amira Z. Ksibe[a,b], Alevtina Mikhaylina[a,b], David J. Scanlan[b], and Rachael Wilkinson[b]

[a] *Department of Chemistry, University of Warwick, UK*
[b] *School of Life Sciences, University of Warwick, UK*

c.blindauer@warwick.ac.uk

The oligotrophic oceans are characterised by extreme scarcity of most macro- and micronutrients, including d-block metal ions (iron, zinc, manganese and others) that are required to maintain most biogeochemical cycles. Nonetheless, photosynthetic picocyanobacteria of the genera *Synechococcus* and *Prochlorococcus* thrive there in abundance and are estimated to contribute a quarter of ocean net primary production [1]. Since both carbon and phosphorus acquisition are thought to require the action of zinc-dependent enzymes, it may be expected that zinc homeostasis is an important process in these smallest but most abundant photosynthetic organisms. Previous genome mining revealed that all sequenced cyanobacterial genomes harbour a gene for a putative zinc sensor, i.e. the zinc uptake regulator Zur [2,3]. This suggests a critical need for regulating zinc homeostasis in these bacteria. We have studied the wild-type and a *zur*-knockout mutant of *Synechococcus* sp. WH9102 under zinc starvation and excess conditions. Both strains grew well at the lowest possible zinc concentration, with the mutant accumulating more cellular zinc than the wild-type (see Figure), as expected as a consequence of de-repression of a Zur-regulated ZnuABC uptake system. The latter rationale was verified by results from RNA-sequencing and quantitative PCR. At moderately high zinc concentration (772 nM), the mutant showed reduced zinc tolerance, but unexpectedly toxicity was not correlated to cellular zinc quotas - on the contrary, the wild-type accumulated significantly more zinc than the mutant at 772 nM, without showing growth impairment. RNA-seq data revealed that under these conditions, transcription of a bacterial metallothionein gene (*bmtA*) was highly upregulated in the wild-type, but not in the mutant. Together with the identification of functional recognition sequences for Zur in the gene promoter regions (Zur boxes) for *znuABC* and *bmtA*, this implies that at high zinc, cyanobacterial Zur is both a repressor and an activator of transcription.



Biophysical characterisation including protein crystallography revealed that *Synechococcus* sp. WH8102 Zur, and by inference all homologous cyanobacterial Zur proteins, harbour a zinc sensory site that differs from previously seen sites in any Fur-family protein. This is accompanied by other unprecedented structural features.

[1] P. Flombaum et al., *Proc. Natl. Acad. Sci. U. S. A.* **110**, 9824–9829 (2013).
[2] J. P. Barnett, A. Millard, A. Z. Ksibe, D. J. Scanlan, R. Schmid and C. A. Blindauer, *Frontiers Microbiol.* **3**, 21 (2012).
[3] A. Mikhaylina, A. Z. Ksibe, D. J. Scanlan and C. A. Blindauer, *Biochem. Soc. Trans.* **46**, 983-1001 (2018).

Structural and Functional Characterization of Heme-Regulated Proteins in Iron Metabolism

Yudai Nishitani[a], Mariko Ogura[a], Hirotaka Okutani[a], Takeshi Uchida[a,b], Yukiko Takeda[c], Kazuhiro Iwai[c], and **Koichiro Ishimori[a,b]**

[a] *Graduate School of Chemical Sciences and Engineering, Hokkaido University, Japan*
[b] *Department of Chemistry, Faculty of Science, Hokkaido University, Japan*
[c] *Molecular and Cellular Physiology, Graduate School of Medicine, Kyoto University, Japan*

koichiro@sci.hokudai.ac.jp

Heme, an iron-porphyrin complex, is a typical cofactor for metalloproteins playing crucial roles in various kinds of biological processes. Recently, however, heme was also found to function as a regulatory or signalling molecule in many regulation systems. Here, we focused on the signalling function of heme in iron metabolism regulated by iron regulatory proteins (IRPs). IRPs control iron metabolism in mammalian cells by binding to the iron-responsive element (IRE) in mRNAs coding proteins for iron uptake, storage and utilization. Two homologue proteins, IRP1 and IRP2, have been reported as IRPs, both of which have a short and consensus amino acid sequence, Cys-Pro, heme regulatory motifs (HRMs), characteristic of heme-regulated proteins. One of the homologues, IRP1 has two HRMs, suggesting that IRP1 can bind heme as the signalling molecule, but the heme binding to IRP1 had not yet been confirmed. We examined the spectroscopic titration of heme to IRP1 and revealed that IRP1 was able to bind two equivalents of heme and the Fe-Cys stretching mode was observed for the resonance Raman spectra of heme-bound IRP1, confirming the ligation of Cys in HRM. To investigate the functional significance of the heme binding to IRP1, we followed the IRE binding activity of IRP1 in the absence and presence of heme by using native polyacrylamide gel electrophoresis (Native PAGE) analysis. In the presence of heme, the IRE binding to IRP1 was inhibited, indicating that heme regulates the IRE binding in IRP1. On the other hand, another homologue, IRP2, has an extra domain including one HRM, the iron-dependent degradation (IDD) domain, not found for IRP1, between domains 1 and 2, and we revealed that IRP2 binds heme to HRM in the IDD domain to induce the oxidation modification of the protein, which would be a trigger for the protein degradation of IRP2 in the ubiquitin-proteasome system. Interestingly, IRP2 also has additional two HRMs corresponding to those in IRP1, and we spectroscopically confirmed that the additional HRMs in IRP2 can also bind heme as found for IRP1. Native PAGE analysis revealed that heme binding to HRMs near the IRE binding site in IRP2 inhibited complex formation with IRE without the oxidative modification, indicating that the function of HRMs varies outside and within the IDD domain in IRP2. However, it should be noted here that the formation of a typical reactive oxygen species (ROS), hydrogen peroxide, which would induce oxidative modification of proteins, was also spectroscopically detected in IDD-domain deleted IRP2 and IRP1. Comparing the heme environmental structures surrounding HRMs, the flexible conformation and many amino acid residues sensitive to ROS were found for the IDD domain, suggesting that such environmental structure in the IDD domain would promote the specific oxidation by ROS. Thus, the environmental structure of HRM regulates the functional significance of heme binding to IRPs as the signalling molecule.

Vanadium compounds activate cell signaling: the importance of speciation

Debbie C. Crans*, Kataryna Kostenkova and Deborah A. Roess

Corresponding author email – Debbie.crans@colostate.edu
Other emails: kostenk@rams.colostate.edu; deborah.roess@colostate.edu

Dept. Chemistry, Colorado State University, Fort Collins, Colorado
Dept. Biomedical Sciences, Colorado State University, Fort Collins, Colorado

First row transition metals include five essential elements and three more elements that have beneficial effects in heath and disease (1). Speciation is found to be critical for cellular action and cellular transport and is closely linked to the environment and form of the metal ion (1). Luteinizing hormone receptors (LHR) signal in cells when treated with bis(maltolato)oxovanadium(IV) (BMOV), bis(ethylmaltolato)oxovanadium(IV) (BEOV), VOSO4 (2), vanadate or polyoxovanadates (3) which decrease membrane lipid packing. Luteinizing hormone receptors (LHR), expressed in physiological numbers less than 30,000 receptors per cell, translocate to and signal within membrane rafts following binding of juman chorionic gonadotropin (hCG) as observed by polarized homo-transferfluorescence resonance energy transfer (home-FRET). Intracellular cyclic adenylate monophosphate (cAMP) levels indicate that only clustered LHR are active and produce the intracellular second messenger, cAMP. To confirm the existence of intact complexes, EPR (2,3) and/or NMR (3) spectra of vanadium compounds in cell media were obtained using 1.0 mM BMOV, BEOV, $VOSO_4$ (2), vanadate and larger polyoxovanadates (3). These data were used to determine the intact complex in a solution to verify the speciation calculations. Effects of BMOV and BEOV were about two-fold greater than solutions prepared from $VOSO_4$ (2). Effects of larger polyoxometalates are found to be more effective than vanadate (3). Studies exploring these and other vanadium compounds as well as other metal compound are explored and our progress reported.

References
(1) “Open questions on the biological role of first-row transition metals?” Debbie C. Crans and Kateryna Kostenkova Chemistry Communications, in press.
(2) “Effects of vanadium(IV) compounds on plasma membrane lipids leads to G protein-coupled receptor signal transduction” Duaa Althumairy, Heide A. Murakami, Dongmei Zhang, B. George Barisas, Deborah A. Roess and Debbie C. Crans *J. Inorg. Biochem.* **2020**, *203*, 110873. doi: 10.1021/acs.inorgchem.9b02848.
(3) “Polyoxometalates Function as Indirect Activators of a G Protein-Coupled Receptor” Duaa Althumairy, Kahoana Postal, B. George Barisas, Giovana G. Nunes, Deborah A. Roess, and Debbie C. Crans Metallomics, 2020, DOI: 10.1039/D0MT00044B, with Front Cover.

Copper stress induces protein aggregation and triggers Hsp33 holdase function through a non-redox mechanism.

Lisa Zuily[a], Nora Lahrach[a], Rosi Fassler[b], Olivier Sénèque[c], Olivier Genest[a], Peter Faller[d], Ursula Jakob[e], Dana Reichmann[b], Marie-Thérèse Giudici-Orticoni[a], and **Marianne Ilbert[a]**

[a] *Unité de Bioénergétique et Ingénierie des Protéines (Institut de Microbiologie de la Méditerranée, CNRS-UMR7281, Aix-Marseille Université, France)*
[b] *Department of Biological Chemistry (The Alexander Silberman Institute of Life Sciences, Safra Campus Givat Ram, The Hebrew University of Jerusalem, Israel)*
[c] *LCBM (CNRS UMR 5249, Univ. Grenoble Alpes, France)*
[d] *Biometals and Biology Chemistry, (Institut de Chimie, CNRS UMR7177, Université de Strasbourg, France)*
[e] *Department of Molecular, Cellular and Developmental Biology University of Michigan, Ann Arbor, MI, USA*

milbert@imm.cnrs.fr

In the cellular environment, protein homeostasis is maintained by a highly dynamic network of molecular chaperones. While their activity and substrate specificity has been extensively studied under a variety of stress conditions, little is known about how chaperones protect proteins during copper stress. Copper is known to generate reactive oxygen species (ROS) via the Fenton reaction, it can also induce protein unfolding. Here, we show that copper cause massive protein aggregation in a ROS independent mechanism. We further focus on the role of the redox-regulated molecular chaperone Hsp33, from *Escherichia coli*, in protecting proteins against copper-induced unfolding. Hsp33 has been shown to sense and respond to protein aggregation triggered by oxidative heat stress using a sophisticated mechanism: with a C-terminal redox switch domain and an adjacent metastable linker region. Here, we show that even under anaerobic conditions and non-stress temperature, copper converts Hsp33 into a highly active chaperone holdase, thus serving as a potent physiological activator of Hsp33. Once activated, Hsp33 effectively prevents copper-induced protein aggregation. These results provide first insights into the role of molecular chaperones during copper stress, and demonstrate how stress-activated chaperones regulate their conversion into effective molecular chaperones.

Structural properties of the transcriptional regulator FurC (PerR) from *Anabaena* sp. PCC7120 and identification of a putative FurC-box

Cristina Sarasa[1*], Emma Sevilla[1], Jorge Guío, Isabelle Michaud-Soret[2], Serge Crouzy[2], María Luisa Peleato[1], Adrián Velázquez-Campoy[1] and María F. Fillat[1]

[1] *Department of Biochemistry and Molecular and Cell Biology and Institute for Biocomputation and Physics of Complex Systems. Faculty of Sciences, University of Zaragoza, Pedro Cerbuna 12, 50009-Zaragoza, Spain.*

[2]*Laboratory of Chemistry and Biology of Metals, University Grenoble Alpes/ CNRS/CEA, CEA-Grenoble. 17 avenue des Martyrs Grenoble cedex 9, 38054-Grenoble, France*

*Presenter's electronic address: crisara@unizar.es

The protein encoded by the *alr0957* gene in *Anabaena* PCC7120 was identified as a member of the Fur family because its small size and the presence of the histidine rich motifs(H2XHX2CX2C). This protein was initially named FurC and afterwards it was suggested to function as the Peroxide Stress Regulator (PerR) in *Anabaena* since it was sensitive to metal-catalyzed oxidation (MCO). FurC/PerR showed the ability to regulate some genes involved in the oxidative stress response, such as the peroxiredoxin A *(prxA) and* alkyl hydroperoxide C *(ahpC),* but also other important genes involved in iron metabolism and photosynthesis. Here we present a biochemical study on the structural insights of the protein and an identification of a putative FurC-box that helped us to extend the knowledge on the regulatory network of FurC. Briefly, we optimized a protocol for the purification of FurC based on a two-step chromatography using Heparin-Sepharose in presence of 10 mM EDTA followed by an anion exchange chromatography. The purified protein contained approximately 1.27 mole of zinc as determined by ICP and was able to bind one mole of manganese per subunit as indicated by Isothermal Titration Calorimetry (ITC). The presence of oligomeric forms dependent on disulphide bridges was analyzed by SDS-PAGE, both in reducing and oxidizing conditions. On the other hand, Size Exclusion Chromatography combined with light scattering and refractometry (SEC-MALLS-RI) demonstrated the presence of different oligomeric states in solution, such as tetramers and dimers, which were not related to the presence of disulphide bridges. Furthermore, a theoretical structural model of a FurC-dimer was performed in accordance with our data. Finally, in order to predict a putative FurC box, the sequences of the promoter regions whose respective genes were found to be directly regulated by FurC in previous works were used as an input for the multiple sequence alignment tool “MEME”. The preliminary FurC-box allowed us to find new putative targets of FurC that were confirmed by Electrophoretic Mobility Shift Assays.

Structural characterization of *Deinococcus radiodurans* Dps2 with manganese: Expression, purification and crystallization

André Alexandre Grangeia Gouveia, Sara TN Silva and Célia V. Romão

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2781-901 Oeiras, Portugal

andre.gouveia.48@itqb.unl.pt

Deinococcus radiodurans is an extremophile gram-positive bacterium known for its remarkable resistance to several conditions such as ionizing radiation, ultraviolet light, desiccation and oxidative stress. Recent studies demonstrated several mechanisms involved in the protection of *D*. *radiodurans* proteome enabling it to provide an efficient response under stress conditions[1] . Among these mechanisms, manganese complexes are proposed to have a crucial role in oxidative stress resistance[2]. Furthermore, intracellular manganese as well as calcium and phosphorous are known to be co localized in electron dense granules in control conditions and upon oxidative stress they are co mobilized throughout the cell. This mobilization is dependent of two **D**NA binding **p**roteins under **s**tarved conditions (Dps), Dps1 and Dps2 which are key players in metal homeostasis[3]. The central aim of this work is to perform a structural characterization of Dps2 in the presence of manganese and manganese plus calcium to elucidate the structure-based mechanism in manganese transport and oxidative stress protection. To achieve this goal Dps2 was expressed and purified in the presence of only manganese and manganese plus calcium. In order to determine the crystal structure of the different proteins loaded with the different metals, crystals were obtained by hanging drop method and data was collected at a synchrotron source.

Interaction of Ferric Uptake Regulator FurA from *Anabaena* sp. PCC7120 with 2-oxoglutarate: a possible link between iron homeostasis and nitrogen metabolism

Jorge Guío[a,b], Cristina Sarasa-Buisan[a,b], Adrián Velázquez-Campoy[a,b], M. Teresa Bes[a,b], María F. Fillat[a,b], M. Luisa Peleato[a,b] and Emma Sevilla[a,b]

[a] *Department of Biochemistry and Molecular and Cell Biology, University of Zaragoza, Spain*
[b] *Institute for Biocomputation and Physics of Complex Systems, University of Zaragoza, Spain*

jguio@unizar.es

FurA (Ferric Uptake Regulator) from the cyanobacterium *Anabaena* sp. PCC 7120 is a master regulator that not only senses iron availability, regulating iron homeostasis, but also controls the expression of genes involved in nitrogen metabolism, photosynthesis and cell morphology. Besides, it has been suggested that FurA could act as a redox sensor protein, due to its disulfide reductase activity and its ability to interact with heme. For that reason, and given its ability to control a large number of cellular processes, it is possible that FurA could be sensing other signal molecules in cyanobacteria, thus acting as a link between different cellular processes. 2-oxoglutarate (2-OG), a metabolite produced in the Krebs cycle, provides carbon skeletons that allow the incorporation of ammonium through the GS-GOGAT cycle, connecting both carbon and nitrogen metabolism. Moreover, 2-OG acts as a signal of carbon/nitrogen balance and previous studies reported that it could modulate DNA-binding activity of the key regulator for nitrogen metabolism NtcA. Since it was previously reported that FurA was able to control the transcription of heterocyst differentiation and nitrogen metabolism, we sought to investigate whether this transcriptional regulator was also able to sense carbon/nitrogen balance via 2-OG. Isothermal Titration Calorimetry (ITC) assays determined that 2-OG was able to interact with FurA and that this interaction depended on the presence of the co-represor metal. Electrophoretic Mobility Shift Assays (EMSA) showed that FurA binding activity to the *ntcA* gene promoter region was modulated by this metabolite, suggesting that, apart from sensing iron availability, FurA could also act as a sensor of carbon/nitrogen balance and potentially integrate iron homeostasis and nitrogen metabolism. Finally, a model of FurA tridimensional structure was built and docked *in silico* with 2-OG, suggesting two putative binding sites, so we constructed two mutants of FurA in which the residues that were predicted to interact with 2-OG were replaced by alanines. These FurA mutants were analyzed by ITC and EMSA assays, showing that Arg70 was involved in 2-OG binding.

Characterization of the stress-induced molecular signal that activates IrrE, a metalloprotease essential for extreme radiation resistance in *Deinococcus* bacteria

Romaric Magerand[a], Laurence Blanchard[a] and Arjan de Groot[a]

[a]*CEA-DRF-BIAM-MEM, UMR 7265 CEA-CNRS-AMU, CEA Cadarache, France*

romaric.magerand@cea.fr

Deinococcus bacteria are extremely resistant to radiation and other DNA damage and oxidative stress-generating conditions. They are able to repair massive DNA damage, including hundreds of double-strand breaks. The extreme resistance involves many factors and several well-regulated mechanisms (1). Compared to radiation-sensitive species, proteins are much better protected against oxidative damage in *Deinococcus* and other radiation-resistant species. This limitation of protein damage has been correlated to a high intracellular concentration of manganese ions (and a high Mn/Fe ratio) and the presence of antioxidant Mn(II) complexes (2). An efficient radiation response mechanism to induce expression of several DNA repair genes is also essential for radiation resistance. This SOS-independent response mechanism is controlled by two proteins: DdrO and IrrE. DdrO is a transcriptional repressor that is cleaved and inactivated by metalloprotease IrrE (3, 4, 5). DdrO cleavage by the constitutively expressed IrrE is stimulated, by an unknown molecular mechanism, when the cells are exposed to radiation or oxidative stress. Cleavage is also induced after a short exposure of the cells to an excess of zinc ions (4), indicating that the zinc shock and the different stress conditions (radiation, desiccation, hydrogen peroxide, …) generate a common molecular signal that activates metalloprotease IrrE. Our current work aims at identifying this molecular signal.

(1) Lim et al, 2019, FEMS Microbiol Rev 43:19-52.
(2) Daly, 2012, DNA Repair 11:12-21
(3) Ludanyi et al, 2014, Mol Microbiol 94:434-449.
(4) Blanchard et al, 2017, MicrobiologyOpen 6:e477
(5) de Groot et al, 2019, Nucleic Acids Res 47:11403-11417.

Overcoming metal intoxication – Elucidating the zinc resistance pathways of *Acinetobacter baumannii*

Saleh Alquethamy[a], Felise G. Adams[b], Marjan Khorvash[b], Victoria G. Pederick[c], Ian T. Paulsen[d], Timothy P. Stinear[a], Bart A. Eijkelkamp[b] and Christopher A. McDevitt[a]

a) *Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia*

b) *College of Science and Engineering, Flinders University, Bedford Park, South Australia, Australia.*

c) *Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide, Adelaide, South Australia, Australia.*

d) *Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney, New South Wales, Australia.*

salquethamy@student.unimelb.edu.au

Acinetobacter baumannii is a Gram-negative nosocomial pathogen associated with significant disease in immunocompromised individuals. Due to the prevalence of carbapenem resistant clinical isolates, the WHO has called for novel antimicrobial therapies to combat this pathogen. The host controls infection by employing multiple insults to kill invading pathogens, including chemical stresses delivered through changes in the abundance of inorganic ions such as zinc. Accordingly, resistance to metal intoxication is crucial for the success of pathogenic bacteria. *A. baumannii* harbours an extensive repertoire of metal ion efflux systems that have not been functionally characterised. Here, we investigated *A. baumannii* putative membrane transport systems and their role in zinc resistance. Analyses of transposon mutant *A. baumannii* strains revealed a role for the resistance nodulation division (RND) transporter CzcCBA in zinc resistance. The importance of this pathway was further probed using a zinc deficient murine infection model, which showed that *A. baumannii* resistance to zinc stress was important for survival in the spleen. Additionally, the cation diffusion facilitator (CDF) transporter CzcD was also observed to contribute to *A. baumannii* zinc resistance. The involvement of CzcCBA and CzcD to resisting other transition metal ion stresses was also investigated. In contrast to CzcD, CzcCBA contributed to cadmium resistance. As did another distinct CDF transporter, CzcE, which was found to be crucial in protecting *A. baumannii* from cadmium intoxication. Collectively, these analyses provide insight into the metal ion resistance mechanisms of *A. baumannii*, and highlight a host niche in which metal ion tolerance is important for infection.

Biochemical and structural characterisation of the *Haemophilus influenzae* PsaA ortholog, HIPsaA

Katherine Ganio[a], Jonathan J. Whittall[b], Ulrike Kappler[c] and Christopher A. McDevitt[a,b]

[a] *Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Australia*
[b] *Department of Molecular and Biomedical Sciences, School of Biological Sciences, The University of Adelaide, Australia*
[c] *Centre for Metals in Biology, School of Chemistry and Molecular Biosciences, University of Queensland, Australia*

kganio@unimelb.edu.au

Haemophilus influenzae is a host-adapted pathogen that colonizes the human nasopharynx that can mediate diseases of the upper and lower respiratory tract. Isolates can be found as encapsulated and non-encapsulated forms which both have similar morphology but produce divergent clinical infection. *H. influenzae* has an array of molecular mechanisms that permit growth and virulence in diverse host niches. Notably, *H. influenzae* can respond to exogenous, host-mediated and endogenous, i.e. metabolically produced, reactive oxygen and nitrogen stresses. Transition metal ions serve crucial roles in bacterial growth, survival and stress response, but the majority of these mechanisms in *H. influenzae* remain to be determined. Bioinformatic analyses of *H. influenzae* 2019 revealed that it encoded two ATP-binding cassette (ABC) transporter solute binding proteins (SBPs) that belonged to the cluster A-I subgroup. Primary sequence analyses suggested that these were orthologs of a manganese-specific (locus tag: C645_00940) and zinc-specific (locus tag: C645_02340) SBPs. Given the central role of manganese in metabolism and resistance to oxidative stress we investigated the biochemical and biophysical properties of C645_00940. We combined recombinant protein purification with *in vitro* metal binding assays to show that C645_00940 was a manganese-binding SBP. Building on this finding, the gene was renamed as HIPsaA due to the functional and structural similarity to *Streptococcus pneumoniae* PsaA. Furthermore, these techniques indicate that this SBP is capable of stably binding other first row transition metal ions including zinc, copper, cobalt and nickel. Collectively, this work provides insight into manganese acquisition in *H. influenzae* and the contribution of this transition metal ion to bacterial virulence.

Metal Sequestration and Antimicrobial Activity of Human Calprotectin are pH-Dependent

Tomer Rosen[a] and Elizabeth M. Nolan[a*]

[a] *Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA*

trosen@mit.edu

During infection, the mammalian host initiates a metal-withholding response against invading pathogens to reduce nutrient availability and thereby limit microbial growth and virulence. Calprotectin (CP) is an abundant metal-chelating innate immune protein that is released at sites of infection and sequesters multiple transition metal ions, including manganese, iron and zinc, from microbial pathogens. The current working model for CP in the extracellular space is based on numerous studies performed at neutral or near-neutral pH. Nevertheless, CP is present at infection and inflammation sites that are expected to be acidic, and such conditions are expected to perturb the metal-binding properties of the protein and thereby influence its functional properties. Here, we present the first evaluation of the coordination chemistry and antimicrobial activity of CP at low-pH regimes. We show that acidic conditions perturb the oligomerization process induced by Ca(II) binding, a critical step for the extracellular functions of CP. Moreover, we demonstrate how acidic pH alters the metal-binding properties and antimicrobial activity of CP, rendering bacterial pathogens less susceptible to the protein. Overall, these findings expand our understanding of the extracellular functions of CP and highlight the importance of considering the molecular complexity of infection sites when seeking to understand the interplay between pathogens and this remarkable metal sequestering protein.

Title: The molecular basis for zinc uptake via *Streptococcus pneumoniae* AdcAII

Abstract:

Streptococcus pneumoniae is a globally significant human pathogen that scavenges essential zinc [Zn(II)] ions from the host during colonization and infection. This is achieved by the ATP-binding cassette transporter, AdcCB, and two solute-binding proteins (SBPs), AdcA and AdcAII. AdcAII has a greater role during the early stages of infection, but the molecular details of how AdcAII acquires Zn(II) ions remain poorly defined. This can be attributed to the inability of crystallographic approaches to determine a high-resolution structure of ligand-free AdcAII. Here, we overcame this issue by systematically mutating each of the four Zn(II)-coordinating residues and performing structural and biochemical analyses on the variant isoforms. Structural analyses of Zn(II)-bound AdcAII variant proteins revealed how specific regions within the SBP undergo conformational changes via their direct coupling to each of the metal-binding residues. Quantitative *in vitro* metal-binding assays, combined with affinity determination and phenotypic analyses, revealed the relative contribution of each coordinating residue to the Zn(II)-binding mechanism. Unexpectedly, the phenotypic growth impact of the mutant *adcAII* alleles did not correlate with SBP affinity, but instead were consistent with the structural perturbations. Taken together, our data show, for the first time, that SBP conformation rather than affinity is the primary determinant of efficacious Zn(II) uptake in *S. pneumoniae*. Collectively, our data reveal a novel metal-binding mechanism for AdcAII and highlight how ligand affinity and protein conformational changes are coupled within ligand-receptor proteins. These mechanistic insights provide a foundation for novel antimicrobial design to disrupt this process in bacterial metal-receptor proteins.

The role of *Clostridium difficile*'s flavodiiron proteins in its ability to survive within the human host

Filipe Folgosa [a], Catarina Alves [a], Maria C. Martins [a] and Miguel Teixeira [a]

[a] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal*

f.folgosa@itqb.unl.pt

Over the years, it has been demonstrated that many anaerobic pathogenic bacteria, such as *Clostridium difficile*, can populate the human gut. As anaerobes, their main challenge is to tackle the exposure to fluctuating concentrations of oxygen. Recently, a number of enzymes were shown to be involved in the response to this stress [1]. Two of these enzymes are known as flavodiiron proteins (FDP). FDPs can act as oxygen reductases, reducing it to water or NO reductases, reducing it to N_2O; some may even use both as substrates [2]. FDP enzymes are constituted by a minimal core unit, composed by a metallo-β-lactamase-like domain, containing the catalytic diiron center and a flavodoxin domain [2,3]. More complex FDPs where found, with multiple extra domains [2]. While one of the *C. difficile*'s FDPs has only the two core domains, the other is very complex, containing an extra short-type rubredoxin domain followed by an NADH:rubredoxin oxidoreductase-like one [4].
The biochemical, redox, spectroscopic (UV-visible, EPR) and catalytic properties of this enzyme and its domain´s constructs will be presented, showing that the whole electron transfer chain from the electron donor, NADH, to its preferential substrate, O2 occurs without the need of external partners. Although redox data pointed to an unclear role for the rubredoxin-like center (Figure1), in this work we demonstrate, by site directed mutagenesis, its relevance for the electron transfer pathway from NADH to the catalytic center.

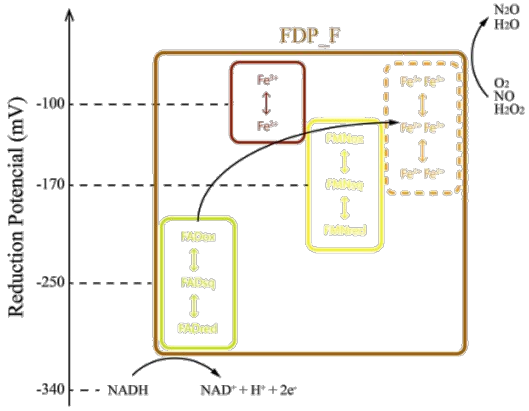


Figure 1 – Reduction potentials diagram for *C. difficile*'s complex FDP.

References

[1] Kint *et al*, *Environm. Microbiol.*, 2017, 19, 1933
[2] Martins *et al*, *Free Rad. Biol. Med.*, 2019, 140, 36
[3] Romão *et al*, *J. Biol. Inorg. Chem.*, 2016, 21, 39
[4] Folgosa *et al*, *Sci. Rep.*, 2018, 8, 10164

New role of copper protein in cancer

Stéphanie Blockhuys and Pernilla Wittung-Stafshede

Biology and Biological Engineering Dept., Chalmers University of Technology, Gothenburg, Sweden

steblo@chalmers.se

Breast cancer progression is associated with increasing copper (Cu) levels in the patient´s serum and cancer tissue. Cu is known to be essential for at least three hallmarks of cancer: proliferative immortality, angiogenesis and metastasis. Yet, it is unclear which Cu-binding proteins are involved in these hallmarks and how they obtain Cu. Earlier, we found that the Cu-chaperone anti-oxidant protein 1 (ATOX1) is upregulated in primary breast cancer tissues, and that ATOX1 accumulates at the lamellipodia borders of migratory breast cancer cells, suggesting a new role of ATOX1 in breast cancer cell migration (*i.e.*, a key phenomenon in metastasis).

To reveal molecular insights in the putative role of ATOX1 in breast cancer cell migration, we performed live cell video microscopy and studied the motility tracks of MDA-MB-231 breast cancer cells as a function of ATOX1 levels. We found that both cell migration velocity and Euclidean distance were reduced upon ATOX1 silencing. Also silencing of ATP7A reduced cell migration suggesting that ATOX1 and ATP7A are on the same pathway regulating cell migration. In addition, in situ proximity ligation assays demonstrated close proximity (<40nm) of ATOX1, ATP7A and lysyl oxidase (LOX) in the cells, whereby the spatial proximity of ATOX1, ATP7A and LOX were interdependent. More, LOX activity measurements in the conditioned medium showed reduced LOX activity for ATOX1 silenced cells. Thus, ATOX1 appears to stimulate breast cancer cell migration via the ATP7A-LOX path whereby Cu delivery from ATOX1 to ATP7A and from ATP7A to LOX are coordinated events.

In addition, upon analysis of the METABRIC database, with ATOX1 mRNA levels and follow-up data for 1904 breast cancer patients, we found that patients with high ATOX1 levels in their primary tumor (i.e. more than 1.3-fold increase above median) have 50% poorer survival than those with low ATOX1 levels. These results indicate that ATOX1 is a potential prognostic biomarker for breast cancer. Preclinical and clinical studies are desired to further examine the correlation between ATOX1 levels in the primary tumor and metastasis likelihood.

Metal-induced aggregation of gamma-crystallins: Insights into the bioinorganic chemistry of cataract disease

Liliana Quintanar, J.A. Dominguez-Calva, G.Palomino, N. Schuth

Department of Chemistry, Center for Research and Advanced Studies (Cinvestav), Mexico.

lilianaq@cinvestav.mx

Cataracts are the leading cause of blindness in the world, and are formed upon aggregation of lens proteins into high molecular weight complexes, causing light scattering and lens opacity. Several studies implicate metals as a potential etiological agent for cataract, while copper and zinc concentrations in the cataractous lenses are increased significantly, as compared to normal lenses. The monomeric human gamma-crystallins are among the most abundant crystallins in the lens and one of the most stable proteins in the human body, but its non-amyloid aggregation is associated with cataracts. In this presentation, the effect of metal ions in the aggregation of these proteins will be discussed. Specifically, Cu(II) and Zn(II) ions induce this aggregation, suggesting site-specific interactions with the gamma-crystallins. The site-specific interactions of copper ions with these crystallins and the mechanisms of copper-induced aggregation will be discussed. These mechanisms include the formation of partially folded intermediates, metal-bridged species, disulfide bridged oligomeric species, and copper-initiated redox processes that lead to oxidative damage of the protein. Metal-induced aggregation could be a physiologically relevant phenomenon; thus, understanding its mechanisms will help elucidate the role of metal ions in the aggregation of human crystallins and their potential involvement in the development of cataracts, providing important insights into the unexplored bioinorganic facet of this disease. This research has been supported by: Conacyt (grant # PN2076 and fellowships to J.A.D.-C., G.P. and N.S.) N.S. is a DFG postdoctoral fellow in Mexico.

Metal ion dyshomeostasis and coagulatory defects in type-1 and type-2 diabetes

Amelie I.S.Sobczak[1], Fladia Phoenix[2], Kondwani Katundu[1], Ruitao Yu[3], Fiona Stefanowicz[4], Samantha J. Pitt[1], Claudia A. Blindauer[5], Ramzi A. Ajjan[2], Alan J. Stewart[1*]

[1]*School of Medicine, University of St Andrews, Medical and Biological Sciences Building, North Haugh, St Andrews, UK.*
[2]*Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK.*
[3]*Northwest Plateau Institute of Biology, Chinese Academy of Sciences, 23 Xinning Road, Xining, China.*
[4]*Scottish Trace Element and Micronutrient Reference Laboratory, Department of Biochemistry, NHS Greater Glasgow and Clyde, Glasgow, UK.*
[5]*Department of Chemistry, University of Warwick, Coventry, UK.*

**Correspondence: Dr Alan J. Stewart, E-mail: ajs21@st-andrews.ac.uk*

Diabetes is a term used to describe a group of conditions that impact upon the body's ability to control blood glucose levels. In type-I diabetes (T1DM), the β-cells in the pancreas responsible for producing insulin are lost, typically from an attack from the immune system, causing insulin deficiency. In type-II diabetes (T2DM), cells become resistant to insulin signalling. Both T1DM and T2DM have wide-ranging consequences for the body as glucose levels are associated with many physiological processes. Individuals with diabetes have an increased risk of cardiovascular disease and coagulatory defects are observed in individuals with both T1DM or T2DM. Our work has revealed that metal ion homeostasis is differentially affected in T1DM and T2DM. For example, HbA1c, a marker for elevated blood glucose, correlates with plasma concentrations of magnesium (negatively) in T1DM and copper (positively) in T2DM. Notably, using a validated turbidimetric assay, the decrease in plasma Mg^{2+} in T1DM was found to be associated with abnormal thrombin-stimulated fibrin clotting or with fibrinolysis. In addition, we found that T2DM is associated with defective plasma Zn^{2+} handling, caused by increased non-esterified fatty acid binding to human serum albumin (HSA) – an interaction which allosterically regulates the ability of the protein to bind Zn^{2+}. Using ITC we reveal that 1-5 mol. eq. of myristate, palmitate, stearate, palmitoleate and palmitelaidate reduce Zn^{2+} binding to HSA. Addition of myristate and Zn^{2+} increase thrombin-induced platelet aggregation in platelet-rich plasma and increase fibrin clot density and clot time in a purified protein system. The concentrations of key saturated and monounsaturated NEFAs positively correlated with clot density in subjects with T2DM (and controls). Collectively, this work increases our understanding of the roles metal ions play in T1DM and T2DM pathogenesis and will have future implications for the management of diabetes.

INTESTINAL COPPER REGULATES FAT ABSORPTION

Svetlana Lutsenko[a], H. Pierson[a], L. Wang [a], A. Muchenditsi[a], B. Kim [b], and M. Ralle[c]

[a] *Department of Physiology, Johns Hopkins University, USA)*
[b] *Department of Animal and Avian Sciences, University of Maryland, USA*
[c] *Department of Molecular and Medical Genetics, OHSU, USA*

lutsenko@jhmi.edu

Small intestine is the major site of dietary copper (Cu) and fat absorption. Recent evidence revealed dysregulation of lipid metabolism in pre-clinical models of Cu disorders. We now show that in healthy intestine dietary Cu markedly modulates processing of nutritional fat and identify a Cu sensitive step. In mice, both low and high Cu in a diet impact the formation of chylomicrons; the effect of Cu deficit is most significant. High Cu appears to shift lipid processing in enterocytes toward triglyceride storage and lipid droplet formation. In contrast, Cu deficiency results in significant changes of lipid profiles and a loss of chylomicrons. Studies in 3D primary enteroids replicate effects of low and high Cu and identify the microtubules stabilization as a Cu-sensitive process. Chelation of Cu increases acetylation of α-tubulin, which regulates the microtubule stability and intracellular distribution of proteins and lipids.

New inhibitors against the [4Fe-4S] quinolinate synthase

Jaione Saez Cabodevilla[a,c], Anne Volbeda[b], Olivier Hamelin[a], Martin Clémancey[a], Jean-Marc Latour[a], Patricia Amara[b], Juan Fontecilla-Camps[b] and **Sandrine Ollagnier[a]**

[a] *LCBM (IRIG, DIESE, Grenoble, France)*
[b] *IBS (IRIG, DBSCI, Grenoble, France)*
[c] *DPM (UGA, St Martin d'Heres, France)*

sollagnier@cea.fr

Nicotinamide adenine dinucleotide (NAD) plays a crucial role in many essential biological reactions. Quinolinic acid is a precursor of this cofactor in most organisms, but its biosynthetic pathway differs among them. In most eukaryotes it is produced from the degradation of *L*-Tryptophan whereas in prokaryotes, it is synthesized from *L*-Aspartate and Dihydroxyacetone phosphate as the result of the concerted action of two enzymes: *L*-aspartate oxidase or NadB and Quinolinate synthase or NadA.[1] Apart of these "de novo" pathways, most of the organisms have salvage pathway(s) to synthesize NAD from small molecules such as nicotinamide or nicotinic acid. However, in some pathogens such as *Helicobater pylori* and *Mycobacteriun leprae,* responsible for gastric cancers and leprosis respectively, no salvage pathway exists and consequently, NadB and NadA constitute interesting antibacterial targets in these pathogens. In the laboratory, we are interested in NadA enzyme which is a [4Fe-4S] enzyme. Its Fe-S cluster that is essential for its activity is coordinated by 3 cysteine ligands and a water molecule.[2] The first *in vitro* and *in cellulo* inhibitor of NadA was described in our laboratory: the dithiohydroxyphtalic acid or DTHPA (Figure 1) inhibits the activity of NadA enzyme by interaction with the cluster through its thiolate groups.[3] However, this molecule presents a lack of specificity both *in vitro* and *in cellulo*. Based on a structure-activity study using DTHPA derivatives, some new inhibitors have been designed (Fig.1). I will present their synthesis, their inhibitory effect on NadA enzyme *in vitro* as well as some spectroscopic and structural data which explain their action mechanism on NadA.

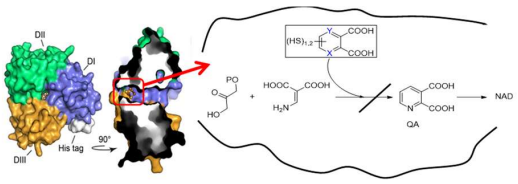


Figure 1: NadA structure showing the tunnel that connects the surface of the protein to the catalytic Fe-S cluster. DTHPA ($(SH)_2$, X=Y=C) and some derivatives as potential better inhibitors of NadA are shown.

[1]: Begley, T.P. *et al.*, Vitam Horm. 2001, 61, 103-119; [2]: Cherrier M.V. *et al.* J. Am. Chem. Soc. 2014, 136, 5253−5256; [3]: Chan, A. *et al.* Angew. Chem. Int. Ed. 2012, 51, 7711 –7714

Modifiable Metal Affinity Chromatography as a Metalloenzyme Surrogate

Lukas Mathew Roth[a], Michael P. Gotsbacher[a] and Rachel Codd[a]

[a]*School of Medical Sciences, The University of Sydney, Australia*

lukas.roth@sydney.edu.au

In 1990, approximately 80% of medicines approved in the U.S. were either natural products or their derivatives[1]. In the early 2000s there was a significant drop in the number of natural products in clinical studies, coinciding with the expansion of high throughput screening (HTS) techniques[1]. However, the limited structural diversity inherent to HTS and the emerging threat of antimicrobial resistance has reinvigorated the focus on exploiting natural products for the discovery and development of new medicines[2].

The adaptation of immobilized metal affinity chromatography (IMAC), a technique originally designed for the isolation of histidine-tagged proteins, has shown promise in isolating and purifying bioactive compounds[3]. IMAC relies on the fundamentals of coordination chemistry to retain and selectively elute compounds with known metal ion affinity. Although originally developed for recombinant protein purification, this simple method has been shown to readily purify hydroxamic acid siderophores, such as desferrioxamine B (DFOB) and other clinical agents, from bacterial cultures[3, 4]. Most IMAC work, in the context of siderophore isolation and purification, has utilized Ni(II) as the metal ion, but there is potential in substituting Ni(II) with other metal ions, such as Cu(II), Fe(III), Ga(III) and Zn(II)[4]. The IMAC columns may consequently act as metalloenzyme surrogates and select for different metabolites as directed by distinct coordination chemistries. This could open up a new platform to discover metalloenzyme inhibitor drug candidates as the isolated metabolites, by virtue of their metal binding affinity, may demonstrate activity against various metalloenzymes.

As an initial proof of concept, we have exposed a mixture of in use metalloenzyme inhibitors to the different IMAC columns with promising results. This work is being followed by the application of supernatant from the marine bacteria *Salinispora tropica* to the IMAC columns. The metabolites isolated by each column will then be analysed by LC-MS and taken through a metabolomics workflow for further selection. Promising prospective metalloenzyme inhibitors will undergo screening using a variety of metalloprotein inhibition assays (e.g. angiotensin converting enzyme, histone deacetylase, and 5-lipoxygenase).

References

[1] J. W. H. Li, J. C. Vederas, *Science Review* **2009**, *325*, 5937, 161-5.
[2] M. G. Moloney, *Trends in Pharmacological Sciences* **2016**, *37*, 8, 689-701.
[3] N. Braich, R. Codd, *The Analyst* **2008**, *133*, 877-80.
[4] G. Gasser. Inorganic Chemical Biology : Principles, Techniques and Applications **2014**. New York: John Wiley & Sons, Incorporated.

Imaging metal complexes in cells

Please leave one blank line

Clotilde Policar,[a] Nicolas Desluc,[a] Hélène Bertrand[a]

Please leave one blank line

[a] *Laboratoire des biomolécules, LBM, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France*

Please leave one blank line

e-mail: clotilde.policar@ens.fr

Please leave two blank lines

Metal complexes are increasingly used for biological applications, as metal-centered probes for imaging or as metal-based drugs.[1, 2] To be active a metal complex must reach its biological target that can be buried in cells. Intracellular quantification, speciation of the metal cation, and intracellular distribution through imaging, as well as the evaluation of the activity directly in a cellular environment are key steps in the design and study of metallo-active bio-molecules.

Imaging is key to the full understanding of the bio-properties of any drug and specific techniques can be used to image these metallo-drugs, including micro X-fluorescence. Applications of imaging using metal-metal-based probes will also be presented. More specifically, probes made of a central metal-CO core, called SCoMPIs (for single core multimodal probes for imaging), can be mapped using unconventional imaging techniques such as IR and X-fluorescence imaging.[6-8]

These topics have been chosen to exemplify a range of approaches at this new frontier in inorganic chemistry.

[1] Farrer N. J., Sadler P. J., in: Alessio E., Bioinorganic medicinal chemistry, Medicinal inorganic chemistry: state of art, new trends and vision of the future, Wiley, Weinheim, 2011, pp. 1-47 — [2] Gilston B. A., O'halloran T., in: Culotta V., Scott R. A., Metals in cells, Mechanisms controlling the metal economy, Wiley, United Kingdom, 2013, — [3] Mathieu E., Bernard A.-S., Delsuc N., Quévrain E., Gazzah G., Lai B., Chain F., Langella P., Bachelet M., Masliah J., Seksik P., Policar C., Inorg. Chem. 56 (2017) 2545-2555 — [4] Bernard A.-S., Giroud C., Ching H. Y. V., Meunier A., Ambike V., Amatore C., Guille Collignon M., Lemaître F., Policar C., Dalton Trans. 41 (2012) 6399-6403 — [5] Policar C., "Mimicking SODs, Why and How: Bio-Inspired Manganese Complexes as SOD Mimics" in: Reboucas J. S., Batinic-Haberle I., Spasojevic I., Warner D. S., St. Clair D., Redox Active Therapeutics, Mimicking SODs, Why and How: Bio-Inspired Manganese Complexes as SOD Mimics, Springer, Switzerland, 2016, pp. 125-16 — [6] Hostachy S., Policar C., Delsuc N., Coord. Chem. Rev. 351 (2017) 172-188 — [7] Hostachy S., Masuda M., Miki T., Hamachi I., Sagan S., Lequin O., Medjoubi K., Somogyi A., Delsuc N., Policar C., Chem. Sci. 9 (2018) 4483-4487 — [8] Clède S., Policar C., Chem. Eur. J. 21 (2015) 942 – 958.

Please leave one blank line

Acknowledgements: This research was funded by the ANR (ANR-15-CE07-0027), PSL université Paris (ANR-10-IDEX-0001-02-PSL), Fondation de la Recherche Médicale (DIE20151234413)

Nickel trafficking in the urease system

Stefano Ciurli

Laboratory of Bio-Inorganic Chemistry, Department of Pharmacy and Biotechnology, University of Bologna, Italy

stefano.ciurli@unibo.it

Urease activity relies on two Ni(II) ions in the active site. The most recent advances achieved by our research group on the structure and function of the proteins involved in nickel catalysis, trafficking and sensing will be discussed. In particular, the structures of *Sporosarcina pasteurii* urease bound to the substrate urea and to various types of inhibitors will be presented, and the derived current view on the enzyme mechanism illustrated. The structural biology of the nickel-chaperones involved in the assembly and maturation of the urease active site as elucidated using crystal structures, NMR and X-ray absorption spectroscopies as well as computational methods will be discussed, and a mechanism for Ni(II) delivery to the urease active site will be proposed. The properties of NikR, a nickel-dependent transcription factor, will be illustrated as determined using both solution and solid-state structural data.

L. Mazzei; M. Cianci; S. Benini; S. Ciurli *Chem. Eur. J.* **2019**, 25, 12145-12158
L. Mazzei; M. Cianci; S. Benini; S. Ciurli *Angew. Chem. Int. Ed*. **2019**, *131*, 7493-7497
L. Mazzei; M. N. Wenzel; M. Cianci; M. Palombo; A. Casini; S. Ciurli *ACS Med. Chem. Lett.* **2019**, *10*, 564-570
L. Mazzei; M. Cianci; U. Contaldo; S. Ciurli *J. Food. Agric. Chem.* **2019**, *67*, 2127-2138
C. A. E. M. Spronk; S. Żerko; M. Górka; W. Koźmiński; B. Bardiaux; B. Zambelli; F. Musiani; M. Piccioli; P. Basak; F. C. Blum; R. C. Johnson; H. Hu; D. S. Merrell; M. J. Maroney; S. Ciurli *J. Biol. Inorg. Chem.* **2018**, *23*, 1309-1330
L. Mazzei; M. Cianci; A. G. Vara; S. Ciurli *Dalton Trans.* **2018**, *47*, 8240-8247
L. Mazzei; M. Cianci; U. Contaldo; F. Musiani; S. Ciurli *Biochemistry* **2017**, *56*, 5391-5404
F. Musiani; D. Gioia; M. Masetti; F. Falchi; A. Cavalli; M. Recanatini; S. Ciurli *J. Chem. Theory Comp.* **2017**, *13*, 2322-2331
L. Mazzei; M. Cianci; F. Musiani; G. Lente; M. Palombo; S. Ciurli *J. Inorg. Biochem.* **2017**, *166*, 182-189
E. Fabini; B. Zambelli; L. Mazzei; S. Ciurli; C. Bertucci *Anal. Bioanal. Chem.* **2016**, *408*, 7971-7980
L. Mazzei; M. Cianci; F. Musiani; S. Ciurli *Dalton Trans.* **2016**, *45*, 5455-5459
L. Mazzei; M. Cianci; S. Benini; L. Bertini; F. Musiani; S. Ciurli *J. Inorg. Biochem.* **2016**, *154*, 42-49
L. Mazzei; O. Dobrovolska; F. Musiani; B. Zambelli; S. Ciurli *JBIC* **2015**, *20*, 1021-1037
S. Benini; M. Cianci; L. Mazzei; S. Ciurli *JBIC* **2014**, *19*, 1243-1261
A. Merloni; O. Dobrovolska; B. Zambelli; F. Agostini; M. Bazzani; F. Musiani; S. Ciurli *BBA Proteins Proteom.* **2014**, *1844*, 1662-1674
M. J. Maroney; S. Ciurli *Chem. Rev.* **2014**, *114*, 4206-4228
B. Zambelli; K. Banasak; A. Merloni; A. Kiliszek; W.R. Rypniewski; S. Ciurli *JBIC* **2013**, *18*, 1005-1017
F. Musiani; E. Ippoliti; C. Micheletti; P. Carloni; S. Ciurli *Biochemistry*, **2013**, *52*, 2949-2954
V. Martin-Diaconescu; M. Bellucci; F. Musiani; S. Ciurli; M. Maroney *JBIC* **2012**, *17*, 353-361
B. Zambelli; F. Musiani, S. Ciurli Met. Ions Life Sci. **2012**, *10*, 135-170.
K. Banaszak; V. Martin-Diaconescu; M. Bellucci; W.R. Rypniewski; M. Maroney; S. Ciurli *Biochem. J.* **2012**, *441*, 1017-1026
B. Zambelli; N. Cremades; P. Neyroz; P. Turano; V. Uverski; S. Ciurli *Mol. BioSyst.* **2012**, *8*, 220-228
S. Benini; M. Cianci; S. Ciurli *Dalton Trans.* **2011**, *40*, 7831-7833
B. Zambelli; F. Musiani; S. Benini; S. Ciurli *Acc. Chem. Res.* **2011**, *44*, 520-530
F. Musiani; B. Bertosa; A. Magistrato; B. Zambelli; P. Turano; V. Losasso; C. Micheletti; S. Ciurli*; P. Carloni *J. Chem. Theory Comp.* **2010**, *6*, 3503-3515

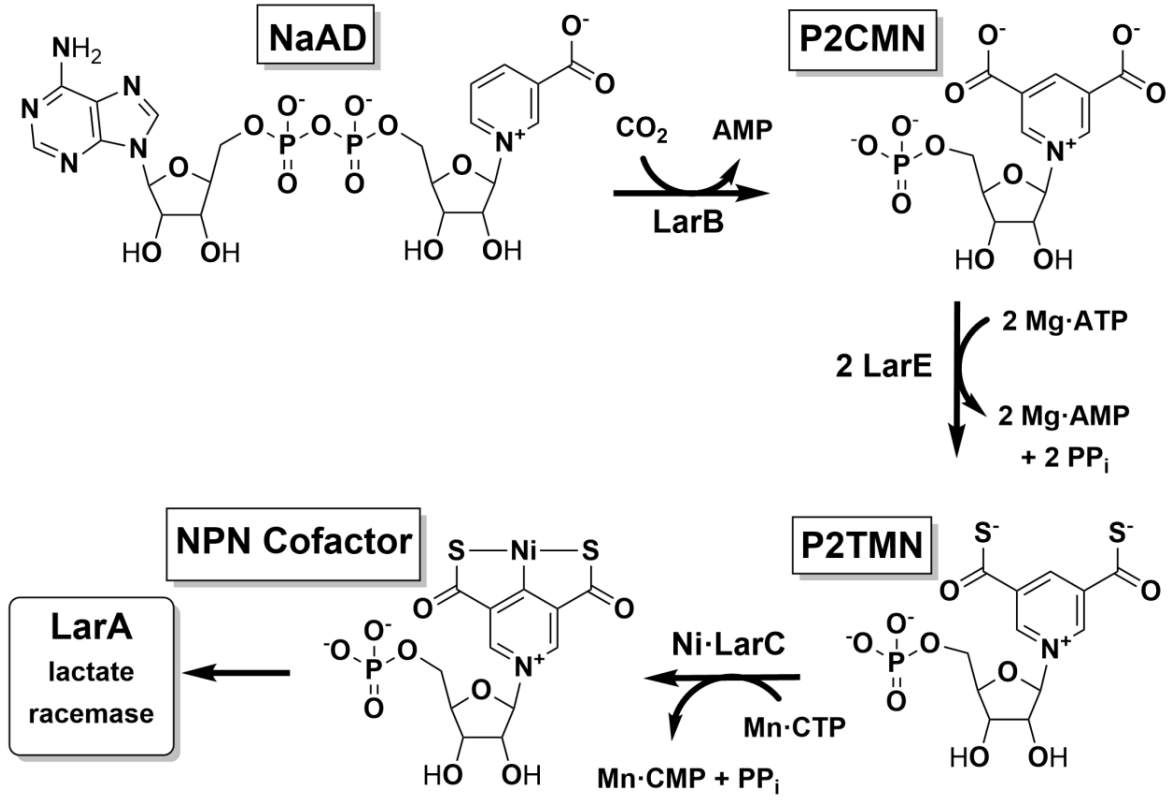
Lactate Racemase and its Novel Nickel-Pincer Nucleotide Cofactor

Robert P. Hausinger[a]

[a] *Departments of Microbiology & Molecular Genetics and Biochemistry & Molecular Biology, Michigan State University, East Lansing, Michigan, USA*

hausinge@msu.edu

Lactate racemase interconverts the L- and D-isomers of lactic acid, a central metabolic intermediate of many cells. The enzyme from *Lactobacillus plantarum*, LarA, harbors a tethered nickel-pincer nucleotide (NPN) coenzyme derived from niacin. Synthesis of the enzyme-bound cofactor requires LarB, a carboxylase/hydrolase of nicotinic acid adenine dinucleotide (NaAD); LarE, a Mg·ATP-dependent sacrificial sulfur insertase; and LarC, a CTP-dependent nickel insertase or cyclometallase. This seminar will summarize recent studies related to NPN synthesis and function.



A structural view of the nickel delivery pathway in urease maturation

Kam-Bo WONG[a]

[a] *Centre for Protein Science and Crystallography, State Key Laboratory of Agrobiotechnology, School of Life Sciences, The Chinese University of Hong Kong*

kbwong@cuhk.edu.hk

Urease is a nickel-containing metalloenzyme that catalyzes the hydrolysis of urea into ammonia and carbon dioxide. To become active, the apo-urease undergoes a maturation process involving the insertion of two nickel ions in its active site. As nickel is a competitive ion at the top of the Irving-Williams series, correct metallation of urease is ensured through specific protein-protein interactions among metallochaperones, namely UreE, UreF, UreG and UreD(H), along the nickel delivery pathway. Our group have been combining structural studies, mutagenesis and biochemical analysis to understand the nickel delivery pathway. Based on our previously determined the crystal structures of UreF/UreH (Fong et al., JBC, 50:43241) and GDP-bound-UreG/UreF/UreH (Fong et al., PLoS Biol, 11:e1001678) complexes, and nickel/GTP-bound UreG dimer (Yuen et al., PNAS, 114:E10890), and our recent structural studies of apo-urease in complex with urease accessory proteins, we have elucidated the mechanism where nickel ions are delivered from one urease assessor protein to another and eventually to urease without releasing the “free” toxic metal to the cytoplasm. This work was supported by grants from the Research Grants Council of Hong Kong (476611, 14117314, AoE/M-05/12, AoE/M-403/16, CRF C6012-17EF, C4041-18EF, C4033-19EF) and Direct Grants from the Chinese University of Hong Kong.

Escherichia coli Repair of Iron Centres YtfE Protein Interacts with the DNA-Binding Protein from Starved cells Dps Protein

Liliana S. O. Silva [a], Simon Andrews[b] and Lígia M. Saraiva[a]

[a] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República EAN, Oeiras, Portugal*

[b] *School of Biological Sciences, Knight Building, University of Reading, Reading, United Kingdom*

lsilva@itqb.unl.pt

RIC (Repair of Iron Centre) proteins, where YtfE is included, belongs to a widespread family of hemerythrin-like di-iron proteins that are present in bacterial and eukaryotic human pathogens. Previously, we showed that the *Escherichia coli* YtfE protects Fe-S containing proteins, such as aconitase and fumarase, that are highly prone to inactivation by the oxidative and nitrosative stresses imposed by the host innate immune system. YtfE restores their activity due to its capacity to provide iron for the reassembling of the iron-sulfur centres.

More recently, we also found that the *E. coli* YtfE interacts with the DNA-binding protein from starved cells. Dps is an iron storage protein of the ferritin superfamily whose function is associated with protection of DNA against oxidative stress. Still, its role in vivo remains largely unclear.

We observed that although the single *ytfE* or *dps* deletion mutant strains show lower aconitase/ fumarase activity, the double *ytfE-dps* mutant does not exhibit a further increased loss of activity. In addition, the double *ytfE-dps* mutant has a lower content of ROS when compared with the single *dps* mutant. Of relevance, a similar behaviour was not observed in strains mutated in other *E. coli* iron storage proteins, such as ferritin and bacterioferritin, or in catalases and peroxidase that also lack *ytfE*. Altogether, we will present results that not only uncovered the first interacting partner of Dps, but also revealed that its interaction with Ytfe is required for the *in vivo* role of the RIC proteins.

Justino MC, Vicente JB, Teixeira M, Saraiva LM. New genes implicated in the protection of anaerobically grown *Escherichia coli* against nitric oxide. J Biol Chem. 2005;280:2636–43.

Overton TW, Justino MC, Li Y, Baptista JM, Melo AMP, Cole J a, et al. Widespread distribution in pathogenic bacteria of di-iron proteins that repair oxidative and nitrosative damage to iron-sulfur centers. J Bacteriol. 2008 Mar;190:2004–13.

Zhao G, Ceci P, Ilari A, Giangiacomo L, Laue TM, Chiancone E, et al. Iron and hydrogen peroxide detoxification proprieties of DNA-binding Protein from starved cells: a ferritin-like DNA-binding protein of *Escherichia coli*. J Biol Chem. 2002;277:27689–96.

Justino MC, Almeida CC, Gonçalves VL, Teixeira M, Saraiva LM. *Escherichia coli* YtfE is a di-iron protein with an important function in assembly of iron-sulphur clusters. FEMS Microbiol Lett. 2006 Apr;257:278–84.

Justino MC, Almeida CC, Teixeira M, Saraiva LM. *Escherichia coli* di-iron YtfE protein is necessary for the repair of stress-damaged iron-sulfur clusters. J Biol Chem. 2007 Apr 6;282:10352–9.

Nobre LS, Garcia-Serres R, Todorovic S, Hildebrandt P, Teixeira M, Latour J-M, et al. *Escherichia coli* RIC is able to donate iron to iron-sulfur clusters. PLoS One. 2014;9:e95222.

Silva LSO, Baptista JM, Batley C, Andrews SC, Saraiva LM. The Di-iron RIC Protein (YtfE) of *Escherichia coli* Interacts with the DNA-Binding Protein from Starved Cells (Dps) To Diminish RIC Protein-Mediated Redox Stress. J Bacteriol. 2018;200:e00527-18.

The role of a *quasi*-conserved domain in flavodiiron NO reductases

Susana Fernandes, Maria C. Martins, Bruno Salgueiro, Filipe Folgosa and Miguel Teixeira

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

scfernandes@itqb.unl.pt

Within the human host, namely in the gut, anaerobes are subjected not only to non-negligible and fluctuating O_2 concentrations, but also to the nitric oxide produced by the innate immune system. Both those diatomic molecules inhibit a plethora of metabolic pathways. In order to survive in that environment, microbes have enzymatic systems that combat NO and O_2 exposure. Flavodiiron Proteins (FDPs) are responsible for the detoxification of those molecules, reducing them to the innocuous N_2O or H_2O, and are present in the three life domains [1].

All FDPs have two core domains: a metallo-β-lactamase like domain, which contains the catalytic diiron site, and a flavodoxin-like domain with a flavin mononucleotide (FMN). The complexity of this family of enzymes expands with the addition of extra domains at the C-terminal side. This is the case of the FDP from *Escherichia coli* that has an additional rubredoxin domain at the C-terminus, and therefore is also called Flavorubredoxin. This protein is a NO-selective FDP (although having a residual O_2 reducing activity), and its crystallographic structure has already been obtained [2]. FDPs have a quite conserved motif in the metallo-β-lactamase domain, GS(T)S(T)YNA. To understand the influence of those residues in the enzyme's activity, we designed five site-directed mutants: S33V, S34V, S33V/S34V, Y35F and N36L.

All mutants were biochemically characterized but, while maintaining the general properties of the wild type enzyme, including their stability, there was a drastic effect in the NO and O_2 reducing activities: the O_2 reducing activity decreased 20-60%, and the NO reducing activity was almost abolished. These results show the importance of that motif for the catalysis of FDPs.

References

[1] M. Martins, C. V. Romão, F. Folgosa, P. Borges, C. Frazão, and M. Teixeira, "How superoxide reductases and flavodiiron proteins combat oxidative stress in anaerobes," *Free Rad. Biol. Med.*, vol. 140, pp. 36–60, 2019.

[2] C. V. Romão *et al.*, "Structure of *Escherichia coli* Flavodiiron nitric oxide reductase," *J. Mol. Biol.*, 2016.

The multidomain flavodiiron protein from *Syntrophomonas wolfei*

Maria C. Martins[a], Filipe Folgosa[a] and Miguel Teixeira[a]

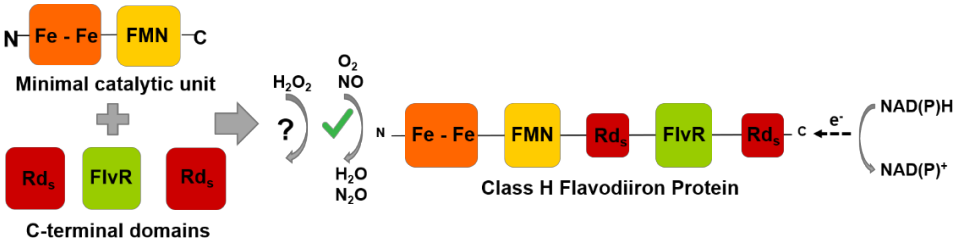
Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

mcmartins@itqb.unl.pt

Syntrophomonas wolfei is an anaerobic bacterium of the *Clostridiales* family, with the ability of oxidizing saturated fatty acids efficiently, in syntrophy with methanogens and other hydrogen/formate-using microorganisms, and because of this, it has been extensively used in bioremediation processes. The genome of this bacterium codifies for a multidomain flavodiiron protein (FDP) belonging to one of the most complex classes of FDPs.[1,2,3]

Flavodiiron proteins constitute a widespread family of enzymes present in all life domains with a crucial role in O_2/ROS detoxification and/or NO detoxification, through the reduction of these species either to H_2O or N_2O, respectively. All the FDPs have a minimal catalytic unit composed by two main domains, a metallo-β-lactamase like domain, harboring the catalytic diiron site (Fe-Fe) and a flavodoxin-like domain with an FMN moiety.[2]

The majority of already characterized FDPs have only this core, but more complex arrangements were found in the genomes of Firmicutes. We recently identified nine classes of FDPs on the basis of the domains' architecture. [3] These complex FDPs have a variety of extra domains following the C-terminal; for example in the case of *S. wolfei* FDP two short-chain rubredoxin domains (Rd_s) and one NAD(P)H:flavin oxidoreductase (FlvR) domain are present.[2,3,4]



In the present work, class H FDP from *S. wolfei* was successfully overexpressed and purified with good yields of cofactor incorporation, 1.6 and 3.2 for flavin and iron per protein, respectively. The kinetic characterization is under progress, but it is already determined a remarkable O_2 reduction activity of 35 s^{-1} and 43 s^{-1} using NADH and NADPH as electron donors respectively. Additionally, it was already verified the capacity for NO reduction activity using NADH as electron donor.

Currently we are completing the kinetic characterization of this FDP with the different substrates and electron donors in order to understand the physiological role of this enzyme, the most complex multidomain FDP characterized until now.

References

[1] Sieber JR, *et al*, Environmental Microbiology, 2010, 12(8), 2289–2301
[2] Martins MC, *et al*, Free Rad Biol Med, 2019, 140, 36-60.
[3] Folgosa F, Martins MC, *et al* FEMS Microbiol Lett,2018, 365, fnx267.
[4] Folgosa, F, Martins, MC, *et al,* Scient. Reports, 2018, 8:10164.

Synthesis of bio-hybrid electrode materials for hydrogen evolution

Afridi Zamader [a,b], Bertrand Reuillard[b], Holly J. Redman[a], Marco Lorenzi[a], Henrik Land[a], Francesca Valetti[c*], Gustav Berggren[a*] and Vincent Artero[b*]

a. Molecular Biomimetic group, Ångström Laboratory, Uppsala University, Sweden
b. Univ. Grenoble Alpes, CNRS, CEA, Laboratoire de Chimie et Biologie des Métaux, France
c. Department of Life Sciences and Systems Biology, University of Torino

Email address of presenting author: afridi.zamader@gmail.com

[FeFe] Hydrogenase enzyme attracted enormous attention for its reversible H_2 production form water with remarkable catalytic rate (6000–9000 molecules H_2 Sec^{-1} per site). Application of such enzymes for making biohybrid devices using the combination of natural (enzymes, cells etc.) and functional artificial (electrodes, semiconductor, nanoparticle etc.) systems stands out as a very promising alternative in recent years. Here, we are preparing Hydrogen evolution catalyst (HEC), which is semisynthetic [FeFe] Hydrogenase(s) with six [2Fe] active site analogues (CO, CN^- and PMe_3 variant) using artificial maturation technique to analyse the effect of tuning electronic and steric factor around active site. In parallel, we are preparing functional mimics of native enzyme, by embedding $[Fe(CO)_3]_2$ (differ by type of bridging ligand) complex with covalently linked polymer using Atomic transfer radical polymerisation (ATRP) technique. We are also studying oxygen resistant [FeFe] hydrogenase (Morra, S. *et.al*. Biochemistry 2016) called *CbA5H* for similar purpose. Finally, to integrate such catalysts (enzyme, biohybrid and bioinspired) for making device, we are developing methodology to anchor them onto nanostructured electrode substrates like-carbon nanotubes .These hybrid materials will be characterized using a range of advanced spectroscopic techniques and their hydrogen evolution activity will be assessed using electrochemical methods coupled to chromatography.

Putative phosphorylation of Escherichia coli anaerobic nitric oxide reductase flavorubredoxin

Silviya R Stateva and Miguel Teixeira

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal; stateva@itqb.unl.pt

Nitric oxide (NO) plays a key role in biology, both beneficial (at nanomolar levels) and adverse (above micromolar levels), as it inhibits multiple metabolic pathways. This adverse effect is used by the human innate immune system to combat pathogens using NO produced by macrophages and neutrophils. Furthermore, this effect is enhanced by the production of reactive oxygen species, also produced by the immune system, which react with NO to produce even more potent weapons. Microorganisms possess an arsenal of enzymatic systems to combat NO stress, being the most extensively characterized in anaerobic conditions the flavodiiron proteins (1), such as the *E.coli* flavorubredoxin (NorV), which reduces NO to nitrous oxide (2). We found recently, using two different *in silico* prediction tools NetPhosBac 1.0 and MPSite that NorV is predicted to have several putative phosphorylation sites. Therefore, we studied the potential phosphorylation of NorV using a phos-tag based system. Here we present preliminary experimental evidences suggesting that NorV is subjected to phosphorylation, a novelty in the field of microbial NO metabolism.

References:

1. Martins, M. C., Romao, C. V., Folgosa, F., Borges, P. T., Frazao, C., and Teixeira, M. (2019) How superoxide reductases and flavodiiron proteins combat oxidative stress in anaerobes. *Free radical biology & medicine*
2. Gomes, C. M., Giuffre, A., Forte, E., Vicente, J. B., Saraiva, L. M., Brunori, M., and Teixeira, M. (2002) A novel type of nitric-oxide reductase. Escherichia coli flavorubredoxin. *The Journal of biological chemistry* **277**, 25273-25276

Insights into determinants of the activity of the flavodiiron NO reductase from *E. coli*

Jéssica Soares, Filipe Folgosa and Miguel Teixeira

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

jessica.soares@itqb.unl.pt

Currently, it is known that nitric oxide (NO) and its derived species are very important molecules for the innate immune system to fight pathogens.[1] The production of NO by the macrophages and neutrophils, provides an efficient defence mechanism against pathogens. However, many pathogens, present the ability to overcome or attenuate those defence mechanisms due to the production of proteins that can effectively perform the scavenging of those molecules. One of those proteins is a flavodiiron protein (FDP) from *E. coli* (flavorubredoxin, FlRd) that catalyses the conversion of nitric oxide (NO) to nitrous oxide (N_2O); FlRd has also a small activity of oxygen reduction to water. FlRd is, thus far, the only known flavodiiron protein that has the NO molecule as the principal substrate.[2]

This FDP is composed by a diiron catalytic centre containing domain, and a flavodoxin like domain – a common core present in all FDPs – and an additional rubredoxin like domain in the C-terminus.[2] This protein possesses a stretch of highly conserved amino acids close to the N-terminus, namely serines 33 and 34, that are putative phosphorylation sites. In order to test that hypothesis, those amino acids were substituted by site-directed mutagenesis, by two aspartates[3], that mimic phosphorylation sites, S33D and S34D.

In this work we present the proteins' quaternary structure, a tetramer in solution, similar to what was observed for the wild-type protein, as well as its reduction activity towards oxygen which showed similar activity when compared to the wild-type FlRd. The determination of the kinetic parameters resulted in a V_{max} of 2.6 and 2.5 s^{-1} and in a K_M of 25.4 and 30.2 μM for S33D and S34D, respectively. Both mutant variants also showed activity for NO and hydrogen peroxide reduction.

References:

1. Wink, D. A. *et al*. *J. Leukoc. Biol.* **89**, 873–891 (2011).
2. Martins, M. C. *et al. Free Radic. Biol. Med.* **140**, 36–60 (2019).
3. Chen, Z. & Cole, P. A. *Curr. Opin. Chem. Biol.* **28**, 115–122 (2015).

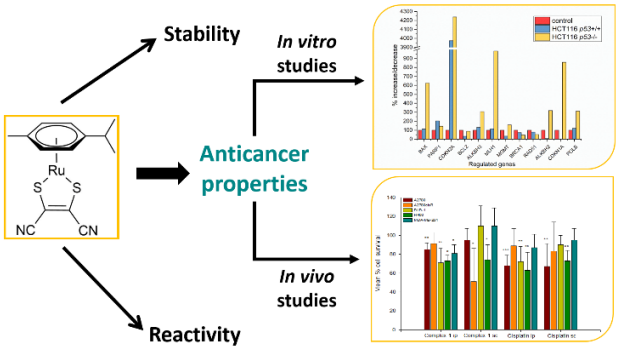
Preclinical Anticancer Evaluation of an Electron-Deficient Organoruthenium(II) Complex

Maria Azmanova[a], Joan J. Soldevila-Barreda[a], Anaïs Pitto-Barry[a], Patricia A. Cooper[b], Steven D. Shnyder[b] and Nicolas P. E. Barry[a]

[a] *School of Chemistry and Biosciences, University of Bradford, United Kingdom*
[b] *Institute of Cancer Therapeutics, University of Bradford, United Kingdom*

M.Azmanova@bradford.ac.uk

There is an urgent need to find novel anticancer therapeutics with different mechanisms of action (MoAs) than platinum(II) drugs, particularly for patients who relapse after having been initially treated with a platinum-containing chemotherapy regimen (*ca.* 50% of all anticancer chemotherapy treatments). In this context, ruthenium-based drugs have demonstrated great potential[1-3] with several complexes exhibiting their anticancer properties *via* MoAs different than nuclear DNA-binding.



Here, we will discuss the *in vitro* cytotoxicity of a 16-e organoruthenium(II) complex towards human lung, colon, and ovarian cancer cells, along with a preliminary investigation of its MoA *via* gene expression studies, cell cycle analysis, and DCFH-DA fluorescence assay. The toxicity and the efficacy of the complex, assessed by a series of *in vivo* studies in mice, including evaluation of the maximum tolerated dose and pharmacodynamics studies, will be discussed.[4]

This project is supported by the Royal Society (University Research Fellowship no. UF150295 to NPEB), the University of Bradford (RDF Award for *in vivo* work), and by the Academy of Medical Sciences (Springboard Award SBF003/1170 to NPEB).

1. Adhireksan, Z.; Palermo, G.; Riedel, T.; Ma, Z.; Muhammad, R.; Rothlisberger, U.; Dyson, P. J.; Davey, C. A. *Nat. Commun.* **2017,** *8*, 14860.
2. Hartinger, C. G.; Jakupec, M. A.; Zorbas-Seifried, S.; Groessl, M.; Egger, A.; Berger, W.; Zorbas, H.; Dyson, P. J.; Keppler, B. K. *Chem. Biodivers.* **2008,** *5*, 2140-55.
3. Barry, N. P. E.; Sadler, P. J. *Chem. Commun.* **2013,** *49*, 5106-5131.
4. Soldevila-Barreda, J. J.; Azmanova, M.; Pitto-Barry, A.; Cooper, P. A.; Shnyder, S. D.; Barry, N. P. E. *ChemMedChem* **2020,** *15*, 982-987.

Appended aromatic moieties determine the Cytotoxicity of Neutral Cyclometalated Platinum(II) complexes derived from 2-(2-Pyridyl)benzimidazole

Monica V., Natalia B., Natalia F-P., Gustavo E. and Begoña G.

[a]*Departamento de Química, Facultad de Ciencias, Universidad de Burgos, Spain*
mvaquero@ubu.es

After the discovery of cisplatin,[1] different families of Platinum(II) complexes have been designed and tested as antiproliferative agents.[2] The substitution of the NH_3 ligands by heterocyclic or alicyclic amines could reduce the toxicity of the platinum complexes[3] compared to cisplatin. In particular, it has been described that the use of bulky amines prevents the deactivation of the platinum complexes in comparison with cisplatin by binding to thiols present in the cell, such as glutathione.[4]

Different Pt(II)-complexes of formula $[PtX_2(N\hat{}N)]$, being (N^N)= 2-(2-pyridyl)benzimidazole derived ligands, have been described in the literature with contrasting results in terms of cytotoxicity, deactivation by thiols, interactions with proteins, etc.[5] that depend principally of the functionalization of the NH group of the pyridylbenzimidazole ligand.

Inspired by these studies we synthesized the first family of neutral Cyclometalated Pt(II) complexes **[Pt-L1]–[Pt-L4]** containing 2-(2-pyridyl)benzimidazole scaffold functionalized with different appended aromatic groups (see Figure 1). We studied the behaviour of these complexes as antitumoral agents against SW480 cells (adenocarcinoma cell line), the interaction with biomolecules as BSA, *d*GMP and DNA concluding the great influence of the appended aromatic moiety on them.[6]

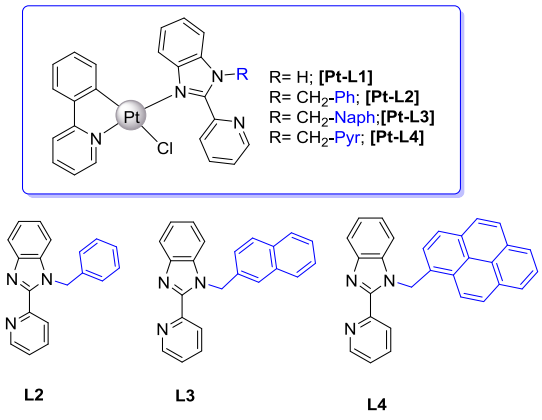


Figure 1

[1]Rosenberg, B.; Van Camp, L.; Krigas, T . *Nature* **1965**, *205*, 698–699.
[2] a) Deo, K. M.; Ang, D. L.; McGhie, B.; Rajamanickam, A.; Dhiman, A.; Khoury, A.; Holland, J.; Bjelosevic, A.; Pages, B.; Gordon, C.; Aldrich-Wright, J. R. *Coord. Chem. Rev.* **2017**, *375*, 148–163. b) Bai, L.; Gao, C.; Liu, Q.; Yu, C.; Zhang, Z.; Cai, L.; Yang, B.; Qian, Y.; Yang, J.; Liao, X.. *Eur. J. Med. Chem.* **2017**, *140*, 349–382.
[3] Cleare, M. J.; Hydes, P. C.; Malerbi, B. W.; Watkins, D. M.. *Biochimie* **1978**, *60*, 835–850.
[4] a) Maji, M.; Karmakar, S.; Ruturaj; Gupta, A.; Mukherjee, A.. *Dalton Trans.,* **2020***, 49,* 2547-2558, b) Kelland, L. R.; Sharp, S. Y.; O'Neill, C. F.; Raynaud, F. I.; Beale, P. J.; Judson, I. R. *J. Inorg. Biochem.* **1999**, *77*, 111–115.
[5] a) Pérez-Arnaiz, C.; Leal, J.; Busto, N.; Carrión, M. C.; Rubio, A. R.; Ortiz, I.; Barone, G.; Díaz De Greñu, B.; Santolaya, J.; Leal, J. M.; Vaquero, M.; Jalón, F. A; Manzano, B. R.; García, B. *Inorg. Chem.* **2018**, *57*, 6124−6134. b) Mansour, A. M.; Shehab, O. R.. *Dalton. Trans.* **2018**, 3459–3468. c) Mitra, K.; Gautam, S.; Kondaiah, P.; Chakravarty, A. R. *ChemMedChem* **2016**, *11*, 1956–1967.
[6] Vaquero, M.; Busto. N; Fernández-Pampín, N.; Espino G; García. B. *Inorg. Chem.* **2020**, *59*, 4961−4971

Anticancer Activity of Neutral Cyclometalated Platinum(II) complexes bearing imidazole derivatives

Natalia Fernández-Pampín[a], Mónica Vaquero[a], Natalia Busto[a] and Begoña García[a]

[a] *Departamento de Química, Facultad de Ciencias, Universidad de Burgos, Spain*

nfpampin@ubu.es

Clotrimazole (**CTZ**) and Bifonazole (**BFZ**) are well known antifungal agents used in mycotic infections like athlete´s foot, vulvovaginal and oropharyngeal candidiasis, between others.[1] Due to the high efficiency and the unremarkable side effects of **CTZ**,[2] this drug was tested against other diseases: Sickle cell disease, Malaria (*Plasmodium*), Chagas (*Trypanosomacruzi*) and Cancer. Therefore, coordination of **CTZ** and **BFZ** to transition metals may be a strategy for enhancing the biological activity of the drugs or modifying their mechanism of action.

There are two examples of Pt(II) complexes containing **CTZ** and none with **BFZ** in bibliography. $K_2[PtCl_4(\mathbf{CTZ})_2]$ was tested against *Trypanosomacruzi*[3] and the *cis*- and *trans*- complexes with formula $[PtX_2(\mathbf{CTZ})_2]$ (X= Cl, I) present activity against six tumor cell lines (prostate, pancreas, breast, colon between others) observing that both complexes showed lower cytotoxicity than cisplatin.[4]

Motivated by these studies we designed and synthesized the first family of neutral cyclometalated complexes containing **CTZ** and **BFZ** (See Figure 1) and we tested their activity as antibacterial and antitumoral agents.

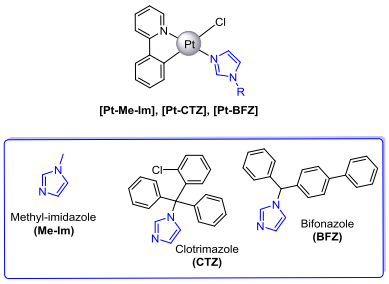


Figure 1. Synthesized Pt(II) complexes bearing imidazol derivatives.

The cytotoxic activity of the complexes was evaluated in lung adeconocarcinoma cells (A549).Only two of them,**[Pt-Me-Im]** and **[Pt-BFZ],** display higher cytotoxicity than cisplatin. By contrast, and in line with the reported results, [4]**[Pt-CTZ]** is less cytotoxic than cisplatin in this tumor cell line. Additionally, the interaction with DNA and the cellular uptake were studied. Briefly, all the complexes interact with double stranded DNA, a commonly identified cellular target for Pt(II) complexes.[5]A lack of correlation between cytotoxicity and cellular internalization was observed for this family of complexes, indicating that can interact with other biological target. In addition, apoptosis studies have shown that cell death is not inhibited by Z-VAD-FMK revealing that the death mechanism is not mediated by caspases.

[1] a) J. M. Torres-Rodríguez, *Arch. Med. Res.***1993**. b) P. D. Crowley, H. C. Gallagher, *J. Appl. Microbiol.***2014**, *117*, 611–617.
[2] S. Kadavakollu, C. Stailey, C. S. Kunapareddy,S. White. *Med. Chem.* **2014**, *4*, 722–724.
[3] R. Sánchez-Delgado, M. Navarro, K. Lazardi, R. Atencio, M. Capparelli, F. Vargas, J. Urbina, A. Bouillez, A. F. Noels, D. Masi, *Inorganica Chim. Acta***1998**, *275-276*, 528-540.
[4] A) T. Gagini, L. Colina-Vegas, W. Villarreal, L. P. Borba-Santos, C. De Souza Pereira, A. A. Batista, M. Kneip Fleury, W. De Souza, S. Rozental, L. A. S. Costa, et al., *New J. Chem.*,**2018**,*42*, 13641. b) M. Navarro, A. R. Higuera-Padilla, M. Arsenak, P. Taylor, *Transit. Met. Chem.***2009**, *34*, 869–875.
[5] F. Trudu, F. Amato, P.Vanahara, T. Pivetta, A.M.Peña-Méndez, J. Havel. *Coord. Chem Rev.***2015**, 13, 79 – 103.

Photoactivation of Dual Antibacterial and Anticancer Activities of New Luminescent 2-Benzoazole-Phenolato Cycloplatinated(II) Complexes

Enrique Ortega, [a] Cristina Pérez-Arnaiz, [b] Venancio Rodríguez,[a] Christoph Janiak,[c] **Natalia Busto**, [b,*] Begoña García[b,*] and José Ruiz[a,*]

[a] *Departamento de Química Inorgánica, Universidad de Murcia and Institute for Bio-Health Research of Murcia (IMIB-Arrixaca), Spain*
[b] *Departamento de Química, Facultad de Ciencias, Universidad de Burgos, Spain*
[c] *Institut für Anorganische Chemie und Strukturchemie, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany*

nbusto@ubu.es

One of the common challenges faced by chemotherapy patients are bacterial infections due to inherent cancer immunodeficiencies, neutropenia, disruption of mucosal barriers…[1] being those caused by Gram-positive bacteria (including multidrug resistant (MDR) strains) the most common infections.[2] As a result, often antibiotics are prescribed during chemotherapy treatment of cancer disease. One of the most successful anticancer agent is Cisplatin whose activity is based on the induction of DNA lesions.[3] The emergence of drug-resistance along with its severe side effects[4] pushed the development of new anticancer drugs.

In addition, Photodynamic Therapy (PDT) has also emerged as a prominent chemotherapeutic anticancer treatment, as it enables spatio-temporal control over drugs activation and consequently, over their biological action and therapeutic effects.[5] Similarly, photodynamic microorganism inactivation (PDI) is also increasingly used both as therapeutic and as prophylactic modalities for the eradication of MDR bacteria strains.[6] In this context, the development of new compounds provided with antibacterial and antitumour activities that can be easily modulated by photoactivation could be an interesting approach in combating bacterial infections in cancer chemotherapy patients.

Three neutral cycloplatinated(II) complexes of the type [Pt(dmba)(L)] (dmba = *N*,*N*-dimethylbenzylamine-κ*N*,κ*C* and L three different 2-benzoazole-phenolato ligands (Fig. 1) were synthesized and characterized. The effect of the hemilabile N^O ligand in both their anticancer and antibacterial activities in the dark and upon blue light irradiation was evaluated with interesting results.

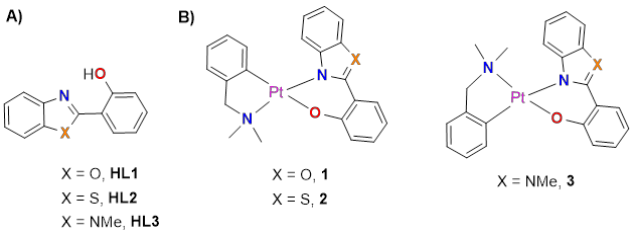


Figure 1. A) Pro-ligands HL and B) Pt(II) complexes.

[1] L. R. Baden, W. Bensinger, M. Angarone, C. Casper, E. R. Dubberke, A. G. Freifeld, R. Garzon, J. N. Greene, J. P. Greer, J. I. Ito, J. E. Karp, D. R. Kaul, E. King, E. Mackler, K. A. Marr, J. G. Montoya, A. Morris-Engemann, P. G. Pappas, K. Rolston, B. Segal, S. K. Seo, S. Swaminathan, M. Naganuma, D. A. Shead, *J Natl Compr Canc Netw* **2012**, *10*, 1412–1445.
[2] E. Montassier, E. Batard, T. Gastinne, G. Potel, M. F. de La Cochetière, Eur. J. Clin. Microbiol. Infect. *Dis.* **2013**,*32*, 841–850.
[3] T. C. Johnstone, K. Suntharalingam, S. J. Lippard, *Chem. Rev.* **2016**, 116, 3436–3486.
[4] L. Kelland, *Nat. Rev. Cancer* **2007**, *7*, 573–584.
[5] Z. Huang, H. Xu, A.D. Meyers, A.I. Musani, L. Wang, R. Tagg, A.B. Barqawi, Y.K. Chen. *Technol. Cancer Res. Treat.* **2008**, 309–320.
[6] N. A. Smith, P. Zhang, S. E. Greenough, M. D. Horbury, G. J. Clarkson, D. McFeely, A. Habtemariam, L. Salassa, V. G. Stavros, C. G. Dowson, P. J. Sadler, *Chem Sci* **2017**, *8*, 395–404.

Reversible BSA aggregation by Cu_2-complexes with metalloproteins active sites as potential antitumoral drug delivery process: a scattering study

Jesica Paola Rada, Ana Maria Percebom and Nicolás A. Rey

Pontifical Catholic University of Rio de Janeiro, Department of chemistry, Brazil

Jesica.paola.rada@gmail.com

Bovine serum albumin, BSA, is a protein present in the blood, known to carry drugs within the biological system. Thus, its interaction with potential drugs has been largely studied, through classic spectroscopies measures.[1] Nevertheless, scattering techniques offer additional and significant information about the structure of the biomolecules when they are exposed to chemical compounds, which enable to understand better the interaction events. Therefore, the use of scattering measurements has rapidly increased in biological and biopharmaceutical sciences.[2] Moreover, hydroxo-bridged dinuclear copper(II) complexes constitute good candidates for prospective antitumor drugs.[3] Especially, copper complexes with a metal sphere simulating the active sites of various metalloproteins (as streptomyces tyrosine,[4] kidney bean PAP[5] and catechol oxidase from sweet potato[6]) have displayed excellent IC_{50} values when tested in different cancer cell lines. The present work extends the research to two 2-thiophene and 2-furan ligands and their copper complexes (Fig. 1).

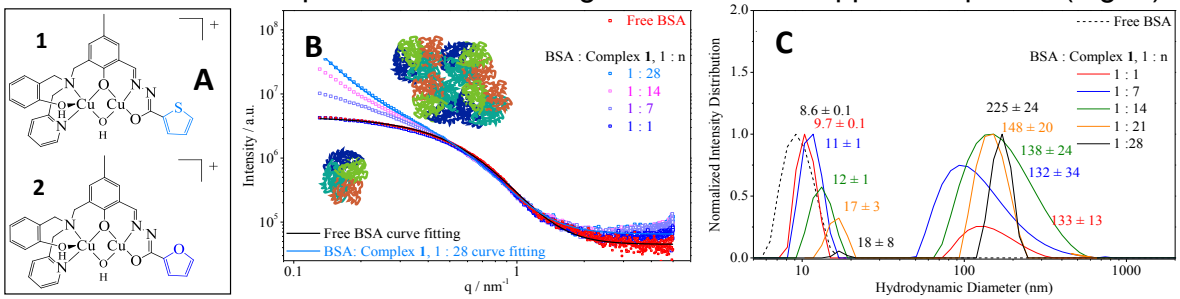


Fig. 1 (A) Dinuclear copper(II) complexes **1** and **2**. (B) SAXS curves and (C) Hydrodynamic diameter of BSA (5×10^{-5} M) in the absence and presence of **1**.

The results of the small angle x-ray scattering, SAXS, of a pure BSA provided a gyration radius, R_g, of 3.5 nm. The **1** and/or **2** induced a slight increase of the size of the protein, and the appearance of an extra population. The curves fitted with Generalized Gaussian Coil model and the results confirmed an aggregation phenomenon. The R_g values were 3.8 and 68 nm, for **1**, and 3.9 and 52 nm for **2**, respectively. For the sake of comparison, the copper salt $Cu(ClO_4)_2{\cdot}6H_2O$ and metal free ligands themselves did not induce protein aggregation. The evidence showed the crucial role of the copper(II) ions coordinated to ligands that mimic the active sites of metalloproteins in the aggregation process. Using Dynamic Light Scattering, DLS technique, confirmed the results obtained by SAXS. With time, it was observed the relaxing of the complexation, turning the BSA to its initial state. These results confirm a reversible aggregation process, which could be probably associated to the transport of potential anticancer copper complexes in blood.

[1] S. Kumar, et. al., *Mol. Struct.* **2017**, *1142*, 293-303. [2] A. P. Minton, *Anal. Biochem.* **2016**, *501*, 4-22. [3] J. P. Rada, et. al., *Inorg. Chem.* **2019**, *58*, 8800-8819. [4] H. Decker, et. al., *Angew. Chem. Int. Ed.* **2006**, *45*, 4546-4550. [5] N. Strater, et. al., *Science.* **1995**, *268*, 1489-1492. [6] P. E. M. Siegbahn, *J. Biol. Inorg. Chem.* **2004**, *9*, 577-590. Authors acknowledge CAPES, FAPERJ, FAPEMIG, FINEP, CNPq, LNLS.

Elemental bioimaging and speciation analysis to track gadolinium retention from MRI contrast agents in the body

Michael Sperling, Stefanie Fingerhut, Rebecca Buchholz, and Uwe Karst

University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstr. 30, 48149 Münster, Germany

e-mail: msperlin@uni-muenster.de)

Metallopharmaceuticals find increasing use as therapeutics and as diagnostic agents in recent years. While platinum-based cytostatics likely are the most important examples, gadolinium-based magnetic resonance imaging (MRI) contrast agents have become a focal point of researchers and governmental agencies in the last decade. The compounds are known to be tolerated well, and to exhibit only few side-effects due their fast excretion kinetics with a half-life of only two hours.

However, in 2006, these compounds started to be associated with a newly discovered disease, nephrogenic systemic fibrosis (NSF), which is only observed for dialysis patients. Furthermore, the disease has only been described for contrast agents with linear ligands, but not for those with macrocyclic ligands. This indicates an influence of the lower kinetic stability of the contrast agents with linear ligands. While the general cause of the disease with a deposition of the gadolinium in the lower parts of the skin has been identified, many details of the pathogenesis are still unknown.

Very recently, small residues of the contrast agents have been discovered to remain in the human brain, and many related questions are currently under investigation. Even despite any current indication for respective pathogenic effects, scientists, manufacturers and regulatory agencies are concerned about these findings.

To investigate this situation, spatially resolved analysis by laser ablation (LA)-ICP-MS [1-5] and synchrotron-based µ-XRF imaging, combined with liquid chromatography (LC) coupled to inductively coupled plasma-mass spectrometry (ICP-MS) and electrospray mass spectrometry (ESI-MS) are used [6].

The results obtained from the different analytical approaches are used to gain information about the processes involved in the retention of gadolinium in different parts of the human body. In this lecture, a brief overview is presented about the development of the respective analytical methods and their application to address the challenges raised above.

[1] D. Clases, M. Sperling, U. Karst, Analysis of Metal-based Contrast Agents in Medicine and the Environment, Trends Anal. Chem., 104, 135-147 (2018).
[2] S. Fingerhut, A.C. Niehoff, M. Sperling et al., Spatially resolved quantification of gadolinium deposited in the brain of a patient treated with gadolinium-based contrast agents, J. Trace Elem. Med. Biol., 45, 125-130 (2018).
[3] S. Fingerhut, M. Sperling, M. Holling et al. , Gadolinium-based contrast agents induce gadolinium deposits in cerebral vessel walls, while the neurophil is not affected: an atopsy study, Acta Neuropathol., 136, 127-138 (2018).
[4] D. Clases, S. Fingerhut, A. Jeibmann, M. Sperling et al., LA-ICP-MS/MS improves limit of detection in elemental bioimaging of gadolinium deposition originating from MRI contrast agents in the skin and brain tissues, J. Trace Elem. Med. Biol., 51, 212-218 (2019).
[5] Radbruch, H. Richter, S. Fingerhut et al., Gadolinium Deposition in the Brain in a Large Animal Model, Invest. Radiol., 54/9, 531-536 (2019)
[6] P. Robert, S. Fingerhut, C. Factor et al., One year Retention of Gadolinium in the Brain: Comparison of Gadodiamide and Gadoterate Meglumine in a Rodent Model, Radiology, 288/2 , 424-433 (2018).

Maternal-developmental undernourishment alters copper homeostasis in the rat kidney and liver

Alma Isabel Santos Díaz,[1*] Abraham Rosas-Arellano,[2] Thomas Kroll,[3] Samuel M. Webb,[3] Marcia Hiriart,[2] Ismael Jiménez Estrada[1] and Fanis Missirlis[1]

[1] Department of Physiology, Biophysics and Neuroscience, Cinvestav, Mexico City, Mexico

[2] Institute of Cellular Physiology, National Autonomous University of Mexico, Mexico City, Mexico

[3] Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Stanford University, Menlo Park, California, USA

*Presenter. alma.santos@cinvestav.mx

We have determined the concentration of Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn in visceral tissue (small and large intestine, lung, kidney, liver and pancreas), skeletal and cardiac muscle (*extensor digitorum longus*, *soleus*, *gastrocnemius* and heart) as well as in central nervous system (spinal cord and brain) of male and female rats fed *ad libitum* or treated with a chronic hypocaloric diet (50% of that consumed by control rats) from 3 weeks before mating, during pregnancy, lactation and continued after weaning until 60 days of postnatal age when the animals were sacrificed. The undernourished rats suffered 70% weight loss compared to their sibling controls. We observed a marked decrease in copper concentration in the kidney of the undernourished rats of both sexes, with a parallel increase in the liver. We discuss our findings in view of a similar response described during infection with fungal or protozoan pathogens in mice. The pancreas of the undernourished rat showed of approximately 40% increase in all elements normalized by the organ's dry weight. Histological examination revealed that exocrine cells maintained their size in the undernourished rat, whereas the islets of Langerhans were significantly fewer and smaller in these animals in relative terms (the pancreas of undernourished animals was smaller primarily due to reduced number of cells). Finally, we noted a tenfold increase in calcium content in the spinal cord of normal rats compared to brain and other organs. We will present X-ray fluorescence images that show the anatomical distribution of this calcium in spinal cord and brain sections. We will also discuss other notable organ-specific differences for specific metals that could be of physiological relevance. Overall, our experiment suggests that severe caloric restriction does not affect in a generalized way the concentration of metals in most organs, with the notable exceptions of the pancreas and, specifically for copper, the kidney and liver.

Metals and morphological synaptic plasticity: insights from correlative super resolution and synchrotron imaging

Richard Ortega[a]

[a]*Chemical Imaging and Speciation, CENBG, CNRS, University of Bordeaux, France*

ortega@cenbg.in2p3.fr

Zinc and copper are involved in neuronal differentiation and synaptic plasticity but the molecular mechanisms behind these processes are still elusive due in part to the difficulty of imaging trace metals together with proteins at the synaptic level. We correlate stimulated emission depletion (STED) microscopy of proteins and synchrotron X-ray fluorescence (SXRF) imaging of trace metals, both performed with 40 nm spatial resolution, on primary rat hippocampal neurons. We achieve a detection limit for zinc of 14 zeptogram (10^{-21} g) per pixel. We reveal the co-localization at the nanoscale of zinc and tubulin in dendrites with a molecular ratio of about one zinc atom per tubulin-αβ dimer. We observe the co-segregation of copper and F-actin within the nano-architecture of dendritic protrusions. In addition, zinc chelation causes a decrease in the expression of cytoskeleton proteins in dendrites and spines. Overall, these results indicate new functions for zinc and copper in the modulation of the cytoskeleton morphology in dendrites, a mechanism associated to neuronal plasticity and memory formation.

Author Index

BioMetals 2020

12th International BioMetals Web-Symposium