Supplementary Materials: Molecular Modelling of the Ni(II)-Responsive *Synechocystis* PCC 6803 Transcriptional Regulator InrS in the Metal Bound Form

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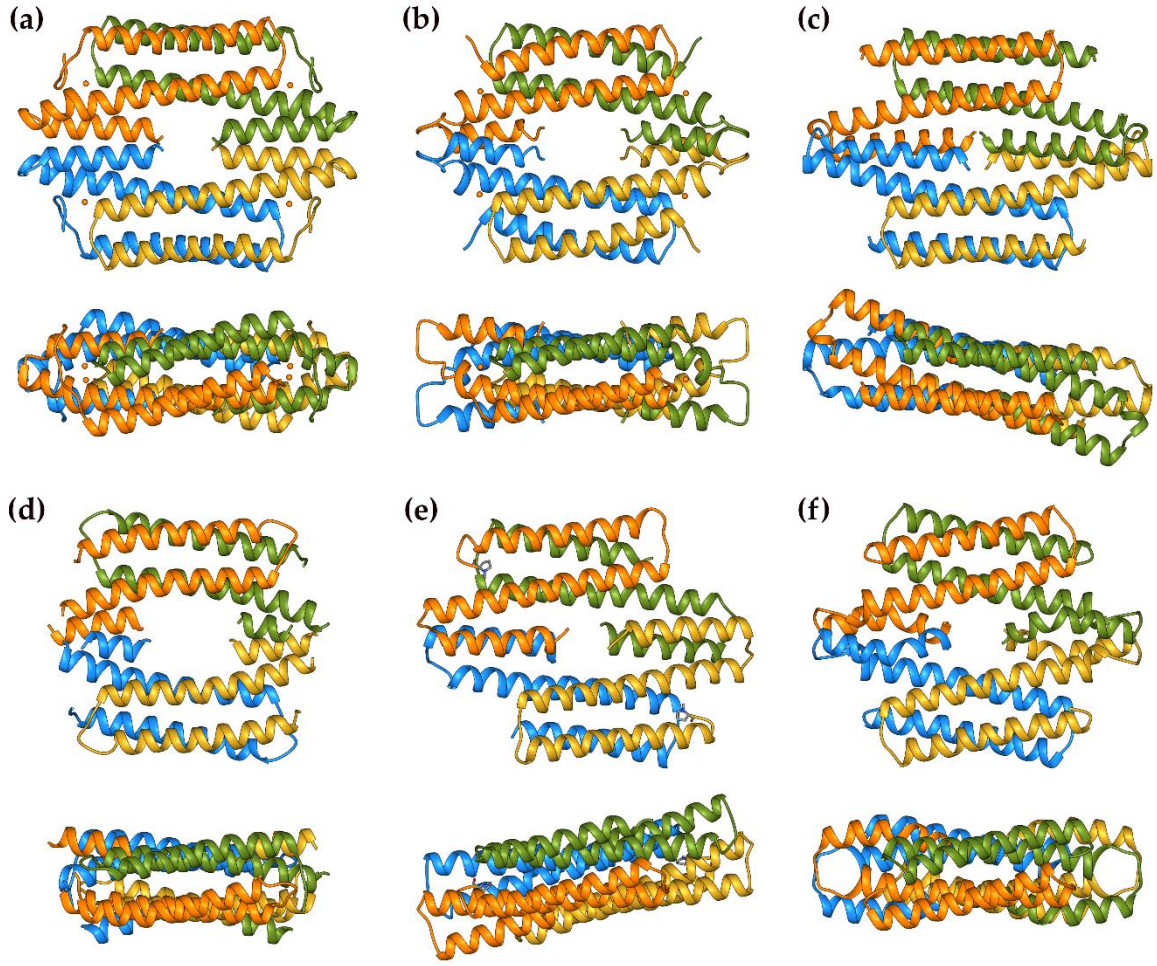

Figure S1. RcnR/CsoR family solved structures. CsoR from (**a**) *Geobacillus thermodenitrificans* NG80-2 (*Gt*CsoR, PDB id: 4M1P) [1], (**b**) *Mycobacterium tuberculosis* (*Mt*CsoR, PDB id: 2HH7) [2], (**c**) *Streptomyces lividans* (*Sl*CsoR, PDB id: 4ADZ) [3], and (**d**) *Thermus thermophiles* HB8 (*Tt*CsoR, PDB id: 3AAI) [4]; together with FrmR from (**e**) *Escherichia coli* (*Ec*FrmR, PDB id: 5LBM, **E**) [5] and (**f**) *Salmonella typhimurium* (strain SL1344) (*St*FrmR, PDB id: 5LCY) [6]. All the structures are reported as ribbons. Chains α, β, α', and β' are coloured in orange, green, light blue and yellow, respectively. Cu(I) ions are depicted as orange spheres, while formaldehyde bound proline residues in *Ec*FrmR is reported as sticks coloured according to atom type. The structure in the bottom panels are rotated by 90° around the horizontal axis with respect to the structure in the top panels.

Table S1. CsoR/RcnR family representative sequences used for the multiple sequence alignment reported in Figure S2.

Protein	UniProt code	Source	Inducer
*Sy*InrS	Q55554	*Synechocystis* PCC 6803	Ni(II)
*Th*InrS	V5V4A7	*Thermosynechococcus* sp. NK55a	Ni(II)
*Ec*RcnR	P64530	*Escherichia coli*	Ni(II)/Co(II)
*Mt*CsoR	P9WP49	*Mycobacterium tuberculosis*	Cu(I)
*Tt*CsoR	Q5SHL1	*Thermus thermophilus*	Cu(I)
*Cg*CsoR	A4QB25	*Corynebacterium glutamicum*	Cu(I)
*Sl*CsoR	Q9KZW5	*Streptomyces lividans*	Cu(I)
*Bs*CsoR	O32222	*Bacillus subtilis*	Cu(I)
*Gt*CsoR	A4INJ9	*Geobacillus thermodenitrificans*	Cu(I)
*Lm*CsoR	Q8Y646	*Listeria monocytogenes*	Cu(I)
*Sa*CsoR	W8UW13	*Staphylococcus aureus*	Cu(I)
*Lf*NcrB	Q06VT2	*Leptospirillum ferriphilum*	Ni(II)/Co(II)
*Mt*RicR	O07434	*Mycobacterium tuberculosis*	Cu(I)
*Ec*FrmR	P0AAP3	*Escherichia coli*	Formaldehyde
*St*FrmR	A0A0H3NLH8	*Salmonella typhimurium* (strain SL1344)	Formaldehyde
*Sa*CstR	[a]	*Staphylococcus aureus* NCTC 8325	HS^-/SO_3^{2-}

[a] Encoded by the complementary strand of nucleotides 37974-38234 in the *S. aureus* strain Newman genome [7].

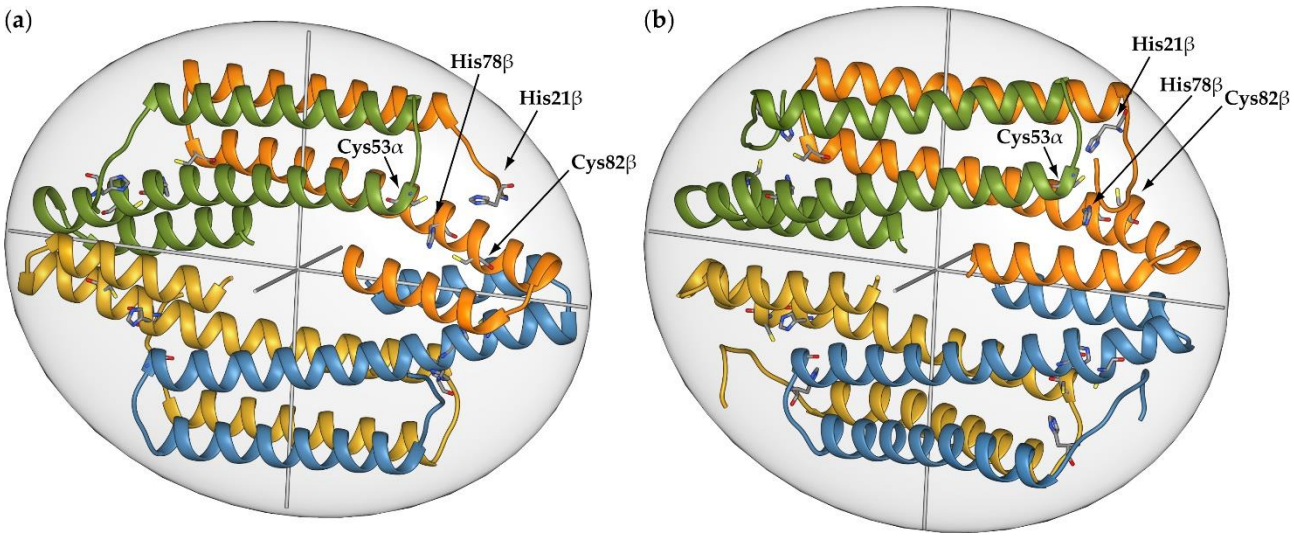

Figure S2. Ribbon diagram and inertia ellipsoid of *Sy*InrS in the apo (**a**) and holo (**b**) conformations. Chains α, β, α', and β' are coloured in orange, green, yellow, and light blue, respectively. Proposed Ni(II) binding residues are reported as sticks coloured accordingly to the atom type. The axes of the inertia ellipsoids are reported as grey sticks.

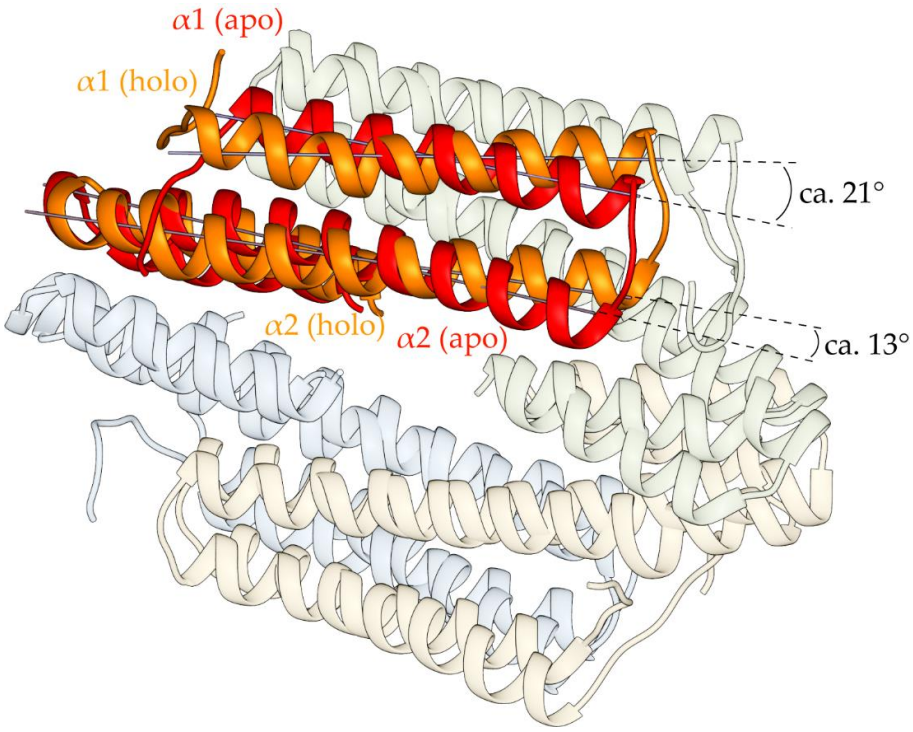

Figure S3. Detail of the α-helices α1 and α2 rotation occurring during the apo to holo conformational transition. Chain α is reported in red and in orange for the apo and the holo conformation, respectively. The other chains were made transparent in order to increase the clarity of the figure. The axes of the α-helices are reported as grey sticks.

Table S2. Structural analysis of the models of Ni(II) bound *Sy*InrS generated starting from the protein in the apo conformation.

Ramachandran plot region	**Apo model**			
	H21(Nδ)-H78(Nε)	**H21(Nδ)-H78(Nε)**	**H21(Nδ)-H78(Nδ)**	**H21(Nε)-H78(Nε)**
Most favoured	92.6%	96.3%	96.3%	93.8%
Additionally allowed	6.2%	3.7%	3.7%	2.5%
Generously allowed	1.2%	-	-	1.2%
Disallowed	-	-	-	2.5%
G-factor	-0.1	0.06	-0.03	-0.03

Table S3. Structural analysis of the models of Ni(II) bound *Sy*InrS generated starting from the protein in the holo conformation.

Ramachandran plot region	**Holo model**			
	H21(Nδ)-H78(Nε)	**H21(Nδ)-H78(Nε)**	**H21(Nδ)-H78(Nδ)**	**H21(Nε)-H78(Nε)**
Most favoured	94.2%	94.2%	93.0%	90.7%
Additionally allowed	5.8%	4.7%	5.8%	9.3%
Generously allowed	-	1.2%	1.2%	-
Disallowed	-	-	-	-
G-factor	0.09	0.03	0.03	0.00

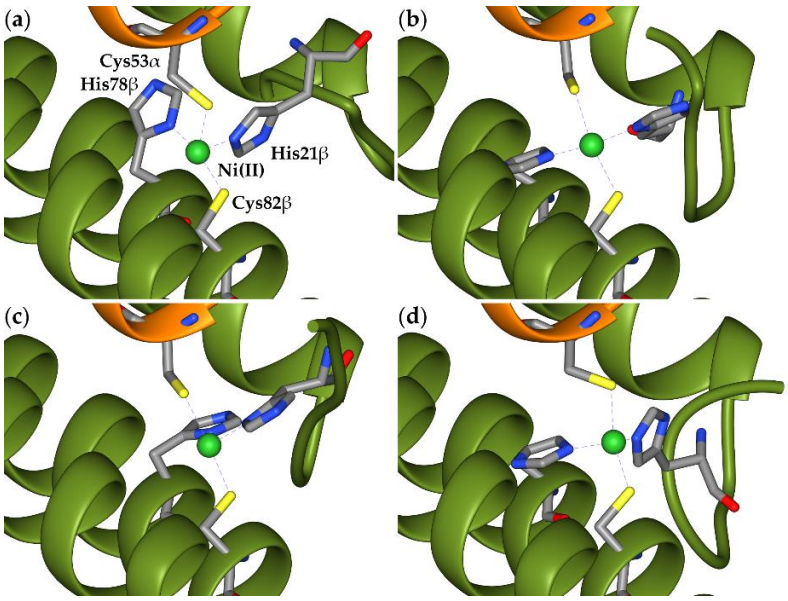

Figure S4. Results for the H21(Nε)-H78(Nδ) (**a**), H21(Nδ)-H78(Nε) (**b**), H21(Nδ)-H78(Nδ) (**c**), and H21(Nε)-H78(Nε) (**d**) modelling of Ni(II) bound *Sy*InrS starting from the protein in the apo conformation. The *Sy*InrS backbone is reported as ribbons coloured by polypeptide chains, with chain α in orange and chain β in green. Putative metal binding residues are reported as sticks coloured according to atom types. The Ni(II) ion is shown as a green sphere.

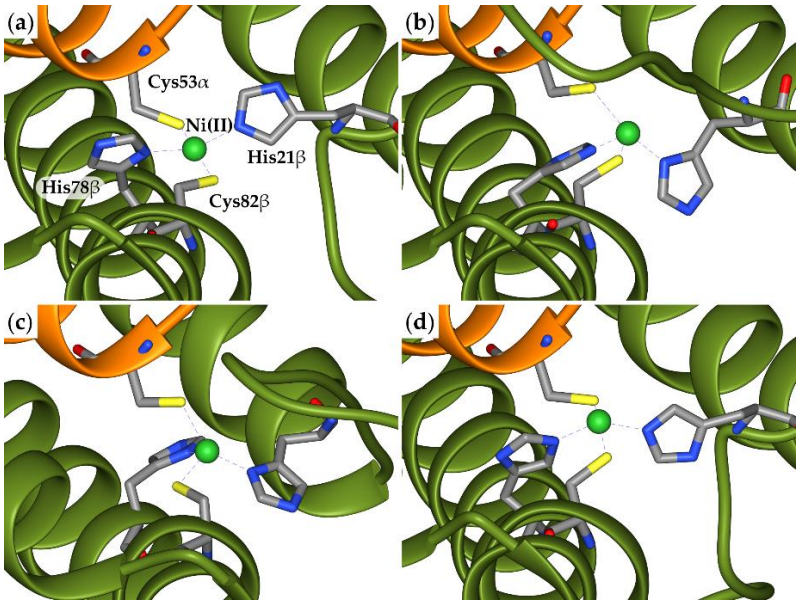

Figure S5. Results for the H21(Nε)-H78(Nδ) (**a**), H21(Nδ)-H78(Nε) (**b**), H21(Nδ)-H78(Nδ) (**c**), and H21(Nε)-H78(Nε) (**d**) modelling of Ni(II) bound *Sy*InrS starting from the protein in the holo conformation. The *Sy*InrS backbone is reported as ribbons coloured by polypeptide chains, with chain α in orange and chain β in green. Putative metal binding residues are reported as sticks coloured according to atom types. The Ni(II) ion is shown as a green sphere.

Supplementary References

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