View Online

Metal sensor proteins: nature's metalloregulated allosteric switches

David P. Giedroc*[a,b] and Alphonse I. Arunkumar[a,b]

Received 4th May 2007, Accepted 13th June 2007
First published as an Advance Article on the web 28th June 2007
DOI: 10.1039/b706769k

Metalloregulatory proteins control the expression of genes that allow organisms to quickly adapt to chronic toxicity or deprivation of both biologically essential metal ions and heavy metal pollutants found in their microenvironment. Emerging evidence suggests that metal ion homeostasis and resistance defines an important tug-of-war in human host–bacterial pathogen interactions. This adaptive response originates with the formation of "metal receptor" complexes of exquisite selectivity. In this perspective, we summarize consensus structural features of metal sensing coordination complexes and the evolution of distinct metal selectivities within seven characterized metal sensor protein families. In addition, we place recent efforts to understand the structural basis of metal-induced allosteric switching of these metalloregulatory proteins in a thermodynamic framework, and review the degree to which coordination chemistry drives changes in protein structure and dynamics in selected metal sensor systems. New insights into how metal sensor proteins function in the complex intracellular milieu of the cytoplasm of cells will require a more sophisticated understanding of the "metallome" and will benefit greatly from ongoing collaborative efforts in bioinorganic, biophysical and analytical chemistry, structural biology and microbiology.

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

Introduction

Transition metal ions are required for life, functioning as cofactors for myriad metalloenzymes that catalyze an extraordinary diversity of biological reactions. As such, cells concentrate metal ions through the activity of specific membrane-associated transporters. Conversely, all metal ions are toxic in excess, and the intracellular availability of each is tightly regulated. Microorganisms have evolved a tremendous sophistication and dedicate considerable regulatory machinery to acquiring, utilizing, trafficking, detoxifying and otherwise managing, the intracellular and extracellular concentrations and types of metal ions encountered in their microenvironments.[1] Toxic heavy metal resistance systems for Hg^{II} and the oxyanions of As are likely billions of years old,[2,3] and evolutionary tinkering of these regulatory systems likely now allows soil- and water-dwelling bacteria to adapt to environments that are heavily polluted with heavy metals from human industrial activity.[4] Understanding how magnetotactic bacteria biomineralize sufficient Fe^{III} to build magnetosomes required for direction

[a]*Department of Biochemistry and Biophysics, Texas A&M University, 2128 TAMU, College Station, TX, 77843–2128, USA*
[b]*Department of Chemistry, Indiana University, 800 E. Kirkwood Avenue, Bloomington, IN 47405–7102, USA. E-mail: giedroc@indiana.edu Fax: +1 812 855 8300; Tel: +1 812 855 9043*

David P. Giedroc

David Giedroc graduated from the Pennsylvania State University in 1980 with a BS in Biochemistry. After a brief stint in Joseph Villafranca's group at Penn State, he earned a PhD in Biochemistry at Vanderbilt University in 1984, where he worked with David Puett on the calcium sensor calmodulin. From 1984–1988, he was an NIH postdoctoral fellow in the late Joseph E. Coleman's laboratory at Yale University, where he worked on zinc-finger DNA binding proteins. From 1988–2007, he was a member of faculty in the Department of Biochemistry and Biophysics at Texas A&M University. He is now Professor of Chemistry at Indiana University.

Alphonse I. Arunkumar

Alphonse Ignatius Arunkumar received BSc and MSc degrees in Chemistry from St. Joseph's College, Bharathidasan University in India. He then pursued his doctoral degree in Biochemistry (1999) at National Tsing Hua University under the guidance of Dr Chin Yu where he studied the structural and biophysical properties of acidic fibroblast growth factors. As a postdoctoral fellow (2000–2004) with Dr Walter Chazin at Vanderbilt University, he studied the molecular basis of multiprotein assemblies particularly the SV40 repliosome. He was then appointed a Research Scientist at Texas A&M University and is currently an Assistant Scientist in the Department of Chemistry at Indiana University.

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

sensing[5] or how Fe-reducing *Geobacter metallireducens*[6] thrives on Fe^{III}-oxides also promises to uncover novel mechanisms of iron scavenging and homeostasis.

For pathogenic bacteria, the challenge may well be greater. These organisms face an ever-changing landscape of metal deprivation and toxicity in the human host, depending on the stage of infection, that is compounded by the oxidative and nitrosidative stress associated with survival inside the phagosomal compartment of infected macrophages. To illustrate, *Mycobacterium tuberculosis*, a human pathogen responsible for two million deaths a year, encodes 28 metal transporter systems and conservatively, ≈15 metal sensor proteins.[7] Not surprisingly, proteins responsible for the acquisition, homeostasis and intracellular trafficking of biologically required metal ions as well as their regulators, *i.e.*, metal sensor proteins, are known virulence factors, deletion of which diminishes or abrogates bacterial survival and/or pathogenesis. Historically, this case has been most strongly made for Fe,[8,9] which is severely limiting and sequestered by host lipocalins, which bind Fe-siderophores secreted by bacteria to scavenge Fe.[10,11] Recent work illustrates the gymnastics that *Staphylococcus aureus*, the causative agent of most wound and hospital-acquired infections, goes to to obtain and utilize heme Fe from the host,[12–15] which itself is superimposed on multiple siderophore-based systems. Genes encoding metal transporter (uptake) components for both Cu^{I} and Mn^{II} are, respectively, virulence factors in food borne infections caused by *Listeria moncytogenes*,[16] *Bacillus anthracis*,[17] the causative agent of anthrax, and *Streptococcus pneumoniae*.[18]

Zn^{II} uptake systems may also play a role in bacterial virulence[19–21] since the availability of free Zn^{II}, like free Fe^{III}, may be limiting[22] or may fluctuate dramatically depending on the specific microenvironment and stage of infection.[23,24] Zn^{II} metalloenzymes are found in biosynthetic pathways required to synthesize complex extracellular structures[25] and maintain intracellular redox status[26] that influence virulence; secreted extracellular zinc metalloproteinases, *e.g.*, lethal factor from *B. anthracis*,[27,28] disable the immune system by blocking intracellular signaling pathways associated with the host defense. Clearly, the Zn^{II} status of the cell is efficiently monitored since multiple Zn^{II}-containing ribosomal proteins (*e.g.*, L31 and S14 in *B. subtilis*) are replaced with a non-Zn^{II} paralog under conditions of Zn^{II} starvation.[29,30] Finally, maintaining Ni^{II} homeostasis is known to be crucial for the gastric colonization of *Helicobacter pylori*, largely due to the action of two Ni^{II} metalloenzymes, hydrogenase, which allows *H. pylori* to use hydrogen as an energy source,[31] and urease, which generates ammonia to maintain a zone of extracellular neutrality in the otherwise acidic environment of the gut;[32] mycobacterial ureases may function similarly by neutralizing the phagosomal pH and inhibiting lysosomal–phagosomal fusion.[33–35]

These few examples illustrate that the degree to which pathogenic bacteria manipulate, exploit and evade host metal-ion status is an important adaptive response to intracellular survival in the mammalian host. This adaptive response is mediated by gene regulatory proteins, coined metalloregulatory proteins[1,36] or metal sensor proteins. These specialized "metal receptor" proteins have evolved metal coordination sites that "sense" specific metals ion(s) by forming specific coordination complexes; this, in turn, functions to activate or inhibit DNA binding or transcription activation, thereby controlling the expression of genes that mediate what must be an exquisitely selective adaptive response (Fig. 1). An emerging consensus summarized in this perspective is that metal selectivity of sensor proteins is dictated strongly by the coordination chemistry of the chelate,[37–39] coupled to the ability of that chelate to drive changes in protein structure and/or dynamics to effect biological regulation.[40]

This perspective will focus on intra-cytoplasmic sensing of transition metal ions in bacteria that employ a single protein that is responsible for both binding metals and regulating gene expression. As such, we will not touch on membrane-associated two-component signaling systems that mediate homeostasis of Mg^{II},[41] transition metal ions, *e.g.*, Cu^{I},[42,43] and resistance to $Cd^{II}/Zn^{II}/Co^{II}$,[44] nor will we discuss an exciting developing story on a Mg^{II}-sensing riboswitch RNA that regulates Mg^{II} uptake.[45–47] In addition, the reader is referred to several recent reviews that

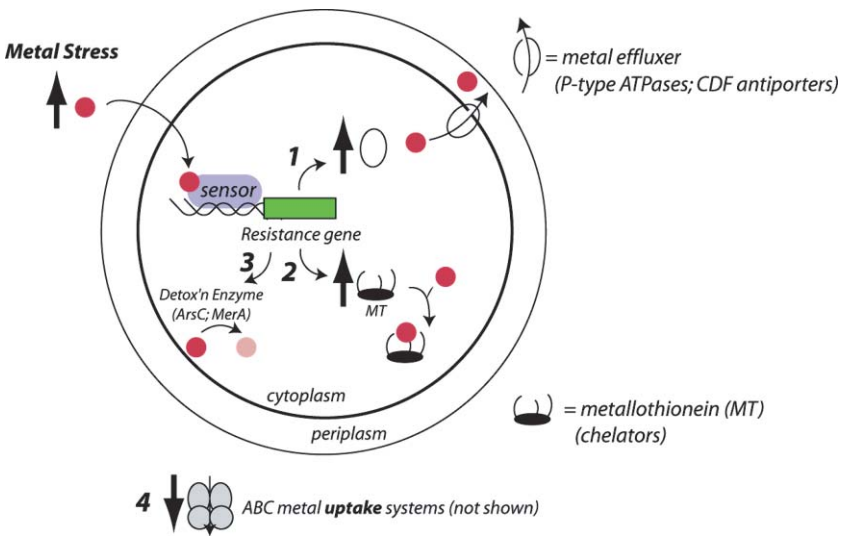


Fig. 1 Cartoon representation of how a generic metal sensor protein affects resistance to toxic concentrations of a particular transition metal ion in the cytoplasm.

 This journal is © The Royal Society of Chemistry 2007

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

place much of what is discussed below in a more comprehensive biological context.[48–50]

The importance of the metallome

Metal-responsive control of gene expression occurs within the context of an organism's metallome, defined as the distribution, identity and quantity of both free (uncomplexed aqueous ions) and complexed metal ions within the cell.[51] Metallomics refers to the study of how the metallome changes in response to some stimulus,[52] and sensitive analytical methods including inductively coupled plasma mass spectrometry (ICP-MS),[52] X-ray fluorescence microscopic imaging,[53] and hybrid metalloproteomics-based strategies for metalloprotein characterization[54,55] are emerging tools to obtain temporal or spatial resolution of the absolute quantities and speciation of multiple elements in a single experiment. Although the metallome remains largely unexplored for the vast majority of organisms and cells, recent evidence suggests that the metallome may well be relatively constant across a range of bacterial species.[56]

The cytoplasm of all cells is a strongly reducing environment maintained by a high ratio of reduced *vs*. oxidized low molecular weight thiols, *e.g.*, glutathione (γ-glutamyl L-cysteinyl glycine) and its conjugates or mycothiols in *M. tuberculosis*,[57] that detoxify reactive oxygen species (ROS) and reactive nitrogen species (RNS).[58,59] In the absence of oxidative stress, these reduced intracellular thiols represent an abundant (≈mM) competing ligand that has a substantial affinity for thiophilic metal ions, in particular Zn^{II} and Cu^{I} and to a lesser degree Ni^{II} and Co^{II}. This reducing environment will further ensure that Mn, Fe and Cu are maintained in the cytoplasm in their reduced oxidation states, Mn^{II}, Fe^{II} and Cu^{I}. This has several consequences. One is that concentrations of free or uncomplexed Cu^{I} and Fe^{II} are kept extremely low so as not to catalyze the production of highly damaging hydroxyl radical.[60] Cu^{I} ions are carefully trafficked inside cells by Cu^{I} chaperones[61,62] which deliver Cu^{I} to acceptor proteins, enzymes and transporters; Fe, on the other hand is stored as Fe^{III} in bacterioferritins[63] and may be trafficked by Dps-like proteins.[64] Secondly, the higher affinity of Cu^{II} relative to Zn^{II} and all other transition metal ions as dictated by the Irving–Williams series,[65,66] is likely of little consequence in the cytoplasm. In contrast, the periplasmic space of gram-negative bacteria is far more oxidizing and this intracellular compartment can be used to sequester excess metal ions, *e.g.*, copper in the more oxidized less toxic Cu^{II} form, either as Cu^{II}/Cu^{I} bimetallated complexes[67–69] or bound to Cu^{II} or Cu^{I}.[70] As required by a more oxidizing environment, Cu^{II} complexes in the periplasm often employ low coordination number thioether (Met) ligation, rather than cysteine thiolates utilized in the cytoplasm.[69,71]

The unique cytoplasmic environment is further shaped by the bacterial metal quota[72] or metallome. Mg^{II} is the most abundant divalent cation, at ≈0.1 M total concentration, with free concentrations in the mM range. Total concentrations of Fe and Zn are next most abundant and are comparable to Ca^{II}, present at $\approx 10^{-4}$ M. Estimates of Cu and Mn are ≈10–50-fold lower (1–10 μM) than Fe/Zn with Co and Ni lower still, in the low μM (Ni^{II}) or sub-μM (Co^{II}) range. A remarkable feature of this picture is that the relative concentrations of free or weakly chelated, kinetically labile aqueous ions, is radically different. Any free Fe^{II} and Mn^{II} will be weakly chelated in the cytoplasm due to their relatively low affinity for thiolate ligands and rapid exchange with other adventitious N/O sites and thus might be present at $\approx 10^{-7}$ M free metal.[51] In order for a cell to detect potential Fe^{II} or Mn^{II} toxicity, the Fe^{II}/Mn^{II}-sensing metalloregulatory proteins might operate at a "set-point" in the μM (or higher) range of free metal; this is, by and large, what has been observed.[73,74] In other words, Mn^{II} and trace Fe^{II}, like Mg^{II} and Ca^{II}, may well equilibrate between free and bound states,[51] and intracellular sensors may well be "tuned" to changes in intracellular concentrations around a particular set-point, reminiscent of the classical Ca^{II} sensor in mammalian cells, calmodulin.[75]

In contrast, the cytoplasm seems to have a tremendous overcapacity to chelate both Cu^{I} and Zn^{II} such that the free concentrations of these metal ions is vanishingly small.[61,72] For toxic Cu^{I}, the reasons for this are generally well-understood,[61] but not so for Zn^{II} which has a relatively low toxicity due to a stable +2 oxidation state and a corresponding lack (to date) of Zn^{II}-specific metallochaperones in the cell. On the other hand, given the considerable extent to which cells concentrate Zn^{II} (*vide supra*), a sizable cytoplasmic pool of free Zn^{II} might compromise the specificity of Fe^{II}/Mn^{II}-sensing and metalloenzyme systems. In any case, the affinity of Cu^{I}/Zn^{II}-specific sensing proteins for their cognate metal might have to be quite high to sense the metal ion in a large pool of competing small molecule and biomolecule (RNA polymerase, ribosome, metalloenzyme) ligands.[38] This is exactly what has been found, with K_{Zn} for *bona fide* zinc sensors in the 10^{12} to 10^{15} M^{-1} range,[72,76] and K_{Cu} in the 10^{18}–10^{21} M^{-1} range.[38,77] As such, Zn^{II} and Cu^{I} will not readily equilibrate between free and bound states, rendering the "set-point" paradigm proposed for Mn^{II} and perhaps Fe^{II} almost certainly not operative. This, in turn, has fueled speculation that Cu^{I}/Zn^{II} sensors, like those that detect heavy metal pollutants Cd^{II}, Hg^{II} and Pb^{II},[78,79] operate under kinetic control, but it is not exactly clear in molecular terms what this means. One possibility is that these extraordinary K_M (association equilibrium constant for metal ion, M) values simply ensure that metal binding is rapid and essentially irreversible in the absence of a metal ligand exchange partner, *e.g.*, a metallochaperone,[80–82] thus protecting the cell by turning on an adaptive response, while minimizing dissociation of the metal from the sensor;[83] a recent investigation of the kinetics of metal binding and dissociation by the Cd^{II}/Pb^{II} sensor CadC is consistent with this picture.[79]

It is not yet clear if the same scenario applies to Co^{II} and Ni^{II}, although it may well be the case for Ni^{II}, given that Ni^{II}-specific metallochaperones are required to metallate Ni^{II} enzymes urease and hydrogenase.[48,84] Furthermore, recent work from *E. coli* suggests that a cytoplasmic Ni^{II} pool detected by the Ni^{II}-specific sensor NikR does not readily equilibrate with a dedicated Ni^{II} pool that enters the cell through the high-affinity *nikABCDE* Ni^{II} transport system that is used exclusively to metallate Ni/Fe hydrogenase.[85] This observation is consistent with the idea, first articulated by Robinson's group,[50] that suggests that intracellular trafficking, sequestration, uptake and efflux of individual metal ions may well vary from cell to cell, and the ability of a sensor to specifically respond to a particular metal ion may be dictated by the intracellular milieu, *i.e.*, the "metallome" characteristic of that organism. A particularly striking demonstration of this is the Ni^{II}/Co^{II}-specific ArsR family sensor *M. tuberculosis* NmtR.[86] NmtR

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

binds metal ions roughly in accord with the Irving–Williams series, $Zn^{II} \geq Ni^{II} >> Co^{II}$; nonetheless, Ni^{II} and Co^{II} derepress NmtR-induced gene expression in a mycobacterial host, *M. smegmatis*, while Co^{II} is the exclusive inducer in cyanobacterial cells (*Synechococcus*).[86] This cytosol-specific selectivity of Co^{II} over Zn^{II} in mycobacteria was found to be determined by two specific features: 1) *M. smegmatis* does not accumulate sufficient Zn^{II} to "turn on" a known Zn^{II} sensor, *Synechococcus* SmtB; and 2) Zn^{II} is a poor allosteric negative regulator of DNA binding relative to Co^{II} or Ni^{II}; X-ray absorption spectroscopy studies reveal that this is because Zn^{II} fails to adopt the octahedral coordination geometry adopted by Co^{II}/Ni^{II}.[37]

Families of metal sensor proteins

There are currently seven major families of metal-sensing transcriptional regulators that have been identified in prokaryotes, and we now have crystallographic structures of one or more representative members in a variety of metallated states for six of these (Fig. 2). In only two families, however, are protein–DNA operator complex structures available, and include the Ni^{II}-DtxR:DNA,[87] Ni^{II}-IdeR:DNA[88] and Ni^{II}-NikR:DNA[89] complexes. Five of the seven sensor families utilize the ubiquitous winged helix domain to bind DNA,[90] formed by an ααααββ secondary structure (Fig. 3a) in which the α2-turn-α3 (HTH) element just N-terminal to the β-wing (β1-β2) makes nucleotide base-specific contacts with the major groove of the DNA operator; the β-wing often makes electrostatic phosphate contacts with the adjacent minor grooves (see Fig. 3b). CsoR has a novel DNA binding fold,[77] while NikR utilizes a dimeric ribbon–helix–helix motif[91] that "reads out" bases in the major groove of the *nik* operator with two N-terminal antiparallel β-strands (ribbons) from each protomer (*vide infra*).

These seven sensor families span the detection of the six primary biologically essential first row transition elements Mn, Fe, Co, Ni, Cu and Zn, as well as Group II d^9 and d^{10} heavy metals Ag/Au and Cd/Hg, respectively. In addition, ArsR and MerR family sensors have been identified that detect the Group IV heavy metal Pb^{II}, as well as Group V trivalent ions As^{III}/Sb^{III} (or As^{V} arsenates) and Bi^{III}. Sensor families are named for the founding member(s) that gave rise to the family. These are the As/Sb sensor *E. coli* R774 ArsR,[92] also referred to as the ArsR/SmtB family, the latter referring to the Zn^{II} sensor *Synechococcus* SmtB,[93] *E. coli* MerR,[94] the Cu sensor *Mycobacterium tuberculosis* CsoR,[77] the Cu sensor *Enterococcus hirae* CopY,[95] the Fe sensor *E. coli* Fur,[96] the Fe sensor *C. diphtheriae* DtxR,[97] and the Ni sensor *E. coli* NikR.[91]

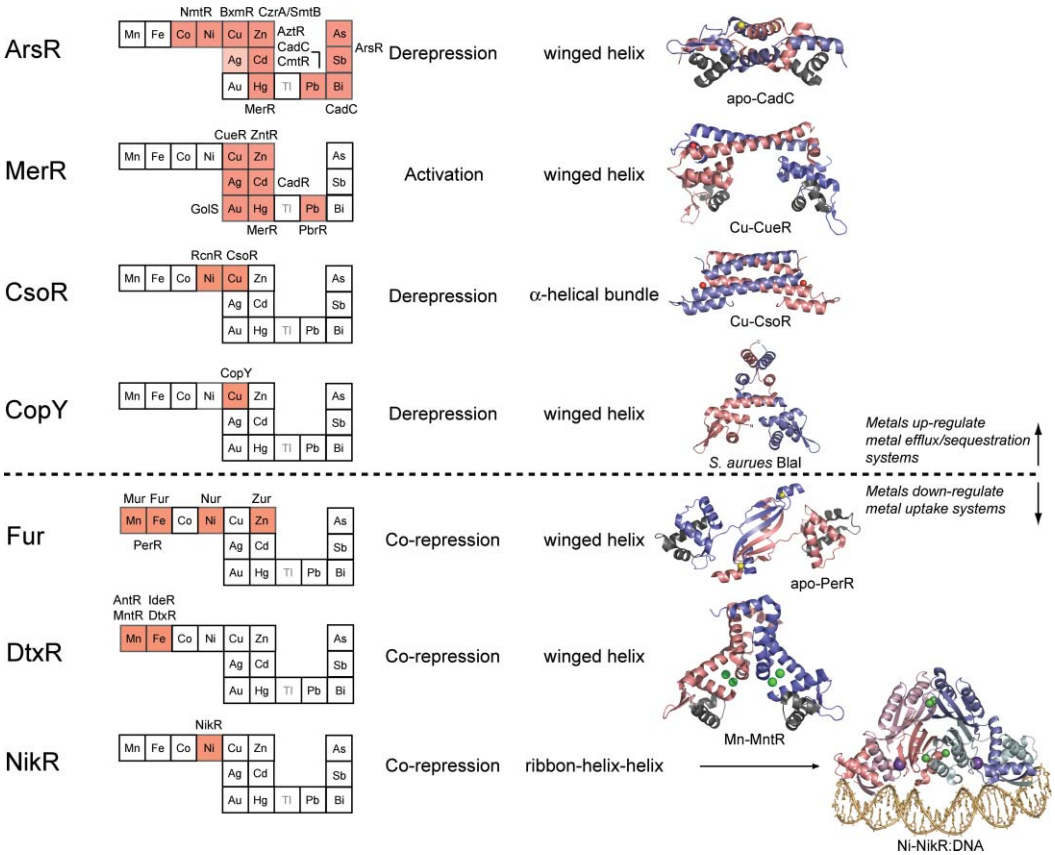


Fig. 2 Structural families of metal sensor proteins. Metals sensed in each case are shaded red, with individual metal sensor proteins that sense the particular metal(s) indicated. See text for details. The mechanism of gene expression is indicated as is the structural unit that mediates operator–promoter DNA binding. Ribbon representations of selected representative members are shown with individual protomers shaded *red* and *blue*. Structures are 1) apo *S. aureus* pI258 CadC with structural α5 Zn^{II} ions *yellow* (1U2W pdb code);[124] 2) Cu^{I} *E. coli* CueR, regulatory Cu^{I} ions *red* (1Q05);[38] 3) Cu^{I} *M. tuberculosis* CsoR, regulatory Cu^{I} ions *red* (2HH7);[77] 4) *S. aureus* BlaI as a model for CopY (1SD4);[104] 5) apo *B. subtilis* PerR, structural Zn^{II} ions *yellow* (2FE3);[150] 6) Mn^{II} *B. subtilis* MntR, Mn_A/Mn_C binuclear cluster ions *green* (2F5F);[107] 7) Ni^{II}-bound *E. coli* NikR-*nik* operator DNA complex, high affinity Ni^{II} ions green, regulatory K^+ ions, purple (2HZV).[89]

 This journal is © The Royal Society of Chemistry 2007

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

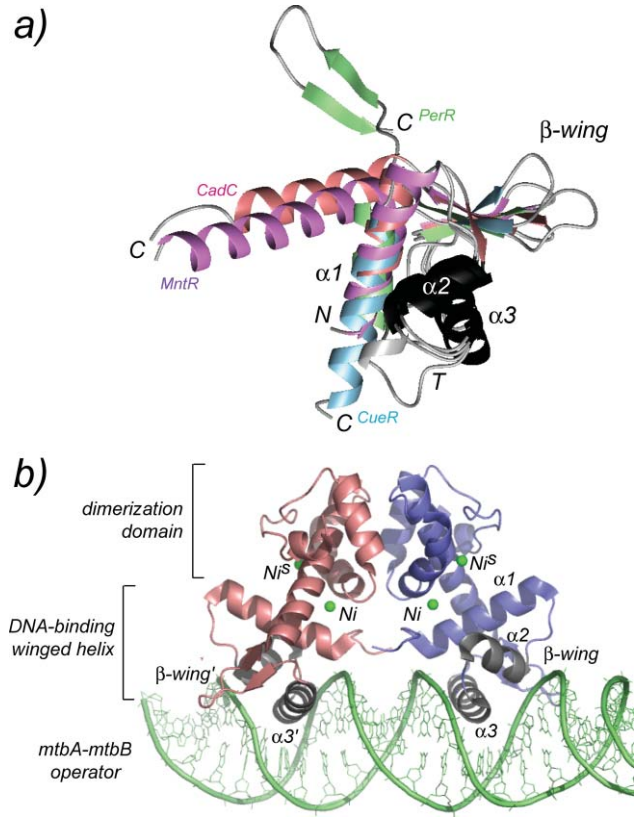


Fig. 3 (a) Ribbon representation of a superposition of the winged helix DNA binding domains (αααββ) of Zn^{II} *S. aureus* pI258 CadC (1U2W; shaded *coral*), Cu^{I} *E. coli* CueR (1Q05; *blue*), apo PerR (2FE3; *green*), and Mn_A-Mn_C *B. subtilis* MntR (2F5F; *violet*). The helix–turn–helix motifs are shaded *black* in each case, with the β-wing toward the back. CueR does not contain an α-helix analogous to N-terminal α1 helix; instead, the α-helix immediately C-terminal to the β-wing is found here. (b) Ribbon representation of the structure of a two-domain version of the Fe-sensor *Mtb* IdeR dimer complexed with two regulatory (Ni) and two structural (Ni^S) Ni^{II} ions and operator DNA (2ISZ).[88] The helix–turn–helix motif is shaded *black* with the α3 helix positioned deep in the major groove; the β-wing is close to the adjacent minor groove.

Metal sensor families above the dotted line in Fig. 2 are involved in metal resistance and function to increase the expression of genes involved in metal detoxification, storage and efflux (see Fig. 1, schemes 1–3); here, the direct binding of a specific ion(s) to a repressor either allosterically inhibits DNA binding, leading to de-repression of regulated genes (ArsR, CsoR and CopY), or allosterically activates transcription initiation by RNA polymerase by remodeling the promoter structure (MerR).[98,99] Those grouped below this line turn off the expression of uptake systems in response to metal excess (Fig 1. scheme 4), where direct binding of metal ion(s) allosterically activates DNA binding; in this case the metal ion functions as an obligate co-repressor. Consistent with this, the Fur, DtxR and NikR structural scaffolds have evolved to sense only the first row transition elements that are required for biological function; in contrast, the ArsR and MerR families contain representative members that have evolved to sense a far wider range of metal ions, including both first row transition elements as well as heavy metal ion xenobiotics, and organic As/Sb/Hg metalloid compounds. ArsR is likely an ancient protein and evolutionary progenitor of the entire ArsR family which conservatively numbers ≥500 members in ≈200 sequenced bacterial genomes, the vast majority of which remain functionally uncharacterized;[100] when one considers the biogeochemical cycling of Hg, *E. coli* MerR[94] may also be a progenitor protein.[101]

Fig. 2, right, shows a ribbon representation of a representative member from individual sensor families, with bound regulatory and structural metal ions highlighted. Only in the case of the Cu^{I} sensor CopY[102,103] is a high resolution structure not yet available. However, CopY is almost certainly a member of the MecI/BlaI family[104] of winged helical β-lactamase repressors (see Fig. 2),[105] uniquely decorated with a C-terminal extension that conserves a pair of Cys–X–Cys sequences projected to coordinate a very stable solvent shielded Cu^{I}_2–S_4 binuclear cluster.[106] Inspection of Fig. 2 reveals several recurring structural features. Firstly, all sensors adopt oligomeric, mostly dimeric, assembly states, with the exception of tetrameric NikR, which functions as a dimer of dimers. This two-fold rotational symmetry allows sensor proteins to interact with two-fold symmetric DNA operator sites (see Fig. 3b). Secondly, metals bind at or near dimer (oligomer) interfaces, and are often coordinated by metal ligand donor atoms that are contributed, or shared, by different protomers (subunits) within the oligomer. Here, metal binding might "freeze out" a high affinity DNA binding conformation, stabilizing a low DNA-binding affinity state(s).[40] If not bound at a protomer interface, regulatory metal ions are coordinated by residues within a single chain, but positioned at an interfacial region between physically separated N-terminal DNA-binding and more C-terminal oligomerization domains. Metal binding here is ideally positioned to anchor the interdomain orientation of the two domains, thereby "freezing in" a high affinity (Fur, DtxR, and NikR families) or allosterically activated (MerR) DNA-binding conformation. The structural and energetic determinants of metal-induced conformational changes are discussed in more detail below.

Coordination chemistry and metal selectivity

Accumulating evidence from a variety of metal-sensing systems is consistent with the proposal that coordination number, geometry, and nuclearity dictates the biological specificity of these metal-regulated switches.[37–39,107] Metal ions that readily adopt a coordination structure compatible with known preferences from fundamental coordination chemistry will drive conformational changes in protein structure and/or dynamics to effect regulation; other metal ions may bind, but form non-native chelate structures that are poorly regulatory. On the other hand, it is now generally accepted that the intrinsic metal ion affinity is a poor predictor of biological metal specificity.[86,50]

A recently reported illustration of this is the Mn^{II}-sensor *B. subtilis* MntR. Like the square planar site[108] in Ni^{II}-specific regulator NikR,[109] the regulatory site in Fe^{II}-regulated Fur,[73] and other metalloproteins that bind divalent transition metal ions,[110] divalent metal ion binding affinities of free MntR tend to roughly follow the Irving–Williams series, with Mn^{II} binding far more weakly than all other divalent metal ions (K_{Mn}≈1 × 10^4 M^{-1}), and Zn^{II} binding over ≈1000-fold more tightly (K_{Zn}≈5 × 10^6 M^{-1}).[74] The MntR homolog from pathogenic *B. anthracis*, AntR, also binds Mn^{II} quite weakly.[111] However, the absolute affinities of

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

these ions are such that only Mn^{II} is likely to reach an intracellular concentration required to activate MntR for DNA binding so as to repress the expression of the Mn^{II} uptake system;[112] the fact that K_{Zn} is six orders of magnitude weaker than *bona fide* Zn^{II}-specific sensors may render intracellular Zn^{II} sensing ineffective. Furthermore, recent structural studies establish that Zn^{II} is unable to form a binuclear Mn^{II} cluster and binds tightly to just one of the two sites (site A) in MntR (see Fig. 6, below).[107] An MntR mutant with relaxed specificity for Mn^{II} over Fe^{II} *in vivo*[113] also binds a single Fe^{II} atom to the A-site.[114] In contrast with Zn^{II}, Cd^{II} which also binds nearly 1000-fold more tightly than Mn^{II} and forms the binuclear complex, is an efficient co-repressor *in vivo*;[107] this may provide *B. subtilis* with a secondary defense against Cd^{II} toxicity.[112] Thus, the relative magnitudes of K_M coupled with the distinct structures of metalloderivatives of MntR collaborate to create a sensor that is selective for Mn^{II}/Cd^{II} over other divalent cations.

On the other hand, the recent discovery of *Salmonella* GolS, a Au^{I}-selective MerR sensor that exhibits 42% amino acid identity to a *Salmonella* CueR in the same organism, provides an illustration of the extent to which nature goes to fine-tune the metal selectivity of a simple S_2 metal coordination chelate.[115] Crystallographic studies of *E. coli* CueR reveal that the coinage metals Cu^{I}, Ag^{I} and Au^{I}, form isostructural coordination complexes[38] and *E. coli* CueR is capable of sensing Cu^{I} and Au^{I} equally well. Remarkably, GolS discriminates between the larger Au^{I} over Cu^{I}/Ag^{I} by over 100-fold, and surgical replacement of the GolS metal binding loop with that of CueR gives rise to significant Cu^{I}-sensing *in vivo*.[115] These studies provide compelling support for the idea that relatively subtle modifications in those residues that make up the metal binding pocket also lead to the evolution of MerR sensors that preferentially detect Cd^{II} (CadR) or Pb^{II} (PbrR) over Zn^{II}, Hg^{II} (MerR[116]) over Cd^{II} (see Fig. 2), and even superoxide *via* a [2Fe-2S] cluster (*E. coli* SoxR).[38,117,118]

The nearly coincident discoveries of *M. tuberculosis* CsoR[77] and *E. coli* RcnR[119,120] also speak of the evolution of distinct metal selectivities on a relatively simple all α-helical scaffold. CsoR is thought to represent the founding member of a large class of Cu^{I}-sensing repressors; indeed, genetic deletion of *csoR* in *B. subtilis* abolishes Cu^{I}-sensing in that organism.[121] CsoR regulates the expression of the Cu-efflux pump that protects *Mtb* from Cu^{I}-toxicity and Cu^{I} stabilizes an allosterically inhibited form of the protein that has weak DNA-binding activity (Fig. 2). Although the mechanism of DNA binding and allosteric inhibition by Cu^{I} is poorly understood,[77] the Cu^{I} is coordinated by one Cys from one protomer (Cys36') and Cys61 and His65 from the other, to from a subunit-bridging S_2N trigonal coordination complex.[77] Substitution of His61 with Ala stabilized a non-native digonal Cys36'–Cys65 S_2 complex reminiscent of *E. coli* CueR;[38] in this case however, H61A CsoR is inactive in Cu^{I}-mediated regulation of the *cso* operator-promoter binding.

We propose that *E. coli* RcnR is a Ni^{II}/Co^{II}-selective member of the fledgling CsoR family that metalloregulates the expression of *rcnA* to maintain Ni^{II} homeostasis in *E. coli*.[119] Although *Mtb* CsoR and *E. coli* RcnR are only distantly related, they are likely derived from a common evolutionary progenitor, a possibility for which is the *nre* metal resistance determinant on megaplasmid pTOM9 from *Achromobacter xylosoxidans* 31A.[122] Although direct metal binding by RcnR has yet to be reported, the work predicts that Cu^{I} and Ni^{II} sensing sites of distinct coordination geometries have evolved on this simple helical scaffold that employ a subset of common metal donor ligands, much like pairs of metal sensors from the ArsR[37] and MerR[38] families.

A variation on this theme occurs in the ArsR family of repressors.[123] Inspection of Fig. 2 reveals that ArsR family proteins collectively sense the widest range of metal ion effectors of any metal sensor protein family. Part of the reason for this is that no fewer than *three* physically separated, subunit-bridging metal receptor sites have evolved on this scaffold (Fig. 4), each named for the secondary structural elements that provide donor atoms to individual metals. These are denoted α3N/α3[124] (also designated metal site 1 in *S. aureus* pI258 CadC[125]), α4C[126] and α5[124] (denoted metal site 2 in *S. aureus* pI258 CadC). A remarkable feature of what appears to be something of a "scatter-shot" picture, is that each metal site, although structurally independent, may well exploit convergent strategies to effect allosteric inhibition of DNA

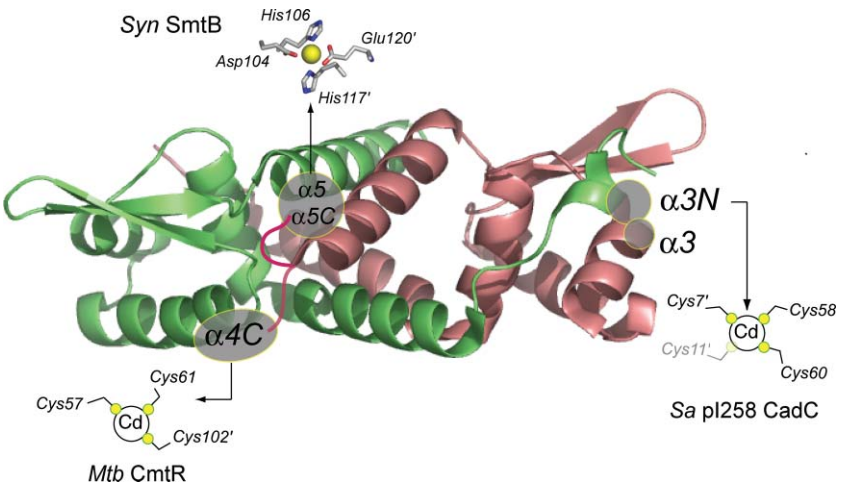


Fig. 4 Ribbon structure of Zn_1-bound *Synechococcus* homodimer SmtB (1R23)[40] with approximate locations of coordination complexes of regulatory metal ions bound to the α3N/α3, α4C and α5/α5C sites as indicated. Schematic representations of Cd^{II} coordination structures for *Mtb* CmtR (S_3 or S_3O)[127] and *S. aureus* pI258 CadC (S_4; Cys11 is a weakly deshielding ligand)[128] that are consistent with spectroscopic measurements are shown, as is the Zn^{II}-sensing α5 site of SmtB.

 This journal is © The Royal Society of Chemistry 2007

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

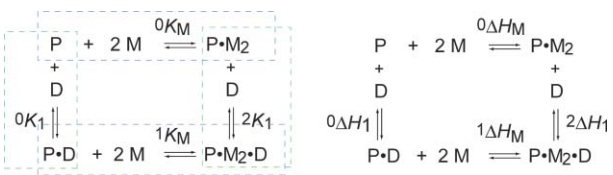
binding. In the case of the α4C sensor *Mtb* CmtR,[126] Cys61 of the Cys57–X_3–Cys61 core Cd^{II}/Pb^{II} binding sequence[127] corresponds precisely to His67 in the α5 Zn^{II} sensor *S. aureus* CzrA (Arg87 in SmtB), whose backbone carbonyl oxygen atom accepts a hydrogen bond from the non-ligating Nε2 face of His97 (His117 in SmtB) of the opposite protomer (see Fig. 5b below).[40] Likewise, our NMR structural studies of CzrA bound to DNA reveal that Val42, the residue in CzrA that corresponds to Val59 next to Cys60, a key allosteric residue in the α3N Cd^{II}/Pb^{II} sensor *S. aureus* pI258 CadC,[128] lies at the protein–DNA interface;[129] if CadC binds to operator DNA in a similar fashion, then Cd^{II}/Pb^{II} binding here might be expected to disrupt key protein–DNA contacts.

Some ArsR sensors, including SmtB and CadC, harbor more than one pair of metal sites, with one pair playing a metalloregulatory role, with the other playing some other role, perhaps structural, but currently not well defined.[128,130] The lone characterized Cu^{I}-sensor in the ArsR family, *O. brevis* BxmR, forms a novel α3N Cu_2–S_4 binuclear cluster with three Cys anchoring this complex;[131] BxmR also contains an α5 site that is exquisitely sensitive to Zn^{II} regulation. BxmR is therefore distinguishable from other closely related ArsR-family sensors in having evolved a metalloregulatory α3N site that can adopt an expanded range of coordination chemistries, while maintaining redundancy in the Zn^{II}-sensing response. This relaxed metal selectivity allows the cyanobacterium *Oscillatoria* to adapt to both monovalent (Cu^{I}/Ag^{I}) and divalent (Zn^{II}/Cd^{II}) metal ion toxicity both *in vivo* and *in vitro*.[132]

Allosteric switching in metal sensor proteins

Theory

Excluding MerR-family sensors, regulatory metal ions allosterically activate or inhibit operator–promoter binding by metal sensor proteins. From classical linkage theory, DNA (D) and metal ion (M) are *i*th and *j*th allosteric ligands, respectively, of the sensor protein, P. Here, P is the minimal oligomeric assembly state that is capable of binding a D molecule containing a single two-fold symmetric operator site, *i.e.*, a homodimer or a NikR tetramer.[133] If P is a homodimer that contains two regulatory metal binding sites per dimer, a simplified coupled equilibrium can be constructed that is characterized by four allosteric "end" states, free P, singly ligated PD, PM_2 species, and "doubly ligated" PDM_2 (Scheme 1).



Scheme 1 Coupled equilibrium scheme illustrating the four "end" allosteric states of a hypothetical sensor protein P that binds two allosteric ligands, DNA D ($i = 1$) and metal ion M ($j = 2$) related to the equilibrium constants (left) and enthalpies (right) that govern the formation of each.

The free energy of allosteric coupling between the binding of the *i*th (D) and *j*th (M ligands) is denoted ${}^{ij}\Delta G_c$ and is simply given by eqn (1)

$$ {}^{ij}\Delta G_c = -RT\ln({}^{j}K_1/{}^{0}K_1) = -RT\ln({}^{1}K_M/{}^{0}K_M) \quad (1) $$

with the equality simply a restatement of the conservation of free energy required by the thermodynamic cycle shown. This equality also illustrates a fundamental feature of all such coupled equilibria, that linkage is reciprocal, *i.e.*, measuring the effect of M on the binding of D to P (the ratio of the vertical manifolds boxed *in green*; Scheme 1) is equal to measuring the influence of D on M binding (defined by the ratio of the horizontal manifolds boxed *in blue*, Scheme 1). In this case, eqn (1) becomes eqn (2)

$$ {}^{12}\Delta G_c = -RT\ln({}^{2}K_1/{}^{0}K_1) \quad (2) $$

The sign on ${}^{12}\Delta G_c$ dictates whether the binding of ligands D and M to P antagonize or enhance the binding of the opposite ligand. If D and M are antagonistic, then ${}^{12}\Delta G_c$ will be positive, indicative of an unfavorable heterotropic coupling free energy; this is clearly the case for the ArsR,[37] CsoR[77] and CopY[102] repressors. On the other hand, if D and M enhance the affinity of one another relative to the P reference state, then ${}^{12}\Delta G_c$ will have a negative sign, indicative of a favorable heterotropic coupling free energy. This clearly characterizes the Fur, DtxR and NikR metal sensor families. The magnitude (positive or negative) is a quantitative indication of the extent to which the two ligands inhibit or reinforce the binding of the other, *i.e.*, the extent to which the binding of the two ligands are thermodynamically linked.

A companion view of ${}^{12}\Delta G_c$ is that this parameter is defined by eqn (3)

$$ {}^{12}\Delta G_c = -RT\ln {}^{12}K_c \quad (3) $$

where ${}^{12}K_c$ is a unitless disproportionation equilibrium constant which defines the extent to which the two singly ligated species PD and PM_2, are populated relative to the free P and doubly ligated PM_2D states (for $i = 1$ and $j = 2$) governed by eqn (4)

$$ \mathrm{P{\bullet}D + P{\bullet}M_2} \overset{{}^{12}K_c}{\rightleftharpoons} \mathrm{P + P{\bullet}M_2{\bullet}D} \quad (4) $$

If this equilibrium lies to the right, then ${}^{12}K_c > 1$ and ${}^{12}\Delta G_c < 0$ indicative of allosteric positive coupling; on the contrary, ${}^{12}K_c < 1$ and ${}^{12}\Delta G_c > 0$ reflects allosteric negative coupling of D and M binding to P. Using these simple relationships, it becomes possible to quantify the allosteric impact that various cognate and non-cognate metal ions have on DNA binding by a particular metal sensor protein. For example, the relative magnitudes of ${}^{12}\Delta G_c$ (Ni^{II}) (+3.3 kcal mol^{-1}) *vs.* ${}^{12}\Delta G_c$ (Zn^{II}) (+2.2 kcal mol^{-1}) for the Ni^{II}/Co^{II}-sensing ArsR family repressor NmtR are opposite to that of the Zn^{II}/Co^{II}-sensor CzrA, as expected for a Ni^{II} sensor that does not detect Zn^{II} *in vivo*.[37] How NmtR tunes its allosteric switch to respond preferentially to Ni^{II} *vs.* Zn^{II} is still under investigation; however, the switch is more effectively turned when the metal binds in an octahedral coordination geometry since Zn^{II} is known to bind tetrahedrally.[37]

A particularly powerful use of this approach allows one to deconvolute the extent to which individual metal ligand donor atoms are required to simply stabilize the metal complex (${}^{0}K_M$ in Scheme 1) *vs.* driving the allosteric switching mechanism (embodied in ${}^{12}\Delta G_c$). Using such an approach, we uncovered a "division of labor" among metal ligand donor atoms in the α5 chelate in CzrA: Asp84 and His97 were found to be key allosteric residues while His86 and His100 could be substituted with non-ligating residues with substantial decreases in K_{Zn} as expected, but with little or no quantitative effect on ${}^{12}\Delta G_c$.[134] These studies

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

established a 1 : 1 correlation between the ability to form a metal site of the native coordination geometry, *i.e.*, tetrahedral, with structural switching within the dimer as revealed by NMR studies, and functional coupling to DNA binding. Similar features appear to characterize the Cd^{II}/Pb^{II} chelates of two other ArsR sensors, CadC[128] and CmtR.[127]

This linkage approach also allows direct elucidation of the underlying energetics associated with the magnitude and sign of $^{12}\Delta G_c$, *i.e.*, the magnitudes and signs of ΔH_c and ΔS_c which correspond to the enthalpic and entropic contributions to the allosteric coupling free energy, respectively. This is important because resolution of ΔH_c and ΔS_c allows one to determine the relative contributions that structural changes (ΔH_c) *vs.* changes in dynamics (ΔS_c) influence the coupling.[135] Isothermal titration calorimetry provides a measure of the global enthalpy change for a reaction, with $^{12}\Delta H_c$ determined from eqn (5) (see Scheme 1, right)

$$^{12}\Delta H_c = {}^{2}\Delta H_1 - {}^{0}\Delta H_1 = {}^{1}\Delta H_M - {}^{0}\Delta H_M \quad (5)$$

and the global $^{12}\Delta S_c$ determined from the Gibbs relationship $^{12}\Delta S_c = (^{12}\Delta H_c - {}^{12}\Delta G_c)/T$. Residue-specific contributions to the global $^{12}\Delta S_c$ can be investigated from an analysis of the residue-specific dynamics over very short (ps–ns),[136,137] intermediate (μs–ms)[138,139] and long (ms–s)[140] timescales by NMR spectroscopy; these experiments allow direct assessment of the relative contributions that backbone conformational entropy, correlated domain motions, and perturbations in the native state conformational ensemble, respectively, make in stabilizing distinct allosteric states accessible to P. This powerful approach can thus be used to define both global and local origins of allosteric regulation, and as discussed below, can provide support for or against mechanistic models that emerge from crystallographic studies.

Recent structural insights into allosteric switching

Below we summarize recent structural findings that provide insights into how metal ions induce conformational changes in protein structure to effect biological regulation embodied in ΔG_c.

ArsR family α5 sensors. The α5 metal site in ArsR family sensors is a tetrahedral, subunit-bridging site composed of either an N_3O or N_2O_2 ligand donor set derived from residues on opposite ends of the C-terminal α5 helix (Fig. 5).[40,124] The crystallographic structures of apo- and Zn_2 forms of SmtB and CzrA reported by Eicken *et al.*[40] as well as companion NMR studies, are consistent with a plausible quaternary structural switching model for allosteric regulation of DNA binding by Zn^{II}/Co^{II} (Fig. 5b). This involves the formation of two successive side chain–main chain, main chain–main chain interprotomer hydrogen bonds that link the α5 metal binding site with the DNA binding α4 (αR) helix thereby stabilizing a "closed" conformation with low DNA binding affinity (Fig. 5a). The key allosteric residue in this model is His117 (His97) whose $N^{\delta 1}$ forms a coordination bond with the metal, the $N^{\varepsilon 2}$ face donating a hydrogen bond to the carbonyl oxygen of Arg87' (His67') across the subunit interface (Fig. 5b).[40] This hydrogen bonding pathway is predicted to make a substantial contribution to the observed $^{12}\Delta G_c$ of ≈+6 kcal mol^{-1}, which occurs with a concomitant rigidification or dampening of the internal dynamics (on the ms–s timescale) of the entire core of the molecule; in striking contrast, the β-wings become highly dynamic.[40] Characterization of single chain, covalently fused dimers of CzrA revealed that filling just one of the two α5 sites stabilized a structurally asymmetric state that is characterized by ≈80% of the allosteric coupling free energy of wild-type CzrA; this reveals that the CzrA switch is not concerted[141] in contrast to conclusions drawn from crystallographic studies alone.[40]

In fact, the degree to which structural changes (embodied in ΔH_c) *vs.* changes in residue-specific dynamics (ΔS_c) participate in driving the ArsR-family α5 site allosteric switch is still an open question, largely due to the lack of understanding of the structure and dynamics of the apo-CzrA-DNA complex (PD in Scheme 1) as well as the ternary PM_2D state. Without this key information, there is considerable uncertainty in making mechanistic projections concerning the molecular basis of allostery on the basis of one or a few crystal structures of a subset of allosteric states (P and PM_2 in this case). Toward this end, we have recently identified solution conditions that allow us to extensively interrogate the structure and residue-specific dynamics of all four allosteric end states of CzrA by NMR spectroscopy.[142]

***B. subtilis* MntR.** MntR binds two metal ions in a binuclear cluster, in either an Mn_A/Mn_B or Mn_A/Mn_C[107] configuration. The Mn_A/Mn_C configuration is thought to be the biologically relevant conformer since these crystals were grown at room

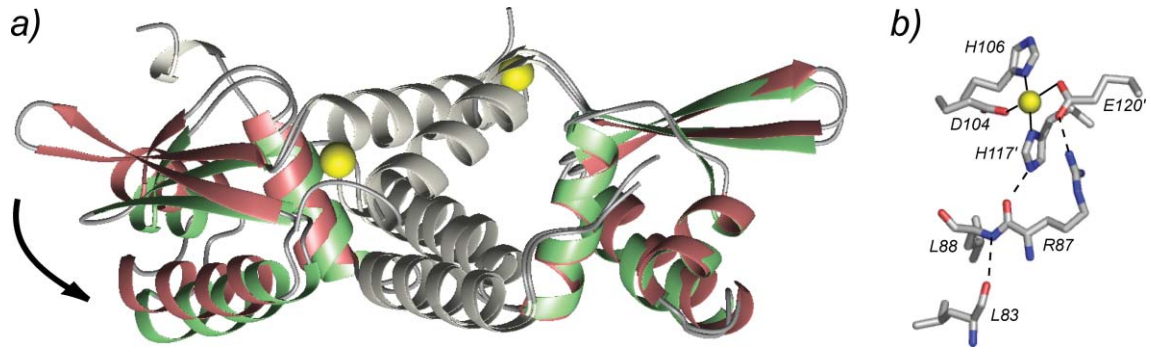


Fig. 5 Allosteric structural changes induced by the binding of regulatory Zn^{II} ions to a representative α5 ArsR-family sensor, *Synechococcus* SmtB. (a) Apo-SmtB (1R1T) superimposed on Zn_2-SmtB (1R22).[40] The *grey*-shaded region comprises the α1 and α5 dimerization α-helices, with the winged helix domains shaded *green* (Zn_2) and *coral* (apo) for the two structures. (b) The α5 chelate structure is shown, as is a quaternary structural hydrogen-bonding pathway that defines a plausible allosteric switch.

 This journal is © The Royal Society of Chemistry 2007

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

temperature and solution EPR experiments are more consistent with a longer (≈4.4 Å) internuclear Mn–Mn distance (Fig. 6) relative the Mn_A/Mn_B[114] conformer.[107,111] A holo-MntR-DNA operator complex structure is not yet available so the possibility exists that DNA might stabilize or enforce a well-defined geometry around the metal ion(s); on the other hand, this was found not to be the case for the related DtsR/IdeR sensors.[143] A recent mechanistic proposal posits that the A-site metal functions as a selectivity filter, which recognizes the coordination geometry of the bound A-site metal and determines whether the C site will be filled; in this model, Mn_C is the activating metal ion in MntR.[107]

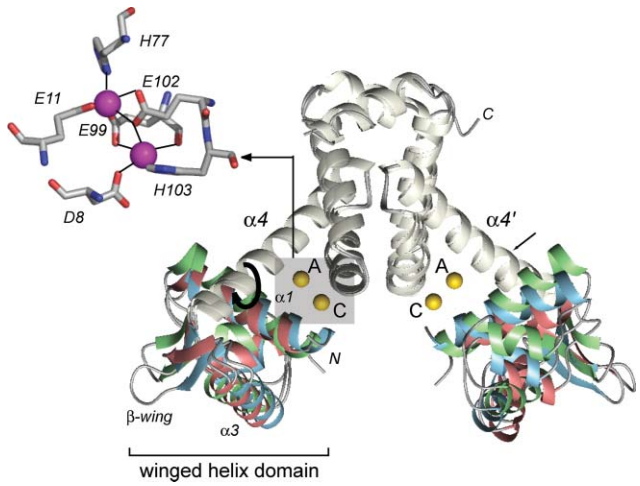


Fig. 6 Allosteric structural changes induced by the binding of regulatory Mn^{II} ions to a representative DtxR-family repressor, *B. subtilis* MntR. Two crystal forms of apo-MntR (2HYF shaded *coral*; 2HYG shaded *green*) and Mn_A/Mn_C MntR (2F5F shaded *blue*) are shown,[107,144] as is the binuclear cluster chelate. This superposition limits were defined by the dimerization domain (shaded *grey*).

Two recent structures of apo-MntR compared to the Zn^{II}, Mn^{II}, Ca^{II} and Cd^{II} structures suggests a quaternary structural mechanism of activation of MntR by metal ions.[144] In particular, the relative dispositions of the N-terminal winged helix and C-terminal dimerization domain change greatly with a pivot point centered at residue 75, mediated by a slight underwinding of residues 72–75 of the α4 linker helix (Fig. 6). This results in a lateral displacement of the winged helix domains by ≈3.4 Å relative to the molecular two-fold axis, coupled with a rigid body rotation of one domain relative to the other. Metal ligands Glu11 (to Mn_A) and Asp8 coordinated to Mn_B/Mn_C from the N-terminal α1 helix play major roles in driving this conformational shift (see Fig. 6).[144] The N-terminus of the α1 helix also appears to adopt a fully helical structure, as a result of forming a coordination bond to Asp8, relative to the apo- and poorly activating Zn^{II} states. A similar proposal has been made for the Fe^{II}-sensor IdeR with base-specific contacts derived from the DNA recognition α3 helix, and electrostatic stabilization provided by residues from the α2 helix and β-wing (see Fig. 3 above).[88]

These structural changes in metal-activated MntR are consistent with the results from hydrogen–deuterium exchange mass spectrometry recently mapped by Cohen and co-workers,[145] although it remains unclear if the two apo-MntR structures trapped by crystallography span the full range of relative orientations of the winged helical and dimerization domains in solution (Fig. 6). As might be expected, the α1, α4 and α5 helices that donate metal ligands to the binuclear cluster become strongly protected from solvent exchange. The greatest effect, however, is in the α4 linker helix, which suggests a high degree of interdomain mobility with metal binding greatly restricting this range of motions. These studies taken collectively are consistent with a model in which the enthalpy of formation of metal ligand coordination bonds is used to drive an unfavorable reduction in the conformational entropy of the repressor, which does not have to be paid upon operator DNA binding. A direct measure of the allosteric coupling enthalpy (ΔH_c) and entropy (ΔS_c) would provide strong support for this model of activation.

Fur family regulators. Fur proteins are found in a wide variety of gram negative prokaryotes and are named for the founding member of this family of proteins, the Fe-regulated repressor, *E. coli* Fur.[96] *E. coli* Fur is a global transcriptional regulator that controls the transcription of over 90 genes which function in iron homeostasis and acquisition, the oxidative stress response, and acid tolerance.[96] The Fur family is now known to include members that sense other metals in the cell, including the Zn^{II}-sensor Zur,[146] the Mn^{II}/Fe^{II}-sensing regulator Mur,[147] the Ni^{II}-selective regulator *S. coelicolor* Nur,[148] and a hydrogen peroxide stress-sensing repressor, PerR, from *B. subtilis*.[149] PerR is capable of binding Fe^{II} or Mn^{II} to a metalloregulatory site which represses the expression of genes that control a response to H_2O_2 and oxidative stress.[150] The mechanism for peroxide-sensing was recently worked out by Lee and Helmann in an elegant series of biochemical experiments.[151] These studies demonstrated that bound Fe^{II} reacts with peroxide, leading to rapid and irreversible metal-catalyzed oxidation of two coordinated His residues, His37 from the DNA binding domain and His91 from the dimerization domain, to 2-oxo-histidine, weakening the affinity of the regulatory site for metal and leading to derepression of regulated genes.

Three crystallographic structures of Fur family proteins have now been solved and include *Pseudomonas aeruginosa* (*Pa*) Fur bound to two activating Zn^{II} ions,[152] *B. subtilis* PerR, with the metalloregulatory metal site empty,[153] and *M. tuberculosis* Zur (annotated as FurB)[154], which was found to contain three Zn^{II} ions in the structure.[155] Fur family regulators contain a C-terminal ββαβα dimerization domain linked to a typical winged helical domain (see Fig. 3) *via* a flexible linker (Fig. 7). Despite some confusion created by the original structure of *Pa* Fur,[152] consensus seems to be emerging as to the role that individual metal ions might play in metalloregulation (activation) of operator-promoter DNA binding. Fig. 7 shows the structure of the apo-PerR dimer, with the winged helical domains shaded *blue* and the C-terminal dimerization domains in *grey*. The structures of one of the two subunits of *Pa* Fur (*coral*) and *Mtb* Zur (*green*) are superimposed on apo-PerR in the C-terminal dimerization domain. PerR,[156] like *E. coli* Zur,[157] *Mtb* Zur and *E. coli* Fur, but not *Pa* Fur, contains what is now known to be a structural tetrathiolate S_4 Zn^{II} site (denoted Zn^S; Fig. 7) formed by a pair of Cys residues derived from the β-hairpin loop between the first two β-strands of the dimerization domain (β3–β4) and a C-terminal Cys–X_2–Cys sequence. In *E. coli* Fur, NMR studies reveal that formation of this Zn^S S_4 chelate strongly stabilizes a functional form of the dimer.[158] Both Zur and *Pa* Fur have two additional bound metals, one of

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

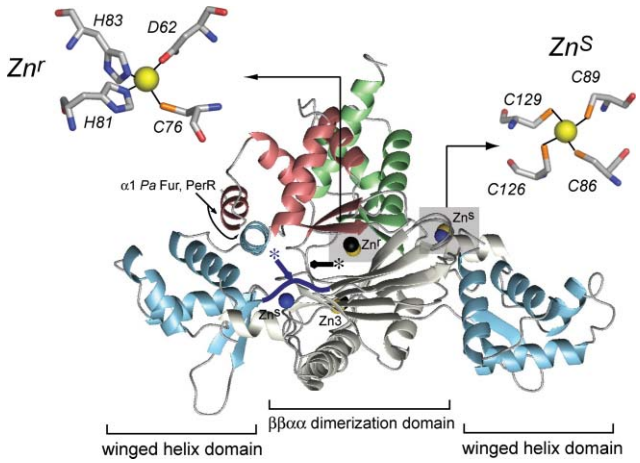


Fig. 7 Allosteric structural changes induced by the binding of regulatory Zn^{II} ions to three representative Fur-family repressors. These include *B. subtilis* PerR homodimer, apo state with structural Zn^{II} ions (Zn^{S}) shaded *blue* (2FE3);[153] one protomer of *M. tuberculosis* Zur (FurB) shaded *green*, with all three Zn^{II} ions shaded *yellow* (Zn^{S}, putative Zn^{r} and Zn3) (2O03);[153] one protomer of *P. aurigenosa* (*Pa*) Fur shaded *coral*, with putative regulatory (Zn^{r}) and other Zn^{II} shaded *black*.[152] *Blue* *, the flexible tether that connects the winged helix and dimerization domains in apo-PerR; *black* *, pivot point in this tether that moves to accommodate metal binding to the presumed regulatory Zn^{r} sites in *Pa* Fur and *Mtb* Zur. Chelate structures of Zn^{S} and Zn^{r} of *Mtb* Zur are also shown.

which (Zn^{r}) is nicely positioned between the two domains. In Zur, Zn^{r} forms a tetrahedral SN_2O coordination complex, with the two His residues (His81, His83) derived from the first β-strand in the dimerization domain, β3, connected to the β-wing *via* a linker, and Asp62 and Cys76 derived from β1 and β2 strands in β-wing itself of the DNA binding domain (Fig. 7). Strikingly, *Pa* Fur contains a bound metal here as well (metal site 2), which is also tetrahedral when bound to Zn^{II} (the Fe^{II} coordination geometry is unknown). Nonetheless, His32 and His89 in this site in *Pa* Fur are absolutely essential for Fe-sensing *in vivo*, and correspond precisely to the two Fe ligands in PerR that are oxidized as a result of peroxide stress.[151] Strikingly, metal binding here appears to orient the two domains into a specific orientation, driven by formation of metal–ligand bonds and the inherent flexibility of the connecting linker between the two domains. Apo-PerR, which lacks a bound metal ion in this site, is in a rather extended conformation, relative to the others; this likely corresponds to an inactive DNA binding conformation. Interestingly, both *Mtb* Zur and *Pa* Fur contain a bound metal (Zn3 in Fig. 7) whose functional role is unclear, but may be adventitiously bound.

These structures are consistent with a simple model of metalloregulation of operator DNA binding in which regulatory metal ions fix the relative dispositions of the DNA-binding and dimerization domains thereby stabilizing a conformation that allows the helix–turn–helix motif to make base-specific contacts with the DNA (see Fig. 3). This model is superficially exactly analogous to the proposal for metal activation in MntR/DtxR/IdeR, although the range of conformational space accessible by apo-Fur family regulators may well be much larger. Forming such a complex in the absence of regulatory metals, while structurally possible, would be strongly opposed by a substantial entropic penalty, *i.e.*, a large $-\Delta S_c$ (see Scheme 1). This might be paid for by the formation of metal coordination bonds that drive the formation of the correct chelate geometry in each case, tetrahedral for Zn^{II}–Zur[157] and distorted octahedral for Fe^{II}–Fur/PerR. Another component of the activation mechanism perhaps unique to Fe^{II}–Fur/PerR invokes folding of the α1 helix, which is an N-terminal appendage on the αααββ winged helix motif (see arrow, Fig. 7). NMR studies of the unactivated *E. coli* Zn^{S} Fur dimer reveal that this helix is not formed, in contrast to the free DNA binding domain and in Zn-bound *Pa* Fur; this folding positions three conserved basic side chains on the same face of the helix that are well positioned to make contacts with the DNA.[158] Recent studies clearly establish that the regulatory metal must by bound to *E. coli* Zn_1 Fur to activate DNA binding.[73]

It will be very interesting to determine the coordination structure around the Ni^{II} in nickel uptake regulator *S. coelicolor* Nur, since unlike Fur, Nur is exquisitely selective for Ni^{II} *in vitro* and *in vivo*.[148] Regulation by Ni^{II}–Nur not only shuts off high affinity Ni^{II} uptake in *S. coelicolor*, but also represses transcription of an Fe–SOD (encoded by *sodF*) allowing Ni–SOD[159] (encoded *sodN*) to function as the major superoxide dismutase under these conditions. Interestingly, in a related *Streptomyces* strain, *S. grisius*, the expression of the *sodF* gene is repressed by an ArsR family repressor (SrnR) that forms an oligomeric complex with a Ni^{II}-sensing protein (SrnQ) of unknown structure; biochemical experiments seem to suggest that SrnR does not bind Ni^{II} directly.[160]

***E. coli* NikR.** NikR is a ribbon–helix–helix DNA binding protein that functions formally as a dimer of dimers. NikR contains a central mixed αβ tetrameric oligomerization domain flanked by homodimeric ribbon–helix–helix DNA binding domains. The tetramerization domain exhibits four-fold rotational symmetry into which is incorporated four Ni^{II} ions bound in a square planar coordination geometry from three His (His87, His89 and His76') and a cysteine residue (Cys95) across the tetramer interface (Fig. 8).[108] This site binds Ni^{II} with pM affinity[161] and allosterically activates operator–promoter binding. *E. coli* NikR also harbors a second set of regulatory or lower affinity Ni^{II} binding sites that further enhance DNA binding affinity and substantially change the structure of the NikR–DNA complex.[161] The coordination structure of these regulatory Ni^{II} sites has recently been probed by element-selective X-ray absorption spectroscopy of two "bimetallic" derivatives of NikR, a Cu^{II}/Ni^{II} hybrid, with Cu^{II} bound to the square planar high affinity site, and Ni^{II} bound to the low affinity sites, and a Ni^{II}/Co^{II} hybrid, with Ni^{II} and Co^{II} bound in the C-terminal and regulatory sites, respectively.[39] These and other experiments clearly establish that only when the C-terminal site adopts a square planar coordination structure, *e.g.*, with Cu^{II} or Ni^{II}, can a unique conformation be induced in NikR such that the lower affinity regulatory sites be filled in a way that they strongly activate *nik* operator–promoter binding.[39,162] The coordination geometry in the regulatory sites was found to be octahedral with six N/O donors in the presence of DNA.[39]

Since 2003 three groups have reported crystallographic structures for NikRs from *E. coli*,[89,133] *H. pylori*[163] and *P. horikoshii*[164] in a number of crystal forms, including the *E. coli* holo-NikR-operator DNA complex.[89] These structures taken collectively reveal that Ni^{II} binding to the low affinity sites dramatically biases the conformational ensemble to adopt a more "closed" *cis*-type

 This journal is © The Royal Society of Chemistry 2007

View Online

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

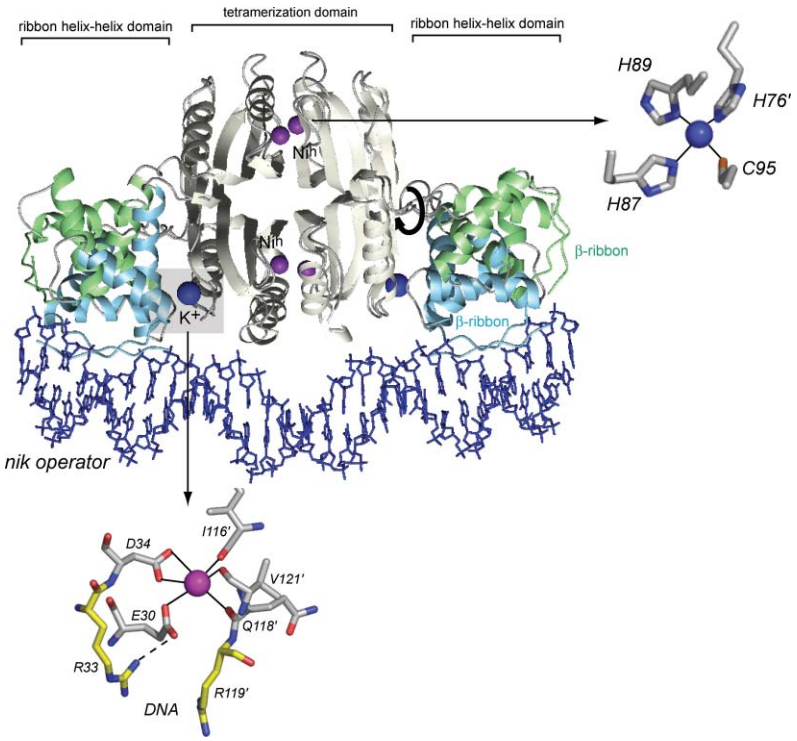


Fig. 8 Structural changes in *E. coli* NikR upon binding regulatory Ni^{II} (K^+) ions to the low affinity sites and operator DNA. The *trans*-configuration of the free Ni^{II}-loaded NikR shaded *green* (2HZA)[133] is compared to the *cis*-configuration conformer of the Ni^{II}–NikR–*nik* operator DNA complex shaded *blue* (2HZV). Regulatory high-affinity Ni^{II} ions are shaded *green*, with regulatory K^+ low affinity metals colored *purple*.[89] Chelate structures are also shown for each metal complex.

conformation relative to *trans*-type or more "open" conformations that are obtained in the absence of regulatory metal ions, but that DNA-binding is required to fully enforce a "closed" *cis* conformation (Fig. 8). Furthermore, the chelate structure of the low-affinity metal sites are not well formed unless holo-NikR is crystallized in the presence of PO_4^- anion[164] or bound to DNA.[89] Remarkably, however, in the DNA complex, a K^+ ion is bound to each of the two regulatory sites, coordinated by two conserved carboxylates (E30, D34) from the ribbon–helix–helix DNA binding domain, and three backbone carbonyl donor atoms (I116, Q118, V121) from the tetramerization domain (see Fig. 8); the conserved Glu30 also helps move a neighboring Arg33 from a "locked" to an "unlocked" conformation to enable specific binding to the operator DNA (Fig. 8).[164] Mutagenesis experiments[89,164] that target E30 and/or D34 are consistent with the identification of these K^+ sites as the regulatory Ni^{II} sites in NikR, as first hypothesized on the basis of the *P. horikoshii* NikR–PO_4^{3-} structure.[164] However, it must be pointed out that this O_6 coordination site, with long (≈3.0 Å) metal–ligand bonds, is a poor match for known Ni^{II} chelates,[165] which also tend to incorporate one or more histidine ligands into an $(N/O)_6$ octahedral coordination complex. Such an atypical coordination geometry for a regulatory metal ion is not unprecedented in metal sensor or metal trafficking proteins,[71] with other metalloregulatory sites characterized by coordination numbers that are lower (Cu^I–S_2 in CueR[38]) or higher (Hg^{II}–S_3 in MerR[116]) than expected. In addition, Cd^{II} complexes of two ArsR-family Cd^{II}/Pb^{II}-sensing repressors CadC (S_4)[78] and CmtR (S_3 or S_3O)[127] are characterized by at least one cysteine thiolate ligand that only weakly deshields the ^{113}Cd nucleus; this is consistent with considerable distortion from regular trigonal planar or tetrahedral symmetry in each case.

In any case, the primary effect of Ni^{II} binding to the low affinity sites in NikR may be functionally analogous to that which occurs in DtxR- and Fur-family regulators, where metal binding quenches interdomain mobility, induces local folding around the chelate, *i.e.*, near subunit-bridging high affinity Ni^{II} ligand His76 in the α3 helix, and stabilizes a conformation of the oligomer that is well-matched to interact specifically with individual half-sites of the DNA operator. As discussed above, this scenario makes the prediction that a large favorable $^{12}\Delta G_c$ in NikR is driven principally by a large and favorable ΔS_c.

Conclusions

Recent structural insights from a wide range of bacterial metal sensor proteins collectively emphasize several common features that characterize these bioinorganic switches. One is that the coordinate covalent nature of metal–ligand bonding is harnessed to drive changes in tertiary and/or quaternary structure and/or dynamics, the full extent of which is not yet fully established for any metal sensor protein. In addition, the central importance of metal homeostasis and resistance to bacterial fitness coupled with the ease with which allostery can apparently be evolved,[166,167] virtually ensures that additional structural scaffolds beyond the seven discussed here will emerge that could accommodate novel "metal receptor" sites. A second emerging theme is that biological metal selectivity, while dictated chiefly by the coordination chemistry of the chelate, is further refined by the metallome within

View Online

which a particular switch operates; this allows an organism to mount a specific adaptive response. Future efforts that more critically evaluate the role that protein motions (dynamics) in solution play in mediating allostery in these systems[138] as well as to determine precisely how allostery is linked to coordination chemistry,[37] should permit a more sophisticated understanding of these fascinating molecules. Concurrently with these efforts, whole organism systems biology and metallomics-based approaches to understand the "inorganic chemistry of the cell"[1], *e.g.*, how metal homeostasis systems impact one another[168] or other stress response systems, including oxidative stress,[117,60] will be required to fully appreciate the extraordinary lengths to which nature has gone to harness the power and minimize the potential damage of metal ions in biology.

Acknowledgements

The authors gratefully acknowledge the NIH (GM042569) and the Robert A. Welch Foundation (A-1295) for support of our work on metalloregulatory proteins. We also wish to thank Hermes Reyes-Caballero and Zhen Ma for critical comments on the manuscript.

References

1 T. V. O'Halloran, *Science*, 1993, **261**, 715.
2 R. Mukhopadhyay, B. P. Rosen, L. T. Phung and S. Silver, *FEMS Microbiol. Rev.*, 2002, **26**, 311.
3 S. Silver and T. Phung, le, *J. Ind. Microbiol. Biotechnol.*, 2005, **32**, 587.
4 D. H. Nies, *Extremophiles*, 2000, **4**, 77.
5 A. Komeili, *Annu. Rev. Biochem.*, 2007, **76**, 351.
6 S. E. Childers, S. Ciufo and D. R. Lovley, *Nature*, 2002, **416**, 767.
7 D. Agranoff and S. Krishna, *Front. Biosci.*, 2004, **9**, 2996.
8 C. Wandersman and P. Delepelaire, *Annu. Rev. Microbiol.*, 2004, **58**, 611.
9 G. M. Rodriguez and I. Smith, *J. Bacteriol.*, 2006, **188**, 424.
10 T. H. Flo, K. D. Smith, S. Sato, D. J. Rodriguez, M. A. Holmes, R. K. Strong, S. Akira and A. Aderem, *Nature*, 2004, **432**, 917.
11 M. A. Holmes, W. Paulsene, X. Jide, C. Ratledge and R. K. Strong, *Structure*, 2005, **13**, 29.
12 S. K. Mazmanian, E. P. Skaar, A. H. Gaspar, M. Humayun, P. Gornicki, J. Jelenska, A. Joachmiak, D. M. Missiakas and O. Schneewind, *Science*, 2003, **299**, 906.
13 E. P. Skaar, M. Humayun, T. Bae, K. L. DeBord and O. Schneewind, *Science*, 2004, **305**, 1626.
14 E. P. Skaar, A. H. Gaspar and O. Schneewind, *J. Biol. Chem.*, 2004, **279**, 436.
15 R. Wu, E. P. Skaar, R. Zhang, G. Joachimiak, P. Gornicki, O. Schneewind and A. Joachimiak, *J. Biol. Chem.*, 2005, **280**, 2840.
16 M. S. Francis and C. J. Thomas, *Microb. Pathog.*, 1997, **22**, 67.
17 O. Gat, I. Mendelson, T. Chitlaru, N. Ariel, Z. Altboum, H. Levy, S. Weiss, H. Grosfeld, S. Cohen and A. Shafferman, *Mol. Microbiol.*, 2005, **58**, 533.
18 J. W. Johnston, D. E. Briles, L. E. Myers and S. K. Hollingshead, *Infect. Immun.*, 2006, **74**, 1171.
19 D. A. Lewis, J. Klesney-Tait, S. R. Lumbley, C. K. Ward, J. L. Latimer, C. A. Ison and E. J. Hansen, *Infect. Immun.*, 1999, **67**, 5060.
20 S. Campoy, M. Jara, N. Busquets, A. M. Perez, De Rozas, I. Badiola and J. Barbe, *Infect. Immun.*, 2002, **70**, 4721.
21 W. Yang, Y. Liu, L. Chen, T. Gao, B. Hu, D. Zhang and F. Liu, *Curr. Microbiol.*, 2007, **54**, 307.
22 J. A. Lindsay and S. J. Foster, *Microbiology*, 2001, **147**, 1259.
23 J. Versieck, *Crit. Rev. Clin. Lab. Sci.*, 1985, **22**, 97.
24 D. I. Thurnham, A. S. Mburu, D. L. Mwaniki and A. De Wagt, *Proc. Nutr. Soc.*, 2005, **64**, 502.
25 D. E. Blair, A. W. Schuttelkopf, J. I. MacRae and D. M. van Aalten, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 15429.
26 A. A. McCarthy, N. A. Peterson, R. Knijff and E. N. Baker, *J. Mol. Biol.*, 2004, **335**, 1131.
27 C. Pezard, P. Berche and M. Mock, *Infect. Immun.*, 1991, **59**, 3472.
28 B. E. Turk, T. Y. Wong, R. Schwarzenbacher, E. T. Jarrell, S. H. Leppla, R. J. Collier, R. C. Liddington and L. C. Cantley, *Nat. Struct. Mol. Biol.*, 2004, **11**, 60.
29 Y. Natori, H. Nanamiya, G. Akanuma, S. Kosono, T. Kudo, K. Ochi and F. Kawamura, *Mol. Microbiol.*, 2007, **63**, 294.
30 E. M. Panina, A. A. Mironov and M. S. Gelfand, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 9912.
31 J. W. Olson and R. J. Maier, *Science*, 2002, **298**, 1788.
32 D. J. McGee and H. L. Mobley, *Curr. Top. Microbiol. Immunol.*, 1999, **241**, 155.
33 A. H. Gordon, P. D. Hart and M. R. Young, *Nature*, 1980, **286**, 79.
34 D. L. Clemens, B. Y. Lee and M. A. Horwitz, *J. Bacteriol.*, 1995, **177**, 5644.
35 K. Sendide, A. E. Deghmane, J. M. Reyrat, A. Talal and Z. Hmama, *Infect. Immun.*, 2004, **72**, 4200.
36 D. M. Ralston and T. V. O'Halloran, *Adv. Inorg. Biochem.*, 1990, **8**, 1.
37 M. A. Pennella, J. E. Shokes, N. J. Cosper, R. A. Scott and D. P. Giedroc, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 3713.
38 A. Changela, K. Chen, Y. Xue, J. Holschen, C. E. Outten, T. V. O'Halloran and A. Mondragon, *Science*, 2003, **301**, 1383.
39 S. Leitch, M. J. Bradley, J. L. Rowe, P. T. Chivers and M. J. Maroney, *J. Am. Chem. Soc.*, 2007, **129**, 5085.
40 C. Eicken, M. A. Pennella, X. Chen, K. M. Koshlap, M. L. VanZile, J. C. Sacchettini and D. P. Giedroc, *J. Mol. Biol.*, 2003, **333**, 683.
41 S. Chamnongpol and E. A. Groisman, *Mol. Microbiol.*, 2002, **44**, 561.
42 D. A. Rouch and N. L. Brown, *Microbiology*, 1997, **143**, 1191.
43 U. E. Ukaegbu, S. Henery and A. C. Rosenzweig, *Biochemistry*, 2006, **45**, 10191.
44 K. Perron, O. Caille, C. Rossier, C. Van Delden, J. L. Dumas and T. Kohler, *J. Biol. Chem.*, 2004, **279**, 8761.
45 M. J. Cromie, Y. Shi, T. Latifi and E. A. Groisman, *Cell*, 2006, **125**, 71.
46 S. Brantl, *Trends Biotechnol.*, 2006, **24**, 383.
47 R. L. Coppins, K. B. Hall and E. A. Groisman, *Curr. Opin. Microbiol.*, 2007, **10**, 176.
48 N. S. Dosanjh and S. L. Michel, *Curr. Opin. Chem. Biol.*, 2006, **10**, 123.
49 J. W. Lee and J. D. Helmann, *BioMetals*, 2007, **20**, 485.
50 S. Tottey, D. R. Harvie and N. J. Robinson, *Acc. Chem. Res.*, 2005, **38**, 775.
51 R. Williams, *Coord. Chem. Rev.*, 2001, **216–217**, 583.
52 J. Szpunar, *Analyst*, 2005, **130**, 442.
53 C. J. Fahrni, *Curr. Opin. Chem. Biol.*, 2007, **11**, 121.
54 J. Lopez-Barea and J. L. Gomez-Ariza, *Proteomics*, 2006, **6**, S51.
55 K. J. Waldron, S. Tottey, S. Yanagisawa, C. Dennison and N. J. Robinson, *J. Biol. Chem.*, 2007, **282**, 3837.
56 L. L. Barton, F. Goulhen, M. Bruschi, N. A. Woodards, R. M. Plunkett and F. J. Rietmeijer, *BioMetals*, 2007, **20**, 291.
57 C. E. Hand and J. F. Honek, *J. Nat. Prod.*, 2005, **68**, 293.
58 K. S. Ung and Y. Av-Gay, *FEBS Lett.*, 2006, **580**, 2712.
59 E. Anes, P. Peyron, L. Staali, L. Jordao, M. G. Gutierrez, H. Kress, M. Hagedorn, I. Maridonneau-Parini, M. A. Skinner, A. G. Wildeman, S. A. Kalamidas, M. Kuehnel and G. Griffiths, *Cell. Microbiol.*, 2006, **8**, 939.
60 D. M. Kurtz, Jr., *J. Inorg. Biochem.*, 2006, **100**, 679.
61 T. D. Rae, P. J. Schmidt, R. A. Pufahl, V. C. Culotta and T. V. O'Halloran, *Science*, 1999, **284**, 805.
62 A. C. Rosenzweig and T. V. O'Halloran, *Curr. Opin. Chem. Biol.*, 2000, **4**, 140.
63 A. Lewin, G. R. Moore and N. E. Le Brun, *Dalton Trans.*, 2005, 3597.
64 S. Shcolnick, Y. Shaked and N. Keren, *Biochim. Biophys. Acta*, 2006, **1767**, 814.
65 H. Sigel and D. McCormack, *Acc. Chem. Res.*, 1970, **3**, 201.
66 J. Frausto da Silva and R. Williams, *The Biological Chemistry of Elements: The Inorganic Chemistry of Life*, Oxford University Press, Oxford, 2nd edn, 2001, p. 575.
67 V. Sendra, D. Cannella, B. Bersch, F. Fieschi, S. Menage, D. Lascoux and J. Coves, *Biochemistry*, 2006, **45**, 5557.
68 L. Zhang, M. Koay, M. J. Maher, Z. Xiao and A. G. Wedd, *J. Am. Chem. Soc.*, 2006, **128**, 5834.
69 K. Peariso, D. L. Huffman, J. E. Penner-Hahn and T. V. O'Halloran, *J. Am. Chem. Soc.*, 2003, **125**, 342.

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

 This journal is © The Royal Society of Chemistry 2007

View Online

70 I. R. Loftin, S. Franke, S. A. Roberts, A. Weichsel, A. Heroux, W. R. Montfort, C. Rensing and M. M. McEvoy, *Biochemistry*, 2005, **44**, 10533.
71 L. A. Finney and T. V. O'Halloran, *Science*, 2003, **300**, 931.
72 C. E. Outten and T. V. O'Halloran, *Science*, 2001, **292**, 2488.
73 S. A. Mills and M. A. Marletta, *Biochemistry*, 2005, **44**, 13553.
74 M. V. Golynskiy, W. A. Gunderson, M. P. Hendrich and S. M. Cohen, *Biochemistry*, 2006, **45**, 15359.
75 J. J. Chou, S. Li, C. B. Klee and A. Bax, *Nat. Struct. Biol.*, 2001, **8**, 990.
76 M. L. VanZile, N. J. Cosper, R. A. Scott and D. P. Giedroc, *Biochemistry*, 2000, **39**, 11818.
77 T. Liu, A. Ramesh, Z. Ma, S. K. Ward, L. Zhang, G. N. George, A. M. Talaat, J. C. Sacchettini and D. P. Giedroc, *Nat. Chem. Biol.*, 2007, **3**, 60.
78 L. S. Busenlehner, N. J. Cosper, R. A. Scott, B. P. Rosen, M. D. Wong and D. P. Giedroc, *Biochemistry*, 2001, **40**, 4426.
79 L. S. Busenlehner and D. P. Giedroc, *J. Inorg. Biochem.*, 2006, **100**, 1024.
80 L. Banci, I. Bertini, F. Cantini, I. C. Felli, L. Gonnelli, N. Hadjiliadis, R. Pierattelli, A. Rosato and P. Voulgaris, *Nat. Chem. Biol.*, 2006, **2**, 367.
81 L. Banci, I. Bertini, S. Ciofi-Baffoni, N. G. Kandias, N. J. Robinson, G. A. Spyroulias, X. C. Su, S. Tottey and M. Vanarotti, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 8320.
82 D. Achila, L. Banci, I. Bertini, J. Bunce, S. Ciofi-Baffoni and D. L. Huffman, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 5729.
83 M. Pruteanu, S. B. Neher and T. A. Baker, *J. Bacteriol.*, 2007, **189**, 3017.
84 H. K. Song, S. B. Mulrooney, R. Huber and R. P. Hausinger, *J. Biol. Chem.*, 2001, **276**, 49359.
85 J. L. Rowe, G. L. Starnes and P. T. Chivers, *J. Bacteriol.*, 2005, **187**, 6317.
86 J. S. Cavet, W. Meng, M. A. Pennella, R. J. Appelhoff, D. P. Giedroc and N. J. Robinson, *J. Biol. Chem.*, 2002, **277**, 38441.
87 A. White, X. Ding, J. C. vanderSpek, J. R. Murphy and D. Ringe, *Nature*, 1998, **394**, 502.
88 G. Wisedchaisri, C. J. Chou, M. Wu, C. Roach, A. E. Rice, R. K. Holmes, C. Beeson and W. G. Hol, *Biochemistry*, 2007, **46**, 436.
89 E. R. Schreiter, S. C. Wang, D. B. Zamble and C. L. Drennan, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 13676.
90 K. S. Gajiwala and S. K. Burley, *Curr. Opin. Struct. Biol.*, 2000, **10**, 110.
91 P. T. Chivers and R. T. Sauer, *Protein Sci.*, 1999, **8**, 2494.
92 M. J. San Francisco, C. L. Hope, J. B. Owolabi, L. S. Tisa and B. P. Rosen, *Nucleic Acids Res.*, 1990, **18**, 619.
93 J. W. Huckle, A. P. Morby, J. S. Turner and N. J. Robinson, *Mol. Microbiol.*, 1993, **7**, 177.
94 N. L. Brown, J. V. Stoyanov, S. P. Kidd and J. L. Hobman, *FEMS Microbiol. Rev.*, 2003, **27**, 145.
95 H. Wunderli-Ye and M. Solioz, *Adv. Exp. Med. Biol.*, 1999, **448**, 255.
96 K. Hantke, *Curr. Opin. Microbiol.*, 2001, **4**, 172.
97 X. Ding, H. Zeng, N. Schiering, D. Ringe and J. R. Murphy, *Nat. Struct. Biol.*, 1996, **3**, 382.
98 T. V. O'Halloran, B. Frantz, M. K. Shin, D. M. Ralston and J. G. Wright, *Cell*, 1989, **56**, 119.
99 A. Z. Ansari, M. L. Chael and T. V. O'Halloran, *Nature*, 1992, **355**, 87.
100 R. P. Saha and P. Chakrabarti, *BMC Struct. Biol.*, 2006, **6**, 24.
101 T. Barkay, S. M. Miller and A. O. Summers, *FEMS Microbiol. Rev.*, 2003, **27**, 355.
102 D. Strausak and M. Solioz, *J. Biol. Chem.*, 1997, **272**, 8932.
103 M. Solioz and J. V. Stoyanov, *FEMS Microbiol. Rev.*, 2003, **27**, 183.
104 M. K. Safo, Q. Zhao, T. P. Ko, F. N. Musayev, H. Robinson, N. Scarsdale, A. H. Wang and G. L. Archer, *J. Bacteriol.*, 2005, **187**, 1833.
105 R. Portmann, K. R. Poulsen, R. Wimmer and M. Solioz, *BioMetals*, 2006, **19**, 61.
106 P. A. Cobine, G. N. George, C. E. Jones, W. A. Wickramasinghe, M. Solioz and C. T. Dameron, *Biochemistry*, 2002, **41**, 5822.
107 J. I. Kliegman, S. L. Griner, J. D. Helmann, R. G. Brennan and A. Glasfeld, *Biochemistry*, 2006, **45**, 3493.
108 P. E. Carrington, P. T. Chivers, F. Al-Mjeni, R. T. Sauer and M. J. Maroney, *Nat. Struct. Biol.*, 2003, **10**, 126.
109 S. C. Wang, A. V. Dias, S. L. Bloom and D. B. Zamble, *Biochemistry*, 2004, **43**, 10018.
110 J. A. Hunt, M. Ahmed and C. A. Fierke, *Biochemistry*, 1999, **38**, 9054.
111 K. I. Sen, A. Sienkiewicz, J. F. Love, J. C. vanderSpek, P. G. Fajer and T. M. Logan, *Biochemistry*, 2006, **45**, 4295.
112 Q. Que and J. D. Helmann, *Mol. Microbiol.*, 2000, **35**, 1454.
113 E. Guedon and J. D. Helmann, *Mol. Microbiol.*, 2003, **48**, 495.
114 A. Glasfeld, E. Guedon, J. D. Helmann and R. G. Brennan, *Nat. Struct. Biol.*, 2003, **10**, 652.
115 S. K. Checa, M. Espariz, M. E. Audero, P. E. Botta, S. V. Spinelli and F. C. Soncini, *Mol. Microbiol.*, 2007, **63**, 1307.
116 L. M. Utschig, J. W. Bryson and T. V. O'Halloran, *Science*, 1995, **268**, 380.
117 P. J. Kiley and H. Beinert, *Curr. Opin. Microbiol.*, 2003, **6**, 181.
118 D. M. Ralston and T. V. O'Halloran, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, **87**, 3846.
119 J. S. Iwig, J. L. Rowe and P. T. Chivers, *Mol. Microbiol.*, 2006, **62**, 252.
120 D. Koch, D. H. Nies and G. Grass, *BioMetals*, 2007, in press.
121 J. Helmann, personal communication.
122 G. Grass, B. Fan, B. P. Rosen, K. Lemke, H. G. Schlegel and C. Rensing, *J. Bacteriol.*, 2001, **183**, 2803.
123 L. S. Busenlehner, M. A. Pennella and D. P. Giedroc, *FEMS Microbiol. Rev.*, 2003, **27**, 131.
124 M. L. VanZile, X. Chen and D. P. Giedroc, *Biochemistry*, 2002, **41**, 9765.
125 J. Ye, A. Kandegedara, P. Martin and B. P. Rosen, *J. Bacteriol.*, 2005, **187**, 4214.
126 J. S. Cavet, A. I. Graham, W. Meng and N. J. Robinson, *J. Biol. Chem.*, 2003, **278**, 44560.
127 Y. Wang, L. Hemmingsen and D. P. Giedroc, *Biochemistry*, 2005, **44**, 8976.
128 L. S. Busenlehner, T. C. Weng, J. E. Penner-Hahn and D. P. Giedroc, *J. Mol. Biol.*, 2002, **319**, 685.
129 A. Arunkumar and D. Giedroc, manuscript in preparation.
130 M. L. VanZile, X. Chen and D. P. Giedroc, *Biochemistry*, 2002, **41**, 9776.
131 T. Liu, X. Chen, M. Li, R. A. Scott and D. Giedroc, manuscript in preparation.
132 T. Liu, S. Nakashima, K. Hirose, M. Shibasaka, M. Katsuhara, B. Ezaki, D. P. Giedroc and K. Kasamo, *J. Biol. Chem.*, 2004, **279**, 17810.
133 E. R. Schreiter, M. D. Sintchak, Y. Guo, P. T. Chivers, R. T. Sauer and C. L. Drennan, *Nat. Struct. Biol.*, 2003, **10**, 794.
134 M. A. Pennella, A. I. Arunkumar and D. P. Giedroc, *J. Mol. Biol.*, 2006, **356**, 1124.
135 N. Popovych, S. Sun, R. H. Ebright and C. G. Kalodimos, *Nat. Struct. Mol. Biol.*, 2006, **13**, 831.
136 J. F. Lefevre, K. T. Dayie, J. W. Peng and G. Wagner, *Biochemistry*, 1996, **35**, 2674.
137 H. Krizova, L. Zidek, M. J. Stone, M. V. Novotny and V. Sklenar, *J. Biomol. NMR*, 2004, **28**, 369.
138 D. Kern and E. R. Zuiderweg, *Curr. Opin. Struct. Biol.*, 2003, **13**, 748.
139 M. Akke, *Curr. Opin. Struct. Biol.*, 2002, **12**, 642.
140 J. J. Englander, G. Louie, R. E. McKinnie and S. W. Englander, *J. Mol. Biol.*, 1998, **284**, 1695.
141 S. Lee, A. I. Arunkumar, X. Chen and D. P. Giedroc, *J. Am. Chem. Soc.*, 2006, **128**, 1937.
142 A. I. Arunkumar, M. A. Pennella, X. Kong and D. P. Giedroc, *J. Biomol. NMR*, 2007, in press.
143 J. A. D'Aquino, J. Tetenbaum-Novatt, A. White, F. Berkovitch and D. Ringe, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 18408.
144 M. A. DeWitt, J. I. Kliegman, J. D. Helmann, R. G. Brennan, D. L. Farrens and A. Glasfeld, *J. Mol. Biol.*, 2007, **365**, 1257.
145 M. Golynskiy, S. Li, V. L. Woods, Jr. and S. M. Cohen, *JBIC, J. Biol. Inorg. Chem.*, 2007, **12**, 699.
146 S. I. Patzer and K. Hantke, *J. Biol. Chem.*, 2000, **275**, 24321.
147 E. Diaz-Mireles, M. Wexler, G. Sawers, D. Bellini, J. D. Todd and A. W. Johnston, *Microbiology*, 2004, **150**, 1447.
148 B. E. Ahn, J. Cha, E. J. Lee, A. R. Han, C. J. Thompson and J. H. Roe, *Mol. Microbiol.*, 2006, **59**, 1848.
149 N. Bsat, A. Herbig, L. Casillas-Martinez, P. Setlow and J. D. Helmann, *Mol. Microbiol.*, 1998, **29**, 189.
150 L. Chen, L. Keramati and J. D. Helmann, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 8190.
151 J. W. Lee and J. D. Helmann, *Nature*, 2006, **440**, 363.

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

View Online

152 E. Pohl, J. C. Haller, A. Mijovilovich, W. Meyer-Klaucke, E. Garman and M. L. Vasil, *Mol. Microbiol.*, 2003, **47**, 903.
153 D. A. Traore, A. El Ghazouani, S. Ilango, J. Dupuy, L. Jacquamet, J. L. Ferrer, C. Caux-Thang, V. Duarte and J. M. Latour, *Mol. Microbiol.*, 2006, **61**, 1211.
154 A. Maciag, E. Dainese, G. M. Rodriguez, A. Milano, R. Provvedi, M. R. Pasca, I. Smith, G. Palu, G. Riccardi and R. Manganelli, *J. Bacteriol.*, 2007, **189**, 730.
155 D. Lucarelli, S. Russo, E. Garman, A. Milano, W. Meyer-Klaucke and E. Pohl, *J. Biol. Chem.*, 2007, **282**, 9914.
156 J. W. Lee and J. D. Helmann, *J. Biol. Chem.*, 2006, **281**, 23567.
157 C. E. Outten, D. A. Tobin, J. E. Penner-Hahn and T. V. O'Halloran, *Biochemistry*, 2001, **40**, 10417.
158 L. Pecqueur, B. D'Autreaux, J. Dupuy, Y. Nicolet, L. Jacquamet, B. Brutscher, I. Michaud-Soret and B. Bersch, *J. Biol. Chem.*, 2006, **281**, 21286.
159 D. P. Barondeau, C. J. Kassmann, C. K. Bruns, J. A. Tainer and E. D. Getzoff, *Biochemistry*, 2004, **43**, 8038.
160 J. S. Kim, S. O. Kang and J. K. Lee, *J. Biol. Chem.*, 2003, **278**, 18455.
161 P. T. Chivers and R. T. Sauer, *Chem. Biol.*, 2002, **9**, 1141.
162 S. L. Bloom and D. B. Zamble, *Biochemistry*, 2004, **43**, 10029.
163 C. Dian, K. Schauer, U. Kapp, S. M. McSweeney, A. Labigne and L. Terradot, *J. Mol. Biol.*, 2006, **361**, 715.
164 P. T. Chivers and T. H. Tahirov, *J. Mol. Biol.*, 2005, **348**, 597.
165 L. Rulisek and J. Vondrasek, *J. Inorg. Biochem.*, 1998, **71**, 115.
166 O. Scholz, M. Kostner, M. Reich, S. Gastiger and W. Hillen, *J. Mol. Biol.*, 2003, **329**, 217.
167 S. R. Luckner, M. Klotzsche, C. Berens, W. Hillen and Y. A. Muller, *J. Mol. Biol.*, 2007, **368**, 780.
168 A. B. Taylor, C. S. Stoj, L. Ziegler, D. J. Kosman and P. J. Hart, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 15459.

Published on 28 June 2007 on http://pubs.rsc.org | doi:10.1039/B706769K
Downloaded on 18 May 2011

 This journal is © The Royal Society of Chemistry 2007