Supporting Information

Ni(II), Hg(II), and Pb(II) Coordination in the Prokaryotic Zinc-Finger Ros87

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Table S1. Dissociation constants for both xenobiotic and native metal ions found in literature for eukaryotic classical zinc finger domains.

Zinc Finger	Binding mode	Zn(II)	Ni(II)	Pb(II)	Buffer and pH	Ref.
Sp1-3	CCHH	$6*10^{-10}$	$4*10^{-6}$		50 mM HEPES, 50 mM NaCl pH 7.0	66
Cp-1	CCHH	$5.7*10^{-12}$ (a)	$1.6*10^{-6}$(b)	$5*10^{-11}$(c)	(a) 100 mM HEPES, 50 mM NaCl pH 7.0	(a) 67
					(b) 100 mM HEPES, 50 mM NaCl pH 7.0	(b) 68
					(c) 10 mM Bis-Tris pH7.0	(c) 38
TFIIIA	CCHH	$1.0*10^{-8}$	$2.3*10^{-5}$		(a) 50 mM HEPES, 50 mM KCl, pH 7.0	4
MTF1-1	CCHH	$2.0*10^{-10}$	$2.1*10^{-5}$		50 mM HEPES, 100 mM NaCl or $NaClO_4$ pH 7.4	62
Sp1-3	CCHH	$5.0*10^{-9}$	$2.9*10^{-6}$		50 mM HEPES, 100 mM NaCl or $NaClO_4$ pH 7.4	62
ZF133-11	CCHH	$2.2*10^{-10}$	$3.2*10^{-6}$		50 mM HEPES, 100 mM NaCl or $NaClO_4$ pH 7.4	62
ZF278-1	CCHH	$6.3*10^{-11}$	$3.5*10^{-6}$		50 mM HEPES, 100 mM NaCl or $NaClO_4$ pH 7.4	62
TFIIIA-F3	CCHH	$4.0*10^{-8}$		$2.0*10^{-9}$	20 mM HEPES pH 7.4	22

Table S2. Experimental ^{N}H and N PCSs measured for Ni(II)-Ros87.

Residue		^{N}H PCS (ppm)	^{15}N PCS (ppm)
ALA	1		
VAL	2		
ASN	3		
VAL	4		
GLU	5	-0.007	0.094
LYS	6	-0.108	-0.371
GLN	7	0.270	0.208
LYS	8	-0.123	-0.082
PRO	9		
ALA	10	-0.122	-0.321
VAL	11	-0.162	-0.827
SER	12	-0.066	-0.414
VAL	13	-0.029	0.091
ARG	14		
LYS	15		
SER	16	-0.031	-0.168
VAL	17		
GLN	18	0.133	0.045
ASP	19	0.103	0.141
ASP	20	0.148	0.605
HIS	21	0.105	0.052
ILE	22		
VAL	23	0.454	1.734
CYS	24		
LEU	25	0.212	0.685
GLU	26		
CYS	27		
GLY	28		
GLY	29		
SER	30	0.584	0.655
PHE	31	0.802	0.630
LYS	32	0.576	1.104
SER	33	0.492	1.731
LEU	34	0.849	1.353
LYS	35	0.364	0.564
ARG	36		
HIS	37		
LEU	38	0.330	0.001
THR	39		

THR	40	-0.329	-0.111
HIS	41	-1.024	-1.105
HIS	42		
SER	43		
MET	44	-0.528	-0.724
THR	45	-0.238	-0.394
PRO	46		
GLU	47	-0.122	-0.208
GLU	48	-0.249	-0.344
TYR	49		
ARG	50	-0.327	-0.348
GLU	51	-0.242	-0.264
LYS	52	-0.220	-0.803
TRP	53	-0.420	-0.403
ASP	54	-0.225	-0.244
LEU	55	-0.050	-0.124
PRO	56		
VAL	57		
ASP	58	-0.028	-0.104
TYR	59	0.005	0.104
PRO	60		
MET	61		
VAL	62		
ALA	63	0.366	0.351
PRO	64		
ALA	65	0.134	0.039
TYR	66		
ALA	67	0.069	0.092
GLU	68	0.010	0.224
ALA	69	0.085	0.019
ARG	70	0.054	0.098
SER	71	0.150	0.078
ARG	72	0.043	0.071
LEU	73	0.026	0.042
ALA	74	0.031	0.097
LYS	75	-0.026	-0.110
GLU	76	-0.019	-0.127
MET	77	-0.020	-0.181
GLY	78	-0.032	-0.094
LEU	79	-0.008	0.005
GLY	80	0.016	0.094
GLN	81	0.010	0.064
ARG	82	0.062	0.041
ARG	83		
LYS	84	-0.002	0.002
ALA	85		
SER	86		
ARG	87	-0.002	-0.020

Figure S1. Primary sequence of Ros protein. The region expressed (Ros87) is colored in red and the residues involved in the zinc coordination are underlined.

1 MTETAYGNAQDLLVELTADIVAAYVSNHVVPVTELPGLISDVHTALSGTS

51 APASVAVNVEKQKPAVSVRKSVQDDHIVCLECGGSFKSLKRHLTTHHSMT

101 PEEYREKWDLPVDYPMVAPAYAEARSRLAKEMGLGQRRKANR

Figure S2. Far-UV CD spectra of Apo-Ros87 (red line), Hg(II)-Ros87 (green), Pb(II)-Ros87 (yellow), Ni(II)-Ros87 (orange) and Zn(II)-Ros87 (violet). All the spectra were acquired at 298K, normalized against the protein concentration and presented as molar ellipticity using the software Spectragryph (F. Menges "Spectragryph - optical spectroscopy software", Version 1.2.9, 2016-2018, http://www.effemm2.de/spectragryph/).

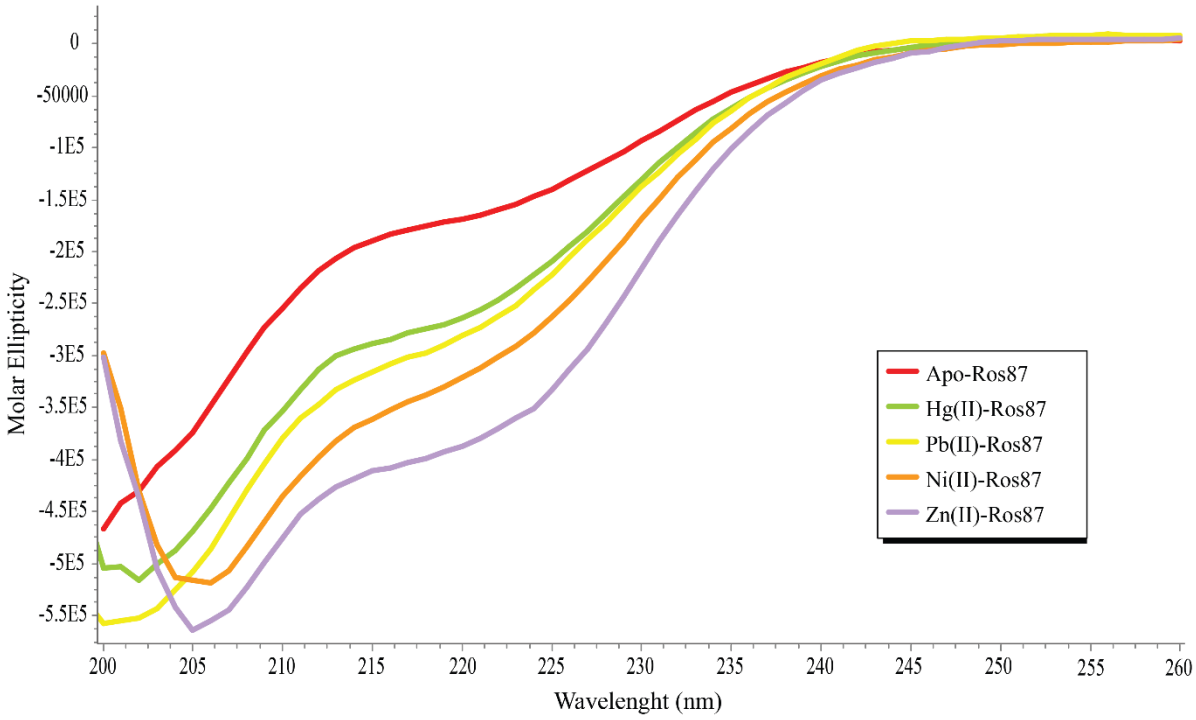

Figure S3. Portions of the ^{1}H-^{15}N HSQC spectrum of apo-Ros87 in presence of 2.4 molar equivalents of Pb(II). The spectrum was acquired using 13.8 ms tranfer delay to allow the coherence transfer from the $H_{\varepsilon 1}$ and $H_{\delta 2}$ histidine side chain protons to $N_{\varepsilon 2}$ and $N_{\delta 1}$ through the $^{2}J_{HN}$ coupling constant. The observed chemical shift patterns indicate that three of the four histidine side chains are mostly in $N_{\varepsilon 2}$-H tautomer forms, whereas the forth appear in a protonated state.

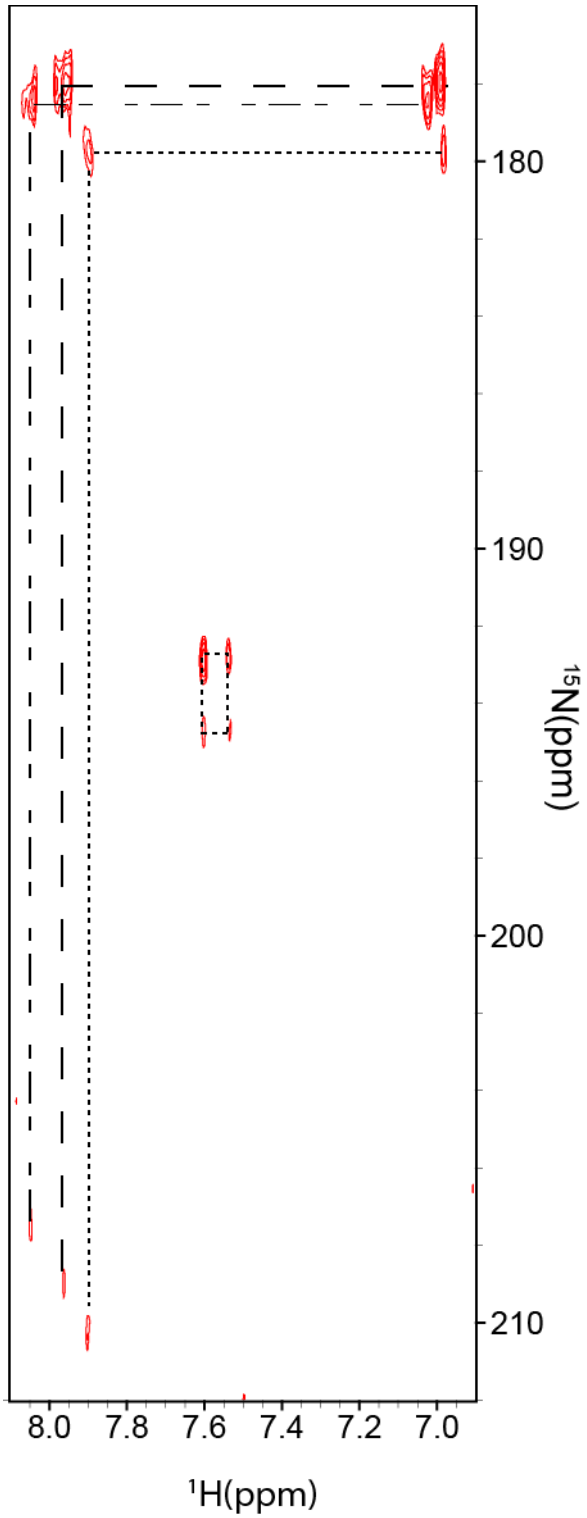

Figure S4. U.V.-Vis spectra of apo-Ros87 titration with $NiCl_2$. The inset shows a magnification of the visible region.