CnrX is the membrane-anchored periplasmic sensor of the CnrYXH complex that contributes to regulate Co and Ni resistance in C. metallidurans CH34. This resistance is induced by the specific release of the ExtraCytoplasmic Function sigma factor CnrH from the CnrYX complex upon sensing of increasing amount of Co or Ni in the environment. We have determined the high-resolution structures of the sensor domain (CnrXs) under the Ni-, Co-, and Zn-bound forms as well as in the apo-form and established the structural basis of metal sensing by CnrX [1]. The Zn-bound form represents the resting state of the complex. While the Zn ion is pentacoordinated in a N3O2 sphere, Ni or Co ions recruit the only methionine (Met123) residue as a sixth ligand to switch on the sensing mechanism. This active site thus offers an original N3O2S1 coordination sphere for Ni or Co where S stands for the thioether sulfur of Met123. We have used a series of spectroscopic techniques to characterize the metal-binding sites of CnrXs [2]. We have investigated the role of the only methionine of CnrX as an unusual ligand in the coordination sphere of Ni or Co by a spectroscopic, theoretical and functional study [3]. Met123 plays a crucial role in metal selectivity and affinity and is a key player of the signal transduction, as the M123A-CnrX derivative is no longer able to propagate the signal of the metal binding on the sensor protein in the CnrYXH complex. The mechanism deduced from these results is in agreement with the concept of metal selectivity and allosteric switching developed for the DNA-binding metal-responsive transcriptional regulators.

(No Image Selected)
Abstract Body: Photosynthesis and carbon fixation have a high demand for metal ions, which are extremely scarce in the open oceans. Yet, marine picophytoplankton, including many cyanobacterial species, survive and carry out photosynthesis in these regions. Carbon fixation in cyanobacteria requires a CO₂ concentrating mechanism that includes a carbonic anhydrase (CA) predicted to be metal-dependent. Most CAs are zinc-dependent, but whether this is also true for cyanobacterial CAs is unknown. Indeed, it is not yet clear if zinc is essential for growth of marine cyanobacteria.

In this respect, bioinformatic mining of cyanobacterial genomes revealed several proteins likely involved in zinc homeostasis, together with evidence for their regulation by the zinc uptake regulator Zur.¹ Notably, this included the metallothionein BmtA in the open ocean strain *Synechococcus* sp. WH8102, suggesting a role for BmtA in response to zinc limitation.

Concurrently, we have developed experimental approaches for the capture and detection of zinc-binding proteins from cyanobacterial cell lysates. Immobilised zinc affinity chromatography in conjunction with mass-spectrometry based proteomics has allowed the identification of the periplasmic protein ZnuA from *Synechococcus* sp. WH8102, likely a component of a high-affinity zinc uptake system operating from periplasm to cytosol. In addition, we discovered an outer membrane protein that not only displays significant zinc-binding ability, but is also up-regulated in response to zinc deprivation. Hence, there is mounting evidence that marine cyanobacteria have highly efficient zinc uptake mechanisms, and in accordance with this suggestion, we have found that *Synechococcus* sp. WH8102 accumulates significant quantities of intracellular zinc even under the most extreme zinc-depleted conditions.

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The mechanism of assembly and in situ repair of biological [4Fe-4S] clusters is poorly understood, but has important implications for understanding cellular Fe homeostasis and diseases associated with oxidative stress. This work focuses on recent advances in understanding the mechanism of oxygen-sensing by the fumarate nitrate reduction (FNR) regulatory protein, and the potential role of A-type proteins and monothiol glutaredoxins in [4Fe-4S] cluster assembly or repair. The observation that the oxygen-induced [4Fe-4S]^{2+}-to-[2Fe-2S]^{2+} cluster conversion in FNR primarily involves sulfide oxidation to yield a [2Fe-2S]^{2+} cluster with partial cysteine persulfide ligation, which can be reversed by addition of Fe(II) under anaerobic conditions in the presence of a dithiol reagent (1), constitutes a new type of reversible cluster conversion. Since similar cluster conversions have been observed for oxygen-sensitive [4Fe-4S] cluster-containing enzymes, e.g. members of the radical-SAM superfamily, these results suggest new mechanisms for the assembly or repair of oxygen-degraded [4Fe-4S] clusters. In addition, the ability of monothiol glutaredoxins to act as primary acceptors of [2Fe-2S] clusters assembled on the IscU scaffold protein and to function as effective cluster donors for A-type proteins (2), indicates that these two classes of proteins are partners in cellular cluster trafficking. Possible mechanisms for the assembly or in situ repair of oxygen-damaged [4Fe-4S] cluster using Fe-bound and [2Fe-2S] or [4Fe-4S] cluster-bound forms of A-type proteins will be discussed.

References
Siderophores, are organic chelators produced by bacteria in order to get access to iron. Pyochelin (Pch) and enantiopyochelin (EPch) are enantiomeric siderophores, with three chiral centers, produced under iron limitation conditions by Pseudomonas aeruginosa and Pseudomonas fluorescens, respectively. After iron chelation in the extracellular medium, Pch-Fe and EPch-Fe are recognized and transported by their specific outer membrane transporters; FptA in P. aeruginosa and FetA in P. fluorescens. Using structural analysis of these transporters, combined with mutagenesis and docking studies we were able to investigate and understand the structural basis of the stereospecific recognition of these enantiomers by their respective transporters. The Pch and EPch binding pockets on FptA and FetA respectively do not share any structural homology but display similar physicochemical properties. The stereospecific recognition of both enantiomers by their corresponding transporters is imposed by the configuration of the siderophore’s chiral C4" and C2" centers. This recognition involves specific hydrogen bonds between the transporters and the siderophores.

Besides, we have also investigate the subcellular distribution in particular areas of the cytoplasm, periplasm, or within the membrane of Pch biosynthesis in P. aeruginosa. We used chromosomal replacement to generate P. aeruginosa strains producing fluorescent fusions with enzymes involved in Pch biosynthesis. Cellular fractionation indicated that a substantial amount of enzymes were located in the membrane fractions. Epifluorescence microscopy imaging showed that the enzymes were mainly clustered at the poles of bacteria, indicating a polar segregation of the proteins. All these data indicate that siderophore biosynthesis does not occur all over the cytoplasm but at the level of the inner membrane and concentrated at the bacterial poles.
Siderophores are produced by microorganisms to acquire essential Fe(III). Whilst the most efficient siderophores are hexadentate, others are only tetradentate but still coordinate to Fe(III) and mediate its uptake [1].

The Fe(III) complexes of hexadentate siderophores are coordinatively saturated and interact with their cognate binding proteins through a combination of electrostatic interactions and hydrogen bonding. The periplasmic binding protein CeuE of Campylobacter jejuni, for example, uses three positively charged arginine residues to attract the negatively Fe(III) complex of the hexadentate enterobactin mimic MECAM. In addition, seven hydrogen bonds are formed, one of which involves a nearby tyrosine residue (Figure 1, left) [2].

Since tetradentate siderophores occupy only four of the six coordination sites of an octahedral Fe(III) centre, we were interested in investigating how the remaining free coordination sites are filled upon protein binding. The crystal structure of ferric 4-LICAM bound to CeuE revealed that two amino acid side chains coordinate directly to the Fe(III) centre (Figure 1, right). A tyrosine residue in the binding pocket moves towards the Fe(III) centre to allow direct coordination, whilst a histidine residue, located on a flexible loop, completes the octahedral coordination sphere [3].

By displaying this previously unobserved switch in binding mode, CeuE is the first siderophore binding protein to provide structural insights into the binding of both tetradentate and hexadentate siderophores. A number of related periplasmic binding proteins have tyrosine and histidine side chains in positions similar to those found to coordinate to Fe(III) in the CeuE structure. Hence, we propose that these two residues act as an adaptor that enables certain binding proteins to capture more than one type of siderophore.

References:

Figure 1: Fe(III) coordinated to the siderophore mimics MECAM (left) and 4-LICAM (right) with key amino acid residues in the binding pocket of CeuE shown.
Abstract Body: High-fidelity, metal-sensing is thought to control the buffered concentration of each metal inside cells which, in turn, is thought to be vital in the control of metal-protein speciation (Nature 2009 460: 823-830, Nature 2008 455: 1138-1142).

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

For Ni(II) (J Biol Chem 2012 287: 12142-12151), for Zn(II) (manuscript in preparation), but not for Co(II) (Metallomics 2013 5: 352-362), metal-selectivity matches the relative affinities of the complement of metal-sensors within the cell. These findings have implications for the nature of the buffered pools of metals available to proteins. They illustrate the merit of considering metal-selectivity in the context of a cellular system.

(No Image Selected)
New Approaches to in vivo Copper Chelation

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Metal Homeostasis and Trafficking

Abstract Body: Its redox activity in cells makes copper an essential element to life whose homeostasis is finely tuned. In mammals, the liver is the place where Cu balance is kept, as hepatocytes excrete excess Cu into the bile (1). The Wilson's disease is a genetic disorder that impairs biliary excretion of Cu because of ATP7B dysfunction. COMMD1 is another protein that also participates to the detoxification process. Defect of one or the other results in a gradual accumulation of Cu in the liver, inducing hepatocyte apoptosis, necrosis and cirrhosis. Current treatments aim at preventing dietary Cu absorption by the body, on the one hand and at chelating Cu from the body overload, on the other hand. However, systemic chelation therapy is not always satisfactory, mainly because of harmful side-effects. In the recent years, bringing together our skills in Cu chemistry and biology, we have developed new chelators specifically targeting intracellular Cu(I) in hepatocytes (2,3). The compounds have two functions, the first one eliciting the second one. Indeed, as prodrugs in the plasma where they have no affinity for Cu, they bind the asialoglycoprotein receptor at the hepatocyte basal membrane. After endocytosis, once inside the cell, the drugs are reduced by the high concentration of glutathione and acquire their high affinity and selectivity for Cu(I).

The proof of concept has been obtained in vitro using WIF-B9 cells which reconstitute a complex epithelium with spaces among cells figuring the bile canaliculi. We have shown that under conditions where there is excess Cu inside the cells, addition of one compound or the other in the culture medium removes the excess of Cu. Our program aims now at testing the compounds in vivo with Atp7b-/- mice kindly given by Dr Lutsenko and that are raised at the CEA.


(No Image Selected)
Abstract Body: Helicobacter pylori is a gram negative bacterium persistently colonizing the stomach of half of the human world population. Infection by this pathogen is associated with chronic gastritis, peptic ulcers and adenocarcinoma. To colonize its unique niche, the acidic stomach, H. pylori strictly depends on nickel. Indeed, H. pylori possesses two nickel-enzymes that are essential for in vivo colonization, [NiFe] hydrogenase and the very abundant virulence factor urease. Thus, H. pylori pathogenicity relies on an important supply of nickel, implying a tight control of its distribution and storage.

H. pylori is equipped with original nickel-binding proteins. HspA, an homolog of the GroES chaperone, contains a unique His-rich C-terminal extension that binds nickel in vitro. We have shown that HspA is involved in intracellular nickel sequestration and detoxification and plays a novel role as a specialized nickel chaperone in hydrogenase maturation. H. pylori also synthesizes two His-rich proteins Hpn and Hpn-2 that were shown in vitro to bind nickel. We investigated their evolutionary history and in vivo role. Hpn and Hpn-2 are paralogous and universally conserved in H. pylori. hpn-2 is restricted to H. pylori whereas hpn is present in the genomes of the gastric urease-positive Helicobacter species. Based on phylogenetic analysis, we propose a scenario for the origin and evolution of Hpn and Hpn-2 involving a genomic inversion leading to the duplication of hpn.

Measurements of metal resistance, intracellular nickel concentrations, urease activities and interactomic analysis showed that (i) Hpn is required for urease activation and acts as a nickel-sequestration protein, while Hpn-2 is not, (ii) Hpn forms homo-multimers in vivo and interacts with Hpn-2, (iii) Hpn-2 is restricting intracellular nickel accumulation and (iv) hpn is epistatic on hpn-2. We propose that the combined Hpn/Hpn-2 activities participate in an oriented pathway of nickel transfer, where Hpn-2 acts first followed by Hpn to transfer nickel to the final acceptor urease, resulting in the control of both nickel accumulation and urease activity.
Abstract Body: To maintain optimal intracellular iron levels, iron transport and storage is tightly regulated in all eukaryotic cells ranging from yeast to humans. However, there are significant gaps in our understanding of iron regulation mechanisms at the cellular and molecular level. We are addressing these gaps by teasing out the molecular details of iron sensing and regulation in the budding yeast *S. cerevisiae* and defining the roles of each component in the iron signaling pathway. In yeast, the monothiol glutaredoxins Grx3 and Grx4, the BolA-like protein Fra2, and the aminopeptidase P-like protein Fra1 function together in an iron-responsive signaling pathway that controls nucleocytoplasmic shuttling of the iron-responsive transcription factor Aft1 and its paralog Aft2. Under iron replete conditions, this pathway induces dimerization of Aft1 (and presumably Aft2), favoring their localization to the cytosol and subsequent deactivation of the iron regulon. Using complementary biophysical and molecular genetic methods, we have demonstrated that Fra2 forms glutathione-ligated, [2Fe-2S]-bridged heterodimers with Grx3 or Grx4 and characterized the Fe-S coordination chemistry of these complexes (1,2). In addition, we now have strong evidence that [2Fe-2S]-Fra2-Grx3 transfers a [2Fe-2S] cluster to Aft2, facilitating Aft2 dimerization. We are currently probing the mechanistic details of this Fe-S cluster transfer process to gain a better understanding of how interactions between Aft1/Aft2, Grx3/4, Fra2, and Fra1 influence their in vivo functions. Since several key proteins in this pathway are conserved in humans and essential for viability, exploiting the yeast system to define their functional and physical interactions will provide a fundamental understanding of their roles in human iron metabolism (3).


Proposed mechanism for Fe-dependent inhibition of Aft1/2. Under iron-replete conditions, the Fra2-Grx3/4 [2Fe-2S]-bridged heterodimer transfers a cluster to Aft1/2 facilitating Aft1/2 dimerization and deactivation of the iron regulon.
Abstract Body: Tissue remodelling is a key response to insult and injury from physical, biological or chemical stress in the body. In these situations the regulation of metal ion homeostasis is often disrupted and may lead to oxidative stress as a result of uncontrolled production of reactive oxygen species by the rogue metal ions. A multitude of biochemical strategies are likely to be employed to combat these stresses, and in many cases metalloenzymes or small molecules containing heavy elements will be involved. Improved understanding of whether particular metals are harmful or beneficial in particular conditions provides the opportunity to mitigate the damage caused by them.

Much of our recent work has focussed on various selenium species and their relationship to disease states involving oxidative stress. Our interest stems from the apparent contradiction that while common forms of dietary selenium likely generate ROS in vivo, epidemiology suggests that their intake reduces incidence of many diseases where oxidative stress is implicated. At the same time, selenium's high atomic number and low background abundance in tissue make it amenable to study using X-ray fluorescence imaging and X-ray absorption spectroscopy to yield spatial and chemical information respectively.

I will discuss the use of these methods to follow Se mobilisation in an animal model of heart attack as well as in the process that leads to ovulation in bovine ovaries, both of which involve rapid tissue remodelling. I will also describe our attempts to relate these results to underlying biochemistry and pose challenges for the future application of the approach to a broader set of conditions and metal ions.
New insights on Copper(I)-alpha Synuclein interactions

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Metal Homeostasis and Trafficking

ABSTRACT BODY:

Alzheimer’s disease (AD), Parkinson’s disease (PD) and transmissible spongiform encephalopathies (TSEs) are neurodegenerative disorders characterized by common cellular and molecular mechanisms, such as protein aggregation and oxidative stress-induced damage. The brain deposits found in AD, PD and TSEs patients are mainly constituted by misfolded aggregated proteins: amyloid β (Aβ), α-synuclein (αS) and prion protein (PrP) respectively. All these proteins share the ability to interact with copper, which plays a crucial role in both protein aggregation and oxidative damage (1). Both Aβ and αS are susceptible to copper-catalyzed oxidation. The reaction involves the reduction of Cu(II) to Cu(I) and the formation of ROS (2). This mechanism is highly selective and site-specific, and involves interactions of the protein with both Cu(II) and Cu(I).

During the last decade the scientific community have put a great deal of effort to characterize copper binding to Aβ, αS and PrP, however very few investigations were specifically focused on Cu(I) (3, 4). In this study the Cu(I)-αS binding features have been explored. Beyond Cu(I), we have also used Ag(I) as a probe. The metal interactions with both the full length protein and model peptides were investigated by means of NMR and CD spectroscopy (5, 6). By monitoring the metal induced effects on αS systems the Cu(I)/Ag(I) binding domains have been identified. The corresponding protein structural rearrangements induced by the metal ions have been investigated as well.

References

Abstract Body: Metals are essential for biological function yet are toxic in excess. As a result, all bacteria have evolved the ability to efficiently scavenge metals from the surrounding environment and control the intracellular bioavailability of these critical components of intracellular metabolism. This process is emerging as a central component of the human host-pathogen interface, referred to as “nutritional immunity.” We have long been interested in the structural basis of regulation and specificity of metal homeostasis and sensing in bacteria, which occurs nearly exclusively at the transcriptional level. Recent structural, thermodynamic and biological studies of zinc, manganese and copper sensing and trafficking in the Gram-positive respiratory pathogen Streptococcus pneumoniae will be discussed. In particular, the impact of a key second coordination shell hydrogen bonding interaction in controlling the magnitude of allosteric positive regulation of DNA operator binding by zinc in the zinc-specific uptake repressor, AdcR (adhesin competence repressor), will be presented. A new structural paradigm for copper resistance and trafficking in S. pneumoniae coupled with a new structure of Cu(I)-specific repressor CsoR from a thermophilic Bacillus species collectively reveal novel insights into Cu(I) coordination chemistry in metal homeostasis. NMR studies of apo-tetrameric Geobacillus CsoR (48 kD) reveal enhanced dynamics around Cu(I)-ligand Cys50; Cu(I)-binding induces a kink in the α2 helix and quenches these dynamics, coupled with folding of the N-terminal “tail” region (residues 12-19) over the Cu(I)-binding site. This folding features an Ile16 N-H/Sγ-Cys79’ hydrogen bond in the second coordination shell, a short Tyr49-Glu95’ side chain hydrogen bond previously implicated in Cu(I)-mediated allostery in other CsoRs, and aromatic stacking of Tyr49 on His12’. The implications of this structure on the mechanism of Ni(II)-sensing by the related CsoR/RcnR family proteins cyanobacterial InrS and E. coli RcnR will be discussed. Supported by a grant from the US NIH (GM042569).
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 sensing, trafficking and catalysis will be discussed [1]. The structures of Sporosarcina pasteurii urease bound to citrate [2] and fluoride will be discussed. Structural data on S. pasteurii and Helicobacter pylori UreE revealed conformational changes involved in metal binding and protein-protein interactions [3,4]. Molecular dynamics calculations on UreG, the first documented cases of intrinsically disordered enzymes [5], indicated rigidity of the active site, justifying its residual catalytic activity, and higher flexibility in regions involved in interactions [6]. H. pylori UreF binds two Ni(II) ions per dimer: the coordination environment of the metal ion was determined using X-ray absorption spectroscopy, while an in vivo assay of urease activation supports a role for Ni(II) binding by UreF in urease maturation. The molecular details of the UreDFGE complex in H. pylori were investigated using computational modeling [7]. The calculated structure suggests a mechanism of Ni(II) binding and release. The properties of NikR, a nickel-dependent transcription factor, will be illustrated as determined using both solution and solid state structural data [8].

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TITLE: New Probes for Weaker Cu(I) Binding Sites Completes a Set of Four That Can Detect Affinities from Nanomolar to Attomolar

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CURRENT CATEGORY: Metal Homeostasis and Trafficking

ABSTRACT BODY:

Abstract Body: Copper plays essential roles in biology, but abnormal interactions are damaging. Reliable quantification of copper-protein interactions will underpin molecular understanding of copper nutrition and toxicity. We have previously established two high affinity probes Bathocuproine disulfonate (Bcs) and Bicinchoninic anion (Bca) capable of in vitro quantification of Cu(I) binding with affinities from pico- to atto-molar concentrations (ref 1).

Quantitative probes are required for Cu(I) binding of lower affinity for proteins and peptides typically associated with neurodegenerative diseases. The present work evaluates two classic Fe(II) ligands Ferene S (Fs) and Ferrozine (Fz) as quantitative probes for Cu(I). Both react with Cu(I) quantitatively to yield well-defined complex anions \([\text{Cu}(\text{Fs})_2]^-\) (\(\lambda_{\text{max}} = 484 \text{ nm, } \varepsilon = 6,700 \text{ M}^{-1}\text{cm}^{-1}\)) and \([\text{Cu}(\text{Fz})_2]^-\) (\(\lambda_{\text{max}} = 470 \text{ nm, } \varepsilon = 4,320 \text{ M}^{-1}\text{cm}^{-1}\)). Formation constants \(\beta_2\) were determined by two approaches: direct metal titration and ligand competition. They provided estimates that consolidated the affinities of the two probes to a unified standard: \((10^{15.1}) \text{ M}^{-2}\) for Fz and \((10^{13.7}) \text{ M}^{-2}\) for Fs; ref 2). The four ligands Bcs, Bca, Fz and Fs in combination form a set of versatile probes capable of detecting and differentiating an extended spectrum of Cu(I) binding affinities from nano- to atto-molar concentrations. Selected examples of quantification of weaker Cu(I) binding in proteins and peptides are provided, including an amyloid-β peptide.

References


![Graph](image-url)

Optimal ranges of [Cu+aq] (expressed as pCu+) buffered effectively by the four Cu(I) probe systems Fs, Fz, Bca and Bcs. Dashed and solid lines indicate that 20% and 100% of total CuI is bound by the ligand.
Abstract Body: Tight-binding metalloenzymes account for many of the transition metal binding sites within a cell. It is becoming increasingly apparent that the number of metals bound in regulatory, trafficking and compartmentalized homeostasis sites is significant as well. Intriguingly, metal ion movement in and out of these sites is not random and appears to regulate key cellular decisions. This movement is controlled by metal-specific transport, metallochaperone and metalloregulatory proteins which conduct a metal to appropriate intracellular targets or sense changes in metal availability and turn on or off specific genes. The latter sites sense changes in free metal concentrations in the femtomolar range for Zn(II) or the zeptomolar range for Cu(I) and adjust the transcription of genes in a manner that keeps the cytosolic concentrations of these free metal ion concentrations at vanishing low levels. Recent structural and mechanistic studies of the CueR and Zur proteins reveal novel biophysical mechanisms for transduction of the signal as well as the molecular basis for the remarkable thermodynamic sensitivity of these metal-sensing molecular switches. While steady-state concentrations of free zinc and copper ions are generally quite low in the mammalian cytosol, this is not necessarily so in other compartments where thermodynamically distinct pools of metal ions can ebb and flow in response to cellular signals. For instance the developmental progression of the mammalian egg and the first step of embryo formation requires a stepwise series of massive zinc fluctuations. A variety of new single cell STEM-EDS/EELS, X-ray fluorescence microscopy methods and fluorescent zinc-specific probes reveal that billions of zinc atoms per cell must undergo dramatic translocations in short time frames, including events known as ‘zinc sparks’. We broadly conclude that biological regulatory mechanisms in the egg, embryo and other cells involve key metal-specific receptors as well as wholesale fluctuations in zinc content and activity in subcellular locations.

(No Image Selected)