Published in final edited form as:

Biochemistry. 2012 October 9; 51(40): 7816–7832. doi:10.1021/bi300981m.

Specific metal recognition in nickel trafficking

Khadine A. Higgins^{1,*}, Carolyn E. Carr¹, and Michael J. Maroney¹

Michael J. Maroney: mmaroney@chemistry.umass.edu

¹Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003, Phone: 413-545-4876

Abstract

Nickel is an essential metal for a number of bacterial species that have developed systems for acquiring, delivering and incorporating the metal into target enzymes, and controlling the levels of nickel in cells to avoid toxic effects. As with other transition metals, these trafficking systems must be able to distinguish between the desired metal and other transition metal ions with similar physical and chemical properties. Because there are few enzymes (targets) that require nickel for activity (*e.g.*, *E. coli* traffics nickel for hydrogenases made under anaerobic conditions and *H. pylori* requires nickel for hydrogenase and urease that are essential for acid viability), the ‘traffic pattern’ for nickel is relatively simple, and nickel trafficking therefore presents an opportunity to examine a system for the mechanisms that are used to distinguish nickel from other metals. In this review, we describe the details known for examples of uptake permeases, metallochaperones and proteins involved in metallocenter assembly, and nickel metalloregulators. We also illustrate the variety of mechanisms, including molecular recognition in the case of NikA protein and examples of allosteric regulation for HypA, NikR and RcnR, employed to generate specific biological responses to nickel ions.

Introduction

Many bacteria require nickel for the production of key enzymes, including ureases, hydrogenases, carbon monoxide dehydrogenases, acetyl coenzyme A synthases, methyl coenzyme M reductases, some glyoxylases and a unique superoxide dismutase (1, 2). Furthermore, many pathogenic bacteria including *E. coli*, *H. pylori*, *B. suis*, *Yersinia* species, *Salmonella*, *Shigella* and *M. tuberculosis* rely on nickel trafficking systems for their survival and pathogenicity (1, 3). *E. coli* and *H. pylori* both require nickel for NiFe-hydrogenases, and in the case of *H. pylori* nickel is also required for urease (4–7). As is the case for other transition metals (8–10), bacteria that utilize nickel have evolved proteins to facilitate the import/export of nickel, and its cellular distribution and incorporation into enzymes in response to the nickel levels in the cell. The proteins involved are collectively referred to as nickel trafficking proteins. Unlike the trafficking systems employed for more common transition metals such as iron, copper and zinc (11), the ‘traffic pattern’ for nickel is relatively simple because bacteria that utilize nickel usually feature only one or two target enzymes. Nonetheless, the nickel trafficking system has all the essential features of any transition metal trafficking system, and thus offers an opportunity to develop a global perspective of the system (Figure 1). Many of the key proteins involved in nickel trafficking have been identified in various organisms. These proteins include importers, exporters, chaperones and accessory proteins involved in enzyme metallocenter assembly, storage proteins and regulators and are summarized in Table 1, which also indicates proteins where structural information is available. In order to function properly, these proteins require a

*Current Address: Department of Chemistry, Indiana University, Bloomington, Indiana 47405

mechanism that allows for discrimination of the metal being trafficked (the cognate metal) from all other metals (non-cognate metals), by finding a way to overcome the binding preferences dictated by the thermodynamics of metal ligand interactions, as reflected in the Irving-Williams series ($K: \text{Co(II)} < \text{Ni(II)} < \text{Cu(II)} > \text{Zn(II)}$). (108, 137, 138) The mechanisms employed in specifically binding Ni(II) ions (Ni(II) recognition) are the focus of this current topics article, and by necessity the focus is on proteins where knowledge of metal-specific protein interactions and functions are available.

Nickel importers

There appear to be two basic strategies involved in the acquisition of nickel by bacteria. The process can be energetically driven by ATP hydrolysis, as in the case of ABC-type permeases, or by passive transport, as in the case of nickel/cobalt transporters, NiCoTs).

The Nik Permease

In *E. coli*, the *nik* operon encodes a nickel ABC-type permease, (139) NikABCDE, that is expressed under anaerobic conditions to satisfy the nickel requirements for NiFe-hydrogenase maturation (12). Hydrogenases carry out the reversible oxidation of hydrogen to protons and electrons, which can be a source of energy (4–6, 12). The transcription of the *nik* operon is positively regulated under anoxic conditions by the fumarate and nitrate regulatory protein, FNR, a transcription regulator of many genes that are linked to anaerobic and fermentative metabolic functions, and negatively regulated by the binding of nickel to the DNA binding protein NikR (140–143). The importer is composed of a periplasmic binding protein, NikA, two trans-membrane proteins, NikB and NikC, and two ATPases, NikD and NikE (12, 13).

Based on the chemistry of other ABC-type transporters, the periplasmic binding protein is likely involved in discriminating nickel from other transition metals. NikA is a 56 kDa monomer that binds Ni(II) ions with a K_d of either of 0.1 or 10 μM (12, 14). NikA can bind other metals including Fe(II), Co(II) and Cu(II), but the binding of these metals to NikA is at least 10-fold weaker than that of Ni(II) (12). The first structural information regarding metal binding to NikA came from X-ray absorption spectroscopy (XAS) data that revealed that the Ni(II) site in NikA is six-/seven-coordinate consisting of 6 N/O-donors at 2.06 Å and a possible S/Cl-donor at 2.57 Å (15).

The first NikA crystal structure was published in 2003 (14), and featured Ni(II) ions bound to the protein. The crystal structure revealed that the overall fold of the protein resembled other periplasmic binding proteins, which have a clam shell structure, except that in the case of NikA the clam shell was open in the presence of the presumed substrate. The first coordination sphere of the Ni(II) was characterized as being composed of five water molecules, one of which formed a hydrogen bond with the side chain of Arg137, and no direct bonds between the protein and the nickel ion (14). The bond distances obtained for the Ni(II)-O bonds in the crystal structure ranged from 2.5 to 3.1 Å (Ave. 2.8 Å), much larger than that obtained from EXAFS analysis (14, 15), and essentially a non-bonded Ni-O distance. A subsequent crystal structure showed NikA bound to $[\text{Fe}(\text{EDTA})\text{H}_2\text{O}]^-$, with the complex in the same protein locus as the previously characterized Ni(II)(H_2O) $_5^{2+}$ ion. (16). Isothermal titration calorimetry (ITC) experiments determined that NikA can bind Ni(II) chelated by EDTA with an affinity of 30 μM (144). The crystallographic data suggested that Ni(II) ions were not the substrate for the transporter, rather that some complex, such as seen in iron-siderophore transport (16, 145), might be required. A third crystal structure of NikA, this time purified from the periplasm in the absence of EDTA showed the nickel center modeled in a square planar geometry and coordinated by three carboxylate ligands from a compound that was modeled as butane-1,2,4-tricarboxylate (BTC) and a histidine residue,

His416 (17). Subsequent mutagenesis studies revealed that His416 is required for nickel uptake (18). However, no evidence of the presence of BTC in *E. coli* has been found, and attempts at synthesizing a Ni(II)-BTC-NikA complex from the apo-protein were unsuccessful (17). Recent evidence shows that $\text{Ni}(\text{H}_2\text{O})_6^{2+}$ ions are not transported by NikABCDE, but that $\text{Ni}(\text{L-His})_2$ is transported (19). The process is stereospecific, as D-His does not support nickel uptake, and involves co-transport of L-His. This complex is likely recognized by the formation of specific H-bonds in the binding site in a manner similar to the recognition of peptides by the peptide transporter OppA, with which NikA shares significant amino acid sequence homologies (Figure 2) (14, 146, 147).

Although NikABCDE may involve recognition of a specific nickel complex by the periplasmic binding protein, NikA, this mechanism is not universal. Recently, an ABC-type transporter, NikZYXWV, was identified in the human pathogen *C. jejuni* (20). Unlike NikA, NikZ does not bind Ni(II) ions chelated by EDTA but binds solvated nickel with a K_d of 0.61 μM . Fluorescence experiments showed that NikL can also bind Co(II) ions (20).

An additional family of ATP-dependent nickel importers has been found in other bacteria, Cbi/NikMNQO, which like NikABCDE has an ATP binding protein and cytoplasmic components. However, unlike NikABCDE they lack a periplasmic binding protein (21).

NiCoT transporters

NiCoTs are a family of secondary nickel and cobalt transporters found in prokaryotes and fungi that mediate the uptake of cobalt and nickel ions into the cell (148). These permeases constitute a distinct structural class of importer that lack the ATPase activity (148). Several NiCoT transporters have been identified in *A. eutrophus* (HoxN) (23), *H. pylori* (NixA) (26), *R. rhodochrous* J1 (NhlF) (28), and *B. japonicum* (HupN) (29). NiCoT permeases have an affinity for both Ni(II) and Co(II) ions, as the name implies, and can be divided into three groups: 1) importers specific for Co(II), 2) importers that can transport both Ni(II) and Co(II) and 3) importers specific for Ni(II) (148). This family of permeases features eight transmembrane helices and conserved Gly-X₅-Glu-His-Ser-Ser-Val-Val and His-X₄-Asp-His sequences (149). The latter conserved motif is associated with nickel binding and is also found in *E. coli* NikC (*vide supra*) (149). Mutagenesis studies showed that mutations made in the His-X₄-Asp-His motif resulted in a decrease in the affinity of HoxN for Ni(II) (24) and NhlF for Ni(II) and Co(II) (25).

Nickel exporters

There is virtually no information about the mechanisms of metal recognition in nickel exporters, where characterized proteins include *H. pylori* CznABC (30), NreB from *A. xylosoxidans* 31A and *E. coli* (31), *E. coli* RcnAB (resistance to cobalt and nickel, formerly YohM and YohN), (32, 33), *P. putida* MdrH (34), *Synechocystis* PCC 6803 NrsD (35) and *L. ferriphilum* NcrAC (36, 37). Mechanisms similar to those employed by importers may apply to exporters as well, though the delivery of Ni(II) to the exporter may well involve accessory proteins as well as small molecular weight complexes.

Chaperones and Accessory Proteins

The proteins involved in metallocenter assembly in nickel enzymes are a somewhat heterogeneous group from both sequence and functional perspectives. Many examples exist where addition of motifs adds functions, such as nickel storage, to one or another accessory protein. Thus, the discussion of these proteins is dependent on the organism involved. The proteins include HypA, HypB, SlyD, UreE, UreG, CooC and CooJ, the first four of which are discussed here.

HypA

Every organism that expresses a NiFe hydrogenase also has a HypA-type nickel metallochaperone to deliver nickel to the apo-H₂ase large subunits. In *E. coli*, HypA, and its homolog HypB, along with HypB and SlyD are responsible for the delivery and incorporation of nickel into three hydrogenases (38, 150). *E. coli* HypA binds two Ni(II) ions per dimer with μ M affinity (39, 41, 42), and co-purifies with one Zn(II) per monomer that binds with nanomolar affinity to two rigorously conserved CXXC motifs. UV-vis studies showed that HypA can also bind Co(II) to the zinc site (39).

Like *E. coli* HypA, *H. pylori* HypA binds Zn(II) and Ni(II) ions, exists in solution as a homodimer, and has been shown to form a heterodimeric complex with HypB (39, 41). Crosslinking studies determined that HypA and UreE (*vide infra*), a urease nickel chaperone, are also capable of forming a heterodimeric complex (43). Of the five histidine residues present in *H. pylori* HypA, only His2 was shown to be vital for nickel binding (41). Differential scanning calorimetry as well as CD thermal melts determined that HypA is stabilized by Ni(II) binding, as the binding of Ni(II) to Zn(II) loaded HypA resulted in $\sim$ 12 °C increase in the melting temperature measured using both techniques (42).

Three structures of HypA proteins have been reported. The first is a NMR structure of the monomeric protein from *H. pylori* (Figure 3) (44), where the N-terminus was modified with additional Gly and Ser residues left after thrombin cleavage of a His-tag, and the other two are crystal structures of monomeric and homodimeric forms from *T. kodakaraensis* (47). The NMR structure of the *H. pylori* HypA monomer reveals that the Ni(II) site is located at the N-terminus and is coordinated by the backbone nitrogen from His2, Glu3 and Asp40 and the imidazole from His2. The use of His2 as a ligand is in agreement with previous studies (41). Other NMR work carried out on an unmodified *H. pylori* HypA homodimeric protein showed that there was dipolar broadening in the N-terminal region (proposed nickel site) upon nickel binding, consistent with a paramagnetic (S=1) five or six-coordinate nickel site (42). It is unclear whether the difference in nickel coordination observed is due to the oligomeric state of the protein or/and the modification of the N-terminal nickel binding site in the monomer structure. The crystal structures of the monomeric and dimeric HypA proteins from *T. kodakaraensis* do not contain Ni(II) ions, but do reveal a domain swap in the homodimeric protein that affects the cysteine coordination of the zinc site (Figure 4) (47). All three HypA structures reveal that the zinc is coordinated by the two conserved CXXC motifs. However, in the dimeric form of the *T. kodakaraensis*, the Zn(II) ion is coordinated by one CXXC motif from each subunit.

XAS studies on the dimeric form of *H. pylori* HypA showed that the unique structural flexibility of the zinc site goes even further, as it appears to be involved in sensing both Ni(II) binding and pH (42, 45). At pH 7.2, the zinc site in the holo-protein (Ni(II) is bound) is tetrahedral with four S-donor ligands (42). However, a decrease in the pH to 6.3 (the internal pH of *H. pylori* under acid shock) results in a ligand substitution involving the replacement of two S-donor ligands on the zinc by two imidazole ligands from histidine residues (42). This structural change could not be replicated by substituting Co(II) for Ni(II) ions [Kennedy, 2006 #304], and so appears to reflect a protein conformational flexibility that is metal specific and may be part of the nickel recognition mechanism. ITC showed that this structural change resulted in a change of nickel binding stoichiometry from one Ni(II) per monomer at pH 7.2 to one nickel per dimer at pH 6.3. Urease activation increases under acidic conditions (151), leading to the suggestion that the changes observed in the HypA zinc site monitor protein conformational changes that facilitate nickel incorporation into urease under acid stress (42). One possibility for the interaction of HypA with the urease metallocenter assembly pathway is suggested by a recent study that shows that HypA competes with UreG for binding UreE (46).

Further studies were carried out using XAS and mutagenesis to probe the connection between the structural changes in the zinc site and the properties of *H. pylori* HypA (42). In a series of mutations involving Cys → Ala or Cys → Asp, alteration of any one of the four cysteines resulted in zinc sites that featured a Zn(Cys)₂(His)₂ coordination environment, bound only one Ni(II) ion per dimer, and no longer sensed nickel binding or pH changes. (42). The data are consistent with the Cys mutations locking the protein into a conformation that is adopted by WT-HypA at low pH. Both of the two CXXC motifs in HypA are flanked by a histidine residue (His79 and His95), a feature unique to *H. pylori* HypA. Mutating either of these residues to Ala also resulted in a protein that did not sense nickel or pH, but where the zinc site resembled that of the WT-HypA zinc site at pH 7.2 (four S-donor ligands). The data from the H79A and H95A mutations suggest that these two histidine residues are ligands in the low pH WT-HypA structure and that the mutation of these residues lock the protein in the conformation adopted by WT-HypA at neutral pH.

HypB

HypB is a P-loop GTPase required for hydrogenase maturation and Ni(II) insertion (41, 49, 50). *E. coli*, *H. pylori* and *M. jannaschii* HypB proteins exist as homodimers in solution (41, 50, 55). HypB also makes heterodimers with HypA (41) and SlyD (*vide infra*), and the HypAB dimers have been shown to exist *in vivo* (53). The details of the interaction between the *H. pylori* versions of HypA and HypB have been determined by NMR (53).

In the reduced form, *E. coli* HypB binds two metal ions per monomer (51). The two metal binding sites are located near the N-terminus and in the GTPase domain of the protein. The CXXCGC motif located near the N-terminus of the protein is associated with the high affinity Ni(II) site that has a K_d in the sub-picomolar range (51). A low affinity metal binding site located in the GTPase domain can bind either nickel or zinc, but has a higher affinity for zinc over nickel (51, 52). The GTPase activity measured is low for *E. coli* HypB, with a k_{cat} of 0.17 min⁻¹ and a K_m of 4 μM (50).

XAS studies carried out on *E. coli* HypB revealed that metal binding status is communicated between the two metal sites. XANES analysis determined that nickel binds to the high affinity site in a four-coordinate planar geometry, and the binding of zinc to the low-affinity site results in a less intense 1s → 4p_z transition and a slightly more intense peak at 8442 eV (white line) that indicates a small distortion in the planar geometry and a reduction in the S-donor ligand content of the nickel site (52). EXAFS analysis reveals that the nickel site is composed of three S-donors at 2.17 Å and one N/O-donor at 1.87 Å. Upon binding zinc in the low-affinity site there is a decrease in the Ni-S bond distance to 2.15 Å and an increase in the Ni-N bond distance to 2.02 Å (52).

Like the nickel site, the zinc site in the G-domain is affected by nickel binding to the high affinity site. XANES analysis revealed that the zinc site is four-coordinate and tetrahedral both with and without nickel in the high affinity site (52). However, the EXAFS analysis revealed that in the absence of nickel, the zinc site contains two Zn(II) ions with an average of two and a half S-donors, one O-donor, one imidazole-ligand and a Zn-Zn interaction (52). However, when nickel binds to the high affinity site, the zinc site becomes more symmetric with an average of two S-donors, one N-donor, one imidazole-ligand and a Zn-Zn interaction (52). These subtle structural perturbations suggest an allosteric recognition mechanism like that found in HypA may operate in HypB as well.

H. pylori HypB protein lacks the N-terminal high-affinity nickel site, and binds Ni(II) ions in a 1:1 stoichiometry with 1.72 μM affinity (53). The protein can bind both nickel and zinc, but zinc competes with nickel for binding, suggesting that the nickel and the zinc sites have ligands in common (54). Mutagenesis studies revealed that Cys106 and His107 located in

the G-domain are essential for metal binding, as mutating these residues resulted in a protein that could no longer bind nickel, and lowered the affinity for zinc by two orders of magnitude (54). Like *E. coli* HypB, GTP hydrolysis by *H. pylori* HypB is slow. Studies on *H. pylori* HypB determined that nickel binding, which causes dimerization, does not affect GTP hydrolysis whereas zinc binding, which does not promote dimerization, inhibits HypB GTPase activity (54). In contrast, other studies have indicated that monomeric HypB is in a low activity state, and that dimerization to a more active form occurs upon binding GTP or nickel (53). Binding Ni(II) ions was proposed to form a “metallic bridge” that stabilizes the active dimer, in which two GTP hydrolysis active sites are formed by the invariant Lys-168 residues (53). However, one study provides data that indicates that apo-HypB (monomer) and the Ni(II) complex (dimer) have about the same activity (k_{cat}/K_M) (54), while the other indicates that the dimeric complex with Ni(II) is more active (53). The origin of these discrepancies is not known.

HypB from *M. jannaschii* also lacks the N-terminal high-affinity nickel site found in the *E. coli* protein. The crystal structure of HypB from *M. jannaschii* (Figure 5) revealed the presence of two metal binding sites involving residues in the G-domain, including a dinuclear zinc site located at the dimer interface, where one zinc is coordinated by three Cys (Cys95 and Cys127 from one monomer and Cys95 from the other monomer) and a water molecule, and the other Zn(II) is coordinated by two Cys (Cys95 and Cys127), one His (His96) residue and a water molecule (55). The zinc sites are bridged by a cysteine residue (Cys95) (55). The structure also revealed that HypB binds guanosine 5'-O-(3-thiotriphosphate) (GTP γ S) at the dimer interface using residues from both monomers (55).

HypB proteins from *B. japonicum* and *R. leguminosarum* feature a histidine rich tail (152–154) that plays a role in nickel storage or buffering (153, 155). *B. japonicum* HypB has 24 N-terminal His residues that can bind up to 9 nickels per monomer with a K_d of 2.3 μ M (152). The protein can also bind other metals including Zn(II), Cu(II), Co(II), Cd(II), and Mn(II) (152). Similar polyhistidine regions have been observed in SlyD (*vide infra*) and other accessory proteins involved in the maturation of urease and carbon monoxide dehydrogenase biosynthesis, UreE (*vide infra*) and CooJ respectively (78, 88, 100).

SlyD

SlyD (sensitive to *lysis D*) (57) was originally identified as a protein that sensitizes *E. coli* to lysis by bacteriophage ϕ X174 (58, 59). SlyD orthologs are found in many species such as *H. pylori*, *T. thermophilus*, *M. thermolithotrophicus*, and *V. cholera* (60, 61, 75, 77, 156). NMR solution structures of *E. coli* SlyD reveals two domains; (60, 62, 75) a peptidyl-prolyl isomerase (PPIase) domain and the “inserted in the flap” (IF) domain. The PPIase domain is related to the FK506-binding protein (FKBP) (57, 58, 157, 158) family of peptidyl-prolyl isomerases and catalyzes the *cis-trans* isomerization of proline peptide bonds (62–64, 159), which is a crucial step in the folding pathway of some proteins (160–162). However, the isomerase function of SlyD has been shown to be nonessential in the role of SlyD in hydrogenase maturation (64, 65). Although loading metal ions into the metal-binding domain is required for hydrogenase assembly (65–67), it has also been shown to disrupt PPIase activity (64). This is consistent with current data that suggests that metal-loaded SlyD exists in a closed conformation (64, 65, 67–70). The IF domain is so termed because it is inserted into the flap region of the FKBP domain (68, 71, 72, 159). This insertion is observed in a small subset of FKBP (62, 158), and is associated with the chaperone function and is involved in protein folding (61, 62, 68, 71, 159, 163).

E. coli SlyD has an unstructured 50 residue C-terminal tail, termed the metal-binding domain (MBD), which contains 28 potential metal-binding residues (15 His, 6 Cys, 7 Asp/Glu) (57, 63, 66, 70) that bind a variety of first-row transition metals with affinities that

follow the Irving-Williams series, but its function is specific to nickel binding *in vivo* (70). This tail is variable among SlyDs from different organisms (60, 61, 75, 164), and allows SlyD to bind multiple metal ions with nanomolar affinity, although the exact number of metal ions has not been definitively determined (60, 63, 64, 66, 69, 70). SlyD participates in hydrogenase maturation through a complex formed with HypB (*vide supra*) via the SlyD IF domain (62, 66, 67, 73, 155). The HypB-SlyD complex forms in the absence of HypA (*vide supra*) or hydrogenase, and the heterodimer formation is disrupted by mutating the two conserved proline residues in HypB (67). Truncation of the MBD of SlyD, or mutations in either of the two metal-binding domains in HypB, results in reduced hydrogenase maturation (52, 67). *E. coli* HypB lacks the histidine rich N-terminal region found in other HypB proteins that can bind multiple nickel ions with high capacity but low affinity [Kaluvarachchi, 2010 #275; Olson, 1997 #361; Rey, 1994 #363; Fu, 1995 #349; Olson, 2000 #335], and this lack of a nickel storage or buffer capacity in *E. coli* HypB is complemented by the metal-binding C-terminal extension found in *E. coli* SlyD (63, 64, 74). This modular model is supported by the observation that $\Delta slyD$ strains have reduced cytoplasmic nickel content and hydrogenase activity compared to the wild-type (65, 66, 74). The ability of the metal-binding domain of SlyD to bind multiple metal ions has also been postulated as the source for Ni(II) ions during hydrogenase active-site assembly (63, 66, 74).

It is thought that one of the roles of the SlyD is to enhance the release of Ni(II) from the high affinity site of HypB (51, 65–67, 73), which binds Ni(II) very tightly (K_d of $(1.3 \pm 0.2) \times 10^{-13}$), and may require some structural change in order to facilitate the release of the Ni(II) ions from the high affinity sites (51). Additionally, studies have shown that the addition of SlyD to HypB with Ni(II) loaded in the high affinity site results in an increase in the catalytic efficiency of GTP hydrolysis by three fold (66). Addition of SlyD to HypB with both the high affinity and low affinity sites occupied also increases the catalytic efficiency of GTP hydrolysis by ten-fold (66). This effect has been traced to metal transfer from the low affinity of HypB site to the metal-binding domain of SlyD (66).

UreE

Synthesis of the urease active site is a complex and highly choreographed process involving several accessory proteins including: UreD, UreE, UreF and UreG. Little is known about UreD, but it has been suggested that this is the first protein that binds to apo-urease (165). UreE is the chaperone that delivers nickel to urease (1, 165). UreF has been suggested to be an activator of the GTPase activity of UreG (94, 166).

UreE from *K. aerogenes* binds 6 nickels per dimer with a K_d of $\sim 9.6 \mu\text{M}$ (78). X-ray absorption spectroscopy and magnetic circular dichroism spectroscopies suggest that the nickel sites have pseudo-octahedral geometry with six N/O-donor ligands, of which three to five are histidines (78). The last 15 amino acids at the carboxyl terminus contain 10 histidine residues (79). Similar to the situation in HypB (histidine rich region located at the N-terminus) and SlyD (histidine rich region located at the C-terminus) proteins, this polyhistidine C-terminus is not a universal feature of UreE orthologues and is not found in either *B. pasteurii* UreE, or in *H. pylori* UreE. *H. pylori* UreE is capable of binding only one Ni(II) or Zn(II) ion per dimer with K_d values of $0.15 \mu\text{M}$ and $0.49 \mu\text{M}$, respectively (88, 89). *B. pasteurii* UreE binds two Ni(II) ions per dimer with a K_d of $35 \mu\text{M}$ (85). NMR and XAS spectroscopies revealed that the nickel sites in *B. pasteurii* UreE are also six-coordinate and involve histidine ligands (85, 86).

The lack of importance of the UreE polyhistidine tail for nickel incorporation into apo-urease was also demonstrated using a truncated UreE from *K. aerogenes*, H144*, where the last 15 residues (including 10 His residues) were removed. This truncated protein was

shown to reduce the number of nickel ions bound to ~2 Ni(II) per dimer (80), like the *B. pasteurii* protein, and its ability to incorporate nickel into urease was unaffected (80).

Other metals including Cd(II), Co(II), Zn(II) and Cu(II) were shown to compete with Ni(II) for the nickel binding site (80). These metals coordinate UreE with distinct coordination geometries, as revealed by a number of spectroscopic techniques that were used to characterize the various coordination environments of the divalent metals bound to H144* UreE. The two Ni(II) sites were found to be pseudo-octahedral with six N/O-donor ligands, are structurally similar to those found in WT-UreE protein, and are distinguished by different numbers of His ligands (81). Mutagenesis revealed that His96, His112 and Asp111 are involved in binding Ni(II) in one of the binding sites, while H110 is involved in the second site (82). In contrast, the two copper sites are tetragonal (CN = 4) with two His ligands each. Additionally, one copper site involved a Cys ligand (81) that was shown by mutagenesis to be Cys79 in H144* UreE (82). Like the nickel sites, the cobalt sites have (N/O)₆ pseudo-octahedral coordination and differed in the number of His ligands. His96 and His110 were also identified as ligands to Co(II). This data shows that different metals are bound to UreE using different coordination numbers and different ligands, and thus the coordination geometry and ligand selection of the bound metal provides a mechanism for metal recognition that is similar to that employed by the metalloregulators NikR and RcnR (*vide infra*).

There are several UreE crystal structures available: 1) *K. aerogenes* (H144*) UreE with copper(II) bound (83), 2) *B. pasteurii* UreE with zinc bound (87), 3) *H. pylori* UreE in three forms: apo, or complexes with copper(II) or nickel (90), and 4) *H. pylori* UreE in three forms: apo, or complexes with zinc or nickel (91). The *K. aerogenes* (H144*) UreE bound three copper(II) ions in three metal sites per dimer. One copper(II) site was located at the dimer interface and was coordinated by His96 from the two subunits, while the copper(II) in the other two sites were coordinated by His112 and His110. All three of these histidine residues were previously identified as nickel binding residues (*vide supra*) (82). Similar to the first metal-binding site in *K. aerogenes*, the zinc bound UreE crystal structure from *B. pasteurii* revealed that zinc was coordinated in a tetrahedral fashion by four conserved His100 residues, one from each subunit in a tetramer (87). The histidine residues coordinated to the metals at the dimer and tetrameric interface of UreE from *K. aerogenes* (His96) and *B. pasteurii* (His100) respectively are conserved in all UreE proteins and are important for urease activation *in vitro* (84) and *in vivo* (82).

The crystal structures of *H. pylori* UreE were determined for the apo-protein as well as complexes with Cu(II) or Ni(II) (90). The structures showed that the apo-protein formed dimers, while the metal-bound protein was tetrameric (dimer of dimers), with the tetramer formed by coordination of the metal ion by His104 residues from each subunit. A second set of *H. pylori* UreE crystal structures were obtained for the apo-protein, and zinc or nickel complexes (91) and implicated the disordered C-terminus in metal binding as well as stabilizing interactions with UreG in the case of the zinc complex (89). These crystal structures revealed that the nickel site is six-coordinate and interacts with His102 from one monomer and His102, His152 and Glu4 from another, as well as a water molecule and one other unknown ligand (91). In contrast, zinc is coordinated in a tetrahedral geometry by pairs of His102 and His152 from each monomer (91). These metal sites were also interrogated by XAS, which confirmed the respective metal geometries and the use of His152 residues that are disordered in the crystal structure (91). Thus, it was shown that the metal ion selectivity of UreE is based on the different metal ion coordination environments that are dictated by the electronic properties of the metal ion in a mechanism that is facilitated by the flexibility of the C-terminal protein region. The cross-talk between UreE, nickel and zinc suggested a specific functional role for different metal complexes of this

UreE protein in regulating the formation of protein-protein complexes involved in enzyme maturation and pointed to a mechanism for nickel transfer involving the H152 ligands, which could be replaced by UreG residues, leading to the transfer of nickel from UreE to UreG.

Metalloregulators

Metalloregulators control the expression of metallothioneins in response to the cellular metal status. Bacterial metallothioneins acquire metals in a process that involves metal binding affinities, access to metals in the cytosol, and protein allostery (10, 138). Several Ni(II)-responsive metallothioneins have been identified including: NikR (140), RcnR (124), NmtR (128), KmtR (131), Nur (132), SrnRQ (134), NcrB (37), Mua (135), NimR (136) and InrS (137). However, information regarding Ni(II) recognition is available for NikR, RcnR, and Nur, and is discussed in detail below.

To control the level of nickel in the cell, *E. coli* utilizes two transcriptional regulators, NikR (also found in a number of bacteria, including *H. pylori*) (115, 122, 123, 140) and RcnR (*vide infra*) (124). The first regulator, NikR, controls the expression of the ABC-type nickel importer, NikABCDE (140), and the second, RcnR regulates the expression of the exporter at higher metal levels, similar to that seen for the zinc-responsive regulatory proteins, Zur and ZntR in *E. coli* (167). In *H. pylori*, NikR also controls the expression of a nickel importer, NixA, a NiCoT permease, as well as a variety of other genes, including the urease structural genes (*ureA-ureB*), nickel uptake factors (*fecA3*, *fipB4*, and *exbB/exbD*), nickel storage genes (*hpn* and *hpn-like*) and genes associated with iron uptake (*fur* and *pfr*) (116).

NikR

NikR is the only member of the ribbon-helix-helix (β - α - α) family of prokaryotic DNA-binding proteins where the function is regulated by metals (105). Nickelated NikR binds to a 28-bp palindromic operator within the NikABCDE promoter, GTATGA-N₁₆-TCATAC, with nanomolar affinity (106, 107). *E. coli* NikR is a homotetramer that binds one nickel ion per monomer, and competition assays determined that NikR binds Ni(II) with picomolar affinity (106, 108). *E. coli* NikR (Figure 6) has two distinct metal binding sites, the “high affinity” site located at the tetramer interface near the C-terminus, and the “low-affinity” site, which is suggested to be located near the interface of the C-terminal domain and the DNA-binding domain (106, 107, 122). In the “high affinity” site, the nickel ions are bound in a four-coordinate planar geometry by the side chains of His87, His89, and Cys95 from one NikR monomer, and by His76 from an adjacent monomer (109). In addition to these residues, there are three other potential ligands (Tyr60, His62 and Glu97) near the high-affinity Ni(II) site (110). NikR can bind a variety of other transition metals *in vitro*, and the binding affinities follow the Irving-Williams series (Co(II) < Ni(II) < Cu(II) > Zn(II)), indicating that there is no unusual affinity leading to a preference for metal binding (108). Nonetheless, the protein responds *in vivo* only to the presence of Ni(II) ions (110). Additionally, the nickel loaded NikR protein was less susceptible to chemical and thermal denaturation (108).

Studies of the metal site structure using XAS showed that each metal adopts a distinct structure (110). The non-cognate metals Co(II), Cu(I), Cu(II) and Zn(II) adopt a variety of alternate geometries and ligands (110). Cobalt binds to the protein forming a six-coordinate complex with N/O-donor ligands, of which three are imidazoles from histidine ligands. Unlike the nickel site, the cobalt site was not coordinated by the S-donor ligand, Cys95. Similar to the nickel site, the copper(II) site was found to be four-coordinate planar with three N/O-donors of which two were imidazole and a S-donor at 2.21(2) Å, ~ 0.08 longer than that of the Ni-S bond distance (2.13(2) Å). Additionally the copper(II) site featured one

less imidazole ligand than the Ni(II) site. Copper(I) and zinc form three-coordinate trigonal and four-coordinate tetrahedral complexes, respectively. These results suggest that metal ion selectivity in NikR is achieved by the coordination number/geometry of the metal-protein complex, as well as ligand selection (110), which could be coupled to protein allosteric changes, an emerging theme in metal recognition by metalloregulators (9).

NikR has other putative nickel binding sites, known as the “low-affinity” sites that bind nickel or other ions (109–111, 117). DNA binding studies demonstrated that nickel binding to a set of low-affinity sites resulted in an increase in the protein’s affinity for DNA (106). Further studies showed that NikR affinity for DNA increased from nanomolar to picomolar in the presence of excess nickel (107). XAS studies performed on heterometallated samples with copper(II) loaded into the high-affinity site and nickel in the low-affinity site reveal that nickel adopts an octahedral geometry with nickel coordinated by two histidine ligands at 1.97(2) Å and four N/O-donors at 2.11(2) Å (110). Furthermore, mass spectrometry and mutagenesis studies identified two histidine residues, His48 and His119, located at the interface of the metal- and DNA-binding domains that participate in the binding of Ni(II) to the low affinity site (112). Crystallography experiments performed on *E. coli* NikR crystals soaked with excess NiCl₂ in the absence and presence of DNA identified six potential low affinity Ni(II) sites on the surface of the protein (113). Most of the sites identified were six-coordinate with N/O-donor ligands and at least one histidine residue coordinated to nickel. Additional studies revealed that K⁺ ions are essential for *E. coli* NikR nickel-responsive DNA binding (111).

Like *E. coli* NikR, *H. pylori* NikR binds four Ni(II) ions in the high-affinity sites with nanomolar affinity (115) in a four-coordinate planar geometry coordinated by the side chains of the corresponding residues-His99, His101 and Cys107 from one metal-binding domain and His88 from another (118). The difference in binding affinity measured in *E. coli* and *H. pylori* NikR may be due to the differences in the methods as well as the assay conditions used to determine the affinities, competition assay versus ITC (168). ITC experiments determined that *H. pylori* NikR can bind up to ten nickel atoms in the low-affinity sites with μM affinity (115).

The crystal structures of NikR protein from *E. coli* (109, 114), *P. horikoshii* (122) and *H. pylori* (119) show that the NikR protein can adopt a variety of conformations that might represent protein allostery that could be coupled to metal ion selection. An open conformation was observed in the *E. coli* and *P. horikoshii* apo-NikR crystal structures where the DNA binding domains are linearly placed on each side of the transmembrane domain (114, 122). A closed *trans*-conformation was observed for nickel bound *P. horikoshii* and *H. pylori* NikRs, where the DNA binding domains are on opposite sides of the transmembrane domain (119, 122). The nickel-bound *E. coli* NikR-DNA structure shows a closed *cis*-conformation where the two DNA binding domains are located on the same side of the transmembrane domain and are bound to DNA (109).

The evidence coupling these conformations to metal selectivity is less clear. Data from small angle x-ray scattering (SAXS) experiments did not support these large conformational changes upon nickel binding to *H. pylori* NikR, as the apo and nickelated protein had very similar scattering patterns (120). Changes in protein dynamics have been proposed based on computational and NMR studies that suggest that NikR in solution is interconverting between the *cis*, open, and *trans* forms, and that the binding of nickel facilitates the interconversion (169).

RcnR

RcnR is a member of the recently characterized CsoR/RcnR family of transcriptional regulators that regulates the expression of the nickel and cobalt exporter, RcnA, and the associated periplasmic protein, RcnB (33, 124). RcnR is a tetrameric α -helical protein that binds to a variety of metals, but only responds by releasing DNA upon binding nickel and cobalt, which have K_d values of 25 and 5 nM, respectively (125). The binding of nickel and cobalt is believed to result in a conformational change in the protein that disfavors the binding of DNA, resulting in the expression of the nickel and cobalt exporter, RcnAB (32, 124). It has been shown that apo-RcnR recognizes a TACT-G₆-N-AGTA sequence, two of which are located in the *rcnA-rcnR* intergenic region (126, 170). Additionally, RcnR interacts with flanking DNA regions (~50 base pairs), leading to DNA wrapping (170). XAS data show that in contrast to NikR, RcnR forms six-coordinate complexes with its cognate metal ions (Ni(II) and Co(II)), and four-coordinate complexes with non-cognate metals (125, 127). Both cognate metals adopt a (N/O)₅S ligand environment that involves coordination of Cys35, the only Cys residue in the protein (125, 127). The sites binding the cognate metals differ in M-S bond distances as well as the number of imidazole ligands. XAS studies determined that Co-S bond distance is 2.31(2) Å, whereas the Ni-S bond distance is 2.62(2) Å (127). Additionally, cobalt binds one more imidazole ligand than nickel (~ 3 for cobalt and ~ 2 for nickel), which uses different His ligands for cobalt to bind to RcnR (*vide infra*) (125, 127). Mutagenesis, lacZ assays and XAS studies determined that nickel and cobalt are distinguished from non-cognate metals by binding to RcnR using the N-terminal amine (125, 127). The nickel and cobalt complexes are further distinguished by the use of His3, which was found by XAS to be a ligand for cobalt, but not for nickel (127).

In addition to Cys35, the N-terminus and His3, RcnR has four other histidine residues (His33, His60, His64 and His67) that are potential metal ligands. Mutagenesis and lacZ assays determined that His3, His64 and Cys35 were necessary for nickel- and cobalt-responsiveness, and His60 was important only for cobalt-responsiveness, indicating further differences in the use of His ligation by the two cognate metals (125). RcnR forms three- or four-coordinate complexes with non-cognate metals, copper(I) and zinc, involving three protein ligands plus one anion from the buffer (127).

Metal responsiveness in RcnR was altered through a H3E mutation, which resulted in a protein that was responsive to Zn(II) ions (127). This mutation alters the zinc site from a four-coordinate site with a (N/O)₂SBr ligand set in WT-RcnR to an unusual six/seven- (with a bidentate carboxylate) coordinate site with a (N/O)₅₋₆S ligand donor-atom set featuring a Zn-S bond distance of 2.61 Å, similar to the Ni-S distance in the WT protein. This result suggested that the H3E mutant protein is responsive to Zn(II) binding because the ion binds to the protein through the same points of attachment as the cognate metals, and thus generates a protein conformation, or change in dynamics, that results in release of DNA.

The interactions that couple metal binding to allosteric response seem to be the essential structural feature in CsoR/RcnR proteins. The copper(I)-responsive CsoR protein from *M. tuberculosis* binds Cu(I) with Cys36, His61 and Cys65 (171), which correspond to Cys35, His60 and His64 in RcnR. Nonetheless, InrS, a new member of the RcnR/CsoR family of proteins that has the CsoR ligand set with a cysteine residue in the position corresponding to His64 in RcnR, was shown to be nickel responsive (137). These results suggest that factors other than the first coordination sphere of the metal are responsible for metal-specific responses in the CsoR/RcnR family of metal responsive transcriptional regulators.

NmtR and KmtR

Two nickel and cobalt responsive transcriptional regulators belonging to the ArsR/SmtB family have been identified in *M. tuberculosis*, NmtR and KmtR (128, 131). *M. tuberculosis* requires nickel for the enzyme urease and a hypothetical nickel-containing glyoxylase I enzyme (8, 130). Although NmtR has no amino acid sequence homology with RcnR and belongs to a distinct class of DNA-binding proteins, it resembles RcnR in several ways. Like RcnR, *M. tuberculosis* NmtR is responsive to both nickel and cobalt binding, controlling the expression of the P-type ATPase, NmtA, by regulating the *nmt* operon via release of DNA upon binding a cognate metal (128, 129). Also in similarity with RcnR, NmtR binds its cognate metals with higher coordination numbers, and non-cognate metals with lower coordination numbers. NmtR binds nickel and cobalt in a six-coordinate and a five-/six-coordinate geometries, respectively, and binds the non-cognate metal ion zinc in a four-coordinate geometry (129). In contrast to RcnR, no Cys ligation is involved in metal coordination, and mutagenesis and metal binding studies identified His3 as an important nickel ligand (129). The H3Q-NmtR mutant protein showed more sensitivity to non-cognate zinc regulation, relative to the cognate metals cobalt and nickel (130). However, unlike RcnR, H3Q-NmtR could regulate protein-DNA interactions in response to nickel binding as well as the WT-NmtR protein (130). A recent NMR structure of apo-NmtR reveals that the apo-protein is in an “open conformation” and adopts a typical winged-helix fold common to the ArsR repressor family (172). Ratiometric pulse chase studies support the binding of the N-terminal amine to nickel because the rate of amidation of the α -amino group of Gly2 is strongly reduced when nickel is bound compared with the apo- and zinc bound protein (172). Molecular dynamics simulations, quantum chemical calculations and mutagenesis studies support a model for nickel binding to NmtR that features a six-coordinate geometry involving the N-terminal amine and the side chains of His3, Asp91, His93, His104 and His107 (130, 172). Thus, there are two examples of metalloregulators that form six-coordinate sites with nickel that involve coordination of the N-terminal amine, which is emerging as key structural determinant in distinguishing nickel ions from other metals.

Nur

S. coelicolor Nur is a nickel-responsive member of the Fur family of transcriptional regulators that controls nickel homeostasis and oxidative stress response (132). Nur negatively regulates the *sodF* gene that encodes FeSOD and the *NikABCDE* gene cluster encoding components of the nickel transporter (132). Nur binds to the promoter regions of these genes in the presence of Ni(II) but not with Fe(II), Zn(II), Co(II), Cu(II), Mn(II) and Cd(II) (132). Nur also plays an indirect role in the expression of the *sodN* gene, which encodes a NiSOD. Members of the Fur family are homodimeric proteins with each monomer containing an N-terminal DNA-binding domain, a C-terminal dimerization domain and a hinge region connecting the two domains (133). The Nur crystal structure (Figure 7) reveals that there are two metal binding sites in each monomer: the M-site and the nickel-site. The M-site, located at the domain interface, can coordinate either nickel or zinc (133). There is some ambiguity regarding the structure of the nickel-site, as the crystal structure showed that the nickel site is octahedral with three histidine ligands, His70, His72 and His61, and three oxygen atoms from malonate and ethylene glycol. Attempts at growing Nur crystals without malonate and dehydrating the crystals confirmed the presence of the three histidine ligands, but they could not identify the electron density for any water molecules.(133) Thus, the structure of the nickel site could be due in part to an artifact of the crystallization conditions.

Conclusion

Several themes regarding nickel recognition in trafficking proteins emerge from these studies. The ABC-type nickel importer, NikA appears to recognize neither Ni(II) ions nor $\text{Ni}(\text{H}_2\text{O})_6^{2+}$ ions, but rather a $\text{Ni}(\text{L-His})_2$ complex that is recognized by the formation of particular H-bonds, and thus resembles recognition of specific peptides in peptide transporters.

Many of the proteins that do bind Ni(II) ions couple allosteric changes to the coordination number and/or ligand set adopted by the nickel complex, the details of which are just beginning to emerge. In a sense, both NikR and RcnR are examples of proteins that utilize extra ligands to create alternative metal-binding sites that overlap the cognate metal binding site, but prevent non-cognate metals from binding in the same fashion as cognate metals. There are also features that appear to distinguish proteins that repress transcription from those that de-repress transcription in response to metal binding. Proteins that repress transcription in response to nickel binding (e.g., NikR and Nur) appear to possess two Ni(II) binding sites; one site with square planar geometry that is unique to Ni(II) coordination chemistry among the biologically significant transition metals, and another site with octahedral geometry. In contrast, proteins where Ni(II) binding leads to de-repression (e.g. RcnR and NmtR) possess one nickel binding site, often recognize other similar metal ions (e.g., Co(II)), and utilize a six-coordinate nickel complex that involves coordination of the N-terminal amine to destabilize the protein-DNA interaction. Additional comparisons with other metalloregulators, such as between RcnR, CsoR and InrS reveal that the nature of the metal complex formed may be relatively unimportant; rather, it is the mechanism that couples the formation of the complex to protein allostery that is the critical feature in the design of nickel metalloregulators.

Protein allostery also plays a role in trafficking proteins other than metalloregulators. Such proteins include the metallochaperone, HypA, where although there is little evidence that the nickel site structure is sensitive to pH, a structural zinc site undergoes ligand substitution in response to binding nickel and changes in pH. The protein structural change that presumably accompanies the change in the zinc site may be critical in directing nickel traffic to aporease under acid stress conditions, a key response required for the acid viability of *H. pylori*. The use of zinc in regulating proteins involved in metallocenter assembly, such as HypA (39, 42, 45), HypB (51, 52), UreE (89) and UreG (173) is another emerging theme.

Acknowledgments

This work was supported by NIH grant R01 GM-069696 (MJM).

References

1. Mulrooney SB, Hausinger RP. Nickel uptake and utilization by microorganisms. FEMS Microbiology Reviews. 2003; 27:239–261. [PubMed: 12829270]
2. Ragsdale SW. Nickel-based Enzyme Systems. Journal of Biological Chemistry. 2009; 284:18571–18575. [PubMed: 19363030]
3. Maier RJ. Use of molecular hydrogen as an energy substrate by human pathogenic bacteria. Biochemical Society Transactions. 2005; 33:83–85. [PubMed: 15667272]
4. Olson JW, Maier RJ. Molecular hydrogen as an energy source for *Helicobacter pylori*. Science. 2002; 298:1788–1790. [PubMed: 12459589]
5. Böck A, King PW, Blokesch M, Posewitz MC. Maturation of hydrogenases. Advances in Microbial Physiology. 2006; 51:1–71. [PubMed: 17091562]
6. Forzi L, Sawers RG. Maturation of [NiFe]-hydrogenases in *Escherichia coli*. Biometals. 2007; 20:565–578. [PubMed: 17216401]

7. Eaton KA, Brooks CL, Morgan DR, Krakowka S. Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. *Infection and Immunity*. 1991; 59:2470–2475. [PubMed: 2050411]
8. Osman D, Cavet JS. Bacterial metal-sensing proteins exemplified by ArsR-SmtB family repressors. *Natural Product Reports*. 2010; 27:668–680. [PubMed: 20442958]
9. Giedroc DP, Arunkumar AI. Metal sensor proteins: nature's metalloregulated allosteric switches. *Dalton Transactions*. 2007:3107–3120. [PubMed: 17637984]
10. Ma Z, Jacobsen FE, Giedroc DP. Coordination chemistry of bacterial metal transport and sensing. *Chemical Reviews*. 2009; 109:4644–4681. [PubMed: 19788177]
11. Martinez-Finley EJ, Chakraborty S, Fretham SJ, Aschner M. Cellular transport and homeostasis of essential and nonessential metals. *Metallomics*. 2012; 4:593–605. [PubMed: 22337135]
12. De Pina K, Navarro C, McWalter L, Boxer DH, Price NC, Kelly SM, Mandrand-Berthelot MA, Wu LF. Purification and characterization of the periplasmic nickel-binding protein NikA of *Escherichia coli* K12. *European Journal of Biochemistry*. 1995; 227:857–865. [PubMed: 7867647]
13. Navarro C, Wu LF, Mandrand-Berthelot MA. The *nik* operon of *Escherichia coli* encodes a periplasmic binding-protein-dependent transport system for nickel. *Molecular Microbiology*. 1993; 9:1181–1191. [PubMed: 7934931]
14. Heddle J, Scott DJ, Unzai S, Park SY, Tame JRH. Crystal structures of the liganded and unliganded nickel-binding protein NikA from *Escherichia coli*. *Journal of Biological Chemistry*. 2003; 278:50322–50329. [PubMed: 12960164]
15. Allan CB, Wu LF, Gu ZJ, Choudhury SB, Al-Mjeni F, Sharma ML, Mandrand-Berthelot MA, Maroney MJ. An X-ray absorption spectroscopic structural investigation of the nickel site in *Escherichia coli* NikA protein. *Inorganic Chemistry*. 1998; 37:5952–5955.
16. Cherrier MV, Martin L, Cavazza C, Jacquamet L, Lemaire D, Gaillard J, Fontecilla-Camps JC. Crystallographic and spectroscopic evidence for high affinity binding of FeEDTA(H₂O)[−] to the periplasmic nickel transporter NikA. *Journal of the American Chemical Society*. 2005; 127:10075–10082. [PubMed: 16011372]
17. Cherrier MV, Cavazza C, Bochot C, Lemaire D, Fontecilla-Camps JC. Structural characterization of a putative endogenous metal chelator in the periplasmic nickel transporter NikA. *Biochemistry*. 2008; 47:9937–9943. [PubMed: 18759453]
18. Cavazza C, Martin L, Laffly E, Lebrette H, Cherrier MV, Zeppieri L, Richaud P, Carriere M, Fontecilla-Camps JC. Histidine 416 of the periplasmic binding protein NikA is essential for nickel uptake in *Escherichia coli*. *FEBS Letters*. 2011; 585:711–715. [PubMed: 21281641]
19. Chivers PT, Benanti EL, Heil-Chapdelaine V, Iwig JS, Rowe JL. Identification of Ni-(L-His)₂ as a substrate for NikABCDE-dependent nickel uptake in *Escherichia coli*. *Metallomics*. 2012
20. Howlett RM, Hughes BM, Hitchcock A, Kelly DJ. Hydrogenase activity in the foodborne pathogen *Campylobacter jejuni* depends upon a novel ABC-type nickel transporter (NikZYXWV) and is SlyD-independent. *Microbiology*. 2012; 158:1645–1655. [PubMed: 22403188]
21. Rodionov DA, Hebbeln P, Gelfand MS, Eitinger T. Comparative and functional genomic analysis of prokaryotic nickel and cobalt uptake transporters: evidence for a novel group of ATP-binding cassette transporters. *Journal of Bacteriology*. 2006; 188:317–327. [PubMed: 16352848]
22. Eberz G, Eitinger T, Friedrich B. Genetic determinants of a nickel-specific transport system are part of the plasmid-encoded hydrogenase gene cluster in *Alcaligenes eutrophus*. *Journal of Bacteriology*. 1989; 171:1340–1345. [PubMed: 2646280]
23. Eitinger T, Friedrich B. Cloning, nucleotide sequence, and heterologous expression of a high-affinity nickel transport gene from *Alcaligenes eutrophus*. *Journal of Biological Chemistry*. 1991; 266:3222–3227. [PubMed: 1847142]
24. Eitinger T, Wolfram L, Degen O, Anthon C. A Ni²⁺ binding motif is the basis of high affinity transport of the *Alcaligenes eutrophus* nickel permease. *Journal of Biological Chemistry*. 1997; 272:17139–17144. [PubMed: 9202033]
25. Degen O, Eitinger T. Substrate specificity of nickel/cobalt permeases: insights from mutants altered in transmembrane domains I and II. *Journal of Bacteriology*. 2002; 184:3569–3577. [PubMed: 12057951]

26. Mobley HL, Garner RM, Bauerfeind P. *Helicobacter pylori* nickel-transport gene *nixA*: synthesis of catalytically active urease in *Escherichia coli* independent of growth conditions. *Molecular Microbiology*. 1995; 16:97–109. [PubMed: 7651142]
27. Fulkerson JF Jr, Garner RM, Mobley HL. Conserved residues and motifs in the NixA protein of *Helicobacter pylori* are critical for the high affinity transport of nickel ions. *J Biol Chem*. 1998; 273:235–241. [PubMed: 9417070]
28. Degen O, Kobayashi M, Shimizu S, Eitinger T. Selective transport of divalent cations by transition metal permeases: the *Alcaligenes eutrophus* HoxN and the *Rhodococcus rhodochrous* NhlF. *Archives of Microbiology*. 1999; 171:139–145. [PubMed: 10201093]
29. Fu C, Javedan S, Moshiri F, Maier RJ. Bacterial genes involved in incorporation of nickel into a hydrogenase enzyme. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:5099–5103. [PubMed: 8197192]
30. Stähler FN, Odenbreit S, Haas R, Wilrich J, Van Vliet AHM, Kusters JG, Kist M, Bereswill S. The novel *Helicobacter pylori* CznABC metal efflux pump is required for cadmium, zinc, and nickel resistance, urease modulation, and gastric colonization. *Infection and Immunity*. 2006; 74:3845–3852. [PubMed: 16790756]
31. Grass G, Fan B, Rosen BP, Lemke K, Schlegel HG, Rensing C. NreB from *Achromobacter xylosoxidans* 31A is a nickel-induced transporter conferring nickel resistance. *Journal of Bacteriology*. 2001; 183:2803–2807. [PubMed: 11292799]
32. Rodrigue A, Effantin G, Mandrand-Berthelot MA. Identification of rcnA (yohM), a nickel and cobalt resistance gene in *Escherichia coli*. *Journal of Bacteriology*. 2005; 187:2912–2916. [PubMed: 15805538]
33. Blériot C, Effantin G, Lagarde F, Mandrand-Berthelot MA, Rodrigue A. RcnB is a periplasmic protein essential for maintaining intracellular Ni and Co concentrations in *Escherichia coli*. *Journal of Bacteriology*. 2011; 193:3785–3793. [PubMed: 21665978]
34. Haritha A, Sagar KP, Tiwari A, Kiranmayi P, Rodrigue A, Mohan PM, Singh SS. MrdH, a novel metal resistance determinant of *Pseudomonas putida* KT2440, is flanked by metal-inducible mobile genetic elements. *Journal of Bacteriology*. 2009; 191:5976–5987. [PubMed: 19648243]
35. Garcia-Dominguez M, Lopez-Maury L, Florencio FJ, Reyes JC. A gene cluster involved in metal homeostasis in the *cyanobacterium Synechocystis* sp. strain PCC 6803. *Journal of Bacteriology*. 2000; 182:1507–1514. [PubMed: 10692354]
36. Tian J, Wu N, Li J, Liu Y, Guo J, Yao B, Fan Y. Nickel-resistant determinant from *Leptospirillum ferriphilum*. *Applied and Environmental Microbiology*. 2007; 73:2364–2368. [PubMed: 17293508]
37. Zhu T, Tian J, Zhang S, Wu N, Fan Y. Identification of the transcriptional regulator NcrB in the nickel resistance determinant of *Leptospirillum ferriphilum* UBK03. *PLoS One*. 2011; 6:e17367. [PubMed: 21387010]
38. Hube M, Blokesch M, Bock A. Network of hydrogenase maturation in *Escherichia coli*: role of accessory proteins HypA and HybF. *Journal of Bacteriology*. 2002; 184:3879–3885. [PubMed: 12081959]
39. Atanassova A, Zamble DB. *Escherichia coli* HypA is a zinc metalloprotein with a weak affinity for nickel. *Journal of Bacteriology*. 2005; 187:4689–4697. [PubMed: 15995183]
40. Chan Chung KC, Zamble DB. Protein interactions and localization of the *Escherichia coli* accessory protein HypA during nickel insertion to [NiFe] hydrogenase. *Journal of Biological Chemistry*. 2011; 286:43081–43090. [PubMed: 22016389]
41. Mehta N, Olson JW, Maier RJ. Characterization of *Helicobacter pylori* nickel metabolism accessory proteins needed for maturation of both urease and hydrogenase. *Journal of Bacteriology*. 2003; 185:726–734. [PubMed: 12533448]
42. Herbst RW, Perovic I, Martin-Diaconescu V, O'Brien K, Chivers PT, Pochapsky SS, Pochapsky TC, Maroney MJ. Communication between the zinc and nickel sites in dimeric HypA: metal recognition and pH sensing. *Journal of the American Chemical Society*. 2010; 132:10338–10351. [PubMed: 20662514]

43. Benoit SL, Mehta N, Weinberg MV, Maier C, Maier RJ. Interaction between the *Helicobacter pylori* accessory proteins HypA and UreE is needed for urease maturation. *Microbiology*. 2007; 153:1474–1482. [PubMed: 17464061]
44. Xia W, Li H, Sze KH, Sun H. Structure of a nickel chaperone, HypA, from *Helicobacter pylori* reveals two distinct metal binding sites. *Journal of the American Chemical Society*. 2009; 131:10031–10040. [PubMed: 19621959]
45. Kennedy DC, Herbst RW, Iwig JS, Chivers PT, Maroney MJ. A Dynamic Zn site in *Helicobacter pylori* HypA: A Potential Mechanism for Metal-Specific Protein Activity. *Journal of the American Chemical Society*. 2006; 139:16–17.
46. Benoit SL, McMurphy JL, Hill SA, Maier RJ. *Helicobacter pylori* hydrogenase accessory protein HypA and urease accessory protein UreG compete with each other for UreE recognition. *Biochimica et Biophysica Acta*. 2012; 1820:1519–1525. [PubMed: 22698670]
47. Watanabe S, Arai T, Matsumi R, Atomi H, Imanaka T, Miki K. Crystal structure of HypA, a nickel-binding metallochaperone for [NiFe] hydrogenase maturation. *Journal of Molecular Biology*. 2009; 394:448–459. [PubMed: 19769985]
48. Chan KH, Lee KM, Wong KB. Interaction between hydrogenase maturation factors HypA and HypB is required for [NiFe]-hydrogenase maturation. *PLoS One*. 2012; 7:e32592. [PubMed: 22384275]
49. Maier T, Lottspeich F, Bock A. GTP hydrolysis by HypB is essential for nickel insertion into hydrogenases of *Escherichia coli*. *European Journal of Biochemistry*. 1995; 230:133–138. [PubMed: 7601092]
50. Maier T, Jacobi A, Sauter M, Bock A. The product of the hypB gene, which is required for nickel incorporation into hydrogenases, is a novel guanine nucleotide-binding protein. *Journal of Bacteriology*. 1993; 175:630–635. [PubMed: 8423137]
51. Leach MR, Sandal S, Sun H, Zamble DB. Metal binding activity of the *Escherichia coli* hydrogenase maturation factor HypB. *Biochemistry*. 2005; 44:12229–12238. [PubMed: 16142921]
52. Dias AV, Mulvihill CM, Leach MR, Pickering IJ, George GN, Zamble DB. Structural and biological analysis of the metal sites of *Escherichia coli* hydrogenase accessory protein HypB. *Biochemistry*. 2008; 47:11981–11991. [PubMed: 18942856]
53. Xia W, Li H, Yang X, Wong KB, Sun H. Metallo-GTPase HypB from *Helicobacter pylori* and its interaction with nickel chaperone protein HypA. *Journal of Biological Chemistry*. 2012; 287:6753–6763. [PubMed: 22179820]
54. Sydor AM, Liu J, Zamble DB. Effects of metal on the biochemical properties of *Helicobacter pylori* HypB, a maturation factor of [NiFe]-hydrogenase and urease. *Journal of Bacteriology*. 2011; 193:1359–1368. [PubMed: 21239585]
55. Gasper R, Scrima A, Wittinghofer A. Structural insights into HypB, a GTP-binding protein that regulates metal binding. *Journal of Biological Chemistry*. 2006; 281:27492–27502. [PubMed: 16807243]
56. Chan KH, Li T, Wong CO, Wong KB. Structural basis for GTP-dependent dimerization of hydrogenase maturation factor HypB. *PLoS One*. 2012; 7:e30547. [PubMed: 22276211]
57. Roof WD, Horne SM, Young KD, Young R. slyD, a host gene required for phi X174 lysis, is related to the FK506-binding protein family of peptidyl-prolyl cis-trans-isomerases. *Journal of Biological Chemistry*. 1994; 269:2902–2910. [PubMed: 8300625]
58. Roof WD, Young R. Phi X174 lysis requires slyD, a host gene which is related to the FKBP family of peptidyl-prolyl cis-trans isomerases. *FEMS Microbiology Reviews*. 1995; 17:213–218. [PubMed: 7669348]
59. Maratea D, Young K, Young R. Deletion and fusion analysis of the phage phi X174 lysis gene E. *Gene*. 1985; 40:39–46. [PubMed: 2936651]
60. Martino L, He Y, Hands-Taylor KL, Valentine ER, Kelly G, Giancola C, Conte MR. The interaction of the *Escherichia coli* protein SlyD with nickel ions illuminates the mechanism of regulation of its peptidyl-prolyl isomerase activity. *FEBS Journal*. 2009; 276:4529–4544. [PubMed: 19645725]

61. Scholz C, Eckert B, Hagn F, Schaarschmidt P, Balbach J, Schmid FX. SlyD proteins from different species exhibit high prolyl isomerase and chaperone activities. *Biochemistry*. 2006; 45:20–33. [PubMed: 16388577]
62. Weininger U, Haupt C, Schweimer K, Graubner W, Kovermann M, Bruser T, Scholz C, Schaarschmidt P, Zoldak G, Schmid FX, Balbach J. NMR solution structure of SlyD from *Escherichia coli*: spatial separation of prolyl isomerase and chaperone function. *Journal of Molecular Biology*. 2009; 387:295–305. [PubMed: 19356587]
63. Wülfing C, Lombardero J, Pluckthun A. An *Escherichia coli* protein consisting of a domain homologous to FK506-binding proteins (FKBP) and a new metal binding motif. *Journal of Biological Chemistry*. 1994; 269:2895–2901. [PubMed: 8300624]
64. Hottenrott S, Schumann T, Pluckthun A, Fischer G, Rahfeld JU. The *Escherichia coli* SlyD is a metal ion-regulated peptidyl-prolyl cis/trans-isomerase. *Journal of Biological Chemistry*. 1997; 272:15697–15701. [PubMed: 9188461]
65. Kaluarachchi H, Altenstein M, Sugumar SR, Balbach J, Zamble DB, Haupt C. Nickel binding and [NiFe]-hydrogenase maturation by the metallochaperone SlyD with a single metal-binding site in *Escherichia coli*. *Journal of Molecular Biology*. 2012; 417:28–35. [PubMed: 22310044]
66. Kaluarachchi H, Zhang JW, Zamble DB. *Escherichia coli* SlyD, more than a Ni(II) reservoir. *Biochemistry*. 2011
67. Leach MR, Zhang JW, Zamble DB. The role of complex formation between the *Escherichia coli* hydrogenase accessory factors HypB and SlyD. *Journal of Biological Chemistry*. 2007; 282:16177–16186. [PubMed: 17426034]
68. Zoldák G, Schmid FX. Cooperation of the Prolyl Isomerase and Chaperone Activities of the Protein Folding Catalyst SlyD. *Journal of Molecular Biology*. 2011; 406:176–194. [PubMed: 21147124]
69. Kaluarachchi H, Sutherland DE, Young A, Pickering IJ, Stillman MJ, Zamble DB. The Ni(II)-binding properties of the metallochaperone SlyD. *Journal of the American Chemical Society*. 2009; 131:18489–18500. [PubMed: 19947632]
70. Kaluarachchi H, Siebel JF, Kaluarachchi-Duffy S, Krecisz S, Sutherland DE, Stillman MJ, Zamble DB. Metal Selectivity of the *Escherichia coli* Nickel Metallochaperone, SlyD. *Biochemistry*. 2011
71. Knappe TA, Eckert B, Schaarschmidt P, Scholz C, Schmid FX. Insertion of a chaperone domain converts FKBP12 into a powerful catalyst of protein folding. *Journal of Molecular Biology*. 2007; 368:1458–1468. [PubMed: 17397867]
72. Zoldák G, Carstensen L, Scholz C, Schmid FX. Consequences of Domain Insertion on the Stability and Folding Mechanism of a Protein. *Journal of Molecular Biology*. 2009; 386:1138–1152. [PubMed: 19136015]
73. Chan Chung KC, Zamble DB. The *Escherichia coli* metal-binding chaperone SlyD interacts with the large subunit of [NiFe]-hydrogenase 3. *FEBS Letters*. 2011; 585:291–294. [PubMed: 21185288]
74. Zhang JW, Butland G, Greenblatt JF, Emili A, Zamble DB. A role for SlyD in the *Escherichia coli* hydrogenase biosynthetic pathway. *Journal of Biological Chemistry*. 2005; 280:4360–4366. [PubMed: 15569666]
75. Löw C, Neumann P, Tidow H, Weininger U, Haupt C, Friedrich-Epler B, Scholz C, Stubbs MT, Balbach J. Crystal structure determination and functional characterization of the metallochaperone SlyD from *Thermus thermophilus*. *Journal of Molecular Biology*. 2010; 398:375–390. [PubMed: 20230833]
76. Low C, Neumann P, Tidow H, Weininger U, Haupt C, Friedrich-Epler B, Scholz C, Stubbs MT, Balbach J. Crystal Structure Determination and Functional Characterization of the Metallochaperone SlyD from *Thermus thermophilus*. *Journal of Molecular Biology*. 2010; 398:375–390. [PubMed: 20230833]
77. Cheng T, Li H, Xia W, Sun H. Multifaceted SlyD from *Helicobacter pylori*: implication in [NiFe] hydrogenase maturation. *J Biol Inorg Chem*. 2012; 17:331–343. [PubMed: 22045417]
78. Lee MH, Pankratz HS, Wang S, Scott RA, Finnegan MG, Johnson MK, Ippolito JA, Christianson DW, Hausinger RP. Purification and characterization of *Klebsiella aerogenes* UreE protein: a

- nickel-binding protein that functions in urease metallocenter assembly. *Protein Sci.* 1993; 2:1042–1052. [PubMed: 8318889]
79. Mulrooney SB, Hausinger RP. Sequence of the *Klebsiella aerogenes* urease genes and evidence for accessory proteins facilitating nickel incorporation. *Journal of Bacteriology.* 1990; 172:5837–5843. [PubMed: 2211515]
 80. Brayman TG, Hausinger RP. Purification, characterization, and functional analysis of a truncated *Klebsiella aerogenes* UreE urease accessory protein lacking the histidine-rich carboxyl terminus. *Journal of Bacteriology.* 1996; 178:5410–5416. [PubMed: 8808929]
 81. Colpas GJ, Brayman TG, McCracken J, Pressler MA, Babcock GT, Ming LJ, Colangelo CM, Scott RA, Hausinger RP. Spectroscopic characterization of metal binding by *Klebsiella aerogenes* UreE urease accessory protein. *Journal of Biological Inorganic Chemistry.* 1998; 3:150–160.
 82. Colpas GJ, Brayman TG, Ming LJ, Hausinger RP. Identification of metal-binding residues in the *Klebsiella aerogenes* urease nickel metallochaperone, UreE. *Biochemistry.* 1999; 38:4078–4088. [PubMed: 10194322]
 83. Song HK, Mulrooney SB, Huber R, Hausinger RP. Crystal structure of *Klebsiella aerogenes* UreE, a nickel-binding metallochaperone for urease activation. *Journal of Biological Chemistry.* 2001; 276:49359–49364. [PubMed: 11591723]
 84. Soriano A, Colpas GJ, Hausinger RP. UreE stimulation of GTP-dependent urease activation in the UreD-UreF-UreG-urease apoprotein complex. *Biochemistry.* 2000; 39:12435–12440. [PubMed: 11015224]
 85. Stola M, Musiani F, Mangani S, Turano P, Safarov N, Zambelli B, Ciurli S. The nickel site of *Bacillus pasteurii* UreE, a urease metallo-chaperone, as revealed by metal-binding studies and X-ray absorption spectroscopy. *Biochemistry.* 2006; 45:6495–6509. [PubMed: 16700560]
 86. Ciurli S, Safarov N, Miletti S, Dikiy A, Christensen SK, Kornetzky K, Bryant DA, Vandenberghe I, Devreese B, Samyn B, Remaut H, van Beeumen J. Molecular characterization of *Bacillus pasteurii* UreE, a metal-binding chaperone for the assembly of the urease active site. *Journal of Biological Inorganic Chemistry.* 2002; 7:623–631. [PubMed: 12072968]
 87. Remaut H, Safarov N, Ciurli S, Van Beeumen J. Structural basis for Ni²⁺ transport and assembly of the urease active site by the metallochaperone UreE from *Bacillus pasteurii*. *Journal of Biological Chemistry.* 2001; 276:49365–49370. [PubMed: 11602602]
 88. Benoit S, Maier RJ. Dependence of *Helicobacter pylori* urease activity on the nickel-sequestering ability of the UreE accessory protein. *Journal of Bacteriology.* 2003; 185:4787–4795. [PubMed: 12896998]
 89. Bellucci M, Zambelli B, Musiani F, Turano P, Ciurli S. *Helicobacter pylori* UreE, a urease accessory protein: specific Ni²⁺- and Zn²⁺-binding properties and interaction with its cognate UreG. *Biochemical Journal.* 2009; 422:91–100. [PubMed: 19476442]
 90. Shi R, Munger C, Asinas A, Benoit SL, Miller E, Matte A, Maier RJ, Cygler M. Crystal structures of apo and metal-bound forms of the UreE protein from *Helicobacter pylori*: role of multiple metal binding sites. *Biochemistry.* 2010; 49:7080–7088. [PubMed: 20681615]
 91. Banaszak K, Martin-Diaconescu V, Bellucci M, Zambelli B, Rypniewski W, Maroney MJ, Ciurli S. Crystallographic and X-ray absorption spectroscopic characterization of *Helicobacter pylori* UreE bound to Ni²⁺ and Zn²⁺ reveals a role for the disordered C-terminal arm in metal trafficking. *Biochemical Journal.* 2012; 441:1017–1026. [PubMed: 22010876]
 92. Voland P, Weeks DL, Marcus EA, Prinz C, Sachs G, Scott D. Interactions among the seven *Helicobacter pylori* proteins encoded by the urease gene cluster. *American Journal of Physiology Gastrointestinal Liver Physiology.* 2003; 284:G96–G106.
 93. Zambelli B, Stola M, Musiani F, De Vriendt K, Samyn B, Devreese B, Van Beeumen J, Turano P, Dikiy A, Bryant DA, Ciurli S. UreG, a chaperone in the urease assembly process, is an intrinsically unstructured GTPase that specifically binds Zn²⁺. *Journal of Biological Chemistry.* 2005; 280:4684–4695. [PubMed: 15542602]
 94. Zambelli B, Musiani F, Savini M, Tucker P, Ciurli S. Biochemical studies on *Mycobacterium tuberculosis* UreG and comparative modeling reveal structural and functional conservation among the bacterial UreG family. *Biochemistry.* 2007; 46:3171–3182. [PubMed: 17309280]

95. Zambelli B, Turano P, Musiani F, Neyroz P, Ciurli S. Zn²⁺-linked dimerization of UreG from *Helicobacter pylori*, a chaperone involved in nickel trafficking and urease activation. *Proteins*. 2009; 74:222–239. [PubMed: 18767150]
96. Boer JL, Quiroz-Valenzuela S, Anderson KL, Hausinger RP. Mutagenesis of *Klebsiella aerogenes* UreG to probe nickel binding and interactions with other urease-related proteins. *Biochemistry*. 2010; 49:5859–5869. [PubMed: 20533838]
97. Jeoung JH, Giese T, Grunwald M, Dobbek H. CooC1 from *Carboxydothemus hydrogenoformans* is a nickel-binding ATPase. *Biochemistry*. 2009; 48:11505–11513. [PubMed: 19883128]
98. Jeoung JH, Giese T, Grunwald M, Dobbek H. Crystal structure of the ATP-dependent maturation factor of Ni, Fe-containing carbon monoxide dehydrogenases. *Journal of Molecular Biology*. 2010; 396:1165–1179. [PubMed: 20064527]
99. Jeon WB, Cheng J, Ludden PW. Purification and characterization of membrane-associated CooC protein and its functional role in the insertion of nickel into carbon monoxide dehydrogenase from *Rhodospirillum rubrum*. *Journal of Biological Chemistry*. 2001; 276:38602–38609. [PubMed: 11507093]
100. Watt RK, Ludden PW. The identification, purification, and characterization of CooJ. A nickel-binding protein that is co-regulated with the Ni-containing CO dehydrogenase from *Rhodospirillum rubrum*. *Journal of Biological Chemistry*. 1998; 273:10019–10025. [PubMed: 9545348]
101. Ge R, Watt RM, Sun X, Tanner JA, He QY, Huang JD, Sun H. Expression and characterization of a histidine-rich protein, Hpn: potential for Ni²⁺ storage in *Helicobacter pylori*. *Biochemical Journal*. 2006; 393:285–293. [PubMed: 16164421]
102. Ge R, Zhang Y, Sun X, Watt RM, He QY, Huang JD, Wilcox DE, Sun H. Thermodynamic and kinetic aspects of metal binding to the histidine-rich protein, Hpn. *Journal of the American Chemical Society*. 2006; 128:11330–11331. [PubMed: 16939237]
103. Seshadri S, Benoit SL, Maier RJ. Roles of His-rich hpn and hpn-like proteins in *Helicobacter pylori* nickel physiology. *Journal of Bacteriology*. 2007; 189:4120–4126. [PubMed: 17384182]
104. Zeng YB, Zhang DM, Li HY, Sun HZ. Binding of Ni²⁺ to a histidine- and glutamine-rich protein, Hpn-like. *Journal of Biological Inorganic Chemistry*. 2008; 13:1121–1131. [PubMed: 18563455]
105. Chivers PT, Sauer RT. NikR is a ribbon-helix-helix DNA-binding protein. *Protein Science*. 1999; 8:2494–2500. [PubMed: 10595554]
106. Chivers PT, Sauer RT. NikR repressor: high-affinity nickel binding to the C-terminal domain regulates binding to operator DNA. *Chemistry and Biology*. 2002; 9:1141–1148. [PubMed: 12401498]
107. Bloom SL, Zamble DB. Metal-selective DNA-binding response of *Escherichia coli* NikR. *Biochemistry*. 2004; 43:10029–10038. [PubMed: 15287730]
108. Wang SC, Dias AV, Bloom SL, Zamble DB. Selectivity of metal binding and metal-induced stability of *Escherichia coli* NikR. *Biochemistry*. 2004; 43:10018–10028. [PubMed: 15287729]
109. Schreiter ER, Wang SC, Zamble DB, Drennan CL. NikR-operator complex structure and the mechanism of repressor activation by metal ions. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:13676–13681. [PubMed: 16945905]
110. Leitch S, Bradley MJ, Rowe JL, Chivers PT, Maroney MJ. Nickel-specific response in the transcriptional regulator, *Escherichia coli* NikR. *Journal of the American Chemical Society*. 2007; 129:5085–5095. [PubMed: 17397155]
111. Wang SC, Li Y, Robinson CV, Zamble DB. Potassium is critical for the Ni(II)-responsive DNA-binding activity of *Escherichia coli* NikR. *Journal of the American Chemical Society*. 2010; 132:1506–1507. [PubMed: 20088519]
112. Wang SC, Li Y, Ho M, Bernal ME, Sydor AM, Kagzi WR, Zamble DB. The response of *Escherichia coli* NikR to nickel: a second nickel-binding site. *Biochemistry*. 2010; 49:6635–6645. [PubMed: 20583753]
113. Phillips CM, Schreiter ER, Stultz CM, Drennan CL. Structural basis of low-affinity nickel binding to the nickel-responsive transcription factor NikR from *Escherichia coli*. *Biochemistry*. 2010; 49:7830–7838. [PubMed: 20704276]

114. Schreiter ER, Sintchak MD, Guo Y, Chivers PT, Sauer RT, Drennan CL. Crystal structure of the nickel-responsive transcription factor NikR. *Nature Structural Biology*. 2003; 10:794–799.
115. Zambelli B, Bellucci M, Danielli A, Scarlato V, Ciurli S. The Ni²⁺ binding properties of *Helicobacter pylori* NikR. *Chemical Communications*. 2007:3649–3651. [PubMed: 17728882]
116. Contreras M, Thiberge JM, Mandrand-Berthelot MA, Labigne A. Characterization of the roles of NikR, a nickel-responsive pleiotropic autoregulator of *Helicobacter pylori*. *Molecular Microbiology*. 2003; 49:947–963. [PubMed: 12890020]
117. West AL, St John F, Lopes PE, MacKerell AD Jr, Pozharski E, Michel SL. Holo-Ni(II)HpNikR is an asymmetric tetramer containing two different nickel-binding sites. *Journal of the American Chemical Society*. 2010; 132:14447–14456. [PubMed: 20863122]
118. Benini S, Cianci M, Ciurli S. Holo-Ni²⁺ *Helicobacter pylori* NikR contains four square-planar nickel-binding sites at physiological pH. *Dalton Transactions*. 2011; 40:7831–7833. [PubMed: 21725560]
119. Dian C, Schauer K, Kapp U, McSweeney SM, Labigne A, Terradot L. Structural basis of the nickel response in *Helicobacter pylori*: crystal structures of HpNikR in Apo and nickel-bound states. *Journal of Molecular Biology*. 2006; 361:715–730. [PubMed: 16872629]
120. Bahlawane C, Dian C, Muller C, Round A, Fauquant C, Schauer K, de Reuse H, Terradot L, Michaud-Soret I. Structural and mechanistic insights into *Helicobacter pylori* NikR activation. *Nucleic Acids Research*. 2010; 38:3106–3118. [PubMed: 20089510]
121. West AL, Evans SE, Gonzalez JM, Carter LG, Tsuruta H, Pozharski E, Michel SL. Ni(II) coordination to mixed sites modulates DNA binding of HpNikR via a long-range effect. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:5633–5638. [PubMed: 22451934]
122. Chivers PT, Tahirov TH. Structure of *Pyrococcus horikoshii* NikR: nickel sensing and implications for the regulation of DNA recognition. *Journal of Molecular Biology*. 2005; 348:597–607. [PubMed: 15826657]
123. Benanti EL, Chivers PT. *Geobacter uraniireducens* NikR displays a DNA binding mode distinct from other members of the NikR family. *Journal of Bacteriology*. 2010; 192:4327–4336. [PubMed: 20581212]
124. Iwig JS, Rowe JL, Chivers PT. Nickel homeostasis in *Escherichia coli* - the rcnR-rcnA efflux pathway and its linkage to NikR function. *Molecular Microbiology*. 2006; 62:252–262. [PubMed: 16956381]
125. Iwig JS, Leitch S, Herbst RW, Maroney MJ, Chivers PT. Ni(II) and Co(II) sensing by *Escherichia coli* RcnR. *Journal of the American Chemical Society*. 2008; 130:7592–7606. [PubMed: 18505253]
126. Blaha D, Arous S, Blériot C, Dorel C, Mandrand-Berthelot MA, Rodrigue A. The *Escherichia coli* metallo-regulator RcnR represses rcnA and rcnR transcription through binding on a shared operator site: Insights into regulatory specificity towards nickel and cobalt. *Biochimie*. 2011; 93:434–439. [PubMed: 21040754]
127. Higgins KA, Chivers PT, Maroney MJ. Role of the N-terminus in Determining Metal-Specific Responses in the *E. coli* Ni- and Co-Responsive Metalloregulator, RcnR. *Journal of the American Chemical Society*. 2012; 134:7081–7093. [PubMed: 22471551]
128. Cavet JS, Meng W, Pennella MA, Appelhoff RJ, Giedroc DP, Robinson NJ. A nickel-cobalt-sensing ArsR-SmtB family repressor. Contributions of cytosol and effector binding sites to metal selectivity. *Journal of Biological Chemistry*. 2002; 277:38441–38448. [PubMed: 12163508]
129. Pennella MA, Shokes JE, Cosper NJ, Scott RA, Giedroc DP. Structural elements of metal selectivity in metal sensor proteins. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:3713–3718. [PubMed: 12651949]
130. Reyes-Caballero H, Lee CW, Giedroc DP. *Mycobacterium tuberculosis* NmtR harbors a nickel sensing site with parallels to *Escherichia coli* RcnR. *Biochemistry*. 2011; 50:7941–7952. [PubMed: 21819125]
131. Campbell DR, Chapman KE, Waldron KJ, Tottey S, Kendall S, Cavallaro G, Andreini C, Hinds J, Stoker NG, Robinson NJ, Cavet JS. Mycobacterial cells have dual nickel-cobalt sensors:

- sequence relationships and metal sites of metal-responsive repressors are not congruent. *Journal of Biological Chemistry*. 2007; 282:32298–32310. [PubMed: 17726022]
132. Ahn BE, Cha J, Lee EJ, Han AR, Thompson CJ, Roe JH. Nur, a nickel-responsive regulator of the Fur family, regulates superoxide dismutases and nickel transport in *Streptomyces coelicolor*. *Molecular Microbiology*. 2006; 59:1848–1858. [PubMed: 16553888]
 133. An YJ, Ahn BE, Han AR, Kim HM, Chung KM, Shin JH, Cho YB, Roe JH, Cha SS. Structural basis for the specialization of Nur, a nickel-specific Fur homolog, in metal sensing and DNA recognition. *Nucleic Acids Research*. 2009; 37:3442–3451. [PubMed: 19336416]
 134. Kim JS, Kang SO, Lee JK. The protein complex composed of nickel-binding SrnQ and DNA binding motif-bearing SrnR of *Streptomyces griseus* represses *sodF* transcription in the presence of nickel. *Journal of Biological Chemistry*. 2003; 278:18455–18463. [PubMed: 12644473]
 135. Benoit SL, Maier RJ. Mua (HP0868) is a nickel-binding protein that modulates urease activity in *Helicobacter pylori*. *MBio*. 2011; 2:e00039–00011. [PubMed: 21505055]
 136. Kidd SP, Djoko KY, Ng J, Argente MP, Jennings MP, McEwan AG. A novel nickel responsive MerR-like regulator, NimR, from *Haemophilus influenzae*. *Metallomics*. 2011; 3:1009–1018. [PubMed: 21952667]
 137. Foster AW, Patterson CJ, Pernil R, Hess CR, Robinson NJ. Cytosolic Ni(II) Sensor in Cyanobacterium: *NICKEL DETECTION FOLLOWS NICKEL AFFINITY ACROSS FOUR FAMILIES OF METAL SENSORS*. *Journal of Biological Chemistry*. 2012; 287:12142–12151. [PubMed: 22356910]
 138. Waldron KJ, Rutherford JC, Ford D, Robinson NJ. Metalloproteins and metal sensing. *Nature*. 2009; 460:823–830. [PubMed: 19675642]
 139. Schneider E, Hunke S. ATP-binding-cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. *FEMS Microbiology Reviews*. 1998; 22:1–20. [PubMed: 9640644]
 140. De Pina K, Desjardin V, Mandrand-Berthelot MA, Giordano G, Wu LF. Isolation and characterization of the *nikR* gene encoding a nickel-responsive regulator in *Escherichia coli*. *Journal of Bacteriology*. 1999; 181:670–674. [PubMed: 9882686]
 141. Eitinger T, Mandrand-Berthelot MA. Nickel transport systems in microorganisms. *Archives of Microbiology*. 2000; 173:1–9. [PubMed: 10648098]
 142. Chivers PT, Sauer RT. Regulation of high affinity nickel uptake in bacteria. Ni^{2+} -Dependent interaction of NikR with wild-type and mutant operator sites. *Journal of Biological Chemistry*. 2000; 275:19735–19741. [PubMed: 10787413]
 143. Wu LF, Mandrand-Berthelot MA, Waugh R, Edmonds CJ, Holt SE, Boxer DH. Nickel deficiency gives rise to the defective hydrogenase phenotype of *hydC* and *fnr* mutants in *Escherichia coli*. *Molecular Microbiology*. 1989; 3:1709–1718. [PubMed: 2695744]
 144. Addy C, Ohara M, Kawai F, Kidera A, Ikeguchi M, Fuchigami S, Osawa M, Shimada I, Park SY, Tame JRH, Heddle JG. Nickel binding to NikA: an additional binding site reconciles spectroscopy, calorimetry and crystallography. *Acta Crystallographica Section D-Biological Crystallography*. 2007; 63:221–229.
 145. Raymond KN, Dertz EA, Kim SS. Enterobactin: an archetype for microbial iron transport. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:3584–3588. [PubMed: 12655062]
 146. Sleight SH, Seavers PR, Wilkinson AJ, Ladbury JE, Tame JR. Crystallographic and calorimetric analysis of peptide binding to OppA protein. *J Mol Biol*. 1999; 291:393–415. [PubMed: 10438628]
 147. Sleight SH, Tame JR, Dodson EJ, Wilkinson AJ. Peptide binding in OppA, the crystal structures of the periplasmic oligopeptide binding protein in the unliganded form and in complex with lysyllysine. *Biochemistry*. 1997; 36:9747–9758. [PubMed: 9245406]
 148. Eitinger T, Suhr J, Moore L, Smith JA. Secondary transporters for nickel and cobalt ions: theme and variations. *Biomaterials*. 2005; 18:399–405. [PubMed: 16158232]
 149. Maroney MJ. Structure/function relationships in nickel metallobiochemistry. *Current Opinion in Chemical Biology*. 1999; 3:188–199. [PubMed: 10226043]

150. Kaluarachchi H, Chan Chung KC, Zamble DB. Microbial nickel proteins. *Natural Product Reports*. 2010; 27:681–694. [PubMed: 20442959]
151. Scott DR, Marcus EA, Wen Y, Singh S, Feng J, Sachs G. Cytoplasmic histidine kinase (HP0244)-regulated assembly of urease with UreI, a channel for urea and its metabolites, CO₂, NH₃, and NH₄⁺, is necessary for acid survival of *Helicobacter pylori*. *Journal of Bacteriology*. 2010; 192:94–103. [PubMed: 19854893]
152. Fu C, Olson JW, Maier RJ. HypB protein of *Bradyrhizobium japonicum* is a metal-binding GTPase capable of binding 18 divalent nickel ions per dimer. *Proceedings of the National Academy of Sciences of the United States of America*. 1995; 92:2333–2337. [PubMed: 7892266]
153. Olson JW, Fu C, Maier RJ. The HypB protein from *Bradyrhizobium japonicum* can store nickel and is required for the nickel-dependent transcriptional regulation of hydrogenase. *Molecular Microbiology*. 1997; 24:119–128. [PubMed: 9140970]
154. Rey L, Imperial J, Palacios JM, Ruiz-Argueso T. Purification of *Rhizobium leguminosarum* HypB, a nickel-binding protein required for hydrogenase synthesis. *Journal of Bacteriology*. 1994; 176:6066–6073. [PubMed: 7928968]
155. Olson JW, Maier RJ. Dual roles of *Bradyrhizobium japonicum* nickel in protein in nickel storage and GTP-dependent Ni mobilization. *Journal of Bacteriology*. 2000; 182:1702–1705. [PubMed: 10692376]
156. Chen T, Li H, Xia W, Sun H. Multifaceted SlyD from *Helicobacter pylori*: implication in [NiFe] hydrogenase maturation. *Journal of Biological Inorganic Chemistry*. 2012; 17:331–343. [PubMed: 22045417]
157. Kang CB, Hong Y, Dhe-Paganon S, Yoon HS. FKBP family proteins: immunophilins with versatile biological functions. *Neurosignals*. 2008; 16:318–325. [PubMed: 18635947]
158. Furutani M, Iida T, Yamano S, Kamino K, Maruyama T. Biochemical and genetic characterization of an FK506-sensitive peptidyl prolyl cis-trans isomerase from a thermophilic archaeon, *Methanococcus thermolithotrophicus*. *Journal of Bacteriology*. 1998; 180:388–394. [PubMed: 9440528]
159. Suzuki R, Nagata K, Yumoto F, Kawakami M, Nemoto N, Furutani M, Adachi K, Maruyama T, Tanokura M. Three-dimensional solution structure of an archaeal FKBP with a dual function of peptidyl prolyl cis-trans isomerase and chaperone-like activities. *Journal of Molecular Biology*. 2003; 328:1149–1160. [PubMed: 12729748]
160. Brandts JF, Halvorson HR, Brennan M. Consideration of the Possibility that the slow step in protein denaturation reactions is due to cis-trans isomerism of proline residues. *Biochemistry*. 1975; 14:4953–4963. [PubMed: 241393]
161. Balbach, J.; Halvorson, HR.; Brennan, M. *Mechanisms of Protein Folding*. 2. Oxford University Press; 2000. Prolyl Isomerization and Its Catalysis in Protein Folding.
162. Schmid FX. Prolyl isomerases. *Advances in Protein Chemistry*. 2002; 59:243–282. [PubMed: 11868274]
163. Jakob RP, Zoldak G, Aumuller T, Schmid FX. Chaperone domains convert prolyl isomerases into generic catalysts of protein folding. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:20282–20287. [PubMed: 19920179]
164. Erdmann, F.; Fischer, G. *Metal Ions in Life Sciences*. John Wiley and Sons; New York: 2007. The nickel-regulated peptidyl prolyl cis/trans isomerase SlyD; p. 501-518.
165. Zambelli B, Musiani F, Benini S, Ciurli S. Chemistry of Ni²⁺ in urease: sensing, trafficking, and catalysis. *Accounts of Chemical Research*. 2011; 44:520–530. [PubMed: 21542631]
166. Park IS, Hausinger RP. Evidence for the presence of urease apoprotein complexes containing UreD, UreF, and UreG in cells that are competent for in vivo enzyme activation. *Journal of Bacteriology*. 1995; 177:1947–1951. [PubMed: 7721685]
167. Outten CE, O'Halloran TV. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science*. 2001; 292:2488–2492. [PubMed: 11397910]
168. Iwig JS, Chivers PT. Coordinating intracellular nickel-metal-site structure-function relationships and the NikR and RcnR repressors. *Natural Product Reports*. 2010; 27:658–667. [PubMed: 20442957]

169. Musiani F, Bertosa B, Magistrato A, Zambelli B, Turano P, Losasso V, Micheletti C, Ciurli S, Carloni P. Computational Study of the DNA-Binding Protein *Helicobacter pylori* NikR: The Role of Ni²⁺. *Journal of Chemical Theory and Computation*. 2010; 6:3503–3515.
170. Iwig JS, Chivers PT. DNA recognition and wrapping by *Escherichia coli* RcnR. *Journal of Molecular Biology*. 2009; 393:514–526. [PubMed: 19703465]
171. Liu T, Ramesh A, Ma Z, Ward SK, Zhang L, George GN, Talaat AM, Sacchettini JC, Giedroc DP. CsoR is a novel *Mycobacterium tuberculosis* copper-sensing transcriptional regulator. *Nature Chemical Biology*. 2007; 3:60–68.
172. Lee CW, Chakravorty DK, Chang FM, Reyes-Caballero H, Ye Y, Merz KM Jr, Giedroc DP. Solution Structure of *Mycobacterium tuberculosis* NmtR in the Apo State: Insights into Ni(II)-Mediated Allostery. *Biochemistry*. 2012; 51:2619–2629. [PubMed: 22394357]
173. Martin-Diaconescu V, Bellucci M, Musiani F, Ciurli S, Maroney MJ. Unraveling the *Helicobacter pylori* UreG zinc binding site using X-ray absorption spectroscopy (XAS) and structural modeling. *J Biol Inorg Chem*. 2012; 17:353–361. [PubMed: 22068961]

Figure 1.

Nickel trafficking in *E. coli* has only one set of target proteins (hydrogenases) but features the required components of all transition metal trafficking systems, an importer (NikABCDE), chaperones and accessory proteins (HypA, HypB and SlyD) used to deliver Ni(II) and incorporate it into hydrogenases, an exporter (RcnAB), and metallo-sensors that control the transcription of the importer (NikR) and exporter (RcnR).

Figure 2. The structure of the peptide transporter OppA with the peptide KK (PDB ID 2RKM) bound via H-bonds between Lys1 and Tyr109, Cys417 and Asp419.

Figure 3. NMR structure of *H. pylori* HypA (PDB ID 2KDX) showing the location of residues involved in nickel coordination: His2, Glu3 and Asp40 (red). The zinc site is shown at the base of the protein and features four S-donor ligands from Cys74, Cys77, Cys91 and Cys94.

Figure 4. The crystal structure of the zinc site in monomeric *T. kodakaraensis* HypA protein (left, PDB ID 3A43) and the protein fold in the dimer illustrating the domain swap. (right, PDB ID 3A44)

Figure 5.

Crystal structure of *M. jannaschii* HypB (PDB ID 2HF8). Left: HypB dimer showing two GTP molecules (red) and two Zn(II) atoms (slate) bound at the dimer interface as well as two Zn(II) atoms (slate) bound at the surface of each monomer. Right: A close up of the zinc site at the dimer interface.

Figure 6.

Top: *E. coli* NikR crystal structure showing the location of the high-affinity sites with nickel (green) bound and the low-affinity sites with K^+ ions (lavender) bound. (PDB ID 2HZV)
 Middle: The high-affinity nickel site with nickel coordinated by the side chains of His87, His89, Cys95 from one subunit and His76 from another. Bottom: The low-affinity site with K^+ coordinated by the side chains of Glu30 and Asp34 from the DNA-binding domain and by the carbonyl oxygens of Ile116, Gln118 and Val121 from the metal binding domain.

Figure 7.

Top: *S. coelicolor* Nur crystal structure highlighting the M-site with zinc (slate) bound and the nickel-site with nickel bound (green). (PDB ID 3EYY). Middle: The M-site with zinc (slate) coordinated by His33, His86, His88 and His90. Bottom: The nickel-site with nickel (green) coordinated by His70, His72, His126, ethylene glycol and malonate.

Table 1

Proteins involved in Nickel Trafficking.

Protein	Organism *	Properties	References
Importers			
NikABCDE	<i>Ec</i>	ABC-type ATPase. <i>Ec</i> NikA is a <i>crystallographically characterized</i> monomeric 56 kDa periplasmic binding protein that transports Ni(L-His) ₂ ; <i>Metal site XAS</i> . NikB and C are transmembrane proteins, NikD and E have ATPase activity.	(12–19)
NikZYXWV	<i>Cj</i>	An ABC-type transporter that binds solvated nickel with $K_d = 0.61 \mu\text{M}$. Also binds Co(II).	(20)
Cbi/NikMNQO	<i>Se</i>	A nickel/cobalt permease.	(21)
HoxN	<i>Ae</i>	A 33.1 kDa permease located on the hydrogenase gene cluster A. Asn31, His62, Asp67, and His68 are critical for permease activity. Cys331 has an important but non-essential function.	(22–25)
NixA	<i>Hp</i>	A monomeric 37 kDa permease that is integral in urease maturation. Residues His44, Asp49, His50, Asp47, Asp55, Asp194, and Asp231 are essential for nickel uptake, while residues Glu198, Asp234, and Glu274 are important but not essential. Residues Phe75, Gly78, His79, Asn127, Trp195, and Ser197 were found to completely abolish nickel uptake and urease maturation. NixA was shown to bind, and possibly import, Co(II), Cu(II), and Zn(II).	(26, 27)
NhlF	<i>Rrh</i>	A NiCoT permease. His74 and His34 are essential for metal transport while His68 and Phe70 are important but not essential.	(25, 28)
HupN	<i>Bj</i>	A 40 kDa nickel permease.	(29)
Exporters			
CznABC	<i>Hp</i>	A cadmium, zinc, and nickel efflux pump. All three genes cause increased sensitivity to cadmium, zinc, and nickel. CznB and CznC are both bind nickel. CznABC modulates urease activity; $\Delta cznA$ and $\Delta cznC$ cause an 8–10 fold increase in urease activity.	(30)
NreB	<i>Ax</i>	Nickel efflux pump with a 42 residue C-terminal polyHis tail.	(31)
	<i>Ec</i>	Confers Ni(II) resistance and responsible for decreased Ni(II) accumulation levels.	(31)
RcnAB	<i>Ec</i>	A nickel and cobalt efflux system whose transcription is controlled by the metalloregulator RcnR. RcnB is a periplasmic protein that does not bind metals.	(32, 33)
MrdH	<i>Pp</i>	A 41 kDa nickel, cadmium, and zinc efflux pump.	(34)
NrsD	<i>S</i>	A nickel efflux pump with a C-terminal polyHis tail.	(35)
NcrAC	<i>Lf</i>	Membrane proteins that form a nickel efflux system.	(36, 37)
Chaperone and Accessory Proteins			
HypA	Monomeric or Homodimeric metallochaperone that delivers Ni(II) to the apo-H ₂ ase large subunits. Contains a structural Zn(II) site that employs highly conserved CXXC motifs. Forms a heterodimers with HypB and UreE.		
	<i>Ec</i>	<i>Metal site XAS</i> . Binds two Ni(II) and two Zn(II) ions per dimer with μM and nM affinity. Each monomer is 13.1 kDa.	(38–40)
	<i>Hp</i>	<i>NMR structure. Metal site XAS</i> . Binds 1 Ni(II) per monomer (25 kDa) & 1 Zn(II) per dimer at pH 7.3. At pH 6.3 two of the Zn(II) S donors are replaced by histidines and there is 1 Ni(II) ion bound per dimer.	(41–46)
	<i>Tk</i>	<i>Crystal Structure</i> . The 31.4 kDa homodimer engages in a domain swap that involves one CXXC motif from each monomer to form the Zn(II) site.	(47)
	<i>Af</i>	Forms a heterodimer with HypB monomer but not HypB dimer. Each monomer is 25 kDa.	(48)
HypB	P-loop GTPase required for H ₂ ase maturation and Ni insertion. It is a homodimer that forms heterodimers with HypA and SlyD.		
	<i>Ec</i>	A 27.2 kDa (monomer) protein that has two metal binding sites; a high-affinity nickel binding site at the N-terminus and a low affinity metal-binding site in the GTPase domain that can bind both nickel and zinc. The former has a CXXCGC motif and binds Ni(II) with sub-pM affinity in a planar site with 3S- and 1 N/O- donor ligands. The later site can	(40, 49–52)

Protein	Organism *	Properties	References
		bind either Ni(II) or Zn(II) with low affinity, but prefers Zn(II) over Ni(II). The Zn(II) site is tetrahedral and consists of two Zn(II) ions. Binding of a second metal perturbs the metal site structure of the monometallated protein.	
	<i>Hp</i>	Lacks the high-affinity site found in of <i>Ec</i> HypB. Binds Ni(II)/Zn(II) with 1:1 stoichiometry with 1.72 μM affinity. Ni(II) does not affect GTP hydrolysis but Zn(II) inhibits it. 27.2 kDa monomer.	(41, 53, 54)
	<i>Mj</i>	<i>Crystal structure.</i> Lacks the high-affinity site found in <i>Ec</i> HypB. Has two metal binding sites with a dinuclear Zn(II) site, one bound by 3Cys (Cys95, Cys127 from one monomer and Cys95 from another) & 1 H ₂ O and the other by 2 Cys (Cys127 and Cys95), 1 His (His96), & 1 H ₂ O. One of the Cys95 bridges the Zn(II) ions. 24.3 kDa monomer.	(55)
	<i>Af</i>	<i>Crystal structure.</i> A 24.7 kDa monomer that undergoes GTP- dependent dimerization.	(56)
	<i>Bj</i>	Possesses 24 N-terminal histidine residues that can bind up to nine Ni(II) ions per monomer (32.8 kDa) with a $K_d = 2.3 \mu\text{M}$.	61, 62
	<i>Rl</i>	Possesses a polyhistidine tail used for nickel storage or buffering. 32 kDa monomer.	63
SlyD	A monomeric peptidyl-prolyl isomerase with chaperone functions involved in Ni(II) delivery to [NiFe]-hydrogenases.		
	<i>Ec</i>	<i>NMR structures.</i> Contains a 50-residue C-terminal tail that binds Ni(II) ions with nM affinity. Forms a complex with HypB with a possible role of transferring Ni(II) to HypB. HypB incubated with SlyD shows a tenfold increase in GTP hydrolysis. 21 kDa monomer.	(57–74)
	<i>Tt</i>	<i>Crystal structure.</i> Lacks the C-terminal polyHis tail found in <i>Ec</i> SlyD. Binds 1 Ni(II) ion per monomer with a $K_d \sim 50 \text{ nM}$.	(75, 76)
	<i>Hp</i>	<i>NMR structure.</i> 20 kDa monomer with a 34 residue polyHis C-terminal tail. Equilibrium dialysis reveals 2.4 Ni(II) per protein with a $K_d = 2.74 \pm 0.26 \mu\text{M}$.	(77)
UreE	Metallochaperone that delivers nickel to apo-urease.		
	<i>Ka</i>	<i>Crystal structures. Metal site XAS.</i> Binds 6 Ni(II) per 35.2 kDa dimer with a $K_d = 9.6 \mu\text{M}$. The two essential Ni sites have pseudo-octahedral geometry with 6 N/O donors, of which are 3–5 histidines. Contains a C-term polyHis tail.	(78–84)
	<i>Bp</i>	<i>Crystal structures. Metal site XAS.</i> Lacks the C-terminal polyHis tail of <i>Ka</i> UreE. Can bind 2 Ni per dimer (41 kDa) with a $K_d = 35 \mu\text{M}$.	(85–87)
	<i>Hp</i>	<i>Crystal structures. Metal site XAS.</i> Lacks the C-terminal polyHis tail of <i>Ka</i> UreE. Can bind 1 Ni/Zn per dimer (38.6 kDa) with $K_d = 0.15/0.49 \mu\text{M}$. Apo-UreE is a dimer and holo- UreE is a tetramer in the solid state.	(46, 88–92)
UreG	P-loop GTPase required for urease maturation.		
	<i>Bp</i>	A 46 kDa dimer with a $k_{cat} = 0.04 \text{ min}^{-1}$. Binds two Zn(II) per dimer with a $K_d = 42 \pm 3 \mu\text{M}$ and a tenfold lower affinity for Ni(II).	(93)
	<i>Mt</i>	A 46.2 kDa dimer with a $k_{cat} = 0.01 \text{ min}^{-1}$. Held together by a disulfide bond.	(94)
	<i>Hp</i>	<i>Metal site XAS.</i> Apo- <i>Hp</i> UreG is a 22 kDa monomer that binds 0.5 Zn(II) per monomer with a $K_d = 0.33 \pm 0.03 \mu\text{M}$. Zn(II) binding, but not Ni(II) binding ($K_d = 10 \pm 1 \mu\text{M}$), causes dimerization. Residues Cys66 and His68 are involved in Zn(II) binding.	(46, 89, 92, 95)
	<i>Ka</i>	Apo- <i>Ka</i> UreG is a 21.9 kDa monomer that binds 1 Zn(II) or Ni(II). Unlike <i>Hp</i> UreG, metal binding does not affect cause dimerization. Residue C72 is involved in Zn(II) binding.	(96)
CooC	A P-loop nickel binding ATPase involved in the incorporation of Ni(II) into carbon monoxide dehydrogenase (CODH). Binds to CooJ and CooT (function unknown) in the process of Ni(II) incorporation into CODH.		
	<i>Ch</i>	<i>Crystal structures.</i> Apo- <i>Ch</i> CooC is a monomer (29 kDa), and dimerizes upon ATP binding. Metal binding occurs in the dimer interface with a CXC motif from each monomer forming a four coordinate binding site, involving cysteine residues C112 and C114. This site binds both Ni(II) and Zn(II) with ~equal affinity.	(98)
	<i>Rr</i>	A 27.8 kDa homodimer that catalyzes ATP and GTP hydrolysis with $K_m = 24.4$ and 26 μM , respectively.	(99)
CooJ	Metallochaperone that is involved in Ni(II) incorporation into carbon monoxide dehydrogenase.		

Protein	Organism *	Properties	References
	<i>Rr</i>	A 12.5 kDa dimer that binds 4 Ni(II) ions per monomer with a $K_d = 4.3 \pm 0.3 \mu\text{M}$. <i>Rr-CooJ</i> has a 15 residue histidine-rich tail similar to that seen in <i>Bj</i> and <i>RMypB</i> , as well as <i>KaUreE</i> .	(100)
Storage/Ni buffer Proteins			
Hpn	<i>Hp</i>	A 7 kDa, 60 amino acid protein, 28 of which are histidine that forms oligomers. Most common oligomeric form is a 20-mer. <i>HpHpn</i> binds 5 Ni(II) per monomer with a $K_d = 7.1 \mu\text{M}$. Ni(II) binding is reversible in the presence of EDTA or acidic pH (below 6.8). The protein contains four cysteines, all of which are involved in metal binding.	(101, 102)
Hpn-like	<i>Hp</i>	A 9 kDa, 72-amino acid protein, 18 of which are histidines and 30 of which are glutamines, that forms oligomers. The most common oligomeric form is a 22($\pm$ 1)-mer. It binds two Ni(II) ions per monomer with a $K_d = 3.8 \pm 0.2 \mu\text{M}$. Ni(II) binding is reversible in the presence of EDTA or acidic pH (below 7.0) It is up-regulated at pH 5.0 by <i>ArsRS</i> .	(103, 104)
Regulators			
NikR		A homotetrameric transcriptional repressor that contains four high-affinity as well as low- affinity metal binding sites.	
	<i>Ec</i>	<i>Crystal structure. Metal site XAS.</i> Recognizes the sequence GTATGA-N ₁₆ -TCATAC within the <i>nikABCDE</i> promoter. Binds one nickel per monomer (15.1kDa) with pM affinity. The high-affinity binding site binds Ni(II) in a planar geometry using His87, His89, and Cys95 from one subunit, and His76 from an adjacent subunit. Nickel bound to low affinity sites increases affinity for DNA from nM to pM. K ⁺ ions are essential for nickel-responsive DNA-binding.	(105–114)
	<i>Hp</i>	<i>Crystal structure.</i> Each 17.1 kDa monomer can bind four Ni(II) ions in high-affinity sites with nM affinity. The planar nickel site is similar to <i>EcNikR</i> . ITC suggests <i>HpNikR</i> can bind up to 10 nickel ions in low-affinity sites with μM affinity.	(115–121)
	<i>Ph</i>	<i>Crystal structure.</i> The high-affinity sites are similar to those in <i>EcNikR</i> . There are four low-affinity sites that are coordinated in a distorted trigonal pyramidal geometry via His64 and Asp65 from one subunit and Asp75 from another subunit.	(122)
	<i>Gu</i>	Recognizes GTG(T/C)TAC-X ₁₃ -GTG(C/T)TAC as the primary DNA sequence and exhibits DNA-wrapping.	(123)
RcnR	<i>Ec</i>	<i>Metal site XAS.</i> 40 kDa tetramer that regulates the expression of the Ni(II)/Co(II) exporters RcnAB. Has K_d values of 25 (Ni) and 5 (Co) nM; binding results in derepression. Recognizes a TACT-G ₆ -N-AGTA DNA sequence. Involves Cys35 in binding all metals; cognate metals are distinguished by six-coordinate (N/O) ₅ S complexation involving the N- terminus. Cobalt and nickel are further distinguished by His3, which is a ligand for Co(II) but not Ni(II)	(124–127)
NmtR	<i>Mt</i>	<i>Metal site XAS. NMR structure.</i> A 25.6 kDa homodimer that controls the expression of the P-type ATPase NmtA. Responds to both Ni(II) and Co(II). Binds one nickel per monomer with K_d values of 0.087 nM and 0.14 nM. Residues Asp 91, His93, His104, His107, His109, and His116 are necessary for Ni(II) or Co(II) recognition. His3 has been found to be important for cognate versus non-cognate distinction. <i>Metal site XAS</i> data indicates that the nickel site is octahedral with three His and three other N/O-donors.	(128–130)
KmtR	<i>Mt</i>	Regulates the expression of an exporter protein. Ni(II) and Co(II) binding results in derepression. His88, Glu101, His110, and His111 are required for Ni(II) and Co(II) recognition.	(131)
Nur	<i>Sc</i>	<i>Crystal structure.</i> Represses <i>sodF</i> (FeSOD). Transcription is activated only in the presence of nickel and occurs even in the presence of EDTA. In the presence of nickel, FeSOD is constitutively expressed; NiSOD expression is abolished. Nur (16.2 kDa) deletion mutants show high levels of nickel accumulation in the cell.	(132, 133)
SmnRQ	<i>Sg</i>	A 99.2 kDa R ₄ Q ₄ octamer that does not require nickel to bind DNA. SmnQ undergoes a conformational change upon binding one nickel.	(134)
NcrB	<i>Lf</i>	Transcriptional regulator of <i>pncrA</i> and <i>pncrB</i> . Binds to the sequence 5'-ATCCCCCTGGGGGGGAT-3' and Ni(II) binding leads to de-repression.	(37)
Mua	<i>Hp</i>	A 36.8 kDa dimer that modulates urease activity by controlling the expression of <i>UreAB</i> without direct DNA binding and imparts nickel resistance. Binds one nickel ion per monomer. A secondary regulator; NikR is the primary regulator.	(135)

Protein	Organism [*]	Properties	References
NimR	<i>Hi</i>	Regulates the expression the Ni(II) permease NikKLMQO based on cytoplasmic nickel availability and pH. Binds 1 nickel per dimer. Is important in the maturation of urease but does not directly control expression of urease genes.	(136)
InrS	<i>S</i>	A 44 kDa tetrameric transcriptional regulator that responds to both Cu(I) and Ni(II) in a 1:1 stoichiometry by de-repressing the cytosolic exporter <i>nrsD</i> . Has an estimated K_d = 2.05 pM.	(137)

^{*} Ae = *Alcaligenes eutrophus*, Af = *Archaeoglobus fulgidus*, Ax = *Achromobacter xylosoxidans* 31A, Bj = *Bradyrhizobium japonicum*, Bp = *Bacillus pasteurii*, Ch = *Carboxydotherrmus hydrogenoformans*, Cj = *Campylobacter jejuni*, Ec = *Escherichia coli*, Gu = *Geobacter uraniireducens*, Hi = *Haemophilus influenza*, Hp = *Helicobacter pylori*, Ka = *Klebsiella aerogenes*, Lf = *Leptospirillum ferriphilum* UBK03, Mj = *Methanococcus jannaschii*, Mt = *Mycobacterium tuberculosis*, Ph = *Pyrococcus horikoshii* OT3, Pp = *Pseudomonas putida* KT2440, Rl = *Rhizobium leguminosarum*, Rr = *Rhodospirillum rubrum*, Rh = *Rhodococcus rhodochrous*, S = *Synechocystis* PCC 6803, Sc = *Streptomyces coelicolor*, Se = *Salmonella enterica* serovar Typhimurium, Sg = *Streptomyces griseus*, Tk = *Thermococcus kodakaraensis*, Tt = *Thermus thermophilus*.