Nickel-responsive transcriptional regulators

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SUPPLEMENTARY INFORMATION

Protein expression and purification

E. coli BL21(DE3) cells harboring the plasmids expressing *Ec*NikR [1], *Ec*RcnR [2], *Sy*InrS [3], *Sc*NuR [4] were grown at 37 °C in LB broth, containing the appropriate antibiotics, until OD_{600} reached 0.5-0.6. Subsequently, protein expression was induced adding isopropyl-β-D-1-tiogalattopiranoside (IPTG) to a final concentration of 0.5-1.0 mM. Protein expressions and purifications were carried out by modifying previously described protocols [1-4]. Protein expression and purity were established by SDS-PAGE using a XCell SureLockTM Mini-Cell Electrophoresis System (Life Technologies) apparatus and NuPAGE 4-12 % Bis-tris acrylamide gels, stained using ProBlue Safe stain (Giotto Biotech).

*Ec*NikR. Protein expression was carried out at 37 °C. After 3.5 hours, the cells were harvested by centrifugation at 11,300 x g for 30 min at 4 °C, and the pellet was resuspended in 40 mL of 20 mM TrisHCl, pH 8.0, containing 500 mM NaCl, 5 mM EDTA, 10 mM $MgCl_2$ and 20 mg mL^{-1} DNase I. Cells were disrupted by two passages through a French Pressure cell (SLM-Aminco) operating at 20,000 psi. The soluble fraction, obtained by centrifugation at 35,000 x g for 30 min, was dialyzed against 20 mM TrisHCl pH 8 to decrease the ionic strength. Subsequently, both DTT and EDTA were added to a final concentration of 2 mM. The protein solution was then loaded onto a MonoQ HR 10/10 column (GE Healthcare) equilibrated with 20 mM TrisHCl pH 8.0, containing 2 mM DTT and 2 mM EDTA. Protein elution was obtained by linearly increasing the ionic strength from 0 to 1 M using NaCl. The fractions containing the protein were collected, buffer was exchanged through dialysis against 20 mM TrisHCl pH 7.0, and the protein solution was further subjected to a similar anionic exchange separation at pH 7.0. Fractions containing the protein were concentrated using 3 kDa MWCO Centricon ultra-filtration units (Millipore) and stored at -80 °C. Immediately before the ITC experiments, the protein was polished loading it onto a Superdex-75 10/300 GL (GE Healthcare) equilibrated with 20 mM HEPES pH 8.0, 150 mM NaCl and eluted in the same buffer. The absence of any metal bound to the purified protein was confirmed by inductively coupled plasma emission spectroscopy (ICP-ES), using a procedure previously described [5].

*Ec*RcnR. Protein expression was carried out at 20 °C. After 48 hours, the cells were harvested by centrifugation at 11,300 x g for 30 min at 4 °C, and the pellet was resuspended in 30 mL of 20 mM HEPES, pH 7.0, containing 0.5 M NaCl, 5 mM TCEP, 5 mM EDTA, 10% v/v glycerol, 1.5 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM $MgCl_2$ and 20 mg mL^{-1} DNase I. Cells were disrupted by two passages through a French Pressure cell (SLM-Aminco) operating at 20,000 psi. The soluble fraction, obtained by centrifugation at 35,000 x g for 30 min, was dialyzed against 20 mM HEPES, pH 7.0, containing 5 mM EDTA, 10% v/v glycerol, 0.1 M NaCl and 1 mM DTT. The protein solution was then loaded onto a SP-sepharose 16/10 column (GE Healthcare) equilibrated with 20 mM HEPES pH 7.0, containing 1 mM TCEP, 0.1 M NaCl, 10% v/v glycerol and 5 mM EDTA. Protein elution was obtained by linearly increasing the ionic strength from 0 to 1 M using NaCl. The fractions containing the protein were collected, concentrated using 3 kDa MWCO Centricon ultra-filtration units (Millipore) and loaded onto a Superdex-75 16/60 column equilibrated with 20 mM HEPES pH 7.0, containing 150 mM NaCl, 1 mM TCEP. The purified protein was stored at -80 °C. Immediately before the ITC experiments, the protein was polished loading it onto a Superdex-75 10/300 GL (GE Healthcare) equilibrated with the same buffer. The absence of any metal bound to the purified protein was confirmed by inductively coupled plasma emission spectroscopy (ICP-ES), using a procedure previously described [5].

*Sy*InrS. Protein expression was carried out at 37 °C. After 16 hours, the cells were harvested by centrifugation at 11,300 x g for 30 min at 4 °C, and the pellet was resuspended in 20 mL of 50 mM phosphate buffer, pH 7.3, containing 0.3 M NaCl, 2 mM DTT, 10 mM imidazole, 10 mM $MgCl_2$ and 20 mg mL^{-1} DNase I. Cells were disrupted by two passages through a French Pressure cell (SLM-Aminco) operating at 20,000 psi. The soluble fraction, obtained by centrifugation at 35,000 x g for 30 min, was loaded onto a 5 mL Ni(II) derived Hi-Trap column equilibrated with 50 mM phosphate buffer pH 7.3, 300 mM NaCl, 10 mM imidazole. Protein elution was carried out using the same buffer with 300 mM imidazole. Fractions containing the eluted protein were collected, concentrated using 3 kDa MWCO Centricon ultra-filtration units (Millipore) and loaded onto a Superdex-75 16/60 column equilibrated with 20 mM HEPES pH 7.9,

containing 300 mM NaCl, 10 mM DTT and 10 mM EDTA. The purified protein was stored at -80 °C. Immediately before the ITC experiments, the protein was polished loading it onto a Superdex-75 10/300 GL (GE Healthcare) equilibrated with 20 mM HEPES pH 7.8, containing 300 mM NaCl and 5 mM TCEP. The absence of any metal bound to the purified protein was confirmed by inductively coupled plasma emission spectroscopy (ICP-ES), using a procedure previously described [5].

*Sc*Nur. Protein expression was carried out at 30 °C. After six hours, the cells were harvested by centrifugation at 11,300 x g for 30 min at 4 °C, and the pellet was resuspended in 30 mL of 20 mM TrisHCl, pH 7.0, containing 0.5 M NaCl, 2 mM DTT, 10 mM $MgCl_2$ and 20 mg mL^{-1} DNase I. Cells were disrupted by two passages through a French Pressure cell (SLM-Aminco) operating at 20,000 psi. The soluble fraction, obtained by centrifugation at 35,000 x g for 30 min, was dialyzed against 20 mM TrisHCl, pH 7.0, containing 5 mM EDTA and 2 mM DTT. The protein solution was then loaded onto a Q-sepharose 16/10 column (GE Healthcare) equilibrated with 20 mM TrisHCl pH 7.0, containing 2 mM DTT and 5 mM EDTA. Protein elution was obtained linearly increasing the ionic strength from 0 to 1 M using NaCl. The fractions containing the protein were collected and ammonium sulfate (AMS) was added to a final concentration of 1 M. The solution was loaded onto a Phenyl Sepharose column, equilibrated with 20 mM TrisHCl pH 7.0, containing 1 M AMS, 2 mM DTT and 5 mM EDTA. Protein elution was obtained by gradually decreasing AMS concentration. Fractions containing the protein were concentrated using 3 kDa MWCO Centricon ultra-filtration units (Millipore) and stored at -80 °C. Immediately before the ITC experiments, the protein was polished loading it onto a Superdex-75 10/300 GL (GE Healthcare) equilibrated with 20 mM HEPES pH 8.0, 150 mM NaCl and eluted in the same buffer. The absence of any metal bound to the purified protein was confirmed by inductively coupled plasma emission spectroscopy (ICP-ES), using a procedure previously described [5].

Calorimetric experiments

Titration experiments were performed at 25 °C using a high-sensitivity VP-ITC microcalorimeter (MicroCal LLC, Northampton, MA). The raw data correspond to the power required by the calorimeter to maintain a constant temperature during and following injection. Each heat pulse corresponds to injection of Ni(II) into the protein solutions. In each titration, 10 µL volumes of a Ni(II) solution were injected into the protein solution in the same buffer (cell volume = 1.4093 mL, stirring speed = 290 rpm) using a computer-controlled 310 µL microsyringe. The reference cell was filled with deionized water. Care was taken to start the first addition after baseline stability had been achieved. A spacing of appropriate time between the ligand injections was applied to allow the baseline to go back to the initial value. For each titration, a control experiment was set up, titrating Ni(II) solution into the buffer alone, under the same conditions. The protein solutions were clear after the titration without any sign of protein aggregation or precipitation. The following conditions were used; concentrations are referred to the oligomer, dimer or tetramer depending on the established oligomerization state in solution:

- *Ec*NikR HA sites - 0.5 mM Ni(II), 1.5 µM protein in 20 mM HEPES pH 8.0, 150 mM NaCl;
- *Ec*NikR LA sites - 0.3 mM Ni(II), 1.0 µM protein pre-incubated with 4.0 µM Ni(II) in 20 mM HEPES pH 8.0, 150 mM NaCl;
- *Ec*RcnR - 1.2 mM Ni(II), 40 µM protein in 20 mM HEPES pH 7.0, 1 mM TCEP, 150 mM NaCl;
- *Sy*InrS - 0.5 mM Ni(II), 10 µM protein in 20 mM HEPES pH 7.8, 5 mM TCEP, 300 mM NaCl;
- *Sc*Nur - 0.15 mM Ni(II), 6.5 µM protein in 20 mM HEPES pH 8.0, 150 mM NaCl.

Integrated heat data were fitted using a non-linear least-squares minimization algorithm to theoretical equations corresponding to different fitting models (one set of site, two sets of sites, sequential binding sites), developed by MicroCal and embedded in the Origin software, used according to the manufacturer's instructions. ΔH (reaction enthalpy change in cal mol^{-1}), K_a (binding constant in M^{-1}) and n (number of binding sites), were the fitting parameters. The reaction entropy was calculated using the relationships $\Delta G = -RT\ln K_a$ (R = 1.9872 cal mol^{-1} K^{-1}, T = 298 K) and $\Delta G = \Delta H - T\Delta S$. The reduced chi-square parameter χ_v^2 ($\chi_v^2 = \chi^2/n$, where n is the degrees of freedom, $n = N_{idp} - N_{par}$, N_{idp} = number of points, and N_{par} = number of parameters floating in the fit) was used to establish the best fit.

The values given for the calculated thermodynamic parameters are apparent, and include contributions not only from metal binding but also from associated events, such as protein dimerization or deprotonation of the cysteines and consequent change in the buffer ionization state. The thermodynamic parameters derived from the best fits are reported in Table SI-1.

Table SI-1. Results of fitting analysis for the ITC experiments performed in the present work on the binding of Ni(II) to different nickel sensors.

***Ec*NikR (HA)**	Fitting model	Two sets of sites
	χ^2_ν	1.3×10^5
	N_1	1.9 ± 0.2
	K_{a1} (M^{-1})	$(4 \pm 2) \times 10^7$
	K_{d1} (nM)	25 ± 12
	ΔH_1 (kcal mol^{-1})	0.8 ± 0.6
	ΔS_1 (cal mol^{-1} K^{-1})	+37.3
	N_2	1.8 ± 0.3
	K_{a2} (M^{-1})	$(2.8 \pm 0.8) \times 10^6$
	K_{d2} (nM)	400 ± 100
	ΔH_2 (kcal mol^{-1})	-16 ± 4
	ΔS_2 (cal mol^{-1} K^{-1})	-23.7
***Ec*NikR (LA)**	Fitting model	One sets of sites
	χ^2_ν	2.1×10^4
	N	4.1 ± 0.1
	K_a (M^{-1})	$(1.4 \pm 0.2) \times 10^6$
	K_d (nM)	700 ± 100
	ΔH (kcal mol^{-1})	-5.6 ± 0.1
	ΔS (cal mol^{-1} K^{-1})	+9.48
***Ec*RcnR**	Fitting model	Two sets of sites
	χ^2_ν	320
	N_1	1.97 ± 0.03
	K_{a1} (M^{-1})	$(6 \pm 5) \times 10^7$
	K_{d1} (nM)	17 ± 14
	ΔH_1 (kcal mol^{-1})	1.78 ± 0.04
	ΔS_1 (cal mol^{-1} K^{-1})	+41.6
	N_2	2.2 ± 0.1
	K_{a2} (M^{-1})	$(5 \pm 4) \times 10^6$
	K_{d2} (nM)	300 ± 200
	ΔH_2 (kcal mol^{-1})	0.11 ± 0.04
	ΔS_2 (cal mol^{-1} K^{-1})	+30.9
***Sc*InrS**	Fitting model	Two sets of sites
	χ^2_ν	2.8×10^4
	N_1	1.7 ± 0.01
	K_{a1} (M^{-1})	$(1.4 \pm 0.3) \times 10^7$
	K_{d1} (nM)	70 ± 15
	ΔH_1 (kcal mol^{-1})	-0.8 ± 0.2
	ΔS_1 (cal mol^{-1} K^{-1})	+30.2
	N_2	2.3 ± 0.1
	K_{a2} (M^{-1})	$(2.2 \pm 0.2) \times 10^5$
	K_{d2} (nM)	4500 ± 400
	ΔH_2 (kcal mol^{-1})	-15.5 ± 0.8
	ΔS_2 (cal mol^{-1} K^{-1})	-27.5
***Sc*Nur**	Fitting model	Two sets of sites
	χ^2_ν	1.86×10^4
	N_1	2.0 ± 0.2
	K_{a1} (M^{-1})	$(1.0 \pm 0.9) \times 10^8$
	K_{d1} (nM)	10 ± 9
	ΔH_1 (kcal mol^{-1})	-6.6 ± 0.1
	ΔS_1 (cal mol^{-1} K^{-1})	+14.5
	N_2	1.6 ± 0.2
	K_{a2} (M^{-1})	$(3.6 \pm 0.6) \times 10^6$
	K_{d2} (nM)	280 ± 50
	ΔH_2 (kcal mol^{-1})	-5.4 ± 0.4
	ΔS_2 (cal mol^{-1} K^{-1})	11.9

Light scattering measurements

*Ec*RcnR (500 µL, 40 µM) in 20 mM HEPES pH 7.0, 150 mM NaCl, 1 mM TCEP was loaded onto a Superdex-75 HR 10/300GL column (GE-Healthcare) pre-equilibrated with the same buffer, and eluted at room temperature at a flow rate of 0.6 mL min^{-1}. The effect of Ni(II) ions on the protein oligomeric state was investigated pre-incubating the protein with stoichiometric concentrations of $NiSO_4$ and using a running buffer containing the same Ni(II) concentration. The column was connected downstream to a multi-angle laser light (690.0 nm) scattering (MALS) DAWN EOS photometer (Wyatt Technology). Quasi-elastic (dynamic) light scattering data (QELS) were collected at a 90° angle using a WyattQELS device. The concentration of the eluted protein was determined using a refractive index detector (Optilab DSP, Wyatt). Values of 0.185 mL g^{-1} for the refractive index increment (dn/dc) and 1.31 for the solvent refractive index were used. Molecular weights were determined using a Zimm plot. Data were analyzed using the Astra software (Wyatt Technology), following the manufacturer's indications.

Homology modeling

The multiple sequence alignment between *Escherichia coli* (*Ec*) RcnR, InrS from *Thermosynechococcus* sp. NK55a (*Ts*InrS) and *Synechocystis* PCC6803 (*Sy*InrS), as well as CsoR from *Mycobacterium tuberculosis* (*Mt*CsoR), *Thermus thermophiles* HB8 (*Tt*CsoR), *Geobacillus thermodenitrificans* NG80-2 (*Gt*CsoR), and *Streptomyces lividans* (*Sl*CsoR) was produced using the Promals3D server [6] (see Figure 2A in the main text). This alignment was then used to calculate 30 structural models of the tetramers of *Ec*RcnR and *Sy*InrS in the *apo* forms using Modeller v9.12 [7] and the *Mt*CsoR dimer and the *Tt*CsoR, *Gt*CsoR, and *Sl*CsoR tetramer structures [PDB codes 2HH7, 3AAI, 4M1P, and 4ADZ, respectively] as templates. Symmetry restraints were imposed in the calculation in order to obtain identical monomers. The best *Ec*RcnR and *Sy*InrS models was selected on the basis of the lowest value of the DOPE score in Modeller [8]. A loop optimization routine was used to refine the regions that showed higher than average energy as calculated using the DOPE score. The results of the PROCHECK analysis [9] for the final models were fully satisfactory (>95% of the residues in the most favored regions of the Ramachandran plot and no residues in the disallowed regions.

Figure 1-SI. Raw titration data of Ni(II) into a solution of *Ec*NikR, representing the thermal effect of 10 µL injections of metal ion solution (1.2 mM) into protein solution (40 µM).

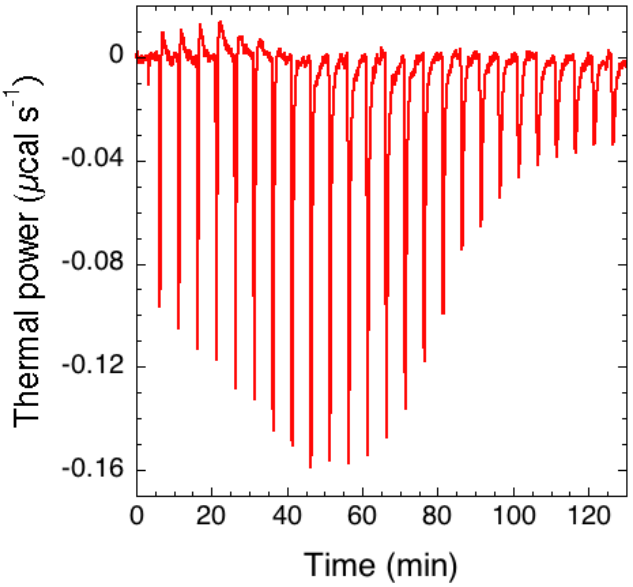
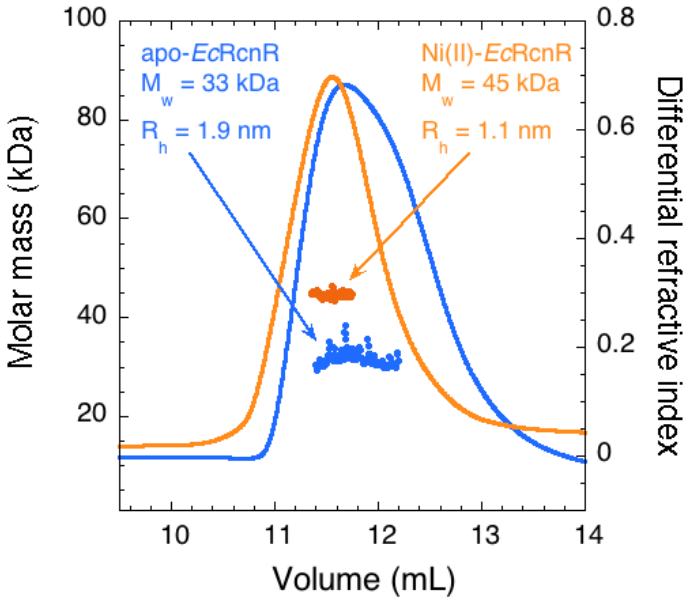


Figure 2-SI. Molar mass distribution plot for *Ec*RcnR in the absence (light blue) and in the presence (orange) of an equimolar amount of Ni(II). The solid lines indicate the traces of the refractive index detector for the protein eluting from the size exclusion column, and the dots represent the weight average molecular weights for each slice (i.e. measured every 0.2 s). The legend indicates the molar mass calculated by MALS and the hydrodynamic radius calculated by QELS, for each form of the eluting protein.



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