# The Coordination Chemistry of Nickel Uptake Regulation in *Escherichia coli*

P.E. Carrington[1], P.T. Chivers[2,3], F. Al-Mjeni[1,4], R.T. Sauer[2], and M.J. Maroney[1]

[1]Department of Chemistry, University of Massachusetts; [2]Department of Biology, Massachusetts Institute of Technology; [3]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine; [4] Department of Chemistry, Sultan Qaboos University, Sultanate of Oman

*Scientists from the University of Massachusetts in Amherst and the Massachusetts Institute of Technology in Cambridge have determined the structure of the high affinity nickel-binding site in NikR, a protein that regulates the uptake of nickel by the gut bacterium Escherichia coli. The scientists show that, in the NikR protein, nickel is bound in a novel four-coordinate planar site consisting of two histidines, one additional oxygen- or nitrogen-donor ligand, and one sulfur-donor (cysteine) ligand. The researchers also noticed that when NikR binds to DNA, the nickel-binding site becomes six-coordinate with ligands made of oxygen and nitrogen donors, but lacking cysteine.*

Authors (from left): Sergio Chai, Faizah Al-Mjeni, Patrick DeCourcy, Paul Carrington, Michael Maroney, Peter Bryngelson, Jennifer Pinkham, and Arthur LaPlante

Microorganisms have developed high-affinity uptake systems to acquire metals, such as iron, copper, and zinc, from the environment. In the gut bacterium *Escherichia coli*, nickel plays a critical role in anaerobic metabolism, but it is usually present at low concentrations in the environment, so *E. coli* synthesizes a protein called NikABCDE, which acquires nickel from the environment and actively transports it into the cell. (The letters A to E label five proteins that assemble to form NikABCDE.)

This protein is produced via transcription of the *nik* operon (chromosomal functional unit acting like a gene) in response to a low oxygen level. When nickel concentration is high enough, the transcription process is repressed by NikR, a protein that appears to regulate nickel uptake in a number of bacteria and archaea.

NikR contains two distinct binding domains: an amino terminal, DNA-binding site and a carboxyl terminal high-affinity nickel-binding site. By using x-ray absorption spectroscopy (XAS) at beamline X9B at the NSLS, we have characterized the structure of the high-affinity nickel-binding site and have shown that the nickel-binding site is sensitive to the DNA-bound state of NikR.

Analysis of x-ray absorption near-edge structure (XANES) revealed that the nickel site is four-coordinate and planar, because it exhibits a 1s ➡ 3d electronic transition near 8332 electronvolts (eV) and a distinct maximum assigned to a 1s ➡ $4p_z$ transition near 8338 eV. This result was confirmed by the analysis of extended x-ray absorption fine structure (EXAFS), which revealed that the distances between nickel and other atoms are typical of planar four-coordinate nickel complexes, and also provided information about the ligands involved.

The nickel-binding domain of NikR contains a number of conserved amino acids that are potential nickel ligands. The structure that emerges from the combined XAS and mutagenesis of four of these amino acids is consistent with the planar four-coordinate $N_2OS$-donor site

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**Contact information**
Michael Maroney, Department of Chemistry, University of Massachusetts, Amherst, MA

Email: maroney@chem.umass.edu

shown in **Figure 2**. Since nickel is the only important biological metal ion that commonly adopts a square planar geometry, the results provide a structural basis for the specificity of NikR toward nickel ions.

Large structural changes were observed when NikR was bound to operator DNA (**Figure 1**). The XANES spectra of the NikR-DNA complexes (left) exhibit somewhat larger, but still small, peaks associated with 1s ➡ 3d electronic transitions near 8332 eV, while the peak assigned to the 1s ➡ $4p_z$ transition near 8338 eV is absent, indicating the presence of a six-coordinate nickel site. EXAFS analysis of the DNA complexes (right) shows that the best fits are obtained for six nitrogen and oxygen donors, including at least two histidine ligands, consistent with the XANES analysis.

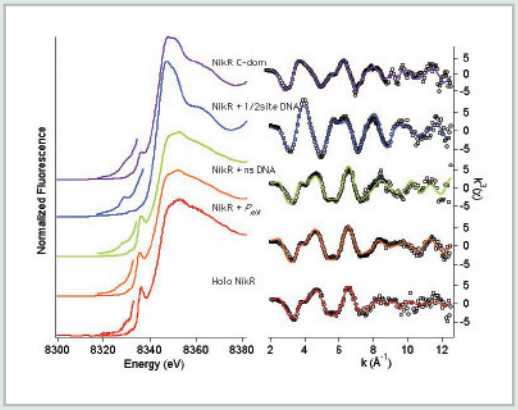


**Figure 1. Nickel K-edge x-ray absorption near-edge spectroscopy (XANES) spectra (left) and unfiltered extended x-ray absorption fine structure (EXAFS) spectra (right). EXAFS data points are represented by open circles and the fit by a solid line.**

NikR appears to be using the coordination chemistry characteristic of nickel not only to selectively bind nickel, but also to change the interaction between nickel and the protein. The change in nickel-coordination geometry has several potential consequences for NikR function, such as its role for NikR in buffering intracellular nickel at very low levels and controlling DNA transcription at higher concentrations of intracellular nickel.

Although the functional consequences of the change in nickel coordination remain to be elucidated, NikR provides a unique example of the role of metal ions in regulating DNA transcription.

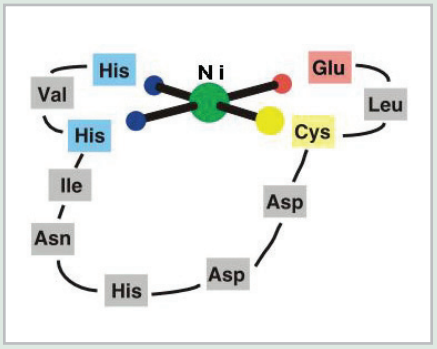


**Figure 2. A model of the high-affinity nickel site in NikR based on x-ray absorption spectroscopy (XAS) and mutagenesis results. Residues are colored according to donor atom types (blue: nitrogen; red: oxygen; and orange: sulfur).**