

Electronic Supplementary Information

***Sinorhizobium meliloti* Nia is a P_{1B-5}-ATPase expressed in the nodule during plant symbiosis and is involved in Ni and Fe transport**

Eliza L. Zielazinski,^{†a} Manuel González-Guerrero,^{†b} Poorna Subramanian,^c Timothy L. Stemmler,^c José M. Argüello^{*d} and Amy C. Rosenzweig^{*a}

^a*Departments of Molecular Biosciences and of Chemistry. Northwestern University, Evanston, Illinois, USA. E-mail: amyr@northwestern.edu*

^b*Centro de Biotecnología y Genómica de Plantas (CBGP), Universidad Politécnica de Madrid, Campus de Montegancedo, Pozuelo de Alarcón, Madrid, Spain.*

^c*Department of Biochemistry and Molecular Biology and the Cardiovascular Research Institute, Wayne State University, School of Medicine, Detroit, Michigan, USA.*

^d*Department of Chemistry and Biochemistry, Worcester Polytechnic Institute, Worcester, Massachusetts, USA. E-mail: arguello@wpi.edu*

[†]These two authors contributed equally to this work.

[§]Electronic supplementary information (ESI) available: Figs. S1, S2, Tables S1, S2, S3

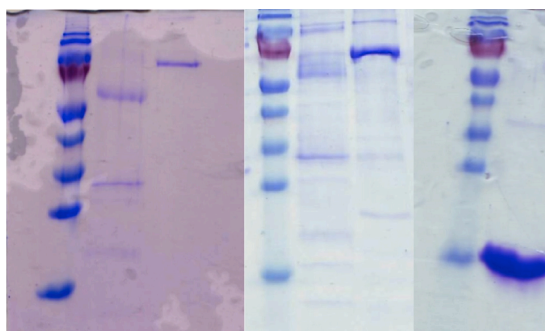


Fig. S1 SDS-PAGE analysis of Nia constructs. Left: Nia (expected molecular mass 85 kDa). Lane 1, 50 mM imidazole elutant from Ni²⁺-chelating column; Lane 2, 150 mM imidazole elutant. Middle: ΔHr-Nia (expected molecular mass 69 kDa). Lane 1, 50 mM imidazole elutant; Lane 2: 150 mM imidazole elutant. Right: purified Nia-Hr (expected molecular mass 18 kDa). Lane markers on all gels from top to bottom: 170 kDa, 130 kDa, 95 kDa, 72 kDa (red), 55 kDa, 43 kDa, 34 kDa, 26 kDa, 17 kDa.

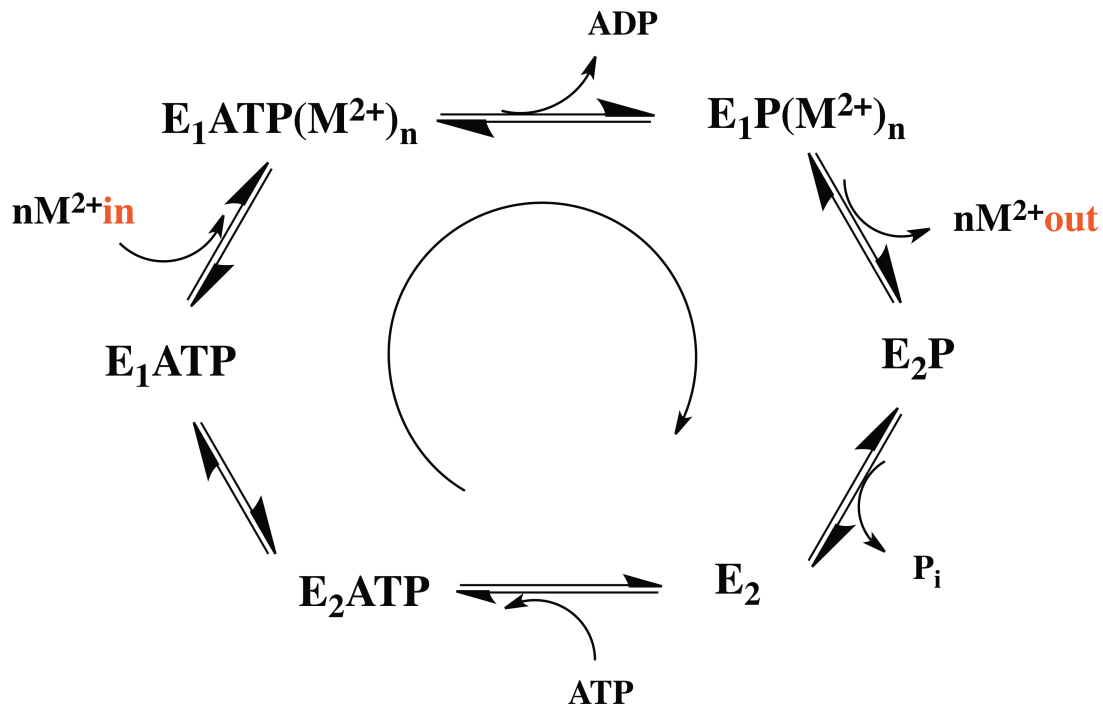


Fig. S2 A generalized P-type ATPase mechanism for a divalent metal substrate (M^{2+}). The cycle shows the movement of the substrate from one side of a cellular membrane (in) to the other (out). E1 refers to a protein conformation in which the metal binding site is accessible to the “in” side of the membrane whereas the E2 state is open to the “out” side. The two states are mediated by covalent phosphorylation (denoted by P) by ATP and dephosphorylation by water.

Metal	WT (nmol metal/mg protein)	<i>nia</i> (nmol metal/mg protein)
Ni	12.55 ± 2.12	4.96 ± 2.73
Fe	47.63 ± 7.23	39.40 ± 1.96
Cu	4.36 ± 0.94	2.12 ± 0.96
Co	0.49 ± 0.20	0.27 ± 0.18
Mn	4.05 ± 1.33	2.19 ± 0.73

Table S1. Metal content of WT and *nia* mutant *S. meliloti* strains when grown in TY medium with no metal supplementation.

Metal	WT (nmol metal/mg protein)	<i>nia</i> (nmol metal/mg protein)
Ni	249.66 ± 31.19	960.88 ± 115.15
Fe	49.36 ± 10.61	34.56 ± 6.31
Cu	0.68 ± 0.16	5.54 ± 1.00
Co	0.12 ± 0.034	0.29 ± 0.15
Mn	1.76 ± 0.21	1.94 ± 0.66

Table S2. Metal content of WT and *nia* mutant *S. meliloti* strains with cells incubated overnight with 1 mM NiCl₂ in TY media.

Metal	WT (nmol metal/mg protein)	<i>nia</i> (nmol metal/mg protein)
Ni	9.28 ± 3.75	6.61 ± 1.13
Fe	152.25 ± 32.18	524.66 ± 85.87
Cu	3.46 ± 1.31	6.03 ± 2.00
Co	0.52 ± 0.09	3.31 ± 0.12
Mn	2.83 ± 0.66	2.79 ± 1.2

Table S3. Metal content of WT and *nia* mutant *S. meliloti* strains with cells incubated overnight with 0.5 mM FeCl₂ in TY media.